CONSERVATION OF A FOSSORIAL GRASSLAND SPECIES (*GEOMYS BURSARIUS*)
THROUGH UNDERSTANDING NICHE REDUCTION, LANDSCAPE GENETICS, AND
PHYLOGENETICS

BY

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DISSERTATION

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ABSTRACT

Considering species conservation through temporal and spatial lenses better identifies threats and can inform management. This is particularly true for grassland species such as the plains pocket gopher (*Geomys bursarius*) that persist in anthropogenically altered landscapes. Here, I first identified ongoing habitat degradation due to agricultural intensification since the 1950s for *G. bursarius* in Illinois. I created species distribution models for *G. bursarius* in Illinois during two eras, the 1950s and the 2010s, and estimated niche shift and niche breadth. *Geomys bursarius* in Illinois exhibited niche shifts and a decrease in niche breadth, with increased occurrence at lower elevations and in sandier soils. The core of the *G. bursarius* distribution in Illinois decreased in suitability, a common phenomenon for species under anthropogenic pressures. Second, I identified genetic structure and gene flow patterns for *G. bursarius* at different spatial scales, and then partitioned gene flow to multiple isolating factors: barriers, distance, and environment. Rangewide, major rivers functioned as barriers, but in Illinois, distance predominantly explained genetic variance. At both spatial scales, soil sand percentage and soil color explained a small proportion of genetic variance. Finally, I inferred a phylogenetic tree for subspecies of *G. bursarius* with a focus on whether *G. b. illinoensis* was monophyletic using mitochondrial genomes (mitogenomes). I found that *G. b. illinoensis* was monophyletic and genetically unique from the other *G. bursarius* subspecies, and that additional subspecies (e.g., *G. b. wisconsinensis*) were genetically unique as well. *Geomys bursarius illinoensis* demonstrated admixture from *G. jugosscicularis* and *G. b. missouriensis*, likely due to retaining ancestral mitogenomes. Overall, *G. b. illinoensis* should be considered an Evolutionary Significant Unit currently facing niche reduction and niche shifts, and management should focus on increasing connectivity and occupancy in regions where *G. bursarius* occurred in the 1950s.
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CHAPTER 1: GENERAL INTRODUCTION

Conservation efforts require a multi-pronged approach, especially in the Anthropocene as habitat loss and climate change continue to decrease genetic diversity (Exposito-Alonso et al. 2022) and cause species declines (Haddad et al. 2015; Bartlett et al. 2016; Fardila et al. 2017; Stewart et al. 2017; Quaglietta et al. 2018; Lino et al. 2019). In particular, approaches that consider temporal dynamics are required to understand historic baselines and current status and threats (Wang 2010; Yannic et al. 2014; Martensen et al. 2017). Understanding ongoing pressures, and identifying potential refugia under climate change, can guide managers (Selwood and Zimmer 2020) as environmental processes continue to threaten taxa-specific conservation goals (Hanson et al. 2020; Exposito-Alonso et al. 2022).

The species niche concept allows for an approach that can quantify a species interaction with the environment and inform conservation goals (Grinnell 1917; Hutchinson 1957; MacArthur and Levins 1967; Guisan and Theurillat 2000). Although habitat loss is a major driver of extinction (Pacifici et al. 2023), Hutchinson’s (1957) concept of an n-dimensional hypervolume allows for quantification of realized niches in which the effects of niche loss or expansion can be separated from the effects of habitat loss (Scheele et al. 2017). It is important to account for continued pressures impacting a species’ realized niche, as interactions with landscapes are dynamic, and how these pressures may impact a species’ persistence (Scheele et al. 2017; McDonald et al. 2018; Rutrough et al. 2019; von Takach et al. 2020; Alexander et al. 2022). The niche reduction hypothesis accounts for novel factors that can limit realized niches such as invasive species (McDonald et al. 2018), disease (Scheele et al. 2019), initial land conversion (Rutrough et al. 2019), climate change (von Takach et al. 2020), and land-use intensification (Alexander et al. 2022). Species distribution modeling can identify shifts and
reductions in the suitability of environmental features associated with a species’ occurrence (Elith and Leathwick 2009; Soberon and Nakamura 2009; Elith et al. 2011) and can identify niche reductions before and after a novel threat (Rutrough et al. 2019; von Takach et al. 2020; Alexander et al. 2022).

Genetic variation is an important component of maintaining biodiversity (DeWoody et al. 2021; Willi et al. 2022), but habitat fragmentation can reduce connectivity between populations, limit gene flow, and produce genetic structure due to isolation (Manel et al. 2003; McRae 2006; Sexton et al. 2014; Wang and Bradburd 2014). Common forms of isolating mechanisms include distance (Wright 1943; Jenkins et al. 2010), barriers (Pfau et al. 2001; Musher et al. 2022), the habitat matrix between occupied patches (Adriaensen et al. 2003; McRae 2006; Zeller et al. 2012), habitat similarities at occupied patches (Sexton et al. 2014; Wang and Bradburd 2014), individual phenotypes (Wang and Summers 2010), or interactions between all of these different drivers (Orsini et al. 2013; Priadka et al. 2019; Muñoz-Valencia et al. 2023). In some cases, individuals can cross longer distances than expected, indicating that species interact with the landscape differently during dispersal than during home range establishment (Centeno-Cuadros et al. 2011; Alexander et al. 2019). However, species with low dispersal distances may face the impacts of local adaptation to habitats similar to the natal patch, isolation by distance, and isolation by barriers in disproportionate ways (Weber et al. 2016; Muñoz-Valencia et al. 2023). Through identifying unique genetic clusters and genetic similarities associated with different environmental drivers, managers can identify management units or areas to improve gene flow for small populations (Segelbacher et al. 2010; Willi et al. 2022).

Finally, although understanding the realized niche and gene flow of a species is important, conservation also requires determining taxonomic relationships and status (Mace 2004; Avise
2009; Willi et al. 2022; Hoelzel 2023). The reason for this is two-fold. First, listing criteria for conservation often requires that a population or subspecies be a monophyletic group (Avise et al. 1987; Moritz 1994, 2002; Coates et al. 2018). Second, understanding historic processes and genetic structure can inform contemporary landscape genetics and can help to tease apart effects of long-term barriers compared to more recent isolating events (Ficetola et al. 2007; Wang 2011; Epps and Keyghobadi 2015), bridging macro- and micro evolutionary processes (Avise 2009; Avise et al. 2016; Rissler 2016). Through understanding the historic colonization and distribution of a taxon, along with determining criteria for identifying subspecies or units worth conserving, management efforts can be targeted for unique evolutionary lineages that are at risk.

Overall, conservation and management require understanding a species distribution and impacts on a species niche, contemporary population structure and connectivity, and taxonomic status and historic genetic signatures. Moreover, determination of conservation units often relies on confirming taxonomic status through nuclear and mitochondrial genetics (Mace 2004; Hoelzel 2023). This approach shows promise particularly for management of species persisting in heavily altered landscapes distributed across a large area.

Applications to a grassland species- Geomys bursarius

Prairies have a long legacy in central North America, shaping range expansion since glacial sheets retreated northward. With glacial recession, a mosaic of soil textures formed, and grasslands became the dominant vegetation (Robertson et al. 1997). However, these prairies also created agriculturally productive soils leading to land conversion from prairies to farmlands (Ramankutty and Foley 1999). Only <0.5% of prairies in upper Midwest persist, with some regions declining to <0.01% of their historic distribution (Robertson et al. 1997). The prairies
that persist in these regions are also noncontiguous and small, often <4 ha with 30% being <0.4 ha (Klopatek et al. 1979). During the Pleistocene, a grassland peninsula extended eastward, allowing western taxa to colonize across the Mississippi River (Smith 1957). Within this peninsula, prairie loss has been particularly severe (Robertson et al. 1997). However, in addition to initial prairie loss, there has been more recent agricultural intensification in which farm size has increased and farming practices have industrialized (Hart 1986; Garcia et al. 1987; Warner 1994; Sulc and Tracy 2007). Understanding responses of fossorial taxa historically associated with prairies can clarify effects of agricultural intensification and identify anthropogenic threats to gene flow.

One grassland species that expanded northward post glaciation, including spreading eastward along the grassland peninsula during the Pleistocene, is the plains pocket gopher (*Geomys bursarius*; Smith 1957; Russell 1968). *Geomys bursarius* ranges from Texas to the southern border of Canada, and from Indiana to New Mexico, occupying a large distribution across the central United States (Connior 2011). *Geomys bursarius* has strong associations with soils likely due to preferences for friability and drainage (Reichman and Seabloom 2002; Connior 2011; Alexander et al. 2022). Soils may also genetically structure gopher populations due to camouflaging pelage against soil color resulting from local adaptation (Hendricksen 1972; Krupa and Geluso 2000). Rivers may have become more of a barrier, potentially due to shifting drainages of the Missouri and Ohio Rivers during the middle Pleistocene, increased flooding, channel incision, and erosion during the Holocene, and channelization during the Anthropocene (Bentley et al. 2016; Fildani et al. 2018; Lark et al. 2019), isolating populations that had expanded along the Pleistocene grassland peninsula (Huang et al. 2020). This could lead to genetic isolation of gophers, as they are poor swimmers (Hickman 1977). Finally, taxonomic
relationships within the *G. bursarius* clade need resolution to clarify its evolutionary history and understand conservation needs (Sudman et al. 2006; Chambers et al. 2009). Here, I assessed conservation of the plains pocket gopher through estimating niche reduction due to agricultural intensification, identifying genetically isolating mechanisms and genetic structure, and clarifying taxonomic status, providing strong support for management of *G. b. illinoensis* as a subspecies.

*Research approach*

Within this dissertation, I aim to provide comprehensive ecological insights with a goal toward informing management decisions of a plains pocket gopher subspecies (*G. b. illinoensis*) and contextualizing the status of *G. b. illinoensis* within the full *G. bursarius* species complex. To accomplish this, I (i) created species distribution models and estimate the realized niche hypervolume of *G. b. illinoensis* in Illinois, (ii) assessed genetic structure and barriers to gene flow for *G. bursarius* and specifically for *G. b. illinoensis* using nuclear markers, and (iii) determined phylogenetic structure of *G. bursarius* using mitochondrial genomes (mitogenomes).

In Chapter 2, I test the niche reduction hypothesis (Scheele et al. 2017) to *G. b. illinoensis* in Illinois. To do this, I estimated suitable habitat using MaxEnt, a presence/pseudo-absence species distribution model (Phillips et al. 2006, 2017), incorporating land cover, soil properties, and precipitation. I modeled *G. b. illinoensis* distributions using historic occurrences (1945-1955) and contemporary occurrences (2013-2019), and then assessed model transferability between the eras. Finally, I estimated niche hypervolumes to identify if the realized niche had shrunk as well as if there had been a shift in suitable habitats. From these analyses, I identified that *G. b. illinoensis* has experienced both a niche reduction as well as niche shifts, with historically occupied sites becoming unsuitable during a period of agriculture intensification.
In Chapter 3, I examine current genetic structure and identify isolating barriers across the *G. bursarius* range as well as specifically for *G. b. illinoensis*, exploring scale-dependent isolating factors. I used microsatellites, including samples from museum specimens and live trapping, to identify genetic clusters (Pritchard et al. 2000; Falush et al. 2003), estimate genetic spatial autocorrelation (Galpern et al. 2014), and partition genetic separation among isolation by barrier, isolation by distance (Wright 1943), and isolation by environment (Wang and Summers 2010; Wang and Bradburd 2014). Because gene flow and genetic structure may be scale dependent (Cushman and Landguth 2010), identifying isolating factors across spatial scales can highlight scale-dependent drivers. In this chapter, I identified major rivers such as the Mississippi, Illinois, and Missouri Rivers acting as isolating barriers across the *G. bursarius* range. Soil properties (i.e., soil sand percent and soil color) also impacted gene flow broadly. Within Illinois, distance was the dominant isolating factor.

Finally, in Chapter 4, I assess the phylogenetic structure of *G. bursarius* using mitogenomes and determined whether subspecies of conservation concern are monophyletic and meet standards for being treated as genetic conservation units (Moritz 1994; Mace 2004). I relied on multiple lines of evidence to determine phylogenetic structure, including haplotype network (Leigh and Bryant 2015), assessed the evolutionary clock (Tamura et al. 2021), and inferred phylogenetic trees (Drummond and Rambaut 2007). I also assessed genetic uniqueness through principal components analysis and admixture (Sand Korneliussen et al. 2014; Meisner and Albrechtsen 2018). I determined that *G. b. illinoensis* is a monophyletic clade with admixture from *G. b. missouriensis* and *G. juggosicularis*, likely a result of historic genetic signatures. However, northern populations like *G. b. wisconsinensis* demonstrated unique genetic signatures but represent a more recent genetic divergence. Although other *G. bursarius* subspecies should
still be clarified (e.g., *G. b. major*), I established that *G. b. illinoensis* is genetically distinct and monophyletic, and provided stronger resolution for the phylogenetic structure of *G. bursarius*. 
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CHAPTER 2: TESTING THE NICHE REDUCTION HYPOTHESIS FOR A FOSSORIAL RODENT (*GEOMYS BURSARIUS*) EXPERIENCING AGRICULTURAL INTENSIFICATION

INTRODUCTION

Habitat loss and fragmentation remain leading threats to biodiversity (Elith and Leathwick 2009; Haddad et al. 2015) as they often decrease population size (Bartlett et al. 2016), genetic diversity (Lino et al. 2019), and area occupied (Stewart et al. 2017; Quaglietta et al. 2018). However, directly linking habitat change to a species’ niche is relatively recent (Scheele et al. 2017). The n-dimensional hypervolume concept describes how multiple environmental factors constrain a species distribution (Hutchinson 1957). There are two primary aspects of the niche: the fundamental niche representing the theoretical niche without limitations to environmental resources, and the realized niche representing a subset of the fundamental niche that incorporates limitations such as interspecific competition and dispersal (Hutchinson 1957; Pulliam 2000). A species can persist at any combination of environmental conditions within the realized niche hypervolume, and, if there were no constraints, within the fundamental niche hypervolume. Considering the niche as a hypervolume has expanded niche-based research to include variation among populations, connections between niche variation and genetic variation (Holt 2009), differing responses of life stages to climate variability (Jackson et al. 2009), and stronger links between niche hypervolume and geographic space (Colwell and Rangel 2009; Soberon and Nakamura 2009). Continued application of the niche concept has recently resulted in the ‘niche reduction hypothesis’ (Scheele et al. 2017).

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The niche reduction hypothesis highlights that novel threats to a species may not be uniform across the species’ realized niche (sensu Hutchinson 1957), thus leading to a reduced niche hypervolume as well as range reduction (Scheele et al. 2017). For example, invasive cats inhabited less complex habitats of the central rock-rat (Zygomys pedunculatus), limiting the central rock-rat’s realized niche to more rugged terrain (McDonald et al. 2018). Due to strong predation specifically in less complex habitats, the prey species’ environmental niche was reduced, omitting niche spaces that were historically occupied in specific environmental conditions. In addition to introduced predators, novel diseases (Scheele et al. 2019) and land conversion (Rutrough et al. 2019) may have effects on a subset of a species’ realized niche.

Understanding niche reduction in agricultural landscapes is a useful next step for global conservation efforts as agriculture is a major driver of landscape change worldwide (Ramankutty and Foley 1998, 1999; Newbold et al. 2015). Initial conversion of landscapes to agriculture may reduce a species’ realized niche (Rutrough et al. 2019), but agricultural expansion and intensification may promote further niche reduction. Agricultural expansion converts high quality habitats into crop fields (Lark et al. 2020) including bringing lands into development as they expire from conservation programs (Morefield et al. 2016). Once a landscape is converted to agriculture, agricultural practices evolve to meet increased yield demands (Hunter et al. 2017), and this agricultural intensification can increase soil compression (Keller and Or 2022) and decrease species richness (Carmona et al. 2020; Raven and Wagner 2021). Although agricultural intensification produces higher crop yields per area farmed, it does not necessarily prevent agricultural expansion (Rudel et al. 2009), and farmers may prioritize land resilient to flooding for agricultural production (e.g., higher elevations; Yang et al. 2021). Continued agricultural
expansion occurs, albeit at a lower rate, in tandem with agricultural intensification (Lin and Huang 2019).

Despite global effects of agriculture on species, the niche reduction hypothesis has only rarely been applied to agricultural land conversion, and to our knowledge, not to agricultural intensification. In one example involving land conversion, a burrowing mammal’s historic range was estimated to be 56% greater than its previously defined historic range, with a shift in the current realized niche due to agricultural development on natural lands (Rutrough et al. 2019). However, it remains unknown whether the niche reduction hypothesis applies more broadly across altered landscapes, especially those experiencing agricultural intensification after most initial land conversion has occurred. If it does, this hypothesis could provide a deeper understanding of niche dynamics and direct land management by identifying drivers of niche reduction or changes in limiting environmental factors.

To further define the scope of the niche reduction hypothesis, we apply the concept to the Midwestern region of the United States, a landscape highly modified by agriculture (Ramankutty and Foley 1998; Ramankutty et al. 1999; Lark et al. 2020). Specifically, Illinois often leads in agricultural intensifying practices in North America (Warner 1994). Farmers in Illinois have transitioned native haying grasses to European species and increased use of annual row crops, synthetic fertilizers, and mechanization (Warner 1994). Between the 1950s and the 1980s, cash-crop farms in Illinois increased in individual size with mid-sized farms growing to ≥400 ha each (Garcia et al. 1987). This increase in farm size is associated with growing corn, expanded soybean production, and a reduction in hay, oats, and pasture (Hart 1986; Warner 1994; Sulc and Tracy 2007). One impact of this agricultural intensification is that fence rows and breaks between crop fields were eliminated (Hart 1986). These landscape changes occurred
heterogeneously across Illinois since the 1950s (Warner 1994; Mankin and Warner 1999), possibly due to farmland-soil and crop yield-soil relationships (Mause 1971; Iverson 1988; Garcia-Paredes et al. 2000). Such spatial heterogeneity may promote niche reductions for species associated with grassland habitat.

Here, we tested for shifts in a species distribution and niche hypervolume between two eras: a historical era post land conversion but before recent agricultural intensification, and a contemporary era reflecting current agricultural practices. We selected a fossorial mammal, the Illinois plains pocket gopher (*Geomys bursarius illinoensis*), as our focal species. It has one of the more restricted ranges of eight subspecies (Fig. 2.1a; Hart 1971; Connior 2011), and is the only subspecies that persists east of the Mississippi River (Hart 1971) and south of the Illinois River (Hoffmeister 1989). Rivers are often boundaries to distributions and may serve as dispersal barriers due to the poor swimming ability of pocket gophers (Komarek and Spencer 1931; Kennerly 1963). The distribution of *G. bursarius* in Illinois has not been studied since 1935 (Mohr 1935), and its environmental niche has never been estimated. Gophers also are ecosystem engineers that contribute greatly to ecosystem processes by altering vegetation composition and biomass and soil properties (Huntly and Reichman 1994; Reichman and Seabloom 2002; Romañach et al. 2005). Therefore, changes in the area occupied or realized niche of gophers will affect functioning of agroecosystems.

Historically, gophers inhabited tallgrass prairie, but now also inhabit other grasslands, some cultivated fields, and urban areas. Native prairies in Illinois have drastically declined with only 0.01% remaining (Robertson et al. 1997). From 1820-1978, there was a reduction from 8.76 million ha to 931 ha, with most prairies converted to agriculture (Iverson 1988). Most (83%) remaining prairies are <4 ha, and 30% are <0.4 ha (Klopatek et al. 1979), creating a highly
fragmented landscape for a species historically associated with prairies. Agricultural conversion and intensification may remove other grassy refuges for burrowing mammals such as bringing road rights-of-way into production (Martin and Heske 2004). Thus, we would expect a niche reduction if specific land cover types within the gopher’s historic niche were disproportionately impacted by agricultural expansion and intensification.

Second, gophers have strong soil texture associations, yet they can occur in a range of soil textures (Connior 2011) including silt loam soils with <1% sand to sandy loam soils (Komarek and Spencer 1931). Species distributions of gophers are related to soil sand and loam percentages likely due to burrow construction and friability, however, these associations range drastically between species (Connior 2011; Warren et al. 2019). Illinois had prairies in sand, black-soil, gravel, and other soil categories (White 1978; Robertson et al. 1997), potentially leading to both landscape and soil associations for gophers. If prairies in specific soil types were lost to agriculture, gophers may exhibit two distinct responses: maintain associations with soils regardless of land cover, or become extirpated from specific soils due to land cover change. Agricultural intensification may also occur on specific soils as farming practices or crop types changed. Hence, we may expect a niche reduction if agricultural intensification impacted soil types non-uniformly.

Third, precipitation in Illinois has increased over time with a 10-year moving average of annual precipitation rising from ~920 mm in 1960 to ~1020 mm in 2011. Extreme precipitation events are also increasing (Dahal et al. 2018). Heavy rains may cause pooling in poorly drained soils (Krichels et al. 2019), leading to flooding of gopher burrows and mortality (Connior and Risch 2010). Thus, changing climate may affect species distributions and realized niches (Wiens et al. 2009)
To address our overarching hypothesis that gophers in Illinois experienced a niche reduction due to agricultural intensification since the 1950s, we developed species distribution models (SDMs) for current and historical eras to identify limiting environmental factors. Next, we created comparable models for the two eras to quantify changes in distribution and environmental associations. Finally, we estimated Hutchinsonian niche hypervolumes to determine if there was a reduction in the gopher’s realized niche over time. We predicted that both the contemporary and historic niches for gophers would include a preference for grasslands, sandy clay loam soils, higher elevations, and sites with lower precipitation and better soil drainage. We expected a decline in the suitability of agricultural land cover since the 1950s due to intensification and a shift to cultivated annual crops. Although grasslands are hypothesized to be the most suitable habitat even contemporarily for gophers, we expected grassland suitability to decrease over time due to continued small-scale land conversion, leaving grasslands in poorer soils and lower productivity areas. Collectively, these changes should result in a narrower niche hypervolume for gophers for the contemporary era relative to the historic era.

**METHODS**

*Contemporary SDM*

We created a contemporary SDM (2008-2019) for pocket gophers in Illinois to assess the current realized niche. SDMs use occurrence records to predict the distribution of species using environmental variables. Presence-pseudoabsence SDM models have increased in use, allowing collation of multiple sources of occurrence records (Valavi et al. 2021). Here, we combined gopher presences ($n = 204$ total, Fig. 2.1b) from four sources: 1) a presence-absence survey developed with stratified sampling ($n = 12$), 2) a presence-only driving survey ($n = 142$), 3)
downloaded presences from the Global Biodiversity Information Facility from 2008-2019 (n = 23), and 4) live-trapping of gophers (n = 27; University of Illinois Urbana-Champaign Institutional Animal Care and Use Committee protocol #17190). For the live-trapping, we followed Sikes et al. (2016) ethical guidelines using both box traps (Connior and Risch 2009) and bucket traps (Moore et al. 2019). Traps were checked every 3 hours. Initially, we conducted a presence-absence survey across the historic range in 2016-2017 and surveyed 75 grassland sites using both walking transects (2-4 transects of 100 m each within grasslands, including linear configurations such as roadside berms) and time-limited searches (15 min). We determined current gopher presences by identifying active gopher mounds (Quinn et al. 2010). Because of low occurrence rates, we shifted to more extensive presence-only surveys in which we drove across much of the historic range (≥2750 km) in 2019 and again identified gopher presences from active mounds.

MaxEnt estimates the relative probability of occurrence by comparing environmental covariates at occurrences to those at background points (Elith et al. 2011). This allows complex transformations to estimate relative habitat suitability and then predict suitability across the study region (Elith et al. 2011). We only modeled within the historic range of gophers in Illinois, with a 10 km buffer, refraining from projecting any models outside of the study area. We retained presences >1 km apart (n = 150) using the ‘spThin’ package (Aiello-Lammens et al. 2015) in R (R Core Team v. 4.1.1) because Geomys bursarius generally have home ranges < 0.5 km² (Zinnel 1992; Connior and Risch 2010).

We created a suite of environmental models at an ~30 m x 30 m resolution incorporating land cover (USDA National Agricultural Statistics Service 2019), soil texture categories, soil drainage class, percent sand (Soil Survey Staff 2018), elevation (U. S. Geological Survey 2006),
and precipitation mean and standard deviation from 2008-2018 (PRISM Climate Group 2014). Land cover included crop types \((n = 39)\), grasslands and pasture types \((n = 7)\) development intensity \((n = 3)\), forest types \((n = 6)\), water \((n = 1)\), and orchards \((n = 7; \text{Table A.3 in Appendix A})\). To ensure that we did not overparameterize the model, we also assessed SDMs using a reduced land cover classification \((n = 7; \text{Fig. A.1-2 in Appendix A})\). Soil texture, drainage class, and percent sand spatially varied predominantly based on past glaciation (Piskin and Bergstorm 1975) with 16 categories for soil texture, 6 categories of drainage ranging from poorly drained to excessively drained (Tables A.1-2 in Appendix A), and sand percent ranging from 0-90%. These environmental variables were rasterized to match the resolution of the elevation raster. There was limited variation in elevation, ranging from 112-281 m with slopes ranging from 0-27.5 degrees. Rainfall data were resampled from an 800 m x 800 m grain to ~30 m x 30 m using the nearest neighbor method. We averaged annual mean precipitation over 10 years (2008-2018), with yearly mean precipitation ranging from 899-1264 mm \((\text{SD} = 103-292 \text{ mm})\). There was higher mean precipitation in the south and higher deviation in precipitation towards the center of the gopher range. All 8 layers had a variance inflation factor \((\text{VIF}) < 2\), and they were not strongly correlated \((r < 0.6)\) and well within acceptable levels of parameter correlation for MaxEnt (Syfert et al. 2013). We further tested multicollinearity of the land cover and soil texture layers by extracting 1000 random cell values and conducting chi-square tests between all the variables \((\text{all } p > 0.25)\).

We ran MaxEnt v3.4.1 across all combinations of the 8 environmental layers and two values of smoothing parameters \((\beta = 1 \text{ or } 2)\) with 10,000 background points and 75% of the thinned occurrence data \((n = 106)\), leaving 25% for model testing. We selected the top models using Akaike’s Information Criterion corrected for small sample size \((\text{AIC}_c)\) in ENMTools.
(Warren et al. 2010), and then calculated area under the curve (AUC) for the training and test data. We also performed a Jackknife analysis to measure the relative contribution of each environmental variable in the model (Elith et al. 2011).

In addition to model testing using 25% of the data, we conducted a field validation of our top model during June-July 2020. We binned predicted habitat suitability into 4 classes (Rhoden et al. 2017) and sampled >20 points in each class: 0-0.059 (Very Low, n = 48; up to the lowest 5% of estimated habitat suitability at gopher occurrences from the contemporary model), 0.06-0.49 (Low, n = 38), 0.5-0.749 (Good, n = 23), and 0.75-1 (Very Good, n = 64). During the initial field validation, we noticed most ‘Very Good’ sites were within parks or lawns within cities and towns, so we included additional randomly selected sites with ‘Very Good’ habitat suitability that were outside of cities or towns (n = 34). To ensure property access, we restricted all validation sites to be near roads. Overall, we surveyed 173 new sites (Fig. 2.1b).

We then used a random forest classification tree to explain why gophers occurred only at a subset of suitable areas or occurred in areas with low estimated habitat suitability (Egly and Larson 2018). We modeled gopher occurrences from the field validation survey as a response to observed land cover recorded during validation surveys, and distance to known gopher occurrence. We included observed land cover in case there was disagreement between observed land cover and the land cover raster layer. We did not include variables used in the MaxEnt model in the random forests to prevent circularity. We assessed classification accuracy by estimating the difference between the out-of-the-bag error rate and parameter permutation across all trees and then normalized by the SD between runs. We estimated node impurity through the Gini index to assess parameter importance (a low Gini index indicates high parameter importance) using the varImpPlot function in the ‘RandomForest’ R package (Breiman 2001).
Historic SDM

We next created a historic SDM using occurrence records from 1945-1955, a period ~45 years after most of the land conversion in Illinois (Robertson et al. 1997). We obtained historic occurrences of *G. bursarius illinoensis* from museum specimens from the Illinois Natural History Survey (Champaign, IL; \( n = 78 \)) and the Field Museum of Natural History (Chicago, IL; \( n = 6 \)). These occurrences were then combined and thinned to be ≥1 km apart \( (n = 56, \text{Fig. 2.1b}) \). Due to limited sample size, we did not withhold 25% of the data for model testing.

For historic land cover, we georeferenced aerial imagery from 1945-1955 (USDA and USGS 2020) of counties within *G. bursarius illinoensis’* range using the georeference tool in ArcMap (Esri ArcMap v. 10.7.1 2019) by creating control points between the digital image and ArcMap satellite base imagery (Esri Digital Globe 2019) at stationary locations (e.g., county borders, and road or river features). We georeferenced 105 aerial images using an average of 41 control points \( (SD = 34) \) and a root mean square error of 102 m \( (SD = 69 \text{ m}) \). We then buffered the thinned historic occurrences by 1 km and digitized the land cover into 9 categories: Agriculture, Agricultural Berm, Forest, Grassland or Pasture, Mild Development, Medium Development, Roads, Water, and Unknown. All the categories were represented in the contemporary model except for agricultural berm and roads. The reduction in land cover classes from the contemporary model was necessitated by our inability to identify crop types or forest types from aerial photography (see Historic vs. Contemporary Models section for comparison models). Mild Development differed from Medium Development by density of buildings. Mild Development could include stand-alone houses or structures in farmland or woods, and Medium Development generally included towns or neighborhoods. We defined Agricultural Berm as a division between two agricultural fields that was not along a road to highlight potential grassland
refugia for *G. bursarius illinoensis*. We used the same raster layers as for the contemporary SDM for soil texture, drainage class, percent sand, elevation, and slope, and we used the 10-year precipitation mean and SD from 1945-1955 (PRISM Climate Group 2014). However, the 10-year precipitation SD had a VIF = 2.7, so models either included mean precipitation or precipitation SD, but not both.

Like the contemporary SDM, we ran MaxEnt models across all combinations of parameters and two smoothing parameters (β = 1 or 2). We were only able to generate 100 background points ≥1 km apart within the historic *G. bursarius illinoensis*’ range because we did not have a range-wide land cover classification and were constrained by our digitization capacity. We hand-digitized land cover from aerial photos within 1 km buffers around these 100 background points to use in MaxEnt. We selected the top models using AICc in ENMTools. We also performed a Jackknife analysis to identify parameter contributions of the top models.

**Historic vs Contemporary SDMs**

SDMs are viewed as temporally static realized niches (Guisan and Theurillat 2000). The niche reduction hypothesis expands the connection between SDMs and the realized niche by incorporating temporal dynamics as well as novel threats to a species. To create comparable models to examine changes in gopher niches between eras, we used the global models with all environmental predictors (hereafter ‘full models’) of the contemporary and historic SDMs (precipitation SD included despite the VIF > 2). We reclassified the contemporary land cover to the 9 land cover types used for the historic SDM (Table A.3 in Appendix A). We then re-ran the full models using the same 100 background points for both the contemporary and historic models and compared parameter contributions with Jackknife analyses. We further compared models by
estimating niche overlap between the contemporary and historic SDMs. We used Warren’s $I$ index, an estimation of niche similarity where 0 indicates no overlap and 1 indicates perfect overlap (Warren et al. 2008), in ENMTools. The niche overlap estimates the spatial overlap of SDM raster layers, providing a spatial estimate of niche similarity, but does not account for variation in contributing parameters. We also used Warren’s $I$ to compare model performance.

To determine specific niche shifts and possible reductions, we compared contemporary and historic gopher occurrences using Wilcoxon-Rank Sum Tests for continuous environmental variables and Pearson’s $X^2$ Tests for categorical variables (soil texture and land cover). If there was a loss of specific niche space, a niche reduction would be supported. However, niche shifts could also occur in which the overall niche volume is constant between eras, but occurrences have shifted to a different predominant environmental space (e.g., gophers occur across a similar range of soil sand percentages, but currently select sandier soils than historically). We also used the “Limiting Factor” analysis in MaxEnt to determine which parameters were limiting at gopher occurrences and broadly across the predicted areas (Elith et al. 2010).

Although creating comparable models is necessary to determine a niche shift, we also wanted to assess model transferability and niche overlap for the best models selected from our initial contemporary SDM and historic SDM (see above). We extracted the predicted habitat suitability for historic occurrences, contemporary occurrences, and background points for each top MaxEnt model. If the contemporary model predicted high suitability for historic occurrences, and the historic model predicted high suitability for contemporary occurrences, there would be temporal stability of the SDMs. For the contemporary model, we also extracted habitat values from absences ($n = 47$) from the presence-absence survey conducted in 2017 to determine if the model predicted known absences to be low habitat suitability. We also visited locations where
gopher specimens were collected between 1945-1981 to determine persistence and contemporary land cover. We confirmed active gopher mounds on foot except along interstates.

*Niche Hypervolumes*

Although the above methods can identify niche shifts related to environmental parameters, we also estimated niche hypervolumes to determine directly if there was a reduction in volume between the two eras. Because non-ordinated categorical variables (e.g., soil texture and land cover) cannot be directly incorporated into hypervolume calculations (Blonder et al. 2018), we ordinated our data. First, we scaled and centered continuous parameters by subtracting the parameter means from the values and then dividing by the parameter SDs. Next, we used PCAMix from the R package ‘PCAmixdata’ (Chavent et al. 2017) for ordination, which allows for mixed data and categorical parameter interpretation (Carvalho and Cardoso 2020). This method integrates principal component analysis with multiple correspondence analysis.

Finally, we calculated the hypervolume using a Gaussian kernel density estimate (KDE) of the first 4 axes to ensure appropriate power given the number of observations (Blonder et al. 2017; Mammola and Cardoso 2020) using the ‘Hypervolume’ R package (Blonder et al. 2018). Although one-class support vector machine (SVM) estimation also is appropriate for calculating realized niche volume (Blonder et al. 2018; von Takach et al. 2020), we opted for Gaussian KDE methods that may better identify holes (Mammola and Cardoso 2020). Given that our data included transformed categorical variables, and our predicted niche loss may be within the center of the niche hypervolume, the tight, binary wrap of SVM (Blonder et al. 2018) may overpredict niche volume for our dataset. Gaussian KDEs have produced appropriate hypervolume estimates for SDMs and for the realized niche (Blonder et al. 2017). We used a kernel bandwidth based on
the Silverman estimator (Silverman 1998). We also report the centroid distance between the two hypervolumes, and the Sørensen similarity to quantify overlap, following recommended best practices (Mammola and Cardoso 2020; Lu et al. 2021).

RESULTS

Contemporary SDM

The top contemporary MaxEnt model (Fig. 2.2a) indicated the gopher’s current distribution is best predicted by land cover, soil texture, and soil drainage ($\text{AUC}_{\text{TRAINING}} = 0.89$). Gophers selected for mildly developed spaces; silty-clay loam, sandy loam, and stratified sand to loam soils; and areas with high drainage capacity (Fig. A.1 in Appendix A). Land cover contributed the most to the model, followed by soil texture, as determined by the jackknife analysis (Fig. A.1 in Appendix A). Agricultural land cover classes all had <0.12 probability of suitable conditions based on the response curves (Fig. A.1 in Appendix A). The contemporary model fit the 25% testing data well ($\text{AUC}_{\text{TESTING}} = 0.90$). Moreover, there was no evidence that the model was overparameterized (Fig. A.1-2 in Appendix A). The alternative model with a reduced number of land cover categories produced similar results, including the same predictors with similar response curves and jackknife analysis, and had a high spatial niche overlap (Warren’s $I = 0.99$) with the selected contemporary model (Fig. A.1-2 in Appendix A).

During field validation, we found 8 novel gopher occurrences in “Very Good” habitat, 1 in “Good” habitat, and 1 in “Low” habitat (Fig. 2.1b). We did not find any gophers in habitat with a “Very Low” suitability classification. Gophers occurred in grassland/pastures ($n = 2$), fallow fields ($n = 1$), developed/open spaces ($n = 2$), and grass berms surrounded by corn ($n = 3$) or soybeans ($n = 3$). The gopher occurrences in “Good” and “Low” habitat had a
misclassification of land cover in which the observed land cover was “fallow field” and “grassland/pasture,” whereas the raster indicated “Developed/Low Intensity” and “Developed/Open Space,” respectfully. All new gopher occurrences were within 2.23 km of a known gopher location. For modeling with the contemporary land cover (raster resolution of 30 m x 30 m), small grass berms were often aggregated with the surrounding land cover, possibly leading the selected contemporary maxent model to underpredict occupancy in these sites. For the classification tree, the residual mean deviance was 0.42 with a misclassification rate of 0.063 (Appendix A.8-A.9 in Appendix A).

Historic SDM

The top historic MaxEnt model (Fig. 2.2b) indicated the gopher’s historic distribution is best predicted by elevation, mean precipitation, and land cover ($\text{AUC}_\text{TRAINING} = 0.68$, Fig. A.3 in Appendix A). Gophers selected for areas with higher elevation, higher precipitation, and along roadsides. Elevation contributed the most to the model, followed by precipitation (Fig. A.6 in Appendix A). However, unlike the contemporary model, agricultural land cover and agriculture berms both had a probable suitability of 0.60 predicted by the response curves (Fig. A.3 in Appendix A). All agricultural land cover was aggregated in these models, so there is limited inference on which types of crops may be more suitable.

Historic vs Contemporary SDMs

The historic and contemporary SDMs had different limiting factors, did not spatially predict similar habitat suitability, and had shifted environmental associations at gopher occurrences. Elevation and precipitation contributed the most to the historic full model ($\text{AUC}_\text{TRAINING} = 0.75$).
whereas soil texture, land cover, and sand percentage contributed the most to the contemporary full model ($\text{AUC}_{\text{TRAINING}} = 0.67$ with 100 background points; Fig. 2.3). The historic and contemporary full models, when restricted to the same 100 background points, had a spatial niche overlap (Warren’s $I$) of 0.92. The best contemporary model and the best historic model had a Warren’s $I$ of 0.79 (Table A.4 in Appendix A). The contemporary full model with 10,000 background points and with 100 background points produced similar models, although there was a decrease in AUC that is expected with lower statistical power. Furthermore, the distance between occurrence records and background points had a similar distribution between the two eras, leading us to conclude that our models are comparable.

There were shifts in the environmental parameters related to gopher occurrences from the 1950s to the contemporary era. Contemporary occurrences had lower elevations ($p < 0.001$), higher mean precipitation ($p < 0.001$), higher standard deviation of precipitation ($p < 0.001$), sandier soil textures ($p < 0.001$), and there was a shift from agricultural areas toward areas dominated by mild development ($p < 0.001$; Fig. 2.4). Historic occurrences from 1945-1955 ($n = 82$) and 1956-1981 ($n = 45$) were predominantly located in agriculture present day (80%, Fig. A.10 in Appendix A) and were not occupied during resurveys in 2017-2018. From the limiting factors analysis, land cover was the primary limiting factor at contemporary gopher occurrences, and mean precipitation was the primary limiting factor at historic occurrences.

Model transferability was low between the historic full model and the contemporary full model with 100 background points (Fig. 2.5). The historic model estimated contemporary gopher occurrences as having only slightly higher habitat values than background points, and the contemporary model estimated historic occurrences as having lower habitat suitability than
background points and contemporary absences. The models were unable to predict suitable occurrences between eras and therefore were not transferable.

**Niche Hypervolumes**

The first 4 dimensions of the PCAMix explained a cumulative 41.9% of the variation of the environmental parameters at contemporary gopher occurrences and a cumulative 48.6% of the variation for the historic occurrences (Fig. A.11 in Appendix A). Both the contemporary and historic niche models identified soil texture as a large contributor to the PCA from the squared loadings (Fig. A.11 in Appendix A). We documented a 16.8% decrease in niche volume between eras (historic volume = 661, contemporary volume = 550) with a centroid distance of 0.482 and a Sørensen similarity of 0.483. Overall, the contemporary niche hypervolume has shifted from the historic niche, and the two niche hypervolumes were only 48.2% similar. We also observed holes within the hypervolumes (Fig. A.12 in Appendix A). Niche hypervolume differences were due to both a niche reduction and a niche shift.

**DISCUSSION**

Our study indicates gophers in Illinois have experienced niche shifts and a niche reduction over time as evidenced by changes in limiting factors, shifts in environmental associations, and a reduced niche hypervolume. By testing the niche reduction hypothesis, we were able to understand how non-uniform spatial responses to threats can underlie a species’ decline and range contraction (Scheele et al. 2017). We observed reduced occurrences of gophers in the interior of their range, similar to patterns for other declining species (Lomolino and Channell 1995). We predicted that gophers would be associated with grasslands, sandy-clay loam soils,
high elevation, low precipitation, and high soil drainage, with reductions in agricultural and grassland suitability over time. Overall, our results supported these predictions, but with further complexities. We start by discussing the documented niche reduction and shift, changes in model contributions and limiting factors for gopher habitat suitability, and the altered environmental-niche associations. Next, we discuss potential species-specific concerns for these niche shifts. We conclude with how the niche reduction hypothesis can be applied more broadly, specifically in other systems with agricultural intensification.

**Niche Reduction and Shifts**

We observed imperfect spatial niche overlap and a reduced niche hypervolume between the historic and contemporary eras. From the SDMs, we documented a shift in environmental drivers from elevation, precipitation, and land cover to land cover, soil texture, and soil drainage based on parameters included in the selected models and the results from the jackknife analyses. The main limiting factor across the gopher range shifted from being mean precipitation to predominantly being land cover. The change from associations with high elevation and precipitation to associations with soil texture and drainage suggests soil-water retention and burrow flooding may be important constraints on gopher distribution. However, increasing precipitation regimes and farm expansion into areas of higher elevation have altered how gophers avoid flooded burrows. Gophers create vertical burrows, likely for drainage, but increased flooding may prevent population establishment or persistence (Davis et al. 1938). Flooding can collapse burrows and force gophers to abandon them (Miller 1957; Thorne and Andersen 1990), decrease burrow excavation rates (Mohr and Mohr 1936), or drown gophers (Kershen 2004). Overall, water dynamics may impact the gopher niche more than previously
thought due to increasing precipitation regimes and agricultural development in areas with adequate drainage.

Although the niche reduction hypothesis can apply to prey responding to biotic interactions including predation (e.g., McDonald et al. 2018), there is limited information on predation for gophers (e.g., Connior and Risch 2010). There may be a wide array of opportunistic predators of gophers, however, American badgers (*Taxidea taxus*) are uniquely able to excavate gopher burrows (Hoffmeister 1989). Monitoring data are lacking to establish whether the distribution or abundance of badgers has changed from historical to contemporary eras in Illinois. However, badgers are a prairie obligate species with unusually large home ranges in the current fragmented landscape (Duquette et al. 2014), and likely have declined over time. During our presence-absence surveys, we detected a single badger burrow, and during our driving surveys, we detected a single road-killed badger. As gophers have shifted to mildly developed areas, cats (*Felis catus*) may increasingly pose predation risks to gophers.

*Species-Specific Concerns*

Crops may have similar restrictions as gophers do in terms of the water table. For example, corn yield relationships vary with the shallow water table (saturated soil within top 2 m of soil profile) in which too much water near the surface may damage crops, even though water at lower soil levels may provide stability during drought (Rizzo et al. 2018). Farmers view land as marginal partially based on likelihood of flooding (Yang et al. 2021), and with increasing precipitation regimes, farmers may view higher elevation as more optimal. This would displace gophers from higher elevations, leading gophers to occupy lower elevations with sandier soils to maintain burrow drainage. Tile drainage, a practice to remove excess subsurface water from croplands,
can alleviate flooding, but most of the drainage is old, implemented for crops with high transpiration (e.g., alfalfa transpires more than soybeans), and is not adequate for increased precipitation from climate change (Castellano et al. 2019). Tile drainage is also not commonly used in the western, sandier areas of the gopher distribution in Illinois, but is used frequently in the northeast region of the gopher range (Rizzo et al. 2018). Recent mapping of tile drainage using geospatial modeling (Valayamkunnath et al. 2020) could increase our understanding of subterranean species distributions in relation to agricultural water management, especially as drainage systems are updated to meet agricultural needs.

Increased precipitation, including more extreme events, from climate change may exclude species from historically occupied areas (Widick and Bean 2019) or extirpate entire populations (McCain and Colwell 2011; Mason-Romo et al. 2018; Wilkening et al. 2019). We observed an increase in precipitation between eras across the background, historic, and contemporary locations, likely due to climate change. Gophers used to persist at the upper limits of precipitation regimes sampled historically (~980 mm), but they now persist at the lower limits of current precipitation regimes (~1020 mm; Fig. 2.4). Future increases in precipitation in the region could further decrease the realized niche for gophers. Interactions between climate change and land cover also may exacerbate risk for species (Williams et al. 2021).

Agriculture has also expanded into more poor-quality lands due to increasing corn prices (Aragon 2019) and has brought conservation lands into use (Morefield et al. 2016; Holland et al. 2020). Soil loss in agricultural systems leads to reduced crop yield and income (Thaler et al. 2021), further increasing the intensification and production on erodible land (Holland et al. 2020). As agricultural practices fluctuate based on economic return, farm expansion and land-use
may further restrict gopher suitability as soils are eroded from previously suitable areas or poor-quality soils are brought into production.

Farm expansion into marginal lands, soil erosion, and changes in agricultural production due to precipitation all may displace gophers, but we observed additional declines in agricultural suitability over time. This reduction of habitat suitability, and occurrences of gophers within agricultural lands, indicates a decrease in niche space similar to other grassland rodents (Nikolić et al. 2019; Rutrough et al. 2019). Since the 1950s, historic sites have remained in agricultural production but had no contemporary gopher occurrences, indicating reduced suitability. Gophers can occupy alfalfa (Hoffman and Choate 2008) and hayfields (Sietman et al. 1994), but alfalfa and hay production in Illinois have declined, replaced with corn and soybeans (Garcia et al. 1987; Warner 1994; Sulc and Tracy 2007). Hence, this decline in habitat suitability for gophers coincides with agricultural practices that increased annual row crops, increased farm size with loss of grassland buffers, and increased soil compaction from heavier machinery (Keller and Or 2022).

**Broader Applications**

Although habitat loss and fragmentation are of conservation concern due to isolating populations and decreasing genetic diversity, how those habitat changes occur can impact species-specific adaptive potential and conservation outcomes. Gophers have strong associations with soil classes (Hoffman and Choate 2008; Connior et al. 2010; Connior 2011; Warren et al. 2017) including creating boundaries between gopher species and subspecies (Genoways et al. 2008; Connior 2011; Henke et al. 2014). Given that gophers in Illinois are becoming increasingly restricted to certain soil properties, genetic-soil associations may be lost if populations within unique soils are
extirpated. Conservation efforts should focus on both gopher persistence in the contemporary soil associations as well as re-establishment within historically occupied soils.

The niche reduction hypothesis can inform conservation recommendations based on historic and contemporary realized niches (Scheele et al. 2017). By identifying niche space that was historically occupied concurrently with the contemporary niche, managers can attempt to restore habitat lost from the historic niche while implementing efforts to conserve existing habitat. For gophers, areas of higher elevation and with silty loam soils can be prioritized for genetic conservation, but areas of high gopher occurrence, such as areas with high sand percentage or in sandy loam soils, could also be prioritized for population conservation.

The niche reduction hypothesis also frames practices of conservation concern as dynamic and is useful for landscapes continuing to change after initial conversion to agriculture. We recommend applying the concept to other systems undergoing agricultural intensification and becoming monocultures (Roesch-McNally et al. 2018). The U.S. Corn Belt has brought more land into production as well as increased yield, but crop type is heavily dependent on economic incentives and crop prices, creating temporally changing landscapes and conservation practices (Lin and Huang 2019). With further studies on niche reduction of species in agriculturally intensifying systems, dynamic strategies incorporating wildlife conservation, sustainable agricultural management, and economics can be developed to meet the complex goals of agroecosystems (Hunter et al. 2017).
Fig. 2.1 (a) *Geomys bursarius* historic range (red line) in Illinois (Hoffmeister 1989) buffered by 10 km with contemporary land cover (gray = development, yellow = corn, dark green = soybean, light green = grassland/pasture). (b) Thinned historic gopher occurrences, thinned contemporary gopher occurrences, contemporary model validation absences, contemporary model validation presences, and the 100 background points used for comparative MaxEnt models across the gopher range in Illinois.
Fig. 2.2 (a) Habitat suitability from the top contemporary species distribution model for pocket gophers across the historic range in Illinois (buffered by 10 km). Parameters included land cover, soil texture, and soil drainage. (b) Habitat suitability from the top historic species distribution model for pocket gophers across the historic range (red line) in Illinois. Parameters included elevation, mean precipitation, and land cover.
Fig. 2.3 Jackknife analysis of parameter contribution representing the gain when a parameter is used in isolation (blue), the decrease in gain when the parameter is omitted (teal), and the gain when all the parameters are included (red) from the comparable (a) contemporary species distribution model, and (b) historic species distribution model for pocket gophers in Illinois.
Fig. 2.4 Comparison of environmental parameter values at historic and contemporary occurrences for pocket gophers in Illinois. Soil texture and land cover are categorical variables (Table A.1.1 & A.1.3). Significant niche shifts are indicated based on Wilcoxon Rank Sum Tests for all parameters except land cover and soil texture, which were evaluated with Pearson’s $X^2$ tests. Gophers now occur at areas with $\sim 1050$ mm (SD = $\pm 150$ mm) of precipitation, elevations at $\sim 150$ m, in silty clay and sandy loam soil texture, well drained and somewhat poorly drained soils, and soils with low and high sand percentage. Gophers also occur in mildly developed areas. Historically, gophers occurred at locations with $\sim 910$ mm (SD = $\pm 90$ mm) of precipitation; elevations at $\sim 220$ m; silty clay and very cobbly silt loam soil texture well drained, moderately well drained, and somewhat poorly drained soils; soils <40% sand percentage, and predominantly in agriculture and along agricultural berms. Although there were niche shifts of most environmental parameters from historic to contemporary occurrences, there was no evidence for a niche shift for slope ($p = 0.34$) or soil drainage ($p = 0.71$).
Fig. 2.5 Predicted habitat suitability for pocket gophers in Illinois from the (a) full contemporary MaxEnt model, and (b) full historic MaxEnt model (with both using 100 background points) at contemporary occurrences, historic occurrences, background locations, and contemporary absences.
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INTRODUCTION

Genetic diversity is a central component of biodiversity (DeWoody et al. 2021). To protect biodiversity, we must first understand how different processes structure genetic variation across temporal and spatial scales (Cushman and Landguth 2010; Hewitt 2004; 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 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 (e.g., Kierepka and Latch 2016a; Priadka et al. 2019; Muñoz-Valencia et al. 2023). Through partitioning genetic variation to these distinct processes, we can gain a holistic understanding of spatial genetic structure (Weber et al. 2016; Muñoz-Valencia et al. 2023).

IBB patterns commonly arise from mountain ranges (Muñoz-Valencia et al. 2023), rivers (Pfau et al. 2001; Mushet 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).

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2 This chapter is in revision at Landscape Ecology
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 (Orsini et al. 2013; Shafer and Wolf 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 compared to 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 to promote genetic diversity.
The plains pocket gopher (*Geomys bursarius*) 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). Rivers and other water bodies are likely effective barriers that prevent gene flow for many subterranean rodents (Visser et al. 2018; Austrich et al. 2020; Mapelli et al. 2020). *Geomys 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). The species also exhibits strong soil associations (Reichman and Seabloom 2002; Alexander et al. 2022).

Burrowing rodents often display local adaptation to soil structure, potentially generating IBE patterns (Barbosa et al. 2021). In fact, soil properties delineate gopher species, acting as distribution boundaries (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. There is little information on how mound creation correlates to time above ground, 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 (~50-100 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. 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 B.1 in Appendix B). 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 1x Sodium Chloride-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). 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 (Fig. B.1 in Appendix B; Esri et al. 2010). We assessed Hardy-Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) 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 $\leq 0.3$ from pairwise comparisons between loci, the loci were retained as this would approximate a 10% linear correlation threshold (Wagner 2022). 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).

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). We then visually inspected assignment of individuals to each cluster using CLUMPAK (Kopelman et al. 2015). 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 STRUCTURE. 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. Geomys bursarius 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 skew clustering.

To further understand how rivers may act as barriers for gophers, we calculated Nei’s F_{ST} (Nei 1987) between a priori regions (see ‘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 (Fig. B.1 in Appendix B, 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 Nei’s $F_{ST}$, we used “pairwise.neifst” 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.

*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, 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. We also included the year of sample collection in case there were temporal impacts on genetic similarities.

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 and calculated Euclidean distances between RGB pixels at each gopher location 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.

**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 ‘Isolation by environment’), separating out *a priori* regions for IBB (see ‘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.

**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. 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 overall loci index association was 0.22 and an $\bar{r}_d = 0.038$. However, because all pairwise $\bar{r}_d$ were $\leq 0.3$, we assumed no linkage between loci. Sample sizes from *a priori* populations ranged from 4-170 (mean = 38, SE = 22). Observed heterozygosity was lower than expected heterozygosity across all loci globally ($Ho = 0.477$, $He = 0.702$, $F_{ST} = 0.167$, $F_{IS} = 0.320$). Rarefied allelic richness ranged from 2.14-2.88 (section B.2 in Appendix B).
**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. 3.1a). 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. 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. 3.1b). 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. 3.2a). 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 (section B.3 in Appendix B).

For the two clusters identified with the thinned sampling method, no further spatial structure resolved in the northern population, indicating that microsatellites could not parse the subspecies *G. b. majusculus, G. b. wisconsinensis, and G. b. bursarius* into separate populations. For the southern cluster, there was added structure with *G. b. illinoensis* clustering separately, and the Mississippi River acting as a boundary (Fig. 3.2b). When *G. b. illinoensis* was analyzed
alone, we identified 2 populations (Fig. 3.1c). However, individuals showed a south-north gradient of admixture indicating that IBD is more likely than IBB for *G. b. illinoensis*.

For *G. bursarius*, Nei’s $F_{ST}$ values ranged from 0.048-0.320 based on *a priori* defined regions divided by major rivers (section B2 in Appendix B). The populations divided by the Canadian River in the southern region had relatively low $F_{ST}$ (0.048), whereas populations divided by the Arkansas, Missouri, Mississippi, and Illinois Rivers all had pairwise $F_{ST} > 0.1$. Nei’s $F_{ST}$ for only *G. b. illinoensis* ranged from 0.036-0.155 based on region (section B.2 in Appendix B).

*Isolation by distance*

For *G. bursarius*, geographic distance was correlated with genetic distance ($r^2 = 0.34$) based on the Moran eigenvector map (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. 3.3a).

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. 3.3b).
**Isolation by environment**

For the IBE models, all VIFs were <2 indicating no multicollinearity. 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 0.37 (Table 3.1). 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 3.1).

**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. 3.4a). 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 \(R^2 = 0.02\)).

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. 3.4b). 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.
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. Isolation by environment minimally explained genetic structure at both scales, although IBE shared variance with IBB and IBD.

*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. *Geomys bursarius* can swim for up to 6.5 minutes and increasing size likely decreases swimming endurance (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 (e.g., Kierepka and Latch 2016b; Waraniak et al. 2019; Burbrink et al. 2022), and the Illinois River is considered the northern range barrier for *G. b. illinoensis* (Hoffmeister 1989; Alexander et al. 2022). Our STRUCTURE analysis clustered populations of *G. bursarius* on either side of the Mississippi River and Missouri River albeit at different hierarchies dependent on sample thinning. The Moran eigenvector maps also showed
the Mississippi and Illinois Rivers as major barriers. Moreover, the low observed heterozygosity supports a Wahlund effect of gopher populations isolated by barriers (Penney and Zimmerman 1976), although $F_{IS}$ indicated that there is likely further population structure within each region.

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, but not complete, isolation of gene flow (Roratto et al. 2015; Painter et al. 2022). This pattern is similar to genetic structure of badgers (*Taxidea taxus*) in Wisconsin, with the Mississippi River acting as a barrier broadly (Kierepka and Latch 2016b), yet the smaller Wisconsin River does not (Kierepka and Latch 2016a).

When restricting the analyses to *G. b. illinoensis* in Illinois and Indiana, STRUCTURE and MEMs did not identify genetic discontinuity based on rivers. Moreover, although IBB explained some of the genetic differentiation through variation partitioning, most variance could not be partitioned between IBB and IBD.

*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). 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).

*Isolation by environment*

Isolation by environment also contributed to genetic structure of *Geomys bursarius*, although minimally compared to other isolating processes. A key concept of IBE is that neutral loci can detect processes being driven by habitat selection or local adaptation. Although local adaptation may impact neutral processes, genetic drift and isolation drive gene flow more. 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 across Geomys species (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. Geomys bursarius 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. Geomys bursarius 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**

Through multiple analyses and genetic distance metrics, we 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. More generally, recognizing genetic-environmental associations are 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 generating 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.
Table 3.1 Top model parameters from redundancy analysis for gene flow due to isolation by environment. 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.

<table>
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<th>PCs</th>
<th>Model parameters</th>
<th>Adjusted R(^2)</th>
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Fig 3.1 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).
Fig. 3.1 cont.
Fig. 3.2 Population assignments from STRUCTURE (left) and number of populations estimated using the Evanno method (right) with 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).
Fig. 3.3 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, $R^2 = 0.17$).
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.

**Fig. 3.4** 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.
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CHAPTER 4: PHYLOGENETIC ASSESSMENT WITHIN A SPECIES COMPLEX OF A SUBTERRANEAN RODENT (GEOMYS BURSARIUS) WITH CONSERVATION IMPLICATIONS FOR ISOLATED SUBSPECIES

INTRODUCTION

Phylogeography is important for understanding the role of historic range expansion and colonization on contemporary genetic structures of taxa and providing context for conservation efforts (Wang, 2010; Epps and Keyghobadi, 2015; Rissler, 2016). Phylogeography and phylogenetics can be particularly useful for clarifying intraspecific genetic variation. Through describing phylogeography of subspecies, we can provide insight into higher levels of taxonomic classification by bridging micro- and macroevolutionary processes (Avise 2009; Avise et al. 2016; Rissler 2016). Such resolution also is needed to clarify whether isolation has produced evolutionary significant units that may require targeted conservation interventions (Moritz 1994; Mace 2004; Hoelzel 2023).

A major driver of genetic variation is range contraction and expansion in response to glacial ice sheets with the Pleistocene glaciation often creating subspecies or population-level genetic structure (Arbogast and Kenagy 2001; Brant and Ortí 2003). In North America, responses to glaciation have shaped species distributions and genomes, with species expanding northward with the retreating glacial sheets (Hewitt 2000). However, with multiple glaciation events, populations expanding northward were likely periodically isolated, or went extinct and experienced recolonization, creating a complex system from which to understand speciation events and intraspecific genetic variation (Russell 1968; Hewitt 2000). As such, species that expanded northward from southern glacial refugia have varying and complicated

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phylogeographic relationships. Populations in the Midwest region often show structuring from northward expansions from glacial refugia in Texas and Mexico (Starkey et al. 2003; Du et al. 2019; Ford et al. 2020; Blais et al. 2021). However, other taxa in the Midwest demonstrate substantial admixture without strong signals from post-glaciation expansion (Barton and Wisely 2012; Ford et al. 2020; Reding et al. 2021). This variation in post-glaciation genetic signatures may be due to different locomotion and dispersal abilities of taxa under changing climates, with taxa facing unique barriers or expansion rates (Feder et al. 2009; Schloss et al. 2012).

Subterranean rodents with low dispersal abilities can retain localized genetic structure from historic range expansion after glaciation (Alexander et al. in review, Elrod et al., 2000). Thus, pocket gophers (Geomyidae) are ideal for understanding phylogeography of a subspecies within a broader species complex (Avise et al. 1979; Sudman et al. 2006; Chambers et al. 2009). Pocket gophers in the genus *Geomys* range broadly across central North America, from northern Mexico to southern Canada, and from Florida to New Mexico (Sudman et al. 2006; Chambers et al. 2009). *Geomys* includes four species groups: *G. bursarius*, *G. breviceps*, *G. personatus*, and *G. pinetis* (Sudman et al. 2006; Chambers et al. 2009). *Geomys bursarius* has eight subspecies that occur across the Great Plains and part of the Midwest (Connior 2011). *Geomys bursarius* likely has undergone both adaptive and non-adaptive speciation (Hart 1971) with glacial events leading to isolated populations and multiple range expansions northward (Russell 1968).

Intraspecific phylogenetics of *Geomys* remain largely unexplored with resolution limited due to a focus on targeted nuclear gene sequencing or specific mitochondrial genes (Elrod et al. 2000, Sudman et al., 2006; Chambers et al., 2009). The subspecies within *G. bursarius* need further examination as some genetic distances show divergence at levels potentially warranting species level delimitation (Baker and Bradley 2006; Chambers et al. 2009).
Here, we expand phylogenetic analyses of pocket gophers by focusing on the *G. bursarius* clade with particular attention to resolving the position of *G. b. illinoensis*. Major waterways like the Mississippi, Missouri, and Illinois Rivers act as barriers across the range of *G. bursarius*, genetically isolating populations (Alexander et al. in review) likely due to the poor swimming ability of gophers (Kennerly 1963). The Illinois River acts as a northern boundary for *G. b. illinoensis*, whereas historic expansion across the Mississippi River followed by genetic isolation offers an excellent opportunity to understand northward range expansion post glaciation (Smith, 1957; Connior, 2011, Alexander et al. in review). Dispersal events across the Mississippi River should be rare, which may promote speciation of *G. b. illinoensis*. Furthermore, *G. b. illinoensis* has a patchy distribution in Illinois due to extensive loss of grassland habitat and ongoing agricultural intensification (Ramankutty and Foley 1999; Augustine et al. 2021), and it has experienced a niche reduction over time (Alexander et al., 2022). Therefore, it is critical to understand the genetic distinctness of *G. b. illinoensis* because it is facing contemporary threats due to landscape change and fragmentation.

We use complete mitochondrial genomes (mitogenomes) and complementary bioinformatic analyses to clarify the relationships of *G. bursarius* subspecies across the central United States, specifically determining if clades such as *G. b. illinoensis* are monophyletic and meet recommendations for being a unique genetic population of conservation concern (Moritz 1994; Mace 2004). Through using well established methods for phylogenetic tree inferences, along with a novel approach using genotype likelihoods, we account for high variation in the mitogenomes and produce holistic insights regarding the phylogeny of *G. bursarius*. Specifically, we (i) generated minimum and median spanning networks to interpret haplotype structure, (ii) inferred phylogenetic trees to determine branch support and establish if subspecies
were monophyletic, and (iii) used a genotype likelihood approach to determine phylogenetic structure of *G. bursarius* to assess genetic uniqueness and admixture of subspecies.

**METHODS**

*Tissue samples and DNA extraction*

We identified *G. bursarius* samples from Alexander et al. (in review) that had high DNA content based on microsatellite amplification to increase the likelihood of reconstructing complete mitogenomes. These samples included 41 toes from museum specimens from the Illinois Natural History Survey collected between 1934 and 1985, and 12 tissue samples from *G. bursarius illinoensis* live-trapped in 2018-2019, following appropriate guidelines (UIUC IACUC #17190; Sikes and The Animal Use and Care Committee of the American Society of Mammalogists, 2016). We sampled eight *G. bursarius* subspecies from across the range, only excluding *G. b. ozarkensis*. All museum samples were identified as subspecies except for five samples from Wisconsin. We assigned these Wisconsin samples as *G. b. wisconsinensis* based on the range map (Connior 2011). We included three samples from other *Geomys* species (1 *G. breviceps dutcheri*, and 2 *Geomys jugossicularis*) as outgroups. *Geomys jugossicularis* was previously considered within the *G. bursarius* clade but was determined to be an independent species (Sudman et al. 2006; Chambers et al. 2009). For full DNA extraction methods, see Alexander et al. (in review). We contracted with Arbor Biosciences (AB; arborbiosci.com; Ann Arbor, MI, USA) to do library preparation, mtDNA-bait enrichment and capture, and genome sequencing. Arbor Sciences created customized mtDNA-baits using a myBaits® kit to enrich for *Geomys* mtDNA prior to sequencing. Baits were customized using a *G. personatus* reference mitogenome (Genbank accession MZ030793.1; Greenia, 2020). To account for potential DNA degradation in
the 44 museum samples, we used 4-plex reactions with dual-round enrichment and subsequent sequencing of ~3 million paired-end reads. The 12 live-trapped individuals with non-degraded DNA were enriched in a single-round using a 12-plex reaction with subsequent sequencing of ~1 million paired-end reads. Enriched libraries were sequenced on the Illumina® NovaSeq platform to produce paired-end reads that were 150 base-pairs in length.

**Assembly and alignment**

We used AdapterRemoval v.2.3.1 (Schubert et al. 2016) to trim consecutive Ns from the 5’ and 3’ ends. We used Burrow-Wheeler Alignment with maximal exact matches (BWA-MEM) v. 0.7.17 (Li and Durbin 2010) to align samples to the *G. personatus* mitogenome. We marked shorter hit splits to estimate Picard similarity in BWA-MEM. We used SAMTools v. 1.12 (Li et al. 2009) to filter, sort, and convert to Binary Alignment/Map (BAM) files. We calculated the breadth of coverage (i.e., the percentage of the genome that had ≥1 X-fold read coverage) and the average depth coverage (the average X-fold number of reads that mapped at any location across the genome).

We then imported the BAM files into Geneious Prime v. 2022.2.1 (Biomatters, Ltd www.geneious.com) and created a mitogenome consensus sequence that contained sites with a minimum depth of 5X reads. In Mega v. 11.03 (Tamura et al. 2021), we manually identified regions with high disagreement and low quality, removing those positions from the mitogenomes. We manually trimmed regions that showed high disagreement, including portions of the D-loop and cyt-b. We individually aligned each read to the COX1 and cyt-b regions (Genbank accession MZ030793.1; Greenia, 2020). COX1 had the lowest adaptive/synonymous replacement (~0), whereas cyt-b is commonly used in phylogenetic reconstruction and also has
low adaptive/synonymous replacement in *Geomys* (Greenia 2020). We generated three datasets: one containing consensus sequences that were manually trimmed to remove disagreements, one that contained reads that were independently aligned to the COX1 gene, and one with reads independently aligned to the cyt-b region.

*Evolutionary substitution model and spanning networks*

We assessed evolutionary substitution models using MEGA 11.0.13 Bayesian information criterion scores for the trimmed mitogenome dataset, COX1, and cyt-b. We then generated a minimum spanning network for the full mitogenome in POPArt (Leigh and Bryant 2015), visualizing haplotype clusters based on regions divided by major riverways that act as dispersal and gene flow barriers (Alexander et al. in review), subspecies, and finally geographic distance. We also visualized median joining trees for the same 4 categories for the COX1 and cyt-b datasets. We did not generate a median joining network for the trimmed mitogenome dataset due to computation limitations associated with high mitogenome variability, but rather used a novel genotype likelihood approach (described below).

*Inferring phylogenetic trees*

We inferred a Bayesian estimate of phylogenetic trees, using the posterior distribution as branch validation. We generated files in Beauti v1.10.4 (Drummond et al. 2012) with the Hasegawa-Kishino-Yano substitution model using a gamma distributed rate variation allowing for invariant sites (HKY+G+I), coalescent constant population size model (Kingman 1982; Drummond et al. 2002). We used an UPGMA tree as an initial tree and a strict clock, meaning different branches experience the same mutation rate. We ran three Monte Carlo Markov Chains (MCMCs) with a
250,000,000-chain length with log outputs every 10,000 iterations. We combined the three runs using LogCombiner v.1.10.4 (Rambaut and Drummond 2018a) and assessed the combined outputs in Tracer v.1.7.2 (Rambaut et al. 2018) with a 10,000 burn-in, relying on effective sample size (ESS) and MCMC pattern. Posterior values and phylogenetic tree structure were compiled in TreeAnnotator v.1.10.4 (Rambaut and Drummond 2018b) and visualized in FigTree v1.4.4. We also inferred tree topology using a maximum likelihood framework, RAxML (Stamatakis 2015), for the trimmed mitogenome, COX1, and cyt-b datasets but with low support, but congruent results (section C.1 in Appendix C).

Genotype Likelihood and Principal Component Analysis of genetic variation

Because of the high frequency of SNPs in the mitogenome datasets, we also used a genotype likelihood approach to filter out rare variants that might be due to authentic differences present in the mitogenomes of a few individuals (or to technical PCR and sequencing errors). Our aim was to increase the strength of the phylogenomic signal by filtering out minimally informative reads present in only a few individuals. A genotype likelihood approach is often used in ancient DNA analyses and is therefore also well suited for our genetic data mainly derived from museum specimens. We used the program ANGSD (Sand Korneliussen et al. 2014) to estimate genotypes. The dataset included only variants that were present in at least 6 of the 56 samples and had p < 0.01, meaning that the retained SNPs were present in ≥10% of the individuals. We used a principal component analysis (PCA) in the program PCAngsd (Meisner and Albrechtsen 2018) to determine the genetic distance between individuals and visualized genetic variation using a custom script in R 4.1.0. As part of the PCAngsd analysis, we also estimated admixture using the
most likely number of populations (K) present based on genotype likelihoods (Skotte et al. 2013).

RESULTS

Sample collection, assembly, and alignment

The samples from 56 individuals (Fig. 4.1) each had a high percentage of reads with a phred score >30 (89.66% ± SE 0.14%). After trimming sections of cyt-b and the D-loop, we retained 14,996 bp of the mitogenome. In other Geomys species, the cyt-b region has been transposed into the nuclear DNA, which can co-amplify when targeting mtDNA (Nitschmann 2023), and may explain the high variation of cyt-b when aligning the full mitogenome. After aligning and trimming specifically for COX1 and cyt-b, we retained 1545 bp and 1067 bp, respectively.

Evolutionary substitution model and spanning networks

The best evolutionary substitution model for the mitogenome and the subregions was the HKY+G+I model. From the minimum spanning tree based on 14,996 bp, G. b. illinoensis clustered uniquely compared to the other subspecies, separated by 412 mutations. Geomys bursarius missouriensis also separated as one of the most distant clusters from G. b. illinoensis, indicating a longer divergence time between G. b. missouriensis and G. b. illinoensis than between other subspecies (Fig. 4.2; section C.2 in Appendix C). Geomys bursarius missouriensis samples clustered with the outgroups, G. jugossicularis and G. breviceps dutcheri. This general pattern was consistent with the median joining trees of COX1 and cyt-b (section C.2 in Appendix C). As expected, COX1 had fewer substitutions than cyt-b due to the conserved nature of the COX1 region.
Phylogenetic trees

Bayesian methods had high support (posterior probability distributions) for phylogenetic tree topology inferred using the trimmed mitogenome dataset (Fig. 4.3). For the Bayesian analysis, all variables had a high Effective Sample Size (ESS) value (all ESS > 46,800), or the number of independent draws from the posterior distribution, except ‘meanRate,’ indicating no correlation between the trees. The meanRate had a low ESS of 17 because we restricted the analysis to be a strict clock with no variation for mutation rate. Consistent with being outgroups, a midpoint rooted Bayesian tree indicated that *G. breviceps dutcheri* is likely an outgroup to all other *Geomys* investigated in this study, *G. jugossicularis* is a sister-clade to all *G. bursarius*, and *G. b. illinoensis* forms a well resolved monophyletic clade. Although most other *G. bursarius* subspecies show geographic partitioning into distinct and well supported clades, *G. b. major* forms a paraphyletic group with individuals clustering with *G. b. industrius* and *G. b. bursarius*, as well as a separate clade containing only *G. b. major*. BEAST outputs identified a *G. b. industrius* sample that grouped with *G. b. major*, and this sample is likely mislabeled based on examining range maps (Connior 2011).

Genotype Likelihood and Principal Component Analysis of genetic variation

We estimated genotype likelihoods for nucleotide variants present in ≥10% of individuals and visualized patterns of genetic variation by region in which the specimens originated (Fig. 4.4). As expected, the variation explained by PC1 and PC2 was low, likely because of the high genetic diversity innately present in the dataset. However, PC1 separated *G. b. illinoensis* from all other individuals, indicating gophers from Illinois have genetic variation that is distinct from other *G. bursarius*. PC1 also separated the two other gopher species, *G. breviceps dutcheri* and *G. 
jugossicularis, toward the left of the PC1 axis. This arrangement provided support that PC1 explains genetic variation in a biologically meaningful way despite the low PC1 contribution (0.3%). PC2 also separated the two other gopher species from other individuals, but also separated G. b. wisconsinensis from other G. bursarius. The separation of G. b. wisconsinensis and other subspecies as belonging to genetically distinct clusters was also supported by the admixture analysis in which individuals had the highest likelihood of being partitioned into eight genetically distinct clusters (Fig. 4.5). Wisconsin individuals had minimal admixture and were grouped with a single admixed individual from Iowa. Geomys bursarius illinoensis mainly had admixture between two groups (Fig. 4.5) that corresponded with individuals from G. b. missouriensis and G. jugossicularis. Geomys breviceps dutcheri was grouped separately and shared limited admixture with only a few other individuals.

DISCUSSION

Multiple analyses indicate G. b. illinoensis experienced genetic isolation and historic divergence from the rest of the G. bursarius species complex. Geomys bursarius illinoensis forms a well-supported, monophyletic clade distinct from other subspecies. However, G. b. illinoensis is still within the clade that diverged from G. jugossicularis. These results further support the scenario of northward expansion post-glaciation with a unique colonization event across the Mississippi River. This range expansion east of the Mississippi River likely occurred farther south than the current G. b. illinoensis range, as fossils of Geomys have been found south of the current distribution (Parmalee and Klippel 1981). Subsequent southern range restriction of G. b. illinoensis was likely due to northward expansion of deciduous forest into plains (Smith 1957; Hart 1971).
Complementary phylogenetic approaches indicate *G. b. illinoensis* is a genetically distinct population. Bayesian tree construction highly supported earlier divergence of *G. b. illinoensis* (clade 1) from the rest of the *G. bursarius* clade (clade 2; Fig. 4.3). The minimum spanning network, the median joining networks, and the mitogenomic PCA also indicate *G. b. illinoensis* is differentiated and has a unique mitogenomic signal. Given *Geomys bursarius illinoensis* had admixture from *G. jugossicularis* and *G. b. missouriensis*, *G. b. illinoensis* likely retains historic mitogenomic traits and shares a common ancestor with *G. b. missouriensis*. Interestingly, these historic signals are not present in all subspecies, with *G. b. wisconsinensis* potentially gaining local adaptive variants that now differentiate it from the ancestral profiles. These analyses resolve *G. bursarius* genetic relationships and expand phylogenetic understanding of a widely distributed species (Elrod et al. 2000; Sudman et al. 2006). Furthermore, our approach based on multiple, independent analyses provides a methodology that may be particularly relevant for study systems with high mitogenomic variation in local populations and that demonstrate similar ancestral dispersal followed by vicariance.

However, our phylogenetic reconstruction raises questions about other *G. bursarius* subspecies. Classification of *G. b. major* may need further assessment because individuals were paraphyletic and grouped with other clades as well as independently. *Geomys bursarius major* included individuals that consistently were identified as basal clades across our phylogenetic inferences, and the admixture results emphasize that most populations share a component with *G. b. major*. This outcome suggests *G. b. major* was a source population or founding ancestral population from which other *Geomys bursarius* subspecies diverged. Given that some of the *G. b. major* samples came from a region that was a glacial refugia, there may be higher haplotype diversity or further divergence there than for samples closer to the *G. b. major* range limits. One
A specimen of *G. b. major* also nested within the *G. b. bursarius* and *G. b. wisconsinensis* clade. That *G. b. major* sample was located the farthest north and may also represent a historic mitogenome retained during northward colonization and subspeciation of *G. b. bursarius* and *G. b. wisconsinensis*.

Post-glacial range expansion into the Midwest varies greatly across taxa, however *G. bursarius* follows a phylogeographic pattern similar to other grassland species (Smith 1957; Huang et al. 2020). A grassland peninsula extended east during the Pleistocene allowing western grassland lineages to cross and establish east of the Mississippi River, including eastward dispersal of gophers (Smith 1957). However, taxa dispersed across the Mississippi River at different times (Soltis et al. 2006; Barrow et al. 2015). Some species like the chorus frog (*Pseudacris* spp.) in Illinois diverged relatively recently (Barrow et al. 2015). These grassland species likely became more isolated as forests moved northward restricting populations to sandy glacial deposits (Smith 1957; Barrow et al. 2015). As anthropogenic land use continues to reduce habitat suitability for these relict western lineages (Barrow et al. 2015; Alexander et al. 2022), conservation of these clades may be required to preserve unique evolutionary genetics.

*Conservation Implications*

Understanding the phylogeographic history of subspecies can inform our understanding of higher taxa relationships and guide conservation goals (Avise et al. 2016; Rissler 2016). As genetic resolution increases and speciation is viewed as a continuous process, however, identification of conservation units requires further deliberation (Coates et al. 2018). We provided evidence that *G. b. illinoensis* is phylogenetically distinct and most distantly diverged from the other *Geomys* subspecies, supporting previous work using nuclear DNA (microsatellites) that *G. b. illinoensis*
is a unique population (Alexander et al. in review). Conservation units under the Endangered Species Act often require historic divergence in both mtDNA and nuclear DNA (Avise et al. 1987; Moritz 1994, 2002; Coates et al. 2018), and *G. b. illinoensis* meets these criteria. Because *G. b. illinoensis* has experienced increased habitat fragmentation and decreased niche breadth within its range (Alexander et al. 2022), we recommend additional monitoring and land management to promote conservation of *G. b. illinoensis*. Given that remnant groups of *G. b. illinoensis* often occur along roadway rights-of-way, roadside management that retains grassland vegetation could increase population connectivity, especially linking areas with sandy soils (Alexander et al. 2022). Roadsides may act as quality habitat but this may depend on the type of road (i.e., paved, gravel, or dirt), and right-of-way management along dirt roads may particularly benefit fossorial rodents (Brock and Kelt 2004). However, roads themselves may function as permeable barriers to subterranean rodents (Esperandio et al. 2019). Road underpasses, particularly as *G. b. illinoensis* is fossorial, may be a promising tool to promote connectivity, but species-specific studies are needed to determine efficacy (D’Amico et al. 2015).

Although we focus on one western lineage of gophers east of the Mississippi River, multiple grassland species in Illinois face conservation threats due to land conversion (Smith 1957; Warner 1994; Barrow et al. 2015), Thus, habitat restoration and providing connectivity between relict prairies and other grasslands should benefit gene flow for multiple species. Furthermore, taxa in Illinois may originate from clades west of the Mississippi River (Smith 1957; Barrow et al. 2015; Williams and Ibrahim 2023), so clarifying whether these eastern populations qualify as Evolutionary Significant Units can aid in prioritizing management actions.
Understanding phylogeographic colonization events contextualizes the evolutionary trajectories and genetic structures in these unique subspecies and populations.
**Fig. 4.1** Spatial distribution of *Geomys bursarius* subspecies and two outgroups (*G. breviceps dutcheri* and *G. jugossicularis*) sampled for mitogenomes. Samples are viewed on the base World Topography Map (ESRI et al. 2017).
Fig. 4.2 Minimum spanning tree for *Geomys bursarius* subspecies and two outgroups (*G. breviceps dutcheri* and *G. jugossicularis*) for the full mitogenomes with the number of mutations along the edges.
Fig. 4.3 Phylogenetic tree under the HKY+G+I model in BEAST with posterior distribution support for each branch. Three separate clades are demarcated (dashed lines).
Fig. 4.4 Principal component analysis in ANGSD showing *G. b. illinoensis* separating from other *Geomys bursarius* subspecies and other *Geomys* species.
Fig. 4.5 Admixture plot with nine genetic clusters from the trimmed mitogenome dataset of the *G. bursarius* clade with two outgroups (*G. jugossicularis* and *G. breviceps dutcheri*).


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CHAPTER 5: SUMMARY AND CONCLUSIONS

Conservation relies on multiple lines of inquiry to make informed decisions, and integrating spatial analyses with genetic approaches is an effective path toward understanding a species in time and place. Relying on multiple approaches is particularly important for species persisting in heavily altered habitats, such as species historically associated with prairies in the Midwest. Following initial land conversion in which <0.01% of historic prairies remained, species may continue to face additional pressures and niche reduction from changing land-use practices, mechanization, and intensification. The niche reduction hypothesis emphasizes that threats such as continued land degradation can decrease the realized niche breadth of declining species. However, to fully understand potential consequences of habitat fragmentation and identify conservation priorities, managers also need to understand genetic structure, gene flow, and taxonomic status.

In this dissertation, I have identified ongoing niche reduction and clarified both genetic and taxonomic structure of the plains pocket gopher (Geomys bursarius). In chapter 2, I identified that a subspecies, G. b. illinoensis, has shifted its distribution to higher elevations and to sandier soils, potentially indicating soil drainage as a driving factor of species persistence. With agricultural intensification, the limiting factors impacting G. b. illinoensis have changed since the 1950s from multiple factors (land cover, precipitation, elevation, soil drainage) to predominantly one factor—land cover. The habitat suitability of agricultural lands has significantly declined. Finally, consistent with a common pattern for species experiencing range loss due to anthropogenic causes, G. b. illinoensis has suffered a reduction in habitat suitability within its central range, with the range edges increasing in suitability.
This ongoing shift and reduction in habitat suitability and niche breadth, including modified soil associations, may further limit gene flow and connectivity. In chapter 3, I used microsatellites to test environmental and distance-based effects on gene flow. For *G. b. illinoensis*, gene flow was predominantly related to distance, indicating that maintaining a network of suitable habitat patches within dispersal distance would allow for gene flow between populations. There also were environmental factors that impacted gene flow, but genetic variation could not be partitioned to a single isolating effect. Rivers proved to be barriers to gene flow, but some genetic variation may also reflect soil properties. As *G. b. illinoensis* continues to experience niche reduction from agricultural intensification and decreased suitability within the core of its range, managing habitat patches to allow gene flow will be increasingly important. From these analyses, I provided evidence and recommendations for management for a subspecies of concern. Yet there still was no evidence that *G. b. illinoensis* was significantly differentiated from other *G. bursarius* subspecies and if it warranted additional conservation resources. If *G. b. illinoensis* is genetically distinct, it could be a conservation priority. This chapter further confirmed that *G. b. illinoensis* is genetically distinct from the rest of *G. bursarius* using neutral nuclear genetic markers.

Prioritization of conservation units also requires monophyly relying on mitochondrial genetics. In chapter 4, I clarified the phylogenetic relationships of the *G. bursarius* subspecies using mitochondrial genomes (mitogenomes). Relying on multiple approaches, I identified that *G. b. illinoensis* is a genetically distinct, monophyletic clade, meeting requirements outlined for being an evolutionary significant unit. The *G. b. illinoensis* subspecies exhibited admixture with a sister taxon *G. jugossicularis*, and another subspecies *G. b. missouriensis*, likely indicating a historic structure. *Geomys bursarius illinoensis* probably dispersed eastward across the
Mississippi River during the Pleistocene, following a grassland peninsula. Other subspecies (i.e., *G. b. wisconsinensis* and *G. b. missouriensis*) demonstrated no admixture, clustering together, potentially indicating local adaptation (*G. b. wisconsinensis*) or retention of historic genetic signatures (*G. b. missouriensis*). Through mitogenomics, I clarified phylogenetic structure of *G. bursarius* and provided evidence that *G. b. illinoensis* meets mitogenomic status that warrants conservation as an evolutionary significant unit.

Integrating habitat suitability and concepts of the realized niche with genetic analyses provides a strong foundation for identifying ongoing ecological processes as well as identifying conservation prioritizations. *Geomys bursarius* has an interesting natural history, where Pleistocene climatic effects allowed development of a grassland peninsula, facilitating eastward expansion across the Mississippi River. However, this population became isolated and genetically divergent. Following anthropogenic land conversion, where grasslands were converted into agricultural fields, continuing changes in farming practices decreased the niche breadth of *G. b. illinoensis* and caused shifts in environmental relationships. *Geomys bursarius illinoensis* is a subspecies meeting the requirements to be considered an Evolutionary Significant Unit and is continuing to face anthropogenic pressures. There is hope, however, for improving habitat, particularly in historically occupied areas, and connectivity between populations would benefit a multitude of grassland species and *G. bursarius* should be considered in grassland management projects within their range.
APPENDIX A: CHAPTER 2 SUPPLEMENTAL MATERIAL

Appendix A.1 Soil texture and soil drainage classifications within the range of *G. bursarius*.

Table A.1 Numeric code for soil texture within the range of *Geomys bursarius* in Illinois

<table>
<thead>
<tr>
<th>Numeric Code</th>
<th>Soil Texture Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Fine sand</td>
</tr>
<tr>
<td>2</td>
<td>Clay loam</td>
</tr>
<tr>
<td>4</td>
<td>Fine sandy loam</td>
</tr>
<tr>
<td>5</td>
<td>Stratified sandy loam to silt loam</td>
</tr>
<tr>
<td>6</td>
<td>Silty clay loam</td>
</tr>
<tr>
<td>7</td>
<td>Silt loam</td>
</tr>
<tr>
<td>8</td>
<td>Sandy loam</td>
</tr>
<tr>
<td>11</td>
<td>Stratified sand to loam</td>
</tr>
<tr>
<td>12</td>
<td>Silty clay</td>
</tr>
<tr>
<td>13</td>
<td>Loam</td>
</tr>
<tr>
<td>14</td>
<td>Very cobbly silt loam</td>
</tr>
<tr>
<td>16</td>
<td>Stratified gravelly sandy loam to silt loam</td>
</tr>
<tr>
<td>17</td>
<td>Very channery loam</td>
</tr>
<tr>
<td>18</td>
<td>Very fine sandy loam</td>
</tr>
<tr>
<td>19</td>
<td>Stratified sand to clay loam</td>
</tr>
<tr>
<td>20</td>
<td>Gravelly coarse sand</td>
</tr>
<tr>
<td>22</td>
<td>Stratified very fine sandy loam to silty clay loam</td>
</tr>
<tr>
<td>26</td>
<td>Variable</td>
</tr>
</tbody>
</table>

Table A.2 Numeric code for soil drainage within the range of *Geomys bursarius* in Illinois

<table>
<thead>
<tr>
<th>Numeric Code</th>
<th>Soil Drainage Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Somewhat excessively drained</td>
</tr>
<tr>
<td>3</td>
<td>Well drained</td>
</tr>
<tr>
<td>4</td>
<td>Moderately well drained</td>
</tr>
<tr>
<td>5</td>
<td>Somewhat poorly drained</td>
</tr>
<tr>
<td>6</td>
<td>Poorly drained</td>
</tr>
<tr>
<td>7</td>
<td>Very poorly drained</td>
</tr>
</tbody>
</table>

Table A.3 Numeric code, the land cover classification from the United States Department of Agriculture National Agricultural Statistics Service 2019, and the reassignment land cover classification for the reduced land cover within the range of *Geomys bursarius* in Illinois

<table>
<thead>
<tr>
<th>Numeric Code</th>
<th>Land Cover</th>
<th>Assigned Reduced Land Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Corn</td>
<td>Agriculture</td>
</tr>
<tr>
<td>2</td>
<td>Cotton</td>
<td>Agriculture</td>
</tr>
<tr>
<td>3</td>
<td>Rice</td>
<td>Agriculture</td>
</tr>
<tr>
<td>4</td>
<td>Sorghum</td>
<td>Agriculture</td>
</tr>
<tr>
<td>5</td>
<td>Soybeans</td>
<td>Agriculture</td>
</tr>
<tr>
<td>12</td>
<td>Sweet Corn</td>
<td>Agriculture</td>
</tr>
<tr>
<td>13</td>
<td>Pop or Orn Corn</td>
<td>Agriculture</td>
</tr>
<tr>
<td>21</td>
<td>Barley</td>
<td>Agriculture</td>
</tr>
<tr>
<td>Numeric Code</td>
<td>Land Cover</td>
<td>Assigned Reduced Land Cover</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------</td>
<td>---------------------------------</td>
</tr>
<tr>
<td>23</td>
<td>Spring Wheat</td>
<td>Agriculture</td>
</tr>
<tr>
<td>24</td>
<td>Winter Wheat</td>
<td>Agriculture</td>
</tr>
<tr>
<td>26</td>
<td>Wheat/Soybeans</td>
<td>Agriculture</td>
</tr>
<tr>
<td>27</td>
<td>Rye</td>
<td>Agriculture</td>
</tr>
<tr>
<td>28</td>
<td>Oats</td>
<td>Agriculture</td>
</tr>
<tr>
<td>29</td>
<td>Millet</td>
<td>Agriculture</td>
</tr>
<tr>
<td>36</td>
<td>Alfalfa</td>
<td>Agriculture</td>
</tr>
<tr>
<td>37</td>
<td>Other Hay/Non-Alfalfa</td>
<td>Agriculture</td>
</tr>
<tr>
<td>42</td>
<td>Dry Beans</td>
<td>Agriculture</td>
</tr>
<tr>
<td>43</td>
<td>Potatoes</td>
<td>Agriculture</td>
</tr>
<tr>
<td>44</td>
<td>Other Crops</td>
<td>Agriculture</td>
</tr>
<tr>
<td>47</td>
<td>Miscellaneous Vegetables &amp; Fruits</td>
<td>Agriculture</td>
</tr>
<tr>
<td>48</td>
<td>Watermelons</td>
<td>Agriculture</td>
</tr>
<tr>
<td>49</td>
<td>Onions</td>
<td>Agriculture</td>
</tr>
<tr>
<td>53</td>
<td>Peas</td>
<td>Agriculture</td>
</tr>
<tr>
<td>54</td>
<td>Tomatoes</td>
<td>Agriculture</td>
</tr>
<tr>
<td>57</td>
<td>Herbs</td>
<td>Agriculture</td>
</tr>
<tr>
<td>58</td>
<td>Clover/Wildflowers</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>59</td>
<td>Sod/Grass Seed</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>60</td>
<td>Switchgrass</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>61</td>
<td>Fallow/Idle Cropland</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>67</td>
<td>Peaches</td>
<td>Orchard</td>
</tr>
<tr>
<td>68</td>
<td>Apples</td>
<td>Orchard</td>
</tr>
<tr>
<td>70</td>
<td>Christmas Trees</td>
<td>Orchard</td>
</tr>
<tr>
<td>71</td>
<td>Other Tree Crops</td>
<td>Orchard</td>
</tr>
<tr>
<td>74</td>
<td>Pecans</td>
<td>Orchard</td>
</tr>
<tr>
<td>76</td>
<td>Walnuts</td>
<td>Orchard</td>
</tr>
<tr>
<td>77</td>
<td>Pears</td>
<td>Orchard</td>
</tr>
<tr>
<td>111</td>
<td>Open Water</td>
<td>Water</td>
</tr>
<tr>
<td>121</td>
<td>Developed/Open Space</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>122</td>
<td>Developed/Low Intensity</td>
<td>Dev Low</td>
</tr>
<tr>
<td>123</td>
<td>Developed/Med Intensity</td>
<td>Dev Med</td>
</tr>
<tr>
<td>124</td>
<td>Developed/High Intensity</td>
<td>Dev High</td>
</tr>
<tr>
<td>131</td>
<td>Barren</td>
<td>grassland/pasture</td>
</tr>
<tr>
<td>141</td>
<td>Deciduous Forest</td>
<td>Forest</td>
</tr>
<tr>
<td>142</td>
<td>Evergreen Forest</td>
<td>Forest</td>
</tr>
<tr>
<td>143</td>
<td>Mixed Forest</td>
<td>Forest</td>
</tr>
<tr>
<td>152</td>
<td>Shrubland</td>
<td>Forest</td>
</tr>
<tr>
<td>176</td>
<td>Grassland/Pasture</td>
<td>Grassland/pasture</td>
</tr>
<tr>
<td>190</td>
<td>Woody Wetlands</td>
<td>Forest</td>
</tr>
<tr>
<td>195</td>
<td>Herbaceous Wetlands</td>
<td>Forest</td>
</tr>
</tbody>
</table>

128
<table>
<thead>
<tr>
<th>Numeric Code</th>
<th>Land Cover</th>
<th>Assigned Reduced Land Cover</th>
</tr>
</thead>
<tbody>
<tr>
<td>205</td>
<td>Triticale</td>
<td>Agriculture</td>
</tr>
<tr>
<td>206</td>
<td>Carrots</td>
<td>Agriculture</td>
</tr>
<tr>
<td>216</td>
<td>Peppers</td>
<td>Agriculture</td>
</tr>
<tr>
<td>222</td>
<td>Squash</td>
<td>Agriculture</td>
</tr>
<tr>
<td>225</td>
<td>Wheat/Corn</td>
<td>Agriculture</td>
</tr>
<tr>
<td>229</td>
<td>Pumpkins</td>
<td>Agriculture</td>
</tr>
<tr>
<td>236</td>
<td>Wheat/Sorghum</td>
<td>Agriculture</td>
</tr>
<tr>
<td>237</td>
<td>Double Crop Barley/Corn</td>
<td>Agriculture</td>
</tr>
<tr>
<td>240</td>
<td>Double Crop Soybeans/Oats</td>
<td>Agriculture</td>
</tr>
<tr>
<td>242</td>
<td>Blueberries</td>
<td>Agriculture</td>
</tr>
<tr>
<td>249</td>
<td>Gourds</td>
<td>Agriculture</td>
</tr>
<tr>
<td>254</td>
<td>Double Crop Barley/Soybeans</td>
<td>Agriculture</td>
</tr>
</tbody>
</table>
Appendix A.2 Area under the curve (AUC), parameter contributions, and jackknife analysis for the selected contemporary model, the selected contemporary model with reduced land cover classification, and the selected historical model.

We did model selection for a suite of models with 63 classifications of land cover to determine if specific crop types were worse for gophers, and then did model selection using a reduced land cover classification ($n = 7$) to ensure we did not overparameterize the model. Both contemporary models identified the same parameters and had similar response curves and jackknife analyses. The two contemporary models had a spatial niche overlap (Warren’s $I$) of 0.99; thus we consider the model with the full land cover classifications to be comparable to a model with fewer land cover classifications.

![Diagram](image)

**Fig. A.1** (a) Area under the curve, and (b – d) response curves where Log Output represents the change in occurrence probability as the environmental parameter is varied for (b) soil texture, (c) land cover, and (d) soil drainage for the top contemporary MaxEnt model for pocket gophers in Illinois. (b) Gophers were more likely to occur in silty clay loam (6), sandy loam (8), stratified sand to loam (11), and variable soils (26). (c) Gophers were more likely to occur in low development areas (121), and not occur in agriculture (yellow), grasslands or pastures (light green), orchards (red), water and wetlands (blue), developed areas (grey), and forests (dark green). (d) Gophers were also likely to occur in very poorly drained (7) and somewhat (2) excessively drained-well drained (3) soils. For full numeric associations of soil texture, land cover, and soil drainage, refer to the Appendix A1.
Fig A.2 (a) Area under the curve, and (b – d) response curves for the selected model using fewer land cover classifications. Log Output represents the change in occurrence probability as the environmental parameter is varied for (b) soil texture, (c) land cover, and (d) soil drainage for the top contemporary MaxEnt model for pocket gophers in Illinois. (b) Gophers were more likely to occur in silty clay loam (6), sandy loam (8), stratified sand to loam (11), and variable soils (26). (c) Gophers were more likely to occur in low development areas, and not occur in agriculture (yellow), forests (dark green), grasslands or pastures (light green), water and wetlands (blue), and medium and highly developed areas (grey). (d) Gophers were also likely to occur in very poorly drained (1) and excessively drained (6) soils. For full numeric associations of soil texture, land cover, and soil drainage, refer to Appendix A1.
Fig. A.3 (a) Area under the curve, and (b – d) response curves where Log Output represents the change in occurrence probability as the environmental parameter is varied for (b) elevation, (c) land cover, and (d) precipitation mean for the top historic MaxEnt model for pocket gophers in Illinois. (b) Gophers were more likely to occur at higher elevation (260 m), (c) along roadways compared to agriculture and agriculture berms (yellow), forests (dark green), grasslands or pastures (light green), low or medium development (grey), or water and wetlands (blue), and (d) in areas with ~980 mm of rainfall.
Fig. A.4 Jackknife analysis of parameter contribution of the top contemporary habitat suitability model for pocket gophers in Illinois. Land cover contributed the most to the regularized training gain and soil drainage contributed the least.

Fig. A.5 Jackknife analysis of parameter contribution of the top contemporary habitat suitability model for pocket gophers in Illinois with reduced land cover classification. Land cover contributed the most to the regularized training gain and soil drainage contributed the least.

Fig. A.6 Jackknife analysis of parameter contribution of the top historic habitat suitability model for pocket gophers in Illinois. Elevation contributed the most to the regularized training gain and land cover contributed the least.
Appendix A.3 Field surveys including validation of contemporary habitat suitability model including the resurvey points (A3.1) random-forest classification tree (A3.2), accuracy and impact on the Gini index (A3.3), and contemporary surveys at historic occurrence locations (A3.4)

![Validation survey presences (white) and absences (black) across the range and predicted contemporary habitat suitability of *G. bursarius illinoensis*.](image)

**Fig. A.7** Validation survey presences (white) and absences (black) across the range and predicted contemporary habitat suitability of *G. bursarius illinoensis*. 
Fig. A.8 Classification tree for field validation points. Positive detections were within 2.2 km of a previously identified occurrence, and observed land cover did not contribute to occurrence probability.

Fig A.9 Results of Random Forest plots from field validation surveys for pocket gophers in Illinois. The variable ‘distance to previously known occurrence’ had the greatest impact on accuracy and the Gini index.
Fig. A.10 Contemporary land cover at historic occurrences of pocket gophers in Illinois from 1945-1955 (black) and 1956-1981 (grey). No individuals were detected at the locations of the historic occurrences during contemporary resurveys.
Appendix A.4 Spatial niche overlap between the historic and contemporary best and global models as well as the impact of the number of background points in a MaxEnt model.

To quantify niche overlap for the best contemporary and historic SDMs, we also compared Warren’s $I$ between the top models. We also wanted to account for the effect of sample size in niche overlap because MaxEnt is sensitive to the number of background points used. Hence, we estimated Warren’s $I$ between: 1) the contemporary full model with 10,000 random background points and reduced land cover categories, 2) the contemporary full model with the specified 100 background points and reduced land cover categories, 3) the selected contemporary model with 10,000 background points and all land cover categories, 4) the historic full model with the specified 100 background points and reduced land cover categories, and 5) the selected historic model with the specified 100 background points and reduced land cover categories.

To determine the impact of the number of background points on our models, and the impact of using full models rather than top performing models, we used Warren’s $I$ to estimate spatial niche overlap. The contemporary full model with 10,000 background points varied the most with the contemporary full model with 100 background points and the selected contemporary model. Because Warren’s $I$ estimates spatial overlap of niche, contemporary models using the full extent have a larger area of predicted SDM values to compare, whereas the historic SDMs are constrained to the 1km buffer around predicted areas we were able to digitize, thus, the contemporary models may be expected to have higher variation. Of note, the selected contemporary and the selected historic models only had a Warren’s $I$ of 0.791.
Table A.4 Warren’s $I$ index, which ranges from 0 to 1 and measures niche similarity for the full contemporary model, the top selected contemporary model, the full historic model, and the top selected historical model. Similarity was quantified for models with all contemporary land cover categories and 10,000 background points (*) and with reduced land cover categories and 100 background points (**).

<table>
<thead>
<tr>
<th></th>
<th>Contemporary Full*</th>
<th>Contemporary Full**</th>
<th>Selected Contemporary*</th>
<th>Historic Full**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Contemporary Full*</td>
<td>0</td>
<td>0.897</td>
<td>0</td>
<td>0.763</td>
</tr>
<tr>
<td>Contemporary Full**</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selected Contemporary*</td>
<td></td>
<td>0.953</td>
<td>0.874</td>
<td>0.763</td>
</tr>
<tr>
<td>Historic Full**</td>
<td>0.763</td>
<td>0.920</td>
<td>0.757</td>
<td>0.791</td>
</tr>
<tr>
<td>Historic Selected**</td>
<td>0.760</td>
<td>0.950</td>
<td>0.791</td>
<td>0.981</td>
</tr>
</tbody>
</table>
Appendix A.5 Principal Component Analysis (PCA) loadings and Niche hypervolume plots

a. Fig A.11 Squared loadings of parameter contributions to the PCA used in estimating niche hypervolume for pocket gophers in Illinois based on (a) contemporary and (b) historic occurrences.
Fig. A.12 Two dimensional hypervolume plots of the first 4 PCA axes for (a) contemporary and (b) historic niche hypervolumes for pocket gophers in Illinois, and (c) the contemporary niche hypervolume compared to the historic niche hypervolume along with their intersection, union, and their respective unique niche hypervolumes for the first 4 PCA axes.
Fig. A.12 cont.
### Table B.1 Sample of subspecies of *Geomys bursarius* by state.

<table>
<thead>
<tr>
<th>Subspecies</th>
<th>Illinois</th>
<th>Indiana</th>
<th>Iowa</th>
<th>Kansas</th>
<th>Missouri</th>
<th>Oklahoma</th>
<th>S. Dakota</th>
<th>Wisconsin</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>wisconsinensis</em></td>
<td>160</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>23</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>21</td>
</tr>
<tr>
<td><em>missouriensis</em></td>
<td>22</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>5</td>
<td>2</td>
<td>32</td>
<td>170</td>
<td>267</td>
</tr>
<tr>
<td><em>majusculus</em></td>
<td>8</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>5</td>
<td>7</td>
<td>9</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td><em>industrius</em></td>
<td>30</td>
<td>22</td>
<td>22</td>
<td>8</td>
<td>30</td>
<td>30</td>
<td>Total</td>
<td>Total</td>
<td></td>
</tr>
</tbody>
</table>

*Subspecies was inferred by a range map (Connor 2011).*
Fig. B.1 Regional boundaries and genetic sample locations (grey) for (a) *Geomys bursarius* and (b) *Geomys bursarius illinoensis* defined by major rivers. Background is the base World Topography Map (ESRI et al. 2017).
Appendix B.2. Hardy-Weinberg Equilibrium, Linkage Disequilibrium, and Population Summary Analyses

Table B.2 P-values from chi-square tests for Hardy-Weinberg Equilibrium for all samples. No locus is out of HWE across populations, indicating that HWE divergence is likely due to population structure. The population bounded by the Illinois River and the Mississippi River deviated, likely due to sample size.

<table>
<thead>
<tr>
<th>Population</th>
<th>GBR06</th>
<th>GBR09</th>
<th>TM1</th>
<th>GBR10</th>
<th>TM6</th>
<th>GBR25</th>
<th>GBR27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flint Hills</td>
<td>0.016</td>
<td>0.05</td>
<td>0.094</td>
<td>0.063</td>
<td>0.551</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Iowa</td>
<td>0.007</td>
<td>0.454</td>
<td>0.003</td>
<td>0.481</td>
<td>1</td>
<td>0.621</td>
<td>0.858</td>
</tr>
<tr>
<td>Kiowa</td>
<td>0.256</td>
<td>0.53</td>
<td>0.382</td>
<td>0.586</td>
<td>0.046</td>
<td>0.382</td>
<td>0.046</td>
</tr>
<tr>
<td>Red Hills</td>
<td>0.667</td>
<td>0.055</td>
<td>0.167</td>
<td>0.479</td>
<td>0.001</td>
<td>0.853</td>
<td>0.757</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>0.423</td>
<td>0.008</td>
<td>0</td>
<td>0.514</td>
<td>0</td>
<td>0.148</td>
<td>1</td>
</tr>
</tbody>
</table>

Table B.3 Sample size (n), observed heterozygosity (Hobs), expected heterozygosity (Hexp), and rarified allelic richness for Geomys bursarius delimited by major rivers.

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>Hobs</th>
<th>Hexp</th>
<th>Ar</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flint Hills</td>
<td>33</td>
<td>0.553</td>
<td>0.679</td>
<td>2.714</td>
</tr>
<tr>
<td>Illinois</td>
<td>170</td>
<td>0.471</td>
<td>0.713</td>
<td>2.733</td>
</tr>
<tr>
<td>Iowa</td>
<td>20</td>
<td>0.448</td>
<td>0.622</td>
<td>2.522</td>
</tr>
<tr>
<td>Kiowa</td>
<td>4</td>
<td>0.500</td>
<td>0.692</td>
<td>2.768</td>
</tr>
<tr>
<td>Red Hills</td>
<td>8</td>
<td>0.510</td>
<td>0.747</td>
<td>2.88</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>32</td>
<td>0.380</td>
<td>0.478</td>
<td>2.140</td>
</tr>
</tbody>
</table>

Table B.4 Inbreeding coefficient (Fis) for each locus across populations. Iowa was fixed at TM6 and Wisconsin was fixed at GBR27.

<table>
<thead>
<tr>
<th>Population</th>
<th>GBR06</th>
<th>GBR09</th>
<th>TM1</th>
<th>GBR10</th>
<th>TM6</th>
<th>GBR25</th>
<th>GBR27</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flint Hills</td>
<td>0.06</td>
<td>0.24</td>
<td>0.27</td>
<td>0.05</td>
<td>0.78</td>
<td>0.17</td>
<td>-0.09</td>
</tr>
<tr>
<td>Illinois</td>
<td>0.23</td>
<td>0.29</td>
<td>0.51</td>
<td>0.20</td>
<td>0.60</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>Iowa</td>
<td>0.66</td>
<td>0.19</td>
<td>0.51</td>
<td>0.11</td>
<td>NA</td>
<td>0.27</td>
<td>0.07</td>
</tr>
<tr>
<td>Kiowa</td>
<td>0.45</td>
<td>0.10</td>
<td>0.22</td>
<td>0.14</td>
<td>1.00</td>
<td>0.22</td>
<td>1.00</td>
</tr>
<tr>
<td>Red Hills</td>
<td>0.33</td>
<td>0.40</td>
<td>0.47</td>
<td>0.17</td>
<td>1.00</td>
<td>0.14</td>
<td>0.16</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>0.00</td>
<td>0.42</td>
<td>0.63</td>
<td>0.16</td>
<td>0.66</td>
<td>0.14</td>
<td>NA</td>
</tr>
</tbody>
</table>
Table B.5  Pairwise Nei’s Fst between populations for Geomys bursarius delimited by major rivers.

<table>
<thead>
<tr>
<th>Population</th>
<th>Flint Hills</th>
<th>Illinois</th>
<th>Iowa</th>
<th>Kiowa</th>
<th>Red Hills</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flint Hills</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Illinois</td>
<td>0.189</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iowa</td>
<td>0.204</td>
<td>0.179</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kiowa</td>
<td>0.107</td>
<td>0.196</td>
<td>0.185</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red Hills</td>
<td>0.107</td>
<td>0.148</td>
<td>0.177</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Wisconsin</td>
<td>0.320</td>
<td>0.267</td>
<td>0.147</td>
<td>0.274</td>
<td>0.272</td>
</tr>
</tbody>
</table>

Table B.6  Pairwise Nei’s Fst between populations for Geomys bursarius illinoensis delimited by rivers with watersheds.

<table>
<thead>
<tr>
<th>Population</th>
<th>Kankakee</th>
<th>Mackinaw</th>
<th>Sangamon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kankakee</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mackinaw</td>
<td>0.155</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sangamon</td>
<td>0.106</td>
<td>0.060</td>
<td></td>
</tr>
<tr>
<td>Vermillion</td>
<td>0.083</td>
<td>0.036</td>
<td>0.039</td>
</tr>
</tbody>
</table>

Fig. B.2  Index of association across all loci for all G. bursarius samples. Although there is significant association, the $\bar{r}_d$ is 0.0385.
Fig. B.3 Pairwise loci index of association for all *G. bursarius* samples. The highest correlation is GBR27 to GBR25 with an $\bar{r}_d$ of 0.237.
Appendix B.3. STRUCTURE spatial population assignment and delta K for a thinned iteration at K=3 with 7 microsatellites and at K=2 without locus GBR06.

a.

b.

Fig. B.4  STRUCTURE population assignment for a thinned trial of Geomys bursarius (n = 137) with a subsampling of G. b. illinoensis (n = 40) for (a) K=3 and 7 microsatellites and (b) K=2 and 6 microsatellites, removing GBR06. Individual assignments viewed on the base World Topography Map (ESRI et al. 2017).
Appendix C: Maximum likelihood analysis using 10,000 bootstraps under the HKY 85 model.

We ran RAxML for the trimmed mitogenome dataset, COX1, and cyt-b. Bootstrap values were used to describe topology support, and trees were visualized in FigTree v1.4.4. For Maximum Likelihood tree inference, we rooted trees based on the midpoint with bootstrap values for branch support (Figure 3). There was poor bootstrap support and phylogenetic resolution of other *Geomys* subspecies, likely due to low sample size and high genetic variation. The phylogenetic patterns derived from the trimmed mitogenome dataset were consistent with those resulting from the COX1 and cyt-b datasets, but with the latter having even less bootstrap support, and thus only present the tree topology for the trimmed mitogenome and not COX1 and cyt-b.
Fig. C.1 RAxML phylogenetic tree of gophers using the trimmed mitogenome with bootstrap values (n = 10,000) along branches under the HKY 85 model.
Appendix C.2. Minimum spanning network of the retained mitogenome and the median spanning networks for COX1 and cyt-b

Fig. C.2 Minimum Spanning Tree based on Regions (Alexander et al. in Review) for the trimmed mitogenomes.
Fig. C.3 Minimum spanning tree using geographic distance to 10 clusters for the trimmed mitogenome.
**Fig. C.4** median joining network by subspecies for COX1 (bp = 1545) from PopArt. Epsilon = 0.
Fig. C.5 Median Joining network by region for COX1 (bp = 1545). Epsilon=0.
Fig. C.6 Median joining network for cyt-b (1067 bp) by species. PopArt, epsilon=0.
Fig. C.7  Median joining network for cyt-b (bp=1067) by region (Alexander et al. in review). PopArt, epsilon=0.