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Genomic Signatures of Decline and Recovery in an Endangered Bat

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Research Article

Keywords: Genetic diversity, recovery, gray bat (Myotis grisescens), admixture, effective population

Posted Date: March 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-8734754/v1

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Abstract

Tremendous population declines may result in the loss of genetic diversity, which can in turn lead to reduced phenotypic health, reduced adaptive potential, and further population declines. In other words, a severe demographic decline may ultimately lead to an extinction vortex. Therefore, understanding genetic diversity is vital for assessing the health of species that have undergone declines, and can even reflect the effectiveness of management actions. We investigated signatures of genetic bottlenecks in a species that has been the subject of focused management efforts since its 1976 listing as endangered in the US, the gray bat (*Myotis grisescens)*. We used whole genome sequencing to calculate genetic diversity metrics, including inbreeding coefficients, Tajima's D, and heterozygosity, and to infer effective population size and structuring. We found a loss of rare alleles and heterozygosity excess, in line with strong declines in relative abundance of the census population. However, we did not detect inbreeding, erasure of population substructure, or low effective population size. Despite an apparent bottleneck, the gray bat appears to have avoided detrimental genetic consequences associated with population declines, likely due to the conservation of connected populations of adequate effective size. Our findings highlight the importance and effectiveness of timely conservation interventions for preserving the genetic health of species. Strong declines in relative abundance (i.e., based on percentage of the initial population) do not necessarily lead to inbreeding when there is still adequate absolute abundance (i.e., number of individuals).

Introduction

The past century has seen an unprecedented and poorly addressed increase in human-caused extinction rates of species, representing a global biodiversity crisis (Scott et al. 2024). Extinction risk increases following large declines in census population sizes (i.e. demographic bottlenecks), which can lead to the loss of genetic diversity and subsequent loss of adaptive potential (Tanaka 1998). Ultimately, these effects can culminate in an extinction vortex and the loss of a species (Brook et al. 2002, Tanaka 2018). Thus, genetic diversity, which allows populations to adapt to new stressors, is vital to track and to manage in vulnerable populations.

Certain scenarios and species characteristics should theoretically preserve genetic diversity in populations experiencing demographic bottlenecks. For instance, mild to moderate population declines with rapid subsequent recovery are less likely to result in genetic bottlenecks than severe declines with slow recovery (Sonsthagen et al. 2017, Lucena-Perez et al. 2021, Olazcuaga et al. 2023). Life history traits of species can also influence the extent of genetic diversity loss. A large initial effective population size can buffer the loss of rare alleles. Additionally, high dispersal ability can contribute to the maintenance of rare alleles and facilitate gene flow (Frankham 1996, Hedrick 2005). However, even being abundant, widespread, and mobile may not be enough to save a species from disastrous genetic decline in the face of external threats, such as habitat loss and fragmentation. For instance, European Nightjars (*Caprimulgus europaeus* L.) suffered genetic diversity loss due to alteration of woodland habitat, despite previously being widespread (Day et al. 2025). Similarly, Passenger Pigeons (*Ectopistes migratorius* L.) exhibited low heterozygosity despite massive population sizes, prior to intensive hunting pressure driving them to extinction (Hung et al. 2014). Many species do not recover genetic diversity even after demographic recovery (e.g., Bortoluzzi et al. 2019, Bozzuto et al. 2019, Robinson et al. 2019, etc.) For example, Northern elephant seals (*Mirounga angustirostris* Gill) and Spotted Kiwis (*Apteryx owenii* Gould) regained population numbers quickly post-bottleneck, but with reduced fitness (Hoelzel et al. 2024), high homozygosity (Ramstad et al. 2013), and continued reliance on conservation interventions.

Though some species suffer lasting negative genetic repercussions after bottlenecks, population declines are not self-fulfilling prophecies. For instance, immigrants from large, genetically healthy source populations can introduce valuable genetic diversity to populations suffering inbreeding depression (e.g., Åkesson et al. 2016), and small populations can create favorable conditions for purging deleterious alleles (e.g., Robinson et al. 2019, Ochoa and Gibbs 2021). Conservation action aims to protect the viability of species through actions such as safeguarding habitat, maintaining dispersal corridors, and sustaining critical population sizes. However, populations of concern often remain genetically vulnerable to collapse, even when protection criteria are met (Tallmon et al. 2004, Bouzat 2010). Thus, it is important to assess genetic diversity when making conservation decisions. The mechanisms that maintain viability are not always well understood and can vary across contexts and taxa (de Kort et al. 2021, Allen et al. 2022, Hoffman et al. 2024). It can also be informative to identify populations that have resisted the loss of genetic diversity through demographic bottlenecks, and their associated life history traits.

The gray bat (*Myotis grisescens* Howell) was among the first species listed under the US Endangered Species Act, following estimated population declines of up to 80% between the mid 19th and mid 20th centuries (Brady et al. 1982). Large, concerted efforts to protect habitat (e.g., gating of sensitive hibernation sites) led to massive demographic recovery since the 1970s, such that the gray bat may now be an example of conservation success (Martin 2007, Adams et al. 2024), although it is still on the endangered species list. The gray bat's relatively low reproductive output (i.e., only one offspring yearly starting at age two) could decrease genetic diversity and increase inbreeding, although the species' promiscuousness and dispersal ability may have the opposite effect. Gray bats, and temperate bat species in general, are also relatively long lived (14–17 years; Harvey 1992). However, the influence of longevity on susceptibility to genetic bottlenecks remain unclear (Bradke et al. 2021, Clark et al. 2024, Gargiulo et al. 2025). The few previously conducted studies of gray bat genetics have found high gene flow between two subpopulations east and west of the Mississippi River Alluvial Plain, and variable but mostly healthy levels of heterozygosity (Lindsay et al. 2015, Nagel et al. 2024). However, no studies have examined gray bat populations north of the Mississippi River Alluvial Plain. Further, the single study assessing genetic bottlenecks in this species used microsatellite-based tests (Lindsay et al. 2015), which can have variable results due to smaller sample sizes (of the genome) and can be sensitive to underlying assumptions (Peery et al. 2012). No study has examined large portions of the gray bat genome through whole genome sequencing (WGS).

We used gray bats to evaluate the genetic effects of a moderate demographic bottleneck with subsequent recovery on a long-lived species. We conducted non-lethal sampling of wild-caught individuals north of the Mississippi River Alluvial Plain. We analyzed genetic diversity and looked for signals of a genetic bottleneck using a thorough suite of tests and statistics. Specifically, we asked whether gray bats show genetic signals of population expansion, contraction, or stability following their demographic bottleneck. We also inferred demographic features, including effective population size and population structure, to ask whether genetic diversity levels in gray bats could be connected to robust reproductive populations or high admixture.

Materials and Methods

Study Site and Sample Collection

We caught gray bats at a privately owned cave in Pike County, Missouri on 20-Oct-2022, in compliance with all animal welfare, state, and federal permits (Missouri State University IACUC #2022-14, MDC Wildlife Collector #60085, and US FWS T&E Recovery #ES04397C, respectively). During this time of the year, gray bats immigrate from bachelor and maternity colonies to hibernation sites, where they mate promiscuously prior to entering hibernation. The cave we sampled is used by gray bats as a minor hibernation site for some individuals during winter and as a temporary stop for other individuals during migration to other hibernation sites. High connectivity and gene flow have been observed across the gray bat range, likely due to dispersal propensity (Lindsay et al., 2015, Holliday et al. 2023, Nagel et al. 2024). Pike County is west-central within the gray bat range (Fig. 1) but likely represents both subpopulations identified by Lindsay et al. (2015).

We obtained tissue samples from 23 adult gray bats using methods originally described by Worthington-Wilmer and Barratt (1996). A sterile, circular biopsy punch (2-mm diameter; Integra, Princeton, NJ, USA) was used to take two tissue punches from the flight membranes on each wing. Samples were immediately stored in microcentrifuge tubes with ethanol (97% concentration) then taken to the lab, where they were kept in a freezer at -20°C until extraction. For extractions, we used a DNeasy Blood and Tissue Kit (Qiagen, Germantown, MD, USA) to extract DNA at Missouri State University, following the standard kit protocols with some modifications. Specifically, to facilitate complete lysis of samples we used additional proteinase K (30–40 uL total per sample) and extended incubation time (24–72 hours) as needed, and to maximize DNA yields we used pre-chilled ethanol (2°C). Final DNA was eluted in water (ddH2O prewarmed to 70°C) and concentrated with a vacufuge.

Sequencing, Filtering, and Alignment

Extracted DNA was sent to The Center for Applied Genomics (Toronto, Canada) for sequencing in 151 base, paired-end fragments on 3 10B lanes of an Illumina NovaSeq X. To assist with low input DNA levels, PCR was used in library preparation. We performed all bioinformatic processes on a Linux workstation at Missouri State University. Adapters were removed from samples with TrimGalore! (V. 0.6.10; Krueger et al. 2015) and cutadapt (V. 5.2; Martin 2011). The quality of entire sequences was assessed with FastQC (V. 0.12.0; Andrews 2010) and the quality of bases was filtered to a Phred33 score of 20 using SAMtools (V.1.21; Danecek et al. 2021).

There was no species-specific reference genome available for the gray bat. Therefore, we aligned our sequences against a reference genome for the cave bat (*M. velifer* [J. A. Allen]; BioProject PRJNA103554; Vazquez et al. 2024) using BWA-mem (V.0.7.19; Li and Durban 2009, 2010). Gray bats are closely related to cave bats (Jones et al. 2002, Agnarsson et al. 2011) and our sequences mapped well to the reference genome.

We removed optical duplicates from the resulting alignments with Picard MarkDuplicates (V.2.0.1; Broad Institute 2019). Indels in the sequences were realigned using GATK IndelRealigner (V.4.3.0.0; Van der Auwera and O'Connor 2020). Finally, we filtered sequences to remove the sex chromosomes and extrachromosomal scaffolds, so that only autosomes were considered for metrics of genetic diversity.

Genetic Diversity

To estimate measures of heterozygosity and homozygosity, we first used the empirical Bayesian approach implemented in ANGSD (V. 0.940; Korneliussen et al. 2014) to obtain a genotype likelihood model. We retained data for all 23 individuals, filtered for sites represented in at least 15 sequences with a minimum depth of 5x and a maximum depth of 60x.We obtained a folded site frequency spectrum from the autosome-only genotype likelihood model with ANGSD realsfs, then used ANGSD thetastat with 50 Kb windows to calculate the number of polymorphic sites (Watterson's theta, θW), the mean number of pairwise differences (nucleotide diversity, π), and genome-wide Tajima's D. Tajima's D compares the relationship of θW and π, with a model population at constant size experiencing only neutral mutation. We calculated individual global and local mean rates of genomic heterozygosity (H), as well as low and high confidence intervals with ROHan (Renaud et al. 2019) using standard parameters in 1-Mb windows.

We used VCFtools (V 0.1.18; Danecek et al. 2011) with the option "–het" to calculate individual expected and observed heterozygosity (H_e and H_o). We calculated individual and per-site inbreeding coefficients (F_{IS} and F_S, respectively) using PCAngsd (Skotte et al. 2013). Inbreeding coefficients compare observed heterozygosity and homozygosity to that expected based on Hardy-Weinberg expected (HWE) proportions. A value of 0 indicates no deviation from HWE, whereas a negative value indicates an excess of heterozygosity, and a positive value indicates an excess of homozygosity.

Population structure and effective population

We used multiple methods to infer ancestry clusters and admixture among individuals. First, we used an iterative approach with PCAngsd (Skotte et al. 2013) to infer a covariance matrix, estimate relatedness, and estimate the number of ancestral populations. We then subset samples to 10 Mb from each chromosome, and used this subset data in NGSadmix (V.32; Meisner and Albrechtsen 2018, 2019) to infer ancestry among individuals. We ran NGSAdmix with 20 replications each of assumed ancestral populations of K = 1–8, using random seeds. To select the best value of K, we calculated ΔK with the NGSAdmix results using the Evanno method (Evanno et al. 2005) and evaluated PCAngsd's automatic K detection. We then assigned individuals to clusters based on Q scores ≥ 0.9. We built structure plots using mean Q values in R. To estimate the strength of structuring between clusters, we used VCFtools to calculate the Weir Cockerham fixation index (F_{ST}).

To estimate the number of reproducing individuals, we calculated recent effective population size (N_e) with linkage disequilibrium in GONE (Santiago et al. 2020), and we estimated long-term effective population size ($N_{e.LT}$) using the equation $\theta W = 4\, N_{e.LT}\, \mu$ (Watterson 1975). For the $N_{e.LT}$ equation, we first obtained the mean θW including runs of homozygosity of all individuals with ROHan. We used a mutation rate (μ) of 2.2×10^{-9}, based on the average mutation

rate of mammals and gray bat life history (Decher and Choate 1995, Kumar et al. 2002). To estimate recent N_e, we considered non-indel sites with a maf of 0.1 and that were sequenced in 90% of samples to create a population ped file. We further filtered the minimum mean and individual depths to 7 and the maximum depths to 60. We ran GONE on a random sample of 20% of sites from the filtered set using standard parameters and used the mean of 40 runs to make N_e estimates.

Results

Sequencing and Alignment

Our overall alignment rate of gray bat sequences was 90.54% (SD ± 0.25%), which we found acceptable. After filtering, the mean depth of coverage was 19.65X ± 2.55 (SD). Coverage was relatively uniform among individuals, and so all 23 individuals sampled were included in all downstream analyses. After filtering and subsetting to autosomes, we considered 1,849,248,362 high quality sites. In the site allele frequency file, which used more aggressive filters including the exclusion of sex chromosomes and extra-chromosomal scaffolds, we considered 14,005,840 total base pairs. In the .ped file, we considered 8,529,672 SNPs.

Genetic Diversity

We calculated multiple metrics of genetic diversity and heterozygosity within the gray bat genome. The autosome-only genome-wide mean θW per site was 0.0542 ± 0.007, with all individuals considered. The mean π per site was 0.067 (SE ± 1e-4). The mean Tajima's D was 0.52 (SE ± 0.002; Fig. 2, Table 1), indicating a scarcity of rare alleles. Across the genome, Tajima's D was normally distributed. Of all sites, 1.17% had Tajima's D values higher than 2, and no sites had values lower than -2.

The mean global genomic rate of heterozygosity (H) of the 23 individuals was 0.00322 (95% CI 0.00301–0.00343; Table 1). Observed heterozygosity was greater than expected heterozygosity (mean H_o = 0.411, mean H_e = 0.376). Indeed, all inbreeding coefficients reflected an excess of heterozygosity based on HWE proportions. At an individual level, mean F_{IS} was -0.23, and at a per-site level mean F_s was -0.18 (Table 1, Fig. 2).

Population structure and effective population

Both methods we used to infer population structure in gray bats indicated multiple populations with high gene flow. Evanno's Delta K resulted in a best fit of 3 population clusters (Table 2), although with the iterative approach implemented in NGSAdmix, we detected just 2 population clusters (Table 1). With K = 2 populations, 65% of individuals were assigned to a single cluster (11 in cluster A; 4 in cluster B; Fig 3a) and structure was extremely weak (weighted F_{ST} = 6.69e-05). With K = 3 populations, structure was weak, only 30% of individuals were assigned to a single cluster, and no individuals were assigned to the third cluster (weighted F_{ST} = 0.00255; 4 in cluster A; 3 in cluster B; 0 in cluster C; Fig. 3b). Individuals assigned to unique clusters when K = 3 were consistently assigned to the same cluster when K = 2. Thus, we decided that 2 subpopulations (with high admixture) is most likely.

Using Watterson's theta, we estimated an $N_{e.LT}$ of 70,738 (95% CI = 68,153–75,000) reproductive individuals (Table 1), which is about half of the minimum recorded census population size from the mid 20th century, and about one tenth of the maximum historical estimates of census population in Missouri (Brady et al. 1982, Martin 2007, Elliott 2008). Estimates of N_e over the past 150 generations show a 94% population decline, with strong declines (≥1% of population loss per generation) starting 108 generations ago and continuing for 37 consecutive generations before hitting the nadir (71 generations/142 years ago, assuming a generation time of 2 years). The 150-generation maximum N_e of 218,297 dropped to a minimum of 13,002. N_e remained at the nadir for 44 generations. Strong increases (≥1% of population per generation) in N_e began 35 generations ago (70 years) and continued until 21 generations ago. For the past 20 generations, contemporary N_e ($N_{e.Cont}$) has remained plateaued at 53,643–59,487 (mean = 57,147), 26.18% of the previous maximum.

Discussion

Despite successful recovery of gray bat population numbers, our analyses show that the species' demographic bottleneck resulted in a loss of rare alleles, heterozygosity excess, and a notable decrease in effective population size. In other words, demographic decline led to a genetic bottleneck characterized by population contraction (Maruyama and Fuerst 1985, Nei 2005). Our estimates of effective population size reaffirm population demographics inferred through census-based methods (e.g., Elliott 2008), with an effective population decline of 94%. However, N_e never dropped below critical thresholds for genetic drift (Shaffer and Samson 1985, Frankham et al. 1996). Near the central area of the gray bat range, we detected multiple subpopulations with high admixture between them. Following a reduction in census size, early conservation actions by managers (e.g., installing bat gates on hibernation sites) and life history traits of the species (e.g., high dispersal, promiscuous mating, and high starting population size) may have helped the gray bat recover demographically and reduced their risk of inbreeding depression, despite a bottleneck.

Our study builds upon previous genetic research on gray bat population structuring and demographic trajectories by Nagel et al. (2024) and Lindsay et al. (2015). Our inference of two subpopulations with high gene flow supports the previously suggested east-west divide (Lindsay et al. 2015 Nagel et al. 2024). Our negative F_{IS} indicates that outbreeding, accompanied by population structure and admixture occurs in this centrally located population. This specifically highlights that central populations might be important conservation priorities because they i) can harbor individuals with genetic makeups of both western and eastern populations, and ii) are likely important for immigration and gene-flow across the range.

We were also able to more clearly identify population trajectories, especially recent ones, compared to previous studies. Like Nagel et al. (2024), we found evidence of large historical effective population sizes ($N_{e.LT}$). Our estimate of 70,738 (95% CI = 68,153–75,000) falls well within the range of that suggested

by Nagel et al. 2024 (95% CI 41,400–243,122), and helps to narrow the estimate. Additionally, we estimated $N_{e.Cont}$ for gray bats for the first time (57,147, with most recent estimates within 20 generations before present) and $N_{e.Bot}$ at the extreme of the bottleneck (just 13,001.9, 62 generations ago). These recent estimates reveal the substantial decline experienced by the species, followed by partial recovery to 26.18% of their historical numbers (in contrast to the overall signal of expansion found by Nagel et al. 2024, and lack of detected bottleneck in Lindsay et al 2015). Our estimates of recent N_e mirror observations of changes in the census population size over time, and indicate that a WGS approach may be especially useful when estimating recent demographic histories for species that, in contrast, have not been well monitored. Similarly, recent work highlights the use of effective population size estimates for differentiating between naturally low populations and those in decline (Liu et al. 2026). On a hopeful note for gray bat recovery, we found recent increases in effective population and no evidence of homozygosity excess associated with inbreeding depression.

Signals of population change

We asked whether gray bats show genetic signs of population decline, expansion, or stability following their demographic bottleneck.

Gray bat census populations have been markedly unstable over the past ~ 200 years (e.g., Brady et al. 1982, Martin 2007, Elliott 2008). After severe declines, the initiation of management actions, such as cave gating, resulted in population expansion (Adams et al. 2024). Historical maximum population sizes, and thus the magnitude of gray bat declines, have been inferred through the sizes of guano piles and stains left by roosting bats on cave ceilings. Our study represents the first genetic inference of gray bat effective population size changes over recent time. Our results support the timelines of previous inferences of population crashes, followed by recovery (Fig. 4), with a severe decline occurring due to cave disturbance around the time of the American Civil War, and recovery beginning as caves became protected habitat under the Endangered Species Act. While both estimates of census population sizes (e.g., from guano piles, Kunz et al. 2009) and effective population size are subject to assumptions and prone to some uncertainty, the similarity of the two estimated histories reflect the same story: one in which gray bats experienced a recent precipitous decline due to anthropogenic factors, and partially recovered following conservation intervention.

As expected from a genetic bottleneck, we found an underrepresentation of rare alleles in modern gray bat populations (based on Tajima's D, F statistics; Table 1; Fig. 2). This loss of rare alleles is characteristic of recent population contraction from a moderate demographic bottleneck (Gattepaille et al. 2013). Gray bats have a high overall rate of global genomic heterozygosity (Table 1) compared to other species (e.g., Prasad et al. 2021), in line with other myotine bats (e.g., Lilley et al. 2020a, b; Vannatta and Carver 2024). Previous studies have found that heterozygosity is positively correlated with fitness, even when a bottleneck has occurred (e.g., Price and Hadfield 2014). This component of genetic health has likely supported gray bats as their populations recover demographically and genetically.

The gray bat is only one of several North American *Myotis* species to have undergone population decline. The Indiana bat (*M. Sodalis* Miller and Allen), like the gray bat, was an early conservation target after undergoing declines related to habitat disturbance. Unlike the gray bat (Bernard et al. 2017), Indiana bats also experienced subsequent, albeit moderate, declines in the early 2000s due to the disease white-nose syndrome (Cheng et al. 2021). Recently, Kwait et al. (2025) conducted a genomic assessment of the species and did not detect a bottleneck associated with white-nose syndrome, nor with historic declines. However, a third species, the little brown bat (*M. Lucifugus* [le Conte]), did show signs of increased genetic drift and reduced genetic diversity due to recent and severe population declines related to white-nose syndrome (Auteri and Knowles 2020). The amount of time from, and severity of, initial decline likely influenced the ability to detect reductions in genetic diversity in each of these species, with the little brown bat's decline being the most recent and most severe, while the Indiana bat's decline was the least severe, though it occurred around the same time as the gray bat's. Different methods of evaluating genetic consequences of decline may also lead to different conclusions, highlighting the need for careful consideration of how sampling and analytic methodologies align with study objectives.

Maintenance of genetic diversity through demographic history, admixture, and life-history traits

We questioned whether admixture and effective population size were explanatory mechanisms of genetic diversity levels in gray bats.

Although we only looked at individuals from a single cave, we found evidence for very weak genetic substructure in the population, with high admixture (K = 2, weighted F_{ST} = 6.69e-05; Fig. 3). Weak structure is understandable, since gray bats regularly travel far distances seasonally (up to 437 km; Tuttle 1976a, Holliday et al. 2023) and males often visit multiple caves to mate promiscuously during the reproductive period (Tuttle 1976b). Additionally, previous habitat losses of up to 50% (Elliott 2008) may have resulted in admixture as gray bats joined new reproductive colonies. Admixture at this single site may also be a result of decreased boundaries for gene flow north of the Mississippi River Alluvial Plain. Regardless, conservation action on both sides of the gray bat range appears to have maintained population connectivity and gene flow between subpopulations. Healthy gene flow and admixture between large subpopulations may have buffered genetic drift and protected against the loss of heterozygosity (Jangjoo et al. 2016, Ceballos et al. 2018).

While strong decreases in census population result in detrimental genetic consequences for affected species, increased extinction threat due to inbreeding depression is most likely to occur when the absolute population size drops below a certain threshold. Because gray bats form dense colonies in limited habitat (95% reproduce and overwinter in only 15 locations), they are extremely vulnerable to habitat disturbance – the loss of even a single cave can lead to substantial population decline. However, they also had historically large census and effective population sizes. This likely meant that, even after population losses of ~ 95% (large declines relative to base population), the absolute number of individuals remained substantial. At the nadir of their bottleneck ($N_{e.Bot}$ = ~ 13,000), gray bats still maintained large effective population sizes compared to many species experiencing bottlenecks associated with substantial inbreeding depression (e.g., N_e = 64–133, Robinson et al. 2016; or N_e = 100, Hoelzel et al. 2024). Theory predicts that populations are most likely to suffer inbreeding depression below very low census population sizes (i.e., < 1000; Frankham et al. 2014), which the gray bat never crossed (Brady et al. 1982,

Shaffer and Samson 1985, Gomulkiewicz and Holt 1995). Thus, we suggest distinguishing between “relative demographic bottlenecks”, in which a population experiences a large drop relative to their original population size (e.g., 90%), and “absolute demographic bottlenecks”, in which a population drops to few individuals (e.g., < 1000). In populations that experience strong absolute demographic bottlenecks, heightened loss of genetic diversity is more likely to lead to inbreeding depression.

Species with slow life histories (i.e., long-lived) may respond to demographic bottlenecks differently than shorter-lived species. However, the direction of, and mechanisms underlying, this effect are unclear. Some evidence points to long-lived species being resistant to genetic bottlenecks (e.g., Hailer et al. 2006), while other studies indicate that these species do not show genetic diversity loss readily in tests (especially m-ratio) and may thus be vulnerable to extinction debt (Peery et al. 2012, Bradke et al. 2021, Clark et al. 2024, Gargiulo et al. 2025). However, most studies on longevity and bottlenecks have used microsatellite tests. Next generation genomic studies on bottlenecks, such as this one, may be especially helpful for understanding the relationship between longevity and the genetic consequences of declines. Anecdotally, the three such studies of *Myotis* species (Auteri and Knowles 2020, Gignoux-Wolfsohn et al. 2021, Kwait et al. 2025) suggest a negative relationship between longevity and resilience to genetic diversity loss. Little brown bats have the longest lifespan of the three species, followed by gray bats, and Indiana bats (Griffin and Hitchcock 1965, Thomson 1982, Decher and Choate 1995). Correspondingly, little brown bats have clear signatures of genetic diversity loss following declines (Auteri & Knowles 2020, Gignoux-Wolfsohn et al. 2021), gray bats have intermediate signatures (this study, Lindsay et al. 2015), and Indiana bats have weak signatures (Kwait et al. 2025). Although this comparison is informal because of differences between the species, scenarios, and studies, the *Myotis* genus may be a useful study system for understanding the links between longevity and susceptibility to genetic diversity loss. The genus is speciose (100 + species) with substantial variation in longevity (5 to 40 + years; Huang et al. 2020), and with many species experiencing population declines.

Conclusions

As humans continue to affect biodiversity across the globe, it is important to recognize that species can recover from severe declines. However, successful conservation, including recovery of genetic diversity, likely hinges on timely and effective intervention. For instance, assisted immigration of bighorn sheep (*Ovis canadensis* Shaw) implemented soon after a demographic bottleneck led to rapid improvements in genetic diversity and survival rates (Poirier et al. 2018). Similarly, the rapid recovery in gray bat effective population — following restriction of human disturbance at critical hibernation sites across the range — shows that timely and well-planned conservation action (taken after a relative bottleneck, but before numbers cross critical thresholds), can be extremely successful. Conversely, if declines are allowed to progress and reach an extreme absolute bottleneck, wherein lower critical thresholds are crossed, populations may experience reductions in genetic diversity from which recovery is either impossible or requires prolonged, even indefinite conservation intervention.

Declarations

Acknowledgements

We thank Victor Piñeiro, Jeanette Bailey, and Aleana Savage, for assistance in the field, Vona Kuczynsa for assistance with field site selection, Lynn Robbins for use of field equipment, the private property owners for access to the site, and Manny Vazquez for providing a reference genome. Deb Finn, Sean Maher, Rebecca Taylor, Shelly Colatskie, Kendra Edge, Carly Trujillo, and Joey Curti provided valuable information and feedback on this project.

Funding: Funding was provided by Missouri State University.

Conflicts of interest: The authors declare that they have no conflicts of interest.

Ethics approval: All research was done in appliance with appropriate IACUC and permitting guidelines (Missouri State University IACUC #2022-14, MDC Wildlife Collector #60085, and US FWS T&E Recovery #ES04397C)

Consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material: Raw sequence data is available on GenBank (SRA Accession: PRJNA1363576)

Code availability: code is available on github https://github.com/marxeao/GrayBatPopGen.

We highlight that demographic bottlenecks do not always lead to genetic collapse. We show the other side of the coin – conservation intervention, and not just destruction, can leave a genetic mark.

Author Contributions: GA and MA-O both conceived the project. MA-O performed data analysis, writing of the original draft, revision of subsequent drafts, and data visualization. GA performed fieldwork, in-house lab work, review and editing of the manuscript, and supervision of the project.

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Tables

Table 1. Main statistics calculated and brief interpretations.

	Tajima's D	F_{ST}	F_{IS}	F_S	H	H_e	H_o	Subp
Value	0.52	0.000067–0.0026	-0.23	-0.18	0.00322	0.376	0.411	2–3
Variation	0.002 SE	n/a	-0.24 – -0.22 (95% CI)	-0.18 – -0.18 (95% CI)	0.00301–0.00343 (95% CI)	0.376–0.376 (range)	0.405–0.416 (range)	n/a
Interpretation	Excess of common alleles	Weak population structure	Heterozygosity excess	Heterozygosity excess	High heterozygosity	High genomic heterozygosity	High heterozygosity/Heterozygosity excess	Popu struc adm occu

Table 2. ΔK calculated using the Evanno et al. (2005) method for different assumed numbers of population clusters (K). Mean best likelihood and standard deviation is presented as well.

K	1	2	3	4	5	6	7	8
ΔK	[a]	[a]	5.90	1.14	0.75	0.65	0.72	0.68
Likelihood ($x10^6$)	-4.62	-4.44	-4.30	-4.18	-4.06	-3.94	-3.83	-3.72
St dev ($x10^3$)	0	0.17	7.77	7.78	7.74	6.12	9.69	5.55

[a]Evanno's method cannot calculate ΔK for 1 or 2 population clusters.

Figures

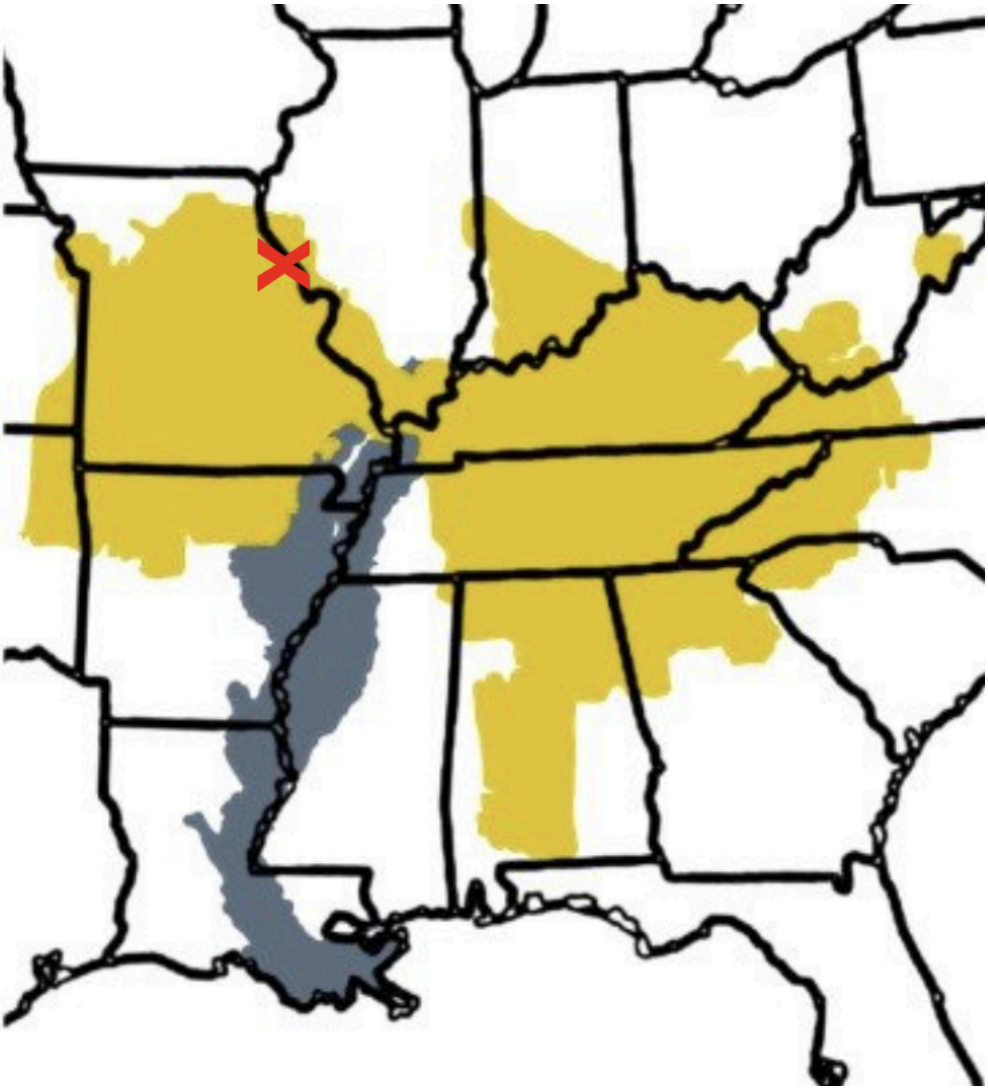


Figure 1

Approximation of the gray bat range, in yellow, within the eastern United States, adapted from the US Fish and Wildlife Service occurrence map (USFWS 2025). The two halves of the range are separated by the Mississippi River Alluvial Plain, shown in gray. The red X shows Pike County's location within Missouri.

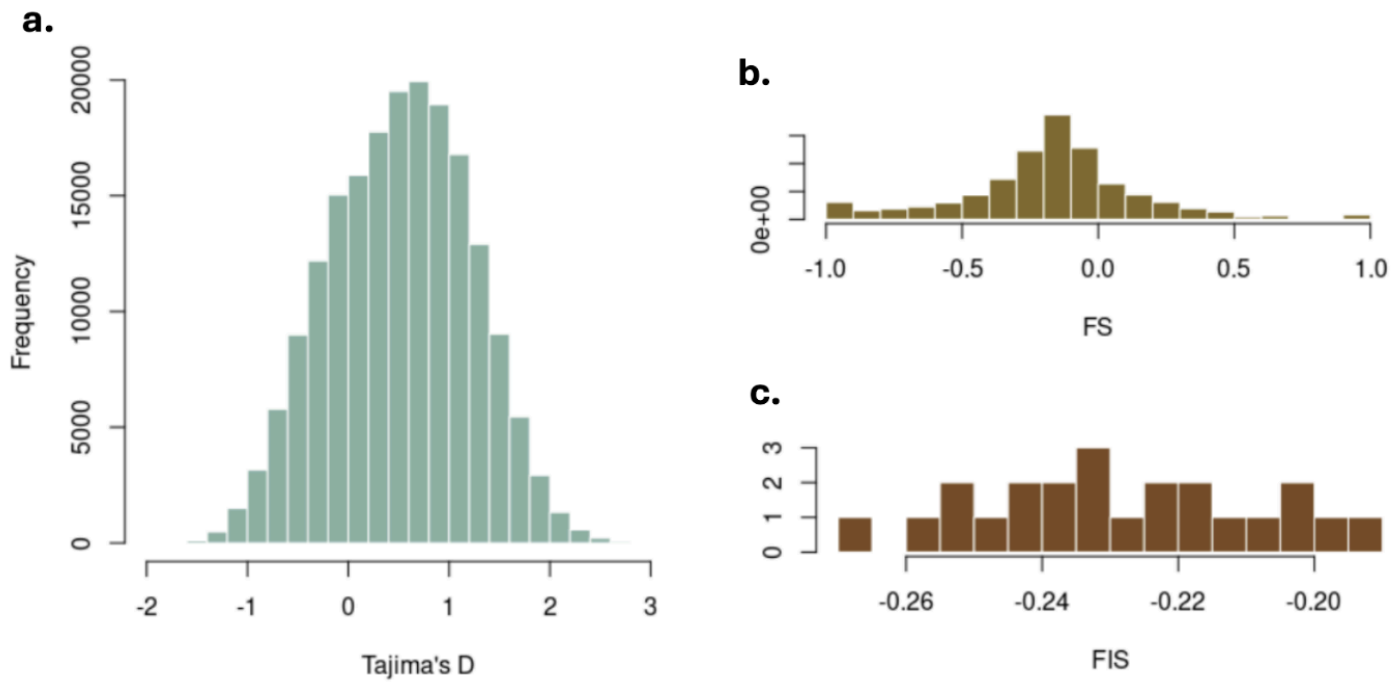


Figure 2

Inbreeding statistics from 23 individuals. a) Tajima's D score calculated in 50,000 base pair windows across the genome, with the frequency at which each score occurred. b) Per-site inbreeding coefficients across the gray bat genome. c) Individuals' inbreeding coefficients.



Figure 3

Assignment of bats to subpopulations by Q score. Each bar represents a single individual. Individuals were assigned to clusters, represented by distinct colors, when Q > 0.9. a: cluster assignment when there were 2 assumed populations. b: cluster assignments when there were 3 assumed populations.

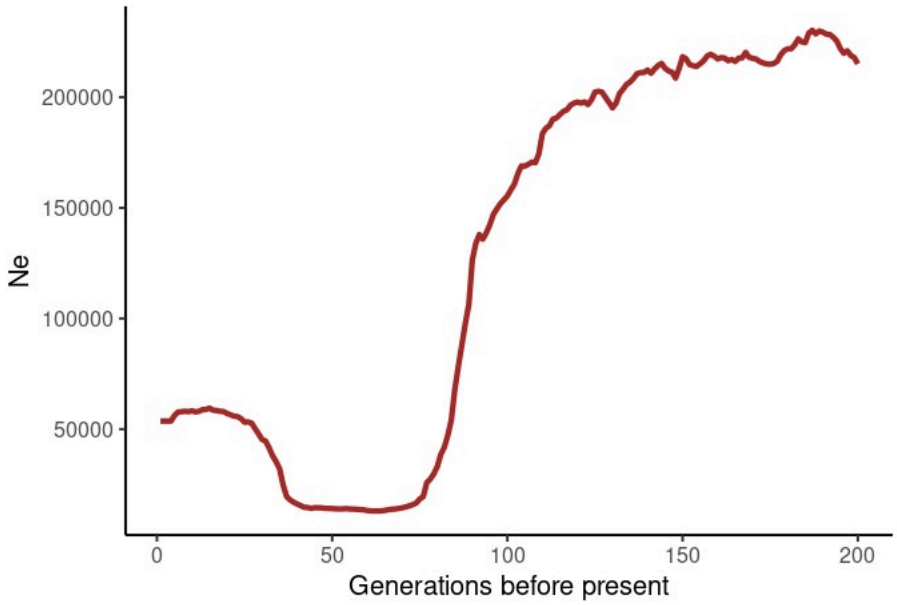


Figure 4

Effective population size of gray bats over the past 200 generations, with more recent time on the left. N_e was calculated in GONE.