



Genetic diversity and historical demography of the imperiled rattlesnake-master borer moth (*Papaipema eryngii*)

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Abstract

Large-scale habitat loss and degradation have triggered a global biodiversity crisis with ecological-specialist species at increased risk of extinction. The Rattlesnake-Master Borer (*Papaipema eryngii*) is a small noctuid moth whose larvae feed exclusively on rattlesnake master (*Eryngium yuccifolium*), a native grassland plant ranging throughout the eastern United States. Widespread loss of grassland habitats has resulted in declines of *P. eryngii* and the species has been considered for listing under the U.S. Endangered Species Act. We analyzed the mitochondrial *COI* barcode sequences of 76 *P. eryngii* from across the species' distribution to examine genetic diversity and structure, and to test for historical changes in population size. We found 26 haplotypes with high haplotype diversity and low nucleotide diversity. Small but significant amounts of genetic differentiation occurred between populations separated by the Mississippi River and among representation units, but > 90% of genetic variation occurred within populations. Isolation by distance was minimal, suggesting a lack of dispersal limitation, at least historically. All historical demographic analyses supported a recent population expansion, likely since the Last Glacial Maximum. Much genetic diversity is present within the range of *P. eryngii*, but the absence of some haplotypes from where they occurred historically suggests efforts to facilitate gene flow among extant populations may be needed to abate the loss of genetic diversity.

Implications for insect conservation This study provides genetic data to inform management actions and future Species Status Assessments. Such data were unavailable for the previous Species Status Assessment, which based representation units on plant hardiness zones.

Keywords Conservation genetics · DNA barcode · Endangered Species Act · Noctuidae · Representation · Species status assessment

Introduction

Large-scale habitat loss and degradation have triggered a global biodiversity crisis, resulting in functionally impaired ecosystems (Butchart et al. 2010; Cardinale et al. 2012; Hooper et al. 2012; Dirzo et al. 2014). Extinction rates associated with biodiversity loss are estimated upwards of 1000× pre-human levels and are typically characterized by an initial loss of ecological-specialist species (Pimm et al. 1995; Barnosky et al. 2011; Clavel et al. 2011; Ceballos et al. 2015). Extinctions of specialists drive communities toward homogenization, leaving only common, tolerant, generalist species (McKinney and Lockwood 1999; Olden et al. 2004; Clavel et al. 2011; Filgueiras et al. 2021). Specialists obligated on other species to meet their requirements are at even greater extinction risk (McKinney 1997; Koh et al. 2004; Biesmeijer et al. 2006; Colles et al. 2009). Within the

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Lepidoptera superfamily Noctuoidea, the Borer Moths (Noctuidae: Hadeninae: Apameini: *Papaipema*) require specific plant species to complete their life cycle. Some species, such as the Stalk Borer (*Papaipema nebris* (Guenée 1852)), are considered agricultural pests because of their generalized host-plant specificity; however, the majority of *Papaipema* species are host-specific on localized and often uncommon plants, making many of conservation concern (Solomon 1988; Goldstein 1999; Goldstein and Fibiger 2005).

The Rattlesnake-Master Borer (*Papaipema eryngii* Bird 1917) is a small (3.5–4.8 cm wingspan) noctuid moth whose larvae feed exclusively on rattlesnake master (Apiales: Apiaceae: *Eryngium yuccifolium* Michaux 1803), a native grassland plant ranging throughout the eastern United States (Bird 1917; U.S.D.A. Natural Resources Conservation Service 2024). The historical range of *P. eryngii* is uncertain, but the species has likely declined significantly due to large-scale habitat loss when grassland ecosystems were converted to agriculture (Bird 1934; Samson and Knopf 1994; Swengel et al. 2011; Bess 2018). Despite the broad distribution of its host plant, *P. eryngii* has been documented from just eight states: Illinois, Iowa, Missouri, Arkansas, Oklahoma, Kansas, Kentucky, and North Carolina. In 2013, the U.S. Fish and Wildlife Service (USFWS) presented their 12-month finding on a petition to list *P. eryngii* under the U.S. Endangered Species Act of 1973 (U.S. Fish and Wildlife Service 2013). At the time, *P. eryngii* was extant at only 16 locations with most localities discovered since 1994. They determined listing was warranted but precluded by higher priority actions and placed *P. eryngii* on the candidate species list. Subsequent surveys during 2013–2018 increased the number of extant *P. eryngii* populations to 55 in six states (U.S. Fish and Wildlife Service 2020a, b). Based on an increased number of extant populations and an analysis of the ability of *P. eryngii* to maintain resiliency, redundancy, and representation through stochastic events and changing environmental conditions, the USFWS determined listing was not warranted in 2020 (U.S. Fish and Wildlife Service 2020a).

Given the small number and fragmented nature of populations of *P. eryngii*, the species may have reduced genetic diversity due to genetic drift, restricted gene flow, and increased inbreeding. Such detrimental genetic factors would put them at increased risk of local extinction and threaten the species' long-term survival. To date, no genetic studies of *P. eryngii* have been conducted. In the status assessment of *P. eryngii* that informed the decision not to list the species, the USFWS used environmental variation across the species' range as an estimate of diversity due to a lack of phenotypic variation among populations and an absence of species-specific genetic information (U.S. Fish and Wildlife Service 2020b). Diversity, which is used to assess representation, is meant to be a proxy for a species' ability to adapt to changing environmental conditions over time (Shaffer et al.

2000; Wolf et al. 2015; Smith et al. 2018). However, this assumes populations are adapted to local conditions, which may not be the case if genetic drift is strong, gene flow is high, or inbreeding is occurring. Data on genetic diversity across the range of *P. eryngii* are needed to evaluate this assumption and inform future Species Status Assessments.

Herein, we examine the genetic diversity and historical demography of *P. eryngii* across the species' range. Specifically, we test for genetic structure, which would indicate restricted gene flow, and we evaluate the hypothesis the species has experienced a historical decrease in population size. The information we present is important for evaluating components of the Species Status Assessment and will also be useful for guiding future management and conservation actions.

Materials and methods

Data collection

We removed a leg from 72 previously collected adult specimens of *P. eryngii* and temporarily placed each into a tube of 100% ethanol for transport to the laboratory. Most specimens were from the private collections of J. Wiker and J. Bess. We also sampled tissue from the head of one freshly collected larval *P. eryngii*. Our oldest specimens were collected in 1991, but 84% were collected since 2008. Our sampling included six of the eight states where *P. eryngii* has been documented; we lacked samples from Iowa and North Carolina, where the species is presumed extirpated (Fig. 1). A list of all samples and their locality and collection information is provided in Supplemental Table S1, available online.

We extracted DNA from tissue samples using the Qiagen DNEasy Animal Blood & Tissue kit (Valencia, CA, USA) following Wilson's (2012) protocol for archival insect specimens. We used polymerase chain reaction (PCR) to amplify a 658 base-pair (bp) portion of the *cytochrome c oxidase I* mitochondrial gene (*COI*) using the *COI* barcode primers for Lepidoptera from Hebert et al. (2004): LepF1 (5'-ATTCAA CCAATCATAAAGATATTGG-3') and LepR1 (5'-TAA ACTTCTGGATGTCCAAAAAATCA-3'). The *COI* barcode is commonly used to identify species in a broad range of taxonomic groups, including lepidopterans, and shows adequate levels of intraspecific variation for use in broad-scale population genetic studies (see Zahir et al. 2017 and references within). Yield and quality of DNA degrade with specimen age, creating PCR problems, especially for longer DNA fragments. For samples that did not work for the above primer set, we attempted to amplify the *COI* barcode fragment in two pieces using the internal *COI* barcode primers for Lepidoptera from Hajibabaei et al. (2006): MLepF1 (5'-GCTTTCCACGAATAAATAATA-3') and MLepR1

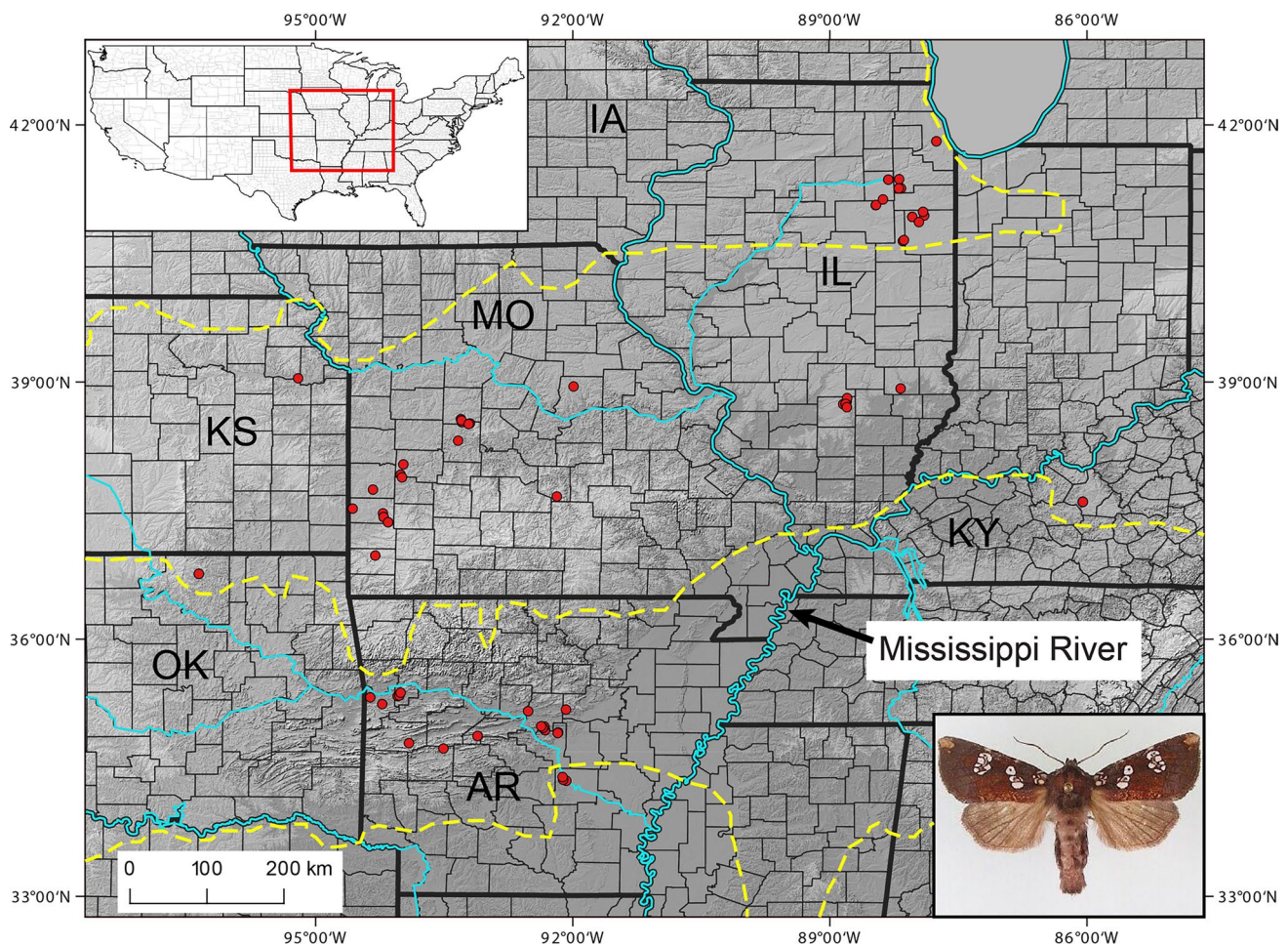


Fig. 1 Locations of genetic samples of Rattlesnake-Master Borer Moth (*Papaipema eryngii*) analyzed in this study plotted on a digital elevation map with major rivers shown. The Mississippi River is labeled and dashed yellow lines delineate the approximate boundaries of the North, Middle, and South representation units. Upper left inset

shows the United States of America with a rectangle demarcating the area displayed. The focal species is pictured in the lower right (Photo by Jim Wiker). State abbreviations are: AR-Arkansas, IL-Illinois, IA-Iowa, KS-Kansas, KY-Kentucky, MO-Missouri, and OK-Oklahoma

(5'-CCTGTTCCAGCTCCATTTTC-3'). In these cases, we paired MLepF1 with LepR1 (~420 bp) and MLepR1 with LepF1 (~300 bp). PCR reactions (25 µl reaction volume) comprised 12.5 µl Promega GoTaq Green Master Mix (Madison, WI, USA), 1.25 µl of each primer (10 mM), 3.5 µl of DNA, and 6.5 µl of water. The thermal-cycler protocol consisted of 95 °C for 1 min, followed by 36 cycles of 95 °C for 30 s, 50 °C for 40 s, and 72 °C for 1 min, and then a final extension phase at 72 °C for 10 min. Successful amplification of the target fragment was verified by gel electrophoresis. PCR products were sent to Eurofins Genomics (Louisville, KY, USA) for purification and sequencing using primers LepF1 and LepR1. For each specimen, we examined and edited sequence chromatograms by eye, trimmed flanking primer sequences, and made contigs from forward and reverse sequences using Geneious v.10.1.3 (Kearse et al. 2012). DNA sequences generated in this study are available

on GenBank under accession numbers PP973552–PP973624 (Supplemental Table S1).

To augment our dataset, we also downloaded three full-length *COI* barcode sequences of *P. eryngii* from the Barcode of Life Datasystem (BOLD; <http://www.boldsystems.org>). These specimens were collected between 1918 and 1938 from the Chicago area in Cook County, Illinois, where the species is now presumed extirpated. We aligned all sequences using the MUSCLE algorithm and visually examined the alignment and translation in Geneious to ensure no gaps or stop codons were present.

Data analysis

To characterize genetic diversity within *P. eryngii*, we calculated haplotype number (h), haplotype diversity (Hd), the number of variable sites (S), nucleotide diversity (π),

and the average number of nucleotide differences (k) using DnaSP v.6 (Rozas et al. 2017). To examine the genetic structure throughout the range of *P. eryngii* and assess the extent to which haplotypes are shared across populations, we inferred a median-joining haplotype network in PopArt (Leigh and Bryant 2015). To evaluate how well we sampled genetic variation in *P. eryngii*, we made a haplotype accumulation curve and estimated the total number of haplotypes using the haploAccum and chaoHaplo functions in the *spider* v.1.5.1 package in R v.4.5.0 (Brown et al. 2012; R Core Team 2025). We calculated the mean and maximum uncorrected pairwise sequence divergence (p -distance) between haplotypes using MEGA v.7 (Kumar et al. 2016).

To evaluate genetic structure, we performed an Analysis of Molecular Variance (AMOVA) in PopArt with 1,000 permutations to test significance (Excoffier et al. 1992). We tested for genetic differentiation across the Mississippi River, a common geographic barrier in many organisms (Soltis et al. 2006; Pyron and Burbrink 2010), and among the three representation units (South, Middle, North) used by USFWS in the Species Status Assessment of *P. eryngii*. We also tested for isolation by distance, a common pattern indicative of dispersal limitation (Wright 1943; Bohonak 1999). For this, we first used our sample latitude and longitude coordinates (WGS84) to calculate a pairwise geographic distance matrix using the Geographic Distance Matrix Generator v.1.2.3 (Ersts 2025). We next calculated a pairwise genetic distance (p -distance) matrix for all 76 samples using MEGA v.7 (Kumar et al. 2016). We then performed a Mantel test using the geographic and genetic distance matrices in GenoDive v.3.06 with 999 permutations to evaluate significance (Meirmans 2020).

We used multiple methods to test for genetic signatures indicative of a historical decrease in population size. First, we calculated Tajima's D (Tajima 1989) and Fu and Li's D^* (Fu 1997). Tajima's D and Fu and Li's D^* are expected to be near zero if population size has been stable. Significant negative values are expected in populations that have undergone recent expansion, whereas significant positive values are expected in populations that recently experienced contraction (Tajima 1989; Fu 1997). Next, we analyzed the mismatch distribution, which compares observed and predicted distributions of pairwise differences to infer historical changes in effective population size (N_e). A unimodal mismatch distribution indicates a recent population expansion, a multimodal (including bimodal) distribution indicates a population decrease or subdivision, and a ragged distribution suggests the population size is stable (Rogers and Harpending 1992). We used the R_2 raggedness statistic of Ramos-Onsins and Rozas (2002) to quantify the smoothness of the observed distribution. The fit of observed values was tested against a null distribution generated using 1,000

coalescent simulations under a constant population size model in DnaSP v.6. (Rozas et al. 2017).

Finally, we constructed a Bayesian skyline plot (Drummond et al. 2005), a genealogical coalescent method that estimates N_e through time without specifying an a priori demographic model (e.g., constant size, exponential growth, or logistic growth). We inferred a skyline plot in BEAST v.1.8.0 (Drummond and Rambaut 2007) under a HKY + Γ substitution model, a strict clock, and a Bayesian skyline tree prior with 8 grouped coalescent intervals. To scale the time axis (X) on the plot, we used a rate of 0.096 substitutions per million years to set the lognormal mean (0.096) and standard deviation (0.05) of the clock rate prior. This rate is based on analyses of *COI* sequence variation in *Parnassius mnemosyne* (Linnaeus 1758) (Lepidoptera: Papilionidae) across central and eastern Europe (Gratton et al. 2008). We chose this rate over the widely used rate of 0.0115 substitutions per million years for mitochondrial DNA (mtDNA) in insects (Brower 1994; Papadopoulou et al. 2010) because it is an intraspecific rate whereas the latter represents an interspecific rate. Rates of mtDNA evolution are time dependent and the use of interspecific rates, which are considerably lower, results in overestimation of the timing of recent events such as those occurring within species (Ho et al. 2005; Ho and Lo 2013; Grant 2015). We also chose this rate because it is based on another species of lepidopteran and rates are known to vary among major insect lineages (Ho and Lo 2013). Model parameters and genealogies were sampled every 3,000th iteration for 30 million generations. We used Tracer v.1.7 (Rambaut et al. 2018) to view $-\ln L$ values and parameter estimates to check for stationarity and ensure all ESS values were > 200 (Drummond and Bouckaert 2015). We then discarded 10% of the initial samples as burn-in and visualized the skyline plot using Tracer v.1.7.

Results

Our DNA alignment contained 600–658 bp of *COI* barcode sequence for 76 *P. eryngii* from Illinois, Kentucky, Missouri, Arkansas, Oklahoma, and Kansas (Fig. 1). Twenty specimens required using the internal PCR primers and the sequencing of two partially overlapping fragments to obtain the full *COI* barcode sequence. Among our 76 sequences, 26 mitochondrial haplotypes were present (h). Haplotype diversity (Hd) was 0.944, nucleotide diversity (π) was 0.00401, the number of variable sites (S) was 28, and the average number of nucleotide differences (k) was 2.35. Based on the median-joining haplotype network, 7 of the 26 haplotypes were shared among two to four states (Fig. 2). Within states, different haplotypes were often separated by haplotypes occurring in other states. Notably, the three haplotypes from the 1918–1938 specimens from the Chicago

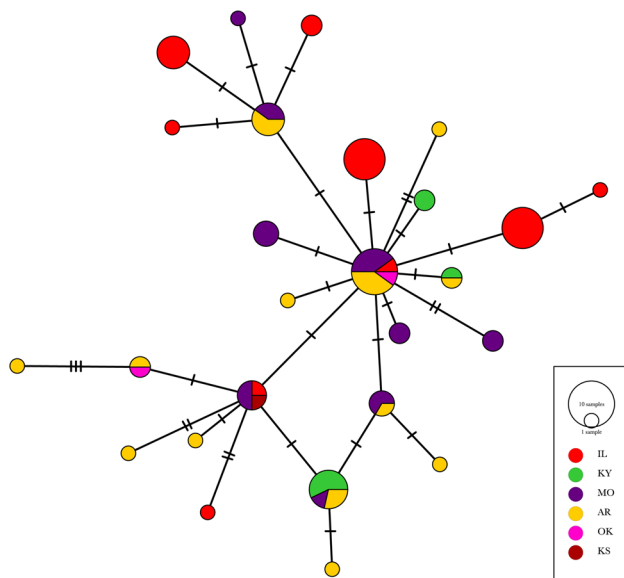


Fig. 2 Median-joining network for 26 haplotypes from 76 *COI* barcode sequences of the Rattlesnake-Master Borer Moth (*Papaipema eryngii*) from Illinois (IL), Kentucky (KY), Missouri (MO), Arkansas (AR), Oklahoma (OK), and Kansas (KS). Each haplotype is represented by a circle in which the area is proportional to its frequency. Hash marks represent mutational steps

area, where *P. eryngii* is likely extirpated, were not found elsewhere in Illinois, but two are still present in other states (one in Kansas and Missouri; one in Arkansas, Missouri, and Oklahoma; Supplemental Table S1). Eight haplotypes were unique to areas east of the Mississippi River, 14 haplotypes were unique to areas west of the Mississippi River, and 4 haplotypes occurred on both sides. The South, Middle, and North representation units contained 13, 13, and 8 haplotypes, respectively, with 7 haplotypes shared between two or more units. Most haplotypes were separated from adjacent haplotypes in the network by a single mutational step and the maximum number of steps between any two haplotypes was seven (Fig. 2). The haplotype accumulation curve was still increasing at 76 samples but was starting to plateau (Supplemental Fig. S1). The Chao1 estimate of the total number of haplotypes was 36 with a 95% confidence interval of 28–70. Based on this, we sampled ~72% (26/36) of the haplotype diversity in *P. eryngii*. Sequence divergence (*p*-distance) between haplotypes averaged 0.55% and maximum sequence divergence between any pair of haplotypes was 1.19%.

AMOVA revealed significant genetic differentiation between populations east and west of the Mississippi River ($\Phi_{ST}=0.031$, $P=0.008$). However, of the total genetic variation present, only 3.1% occurred between populations on opposite sides, whereas 96.9% occurred within populations on the same side. Similarly, AMOVA revealed significant genetic differentiation among the three USFWS

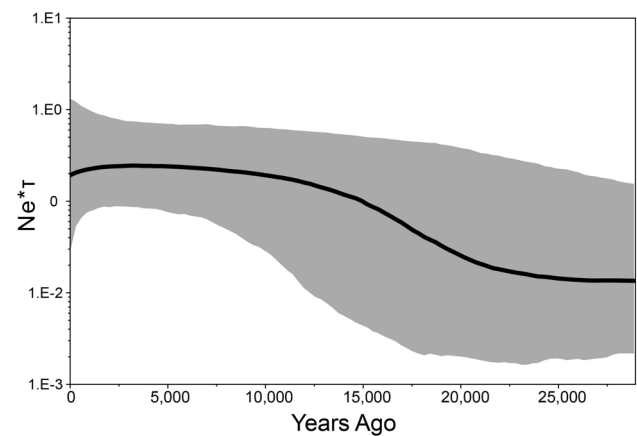


Fig. 3 Bayesian skyline plot showing inferred changes in effective population size (N_e) in Rattlesnake-Master Borer Moth (*Papaipema eryngii*) over the past ~30,000 years

representation units ($\Phi_{ST}=0.096$, $P<0.001$); however, only 9.6% of the total genetic variation occurred among units with 90.4% occurring within units. A Mantel test revealed a weak but significant correlation between geographic and genetic distance ($r=0.10$, $P_{one-tailed}=0.001$), suggesting minimal influence of isolation by distance in the genetic differentiation of populations.

Tajima's D (-1.89 , $P=0.009$) and Fu and Li's D^* (-2.52 , $P=0.035$) both supported a recent population size increase in *P. eryngii*. The mismatch distribution was distinctly unimodal ($R_2=0.040$, $P=0.015$), indicating a recent population expansion. The Bayesian skyline plot showed a marked population size increase from ~22,500 to ~12,500 years ago and then a slower increase until ~2,500 years ago after which it decreased slightly toward the present (Fig. 3). The 95% highest posterior density estimates are wide at older time scales and widen slightly again ~2,000 years ago to the present, indicating increased uncertainty during these times, but the overall pattern supports a population expansion since the Last Glacial Maximum (~21,000 years ago).

Discussion

Our results revealed high haplotype diversity and little genetic structure across the range of *P. eryngii*. A small, but significant, amount of genetic differentiation occurred between *P. eryngii* populations on opposite sides of the Mississippi River, but ~97% of genetic variation occurred within populations on the same side. We also found minimal isolation by distance, indicating a lack of dispersal limitation, at least historically. Our haplotype network showed several mitochondrial haplotypes are shared among geographically distant populations (e.g., Illinois and Oklahoma), indicating these populations are currently

or were recently connected by gene flow. Given the massive loss and fragmentation of prairie habitats in the region (Samson and Knopf 1994), these widely disjunct populations are not likely exchanging migrants presently. Sampling more individuals per population would improve estimates of population-level genetic diversity and using either faster-evolving genetic markers such as microsatellites or genome-scale data (e.g., RADseq, GBS, WGS) would provide better measures of contemporary gene flow among populations.

Historically, *P. eryngii* was likely distributed in open habitats throughout the central and southeastern United States, and extensive conversion of these systems to agriculture led to wide-scale losses of populations, resulting in the current disjunct clusters of remnant populations (Bird 1934; Samson and Knopf 1994; Swengel et al. 2011; U.S. Fish and Wildlife Service 2013, 2020). Contrary to our hypothesis, we did not find evidence of a widespread population decline in *P. eryngii*; instead, our analyses indicated a recent population expansion. Given that loss and fragmentation of open habitats in the region have been concentrated in the past 200 years, the mutation rate of mtDNA is likely too slow to detect such a recent change in population size. mtDNA is likely capturing information about population size changes over older time scales such as the past 10,000 to a few million years (Avise et al. 1987). Although grasslands expanded across much of central North America in the late Miocene (8–5 million years ago), repeated glaciations during the Pleistocene (2.5 million–11,500 years ago) caused broad-scale vegetation changes (Axelrod 1985; Webb et al. 2003; Anderson 2006). The timescale on our skyline plot inferred the population size increase in *P. eryngii* occurred ~22,500 to ~12,500 years ago, which corresponds to the period after the Last Glacial Maximum (~21,000 years ago) when grassland habitats expanded following the retreat of glacial ice sheets (Anderson 2006; Grant 2015). Notably, tallgrass prairie as we know it currently, did not exist before the Holocene (11,500 years to present; Anderson 2006). Although *P. eryngii* and its host plant, rattlesnake master, occur in the tallgrass prairie region today, both also occur in savanna, flatwood, glade, and open woodland habitats in Arkansas, Kentucky, Missouri, and North Carolina (historically). Rattlesnake master and *P. eryngii* were likely both pushed south to the U.S. coastal plain during the Last Glacial Maximum and then expanded northward as the climate warmed. The low nucleotide diversity, high haplotype diversity, and lack of genetic structure in *P. eryngii* are consistent with a recent population expansion following a period of low effective population size. Although we were unable to detect a genetic signature of a population decline in *P. eryngii* over the past 200 years, our results suggest that any such decline is not simply an extension of a long-term downward trend due to natural causes.

We estimated 36 haplotypes exist within *P. eryngii*, of which we sampled 26 (~72%). Thus, it appears that much of the historical genetic diversity in *P. eryngii* remains present, likely due to the recency of fragmentation or gene flow from undocumented populations. *Papaipema eryngii* is notoriously difficult to detect (U.S. Fish and Wildlife Service 2020b) so it may persist in some unsampled grassland fragments. Targeted surveys of appropriate grassland fragments in the study region may help to find additional populations. Nonetheless, the current absence of some haplotypes that were present in Illinois historically (1918–1938) suggests efforts to facilitate gene flow among extant populations may be needed to abate the loss of genetic diversity due to genetic drift and inbreeding. Habitat corridors connecting populations could be established, and suitable restorations within corridors could be repatriated with *P. eryngii*. Adult *P. eryngii* can disperse at least 3.2 km in their 10–14 day life span (Wiker 2017). Minimal isolation by distance suggests they are capable of dispersal in areas of contiguous suitable habitat. Reciprocal translocations should also be considered between extant populations in cases where a suitable habitat corridor between populations cannot be established. The small divergences among haplotypes and low genetic differentiation among populations suggest any nearby population could potentially serve as a source for repatriation efforts. However, adaptive genetic differences may exist between populations that we could not evaluate with our *COI* barcode locus. For this reason, phenotypic traits, physiological tolerances, and phenology of populations should be closely examined before any translocations, especially over great distances where environmental conditions are likely to differ, to ensure maladapted alleles are not introduced.

In 2020, USFWS determined listing *P. eryngii* was not warranted based in part on an analysis of the ability of *P. eryngii* to maintain resiliency, redundancy, and representation through stochastic events and changing environmental conditions (U.S. Fish and Wildlife Service 2020a, b). Representation refers to a species' ability to adapt over time to a changing environment and is often measured by the extent of genetic and ecological diversity within and among populations (Shaffer et al. 2000; Wolf et al. 2015; Smith et al. 2018). In the absence of species-specific genetic information, USFWS used environmental variation across the range of *P. eryngii* as a proxy. Specifically, they used plant-hardiness zones to divide extant populations into three latitudinal representation units, and then evaluated resiliency and redundancy under this framework (U.S. Fish and Wildlife Service 2020b). Populations in different environmental settings often have unique genetic, ecological, and behavioral variation, thus dividing a species' range into mutually exclusive geographic areas that capture this underlying diversity is a reasonable approach for determining representation (Wolf et al. 2015; Smith et al. 2018). However, this assumes

populations are adapted to local conditions, which may not be the case if genetic drift is strong, gene flow is high, or inbreeding occurs.

We found genetic diversity is high within the three representation units with little genetic differentiation among units, indicating recent gene flow and/or population expansion. Furthermore, some extant populations may have lost diversity through genetic drift. Our results cast doubt on the assumption that populations are locally adapted and indicate populations across the species' range are genetically similar to one another. Genetic diversity is thought to be related to a species' ability to adapt to changing environments (Barrett and Schluter 2008; Markert et al. 2010); however, because *COI* is a neutral (or near-neutral) genetic marker and has no direct effect on fitness, it may not be a good indicator of a species' adaptive potential. Neutral markers may provide information about drift, gene flow, and inbreeding, evolutionary forces that can oppose local adaptation, but they are not directly linked with genetic variation and population differentiation in adaptive traits (Holderegger et al. 2006; Forister et al. 2008; Teixeira and Huber 2021). Geographic variation in phenotypic traits of *P. eryngii*, including physiological traits, and the heritability of those traits should be examined to evaluate adaptive genetic diversity and local adaptation. Larval *P. eryngii* hatch up to 1 month earlier and adults emerge from their pupae ~ 1 month later in southern populations compared to northern populations (Bess 2018; U.S. Fish and Wildlife Service 2020b). This variation is likely related to temperature (i.e., due to phenotypic plasticity), but there could be genetic-based variation as well.

Rattlesnake master, the host foodplant of *P. eryngii*, occurs throughout the central and southeastern United States, but *P. eryngii* is seemingly absent from latitudes south of central Arkansas (Bess 2018; U.S. Fish and Wildlife Service 2020b; U.S.D.A. Natural Resources Conservation Service 2024). The rattlesnake master varieties/species present in portions of the southeastern United States, including *Eryngium yuccifolium synchaetum*, differ in morphology and ecological requirements (e.g., frequent fire) from the variety in Arkansas and the midwestern states (*Eryngium yuccifolium yuccifolium*) that might make them less suitable host plants (Bess 2018; U.S.D.A. Natural Resources Conservation Service 2024). Alternatively, the warmer temperatures at these southern latitudes may limit the distribution of *P. eryngii*. Larval *P. eryngii* aestivate during the hottest portions of summer and resume feeding only when temperatures cool, which occurs later at the southern range edge in Arkansas and Kentucky (Bess 2018). This observation indicates high temperatures restrict activity and growth in *P. eryngii*, which could be a problem with current climate-warming trends. The plant hardiness zones used by USFWS in the Species Status Assessment were from 2009 (U.S. Fish and Wildlife Service 2020b) and the newer 2023 zones

(U.S.D.A. Agricultural Research Service 2023) shifted all populations of *P. eryngii* in the southern part of the species' range in Arkansas from Zone 7 to Zone 8. Notably, the two populations in Zone 8 in the 2009 map are considered extirpated (Bess 2018; U.S. Fish and Wildlife Service 2020b). Studies of larval thermal ecology and tolerance limits as well as monitoring of Arkansas populations are needed to understand and document any effects of climate warming on *P. eryngii*. If population declines and extirpations are observed, then USFWS should reassess the status of *P. eryngii*, incorporating our genetic data and the changes in plant hardiness zones.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10841-025-00698-0>.

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Data availability Genetic data generated in this study are openly available in GenBank at <https://www.ncbi.nlm.nih.gov/genbank/> under accession numbers PP973552–PP973624. Locality data including GPS coordinates (0.01 decimal degrees) of sampled specimens are available in the supplementary material available online. Requests for more precise locality data can be made to the corresponding author.

Declarations

Ethical approval Not applicable.

Conflict of interest The authors declare no competing interests.

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