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Research Article

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Disentangling genetic diversity of *Myotis septentrionalis*: population structure, demographic history, and effective population size

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Abstract

Myotis septentrionalis (Northern Long-eared Bat) has recently suffered a >90% decline in population size in North America due to white-nose syndrome (WNS). We assessed genetic diversity, population structure, current effective population size, and demographic history of *M. septentrionalis* distributed across the United States to determine baseline levels pre-WNS. We analyzed RADseq data from 81 individuals from Kentucky, Louisiana, Michigan, Minnesota, North Carolina, Oklahoma, and Wisconsin. Additionally, we examined population genetic structure using discriminant analysis of principal components, fastStructure, and STRUCTURE. We then estimated effective population size and demographic history using fastsimcoal2. Similar levels of genetic diversity were found across all samples. We found no population genetic structure in the varied analyses from these contemporary samples. The best model for demographic history estimated a rapid population expansion followed by a slower expansion approximately 340,000 years ago. The vagility of *M. septentrionalis*, along with male dispersal and random mating, may provide a buffer against serious bottleneck effects stemming from rapid population declines due to WNS. This research provides a baseline for tracking and monitoring the influence of WNS on genetic diversity such as potential reduced diversity or increased population structuring in the future.

Key words: bats, demographic history, effective population size, fastsimcoal2, fastStructure, genetic diversity, *Myotis septentrionalis*, population structure, STRUCTURE, white-nose syndrome.

Desenmarañando la diversidad genética de *Myotis septentrionalis*: estructura poblacional, historia demográfica y tamaño efectivo de la población

Resumen

Myotis septentrionalis ha sufrido recientemente una disminución de >90% en el tamaño poblacional en Norte América debido al síndrome de la nariz blanca (SNB). Evaluamos la diversidad genética, estructura poblacional, el tamaño actual poblacional efectivo y el historial demográfico de *M. septentrionalis* distribuidos en los Estados Unidos para determinar la información base de la especie previo al SNB. Analizamos los datos de RADseq de 81 individuos de Kentucky, Luisiana, Michigan, Minnesota, Carolina del Norte, Oklahoma y Wisconsin. Adicionalmente, examinamos la estructura genética de la población utilizando el análisis de componentes principales discriminatorio, fastStructure y STRUCTURE. Consecuentemente, estimamos el tamaño efectivo poblacional y el historial demográfico utilizando fastsimcoal2. Se encontraron niveles similares de diversidad genética en la totalidad de las muestras. Encontramos una estructura genética poblacional débil en varios análisis de estas muestras contemporáneas. El mejor modelo de historia demográfica estimó una rápida expansión poblacional seguido de una expansión más lenta hace aproximadamente 340,000 años. La vagilidad de *M. septentrionalis*, junto con la dispersión de los machos y el apareamiento aleatorio, puede servir como amortiguador contra los graves efectos de los cuello de botella derivados de la rápida disminución poblacional de esta especie debido a SNB.

Palabras clave: diversidad genética, estructura poblacional, fastsimcoal2, historia demográfica, murciélagos, síndrome de la nariz blanca (SNB), STRUCTURE, tamaño efectivo de la población.

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A number of North American bat species are facing a serious and immediate threat: white-nose syndrome (WNS), a fungal infection caused by *Pseudogymnoascus destructans* (Blehert et al. 2009; Gargas et al. 2009; Lorch et al. 2011; Minnis and Lindner 2013). This fungus has been identified on the outer epidermis of 18 bat species in Canada and the United States (White-Nose Syndrome Response Team 2021) and 12 of these species have developed WNS (manifesting as skin lesions, decreased body weight, and mass mortalities). The Northern Long-Eared Bat (*Myotis septentrionalis*) has been particularly adversely affected by WNS potentially due to behaviors such as hibernating in cave crevices promoting indirect (bat–cave substrate–bat) and density-dependent direct (bat–bat) transmission (Langwig et al. 2012; Zukal et al. 2014). Consequently, WNS was recently classified as an extreme and pervasive threat to *M. septentrionalis* (Cheng et al. 2021). *Myotis septentrionalis* formerly was widely distributed throughout the eastern and midwestern United States and southeastern Canada (Caceres and Barclay 2000). This species typically roosts in cavities in trees and snags (Silvis et al. 2016) and hibernates in caves, mines, other emergent rock features and in forests in the southern portion of its distribution during the winter (Caceres and Barclay 2000; Jordan 2020; White et al. 2020). Although once common, *M. septentrionalis* is now listed as endangered in Canada (COSEWIC 2013) under the Species at Risk Act and in the United States (United States Fish and Wildlife Service 2015, 2022) under the Endangered Species Act of 1973, with recent studies reporting over 90% decline in abundance across 17 states and 2 Canadian provinces (United States 1983; United States Fish and Wildlife Service 2015, 2022; Cheng et al. 2021). As such, determining baseline levels of genetic diversity before WNS impacted the genetics of *M. septentrionalis* allows for assessing how WNS influences genetic diversity in the future.

Genetic diversity is a complex concept encompassing genetic population structure, demographic history, and effective population size and is a critical facet in assessments of population viability of species. Population structure influences genetic diversity in multiple ways. Smaller isolated populations with little gene flow can be heavily impacted by genetic drift and inbreeding (Nei et al. 1975; Templeton 1980; Hedrick and Miller 1992; Landguth et al. 2010; Frankham 2018). Alternatively, species that form large continuous distributions may exhibit low levels of genetic differentiation (Burland and Wilmer 2001) and high genetic diversity leading to increased disease resistance, adaptability to environmental stressors, and phenotypic plasticity (Booy et al. 2000). Additionally, adaptations (e.g., true flight in bats, migration, and hibernation) can influence population structure (Burland and Wilmer 2001) and genetic diversity for a species.

Demographic history also plays a role in genetic diversity. Postglacial range expansions may have increased gene flow among previously separated populations (Sakaguchi et al. 2011), whereas recent range expansions into novel habitat may lower genetic diversity due to reduced gene flow into peripheral populations (Pierce et al. 2017; Rougemont et al. 2020). Recently established populations may experience founder effects (Mayr 1954; Templeton 1980), bottlenecks may cause loss of rare alleles due to genetic drift (Nei et al. 1975; Chakraborty and Nei 1977; Wisely et al. 2002), and population explosions can lead to a rapid accumulation of rare alleles (Keinan and Clark 2012). Therefore, the demographic history of a species affects its current effective population size due to changes in allele frequencies (Trask et al. 2017).

Population size is important in determining extinction risks because smaller populations experience higher rates of inbreeding and are more vulnerable to the effects of genetic drift (Nei et al. 1975). Unfortunately, census size (current number of reproductive adults) can be difficult to estimate and does not consider other factors such as uneven sex ratios or polygamy as contributing to population viability. Monitoring bats is especially challenging due to their life-history strategies (small body size, nocturnal behavior, unknown or inaccessible roosting preferences, and high vagility) making census count studies particularly difficult (O'Shea and Bogan 2003; Wright et al. 2021). Unlike other cave-hibernating bats in eastern North America, *M. septentrionalis* numbers are often undercounted due to cryptic roosting even where swarm surveys suggest that they are common (Raesly and Gates 1986). Alternatively, effective population size reflects the census size and demographic history of a population and can more accurately indicate the ability of a species to adapt to environmental stressors (Wright 1931; Kimura and Crow 1963; Frankham 1995; Harmon and Braude 2010; Wright et al. 2021); thus, many research studies use effective population size to predict population viability (Mace et al. 2008; Frankham et al. 2014; Vonhof and Russell 2015; Trask et al. 2017).

For this study, we investigated pre-WNS genetic diversity of the endangered *M. septentrionalis* to determine population structure across multiple states in the United States in order to evaluate its potential to withstand challenges to survival—including WNS—anthropogenic forces, and environmental changes. Additionally, we estimated current effective population size and demographic history of the species to further understand genetic diversity across the Midwest and Southern regions of the United States. This research will provide a baseline for future studies examining the effects of WNS on genetic diversity.

Materials and methods.

We obtained DNA samples from wing biopsies (3-mm tissue samples from plagiopatagium or wing membrane) preserved in 95% ethanol from 74 *M. septentrionalis* that were captured across the midwestern and southern regions of the United States and released on site. Liver samples of 13 additional individuals were included from the Museum of Texas Tech University Natural Sciences Research Laboratory (Lubbock, Texas) that were collected before the species became federally protected. Bats were identified based on morphological features by trained researchers (Lowery 1974; Jones et al. 1981; Caceres and Barclay 2000; Stevens et al. 2017). All bats were captured according to an Institutional Animal Care Use Committee (IACUC) protocol approved by Texas Tech University (TTU-IACUC # 14032-04) or United States Forest Service (IACUC # 2016-001) and followed American Society of Mammologists guidelines (Sikes et al. 2016).

DNA sequencing.

We isolated DNA with a blood and tissue analysis kit (Qiagan DNeasy Blood & Tissue Kit; Qiagen, Hilden, Germany) following the recommended protocol including centrifuging for an additional minute after discarding flow-through after the second wash (to avoid ethanol carryover) and repeating the elution step (to maximize DNA yield). After isolation, we quantified DNA concentrations with a fluorometer (Qubit 3.0 Fluorometer by ThermoFisher) and sent samples to a biotechnology lab (Admera Health, South Plainfield, New Jersey) to be sequenced. We used double-digest restriction site-associated DNA sequencing (ddRADseq) to identify single nucleotide polymorphisms (SNPs) in which base variability is detected along loci using 2 restriction enzymes, EcoRI-MspI (Peterson et al. 2012). Fragments were sequenced to generate 150-bp paired-end reads (Illumina NextSeq; San Diego, California). A quality-control software (AfterQC) was used to remove bases with Q-scores < 30 (sequencing accuracy < 99.9%) and low-quality reads

such as those having >5 N's (unknown nucleotide) or those that were less than 35 bp in length (Chen et al. 2017).

We aligned paired reads to a chromosome-length *M. septentrionalis* genome assembly (Dudchenko et al. 2017) using Burrows–Wheeler Alignment tools (BWA; Li and Dur 2009) and Sequence Alignment/Map (SAMtools; Li et al. 2009). We then identified and built loci using the *gstacks* module (Stacks v 2.52; Catchen et al. 2013). Poor-quality alignments or those with mapping errors were discarded. We used Stacks' *population* module to retain only loci found in at least 80% of samples—the recommended *80% rule* is considered generally effective for identifying polymorphic loci (Paris et al. 2017) and then we randomly selected 1 SNP from each locus to prevent processing linked SNPs, which can lead to significant biases in downstream analyses (Raj et al. 2014). Individuals that were missing more than 25% of their loci were removed and *gstacks* and *populations* were repeated with the remaining individuals (O'Leary et al. 2018).

Genetic analyses.

We estimated nucleotide diversity (π) as well as observed and expected levels of heterozygosity (the possession of 2 different alleles for a particular locus). Additionally, we calculated the inbreeding coefficient (F_{IS}) for each subpopulation and the fixation index (F_{ST}) across all samples. The F_{IS} statistic measures the proportion of genetic variation within an individual relative to the subpopulation, whereas F_{ST} measure the genetic variation within the subpopulation relative to the total population (Wright 1951; Weir and Cockerham 1984).

Population structure.

Assuming that individuals from different populations share a proportion of their genome from a common ancestor, we investigated genetic structure by assessing shared patterns of genetic variation among individuals. We used multiple methods to identify the number of genetic clusters (K): discriminant analysis of principal components (DAPC), fastStructure, and STRUCTURE. DAPC is a multivariate approach that transformes genetic data (centered and scaled) and conducts a principal component analysis followed by a discriminant analysis on the principal component (PC) scores to describe differences in diversity among populations (Jombart et al. 2010). The DAPC was conducted using "adegenet" (Jombart and Ahmed 2011) and plotted with a public repository script (Jenkins 2021) in R (R Development Core Team 2011). We determined the number of PCs to retain using a cross-validation method which uses 90% of observations as a training set to test the other 10% of observations (Jombart and Ahmed 2011).

FastStructure estimated the number of genetic clusters and identified levels of admixture among subpopulations, but with an algorithm 2 orders of magnitude faster than STRUCTURE making it a good choice for large SNP data sets (Pritchard et al. 2000; Raj et al. 2014). We ran fastStructure to assess potential genetic clusters (K = 1 through K = 10) for 10 independent runs and the mean log-marginal likelihood for each K was calculated (Raj et al. 2014). The K with the highest mean likelihood determined the most likely number of clusters.

Based on fastStructure results, we ran STRUCTURE on a smaller range of K (e.g., K = 1 through K = 5) to detect fine-scaled clustering that fastStructure may have missed (Pritchard et al. 2000; Stift et al. 2019) for 10 independent runs for each K. We selected the admixture model with 50,000 burn-in replicates and 500,000 Markov chain Monte Carlo iterations with no prior location information (Pritchard 2007; Gilbert et al. 2012). To determine the number of genetic clusters for STRUCTURE, we used the Pritchard method in which the number of clusters with the greatest mean likelihood was chosen (Pritchard et al. 2000) and the Evanno method in which the number of clusters was based on the second-order rate of change (Evanno et al. 2005). Both methods were analyzed using StructureHarvester (Earl and vonHoldt 2012). Finally, we plotted the model with the optimal K using Clumpp (Jakobsson and Rosenberg 2007) and DISTRUCT (Rosenberg 2004) as well as estimated individual membership coefficients for each cluster.

Demographic history and current effective population size.

Site frequency spectra (SFS) describe distributions of derived allele frequencies for variant genomic sites following the general pattern of many rare alleles with a few that are common (Braverman et al. 1995). If the ancestral allele is unknown, then a folded SFS is used by determining the less frequent, or minor, allele (Bustamante et al. 2001). The frequency of minor alleles, and therefore specific shape of the SFS, indicates population demographic history (i.e., bottlenecks, growth, or decline) as well as current and historical effective population sizes (Marth et al. 2004; Excoffier et al. 2013; Sovic et al. 2016). For example, populations that have undergone a bottleneck will have lost many rare alleles due to genetic drift while populations that are currently experiencing population growth will have more rare alleles than expected (Nei et al. 1975).

Because the ancestral allele was unknown, we used the minor allele frequency (MAF), and assumed that the derived allele is the less frequent allele, to create a folded SFS (Fu 1995; Marth et al. 2004). To calculate the MAF, we reran *populations* retaining all SNPs and then used *vcf2sfs* R script to create the folded SFS (Liu et al. 2018). We then used fastsimcoal2 (v2.6) to estimate current effective population size (as of 2018 which was the date of last sample taken) and the likelihood of 3 demographic models: constant population size, consistent population growth or decline, and historical bottleneck or explosion, i.e., rapid decline or expansion followed by a slower expansion (Supplementary Data SD1; Excoffier et al. 2013). Fastsimcoal2 estimated the likelihood of a model (e.g., a recent bottleneck) by comparing the observed folded SFS to the expected SFS for the model by performing coalescent simulations (Excoffier et al. 2013).

For each demographic model, we tested 2 mutation rates: 2.2e-9 commonly used for mammals (Kumar and Subramanian 2002) and 2.5e-8 reported in humans (Nachman and Crowell 2000), which was used in a previous bat demographic study (Vonhof and Russell 2015). Each model was run independently 100 times for 500,000 iterations and 100 optimization cycles. The Akaike Information Criterion and likelihood distributions were compared to determine the best model. Parameter estimates (i.e., current effective population size, time since historical events, historical effective population size) were bootstrapped to obtain 95% confidence intervals for each parameter.

Results

DNA sequencing.

We retained 81 individuals from Kentucky (n = 12), Louisiana (n = 29), Michigan (n = 1), Minnesota (n = 5), North Carolina (n = 12), Oklahoma (n = 20), and Wisconsin (n = 2) to include samples across the *M. septentrionalis* distribution (Fig. 1). Samples were collected from 2007 to 2014 (Supplementary Data SD2). Kentucky and Michigan were sampled across the leading edge of WNS invasion; all other samples were collected before WNS detection (White-Nose Syndrome Response Team 2021). We genotyped 1,214,855 loci (Supplementary Data SD3) with 3,401,692.5 reads per individual and an effective per-sample coverage of 8.0× (range: 3.6 to 48.0×) which is consistent with previous low-coverage RADseq bat studies (Auteri and Knowles 2020; Pinzari et al. 2020).

Genetic analysis.

Due to low sample sizes, we combined Michigan, Minnesota, and Wisconsin into 1 northern group (MMW, n = 8) for these analyses. Genetic statistics did not differ greatly among the states (Table 1). Nucleotide diversity ranged from 0.000120 (Kentucky and Oklahoma) to 0.000139 (Louisiana and the northern states). Observed heterozygosity for all sites (variant and invariant) ranged from 0.000086 (Kentucky) to 0.000115 (northern states) with expected heterozygosity levels ranging from 0.0000114 (Kentucky) to 0.000137 (Louisiana). The northern states had the lowest levels of inbreeding (F_{IS} = 0.000077) followed by Oklahoma (F_{IS} = 0.0000118), whereas Louisiana had the highest levels (F_{IS} = 0.000226). The greatest genetic differentiation was found between northern states (MMW) and North Carolina with F_{ST} = 0.0416 (Table 2) and the least genetic differentiation was between Louisiana and Oklahoma (F_{ST} = 0.0242).

Population structure.

To investigate population structure, we analyzed 57,651 loci using DAPC, fastStructure, and STRUCTURE. There was very little structure based on the DAPC with the first and second axes accounting for 3.15% and 2.46% of the variation, respectively (Fig. 2). For fastStructure, all independent runs selected K = 1, most likely meaning that only 1 ancestral genetic cluster was identified for all individuals (Fig. 3). The Pritchard and Evanno methods for STRUCTURE both supported the presence of 3 ancestral genetic clusters (Fig. 4). Although STRUCTURE supported 3 genetic clusters, all individuals were assigned to 1 cluster (Fig. 5).

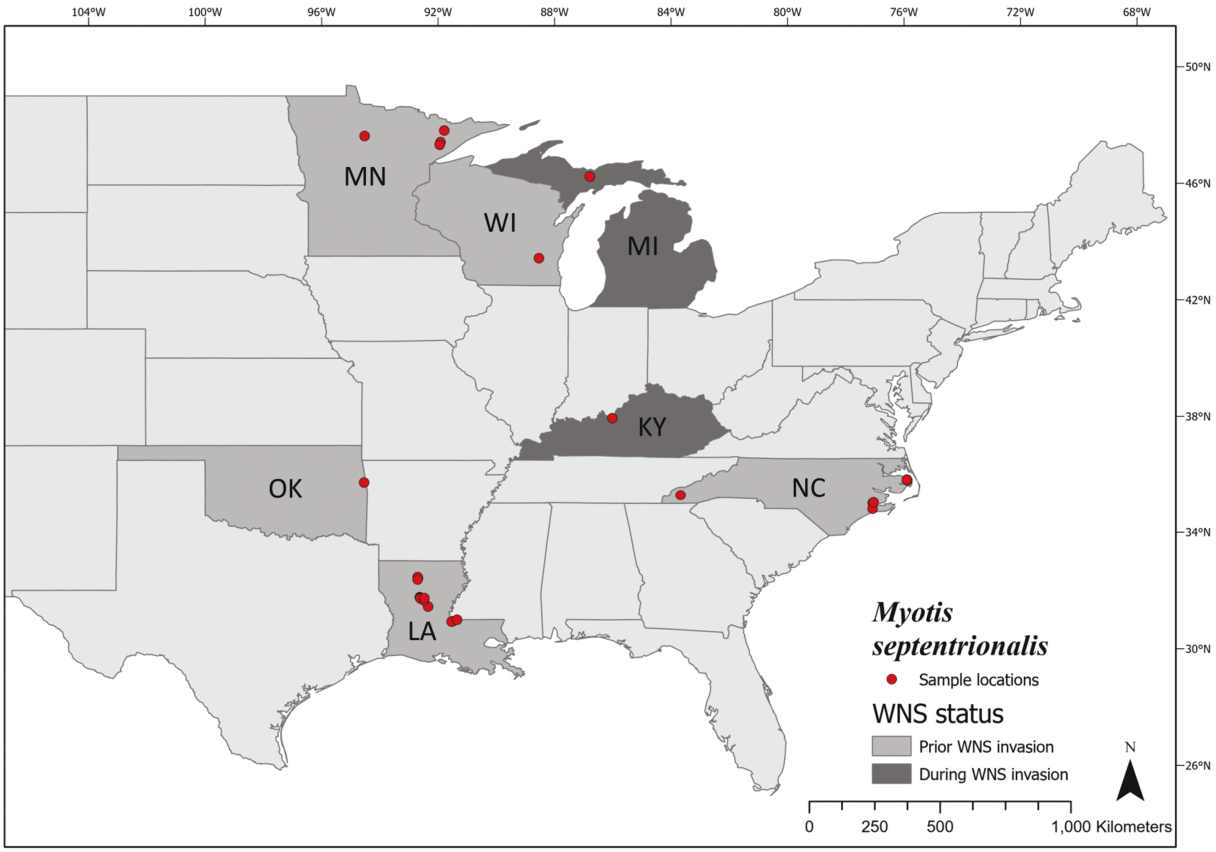

Fig. 1. Sample locations for *Myotis septentrionalis* (Northern Long-eared Bat) in Kentucky (KY), Louisiana (LA), Michigan (MI), Minnesota (MN), North Carolina (NC), Oklahoma (OK), and Wisconsin (WI). Light gray states represent locations sampled prior to WNS invasion and dark gray states represent locations sampled during initial WNS invasion.

Table 1. Genetic statistics for *Myotis septentrionalis* in Kentucky (KY), Louisiana (LA), Michigan/Minnesota/Wisconsin (MMW), North Carolina (NC), and Oklahoma (OK). Statistics include number of samples (*N*), observed and expected heterozygosity, nucleotide diversity (*Pi*), and inbreeding coefficient (F_{IS}). Samples were collected from 2007 to 2018.

State	N	Total sites	Variant sites	Obs Het	Exp Het	Pi	F_{IS}
KY	12	42,327,663	57,648	0.000086	0.000114	0.000120	0.000136
LA	29	42,327,664	57,648	0.000102	0.000137	0.000139	0.000226
MMW	8	42,327,655	57,641	0.000115	0.000129	0.000139	0.000077
NC	12	42,327,662	57,648	0.000088	0.000116	0.000123	0.000135
OK	20	42,327,662	57,648	0.000099	0.000117	0.000120	0.000118

Demographic history and current effective population size.

Likelihoods for individual runs were similar for each demographic model indicating convergence on a global maximum for parameters rather than hitting local maxima in parameter space. The mutation rate of 2.2e-9 had higher likelihoods for each of the given demographic histories over the corresponding model with the higher mutation rate of 2.5e-8 (Table 3). The most supported model was the bottleneck/explosion-9 model which estimated the current effective population size to be 828,338 (821,096 to 829,559) individuals. This model estimated an ancestral population of 73,073 (67,895 to 78,910) individuals experienced a rapid population explosion with a growth rate of 0.343 (0.316 to 0.370) followed by a slower population growth of 0.088 (0.082 to 0.095) approximately 340,000 years ago assuming a 1.2-year generation time (Bhak et al. 2017).

Discussion

Similar levels of nucleotide diversity and heterozygosity were found among individuals throughout the Midwest and Southern regions of the United States even for bats captured in the upper Midwest, the leading edge of WNS at the time of sampling. Inbreeding within locales was greatest for Louisiana, possibly due to the Kisatchie National Forest being isolated from most of the remainder of the *M. septentrionalis* distribution with the nearest known populations approximately 160 km away in Oklahoma and Arkansas. Individuals in Louisiana and Oklahoma were most similar based on F_{ST} values (Table 3) possibly because these locations had the largest sample sizes, and the higher F_{ST} found among other sites was due to a sampling artifact.

We found no evidence of population genetic structure for *M. septentrionalis* when considered as a whole. Despite the likelihood estimates, STRUCTURE did not identify multiple clusters (see Fig. 5). This pattern (higher value of *K* chosen yet all individuals belonging to 1 cluster) has been seen in previous studies of highly mobile species (Quintela et al. 2014; Ito et al. 2020).

Prior to this study, only 1 analysis of *M. septentrionalis* using RADseq existed (Grimshaw et al. 2022). In that work, weak structure unrelated to geography was found for 3 species of bats in Louisiana: *Eptesicus fuscus*, *M. austroriparius*, and *M. septentrionalis*. However, *M. septentrionalis* genetic diversity has been investigated using microsatellite data. Fine-scaled structuring was found across 2 Canadian provinces (New Brunswick and Nova Scotia), but the clustering was unrelated to geographic location or distance among colonies (Johnson et al. 2015). Prior to WNS, population structure was indistinguishable for this species within West Virginia across spatial scales as well as between West Virginia and New York (Johnson et al. 2014). Similarly, Gorman et al. (2022a) found little population structure for *M. septentrionalis* from coastal Massachusetts to coastal North Carolina post-WNS. Weak or absent structure has also been found on microsatellite data for *M. lucifugus*, which has a similar life history and annual cycle as *M. septentrionalis* (Fenton and Barclay 1980; Caceres and Barclay 2000; Dixon 2011; Miller-Butterworth et al. 2014). Our results are consistent with these previous studies in suggesting that there is little to no genetic structuring this species.

That said, we recognize the limitations of our study. First, there were unequal sample sizes with 3 states having 5 or fewer individuals (Michigan, Minnesota, and Wisconsin), 2 states having 12

Table 2. Genetic differentiation (F_{ST}) for *Myotis septentrionalis* in Kentucky (KY), Louisiana (LA), Michigan/Minnesota/Wisconsin (MMW), North Carolina (NC), and Oklahoma (OK). Samples were collected from 2007 to 2018.

	KY	LA	MMW	NC
KY				
LA	0.0261			
MMW	0.0399	0.0292		
NC	0.0369	0.0269	0.0416	
OK	0.0263	0.0242	0.0286	0.0274

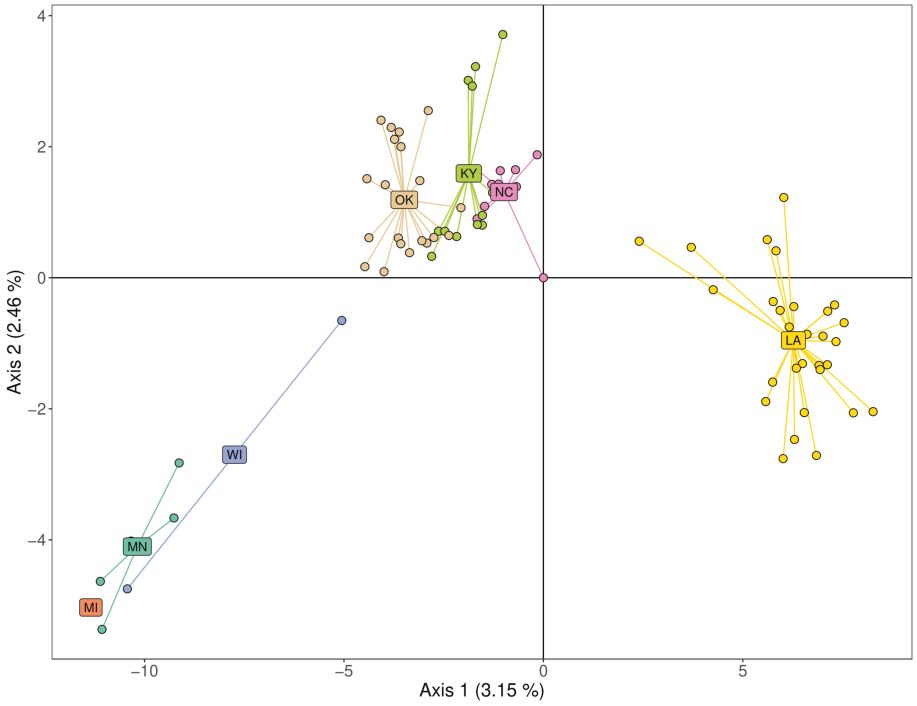

Fig. 2. DAPC results for *Myotis septentrionalis* (Northern Long-eared Bat) in Kentucky (KY), Louisiana (LA), Michigan (MI), Minnesota (MN), North Carolina (NC), Oklahoma (OK), and Wisconsin (WI). Axis 1 accounts for 3.15% of variation and Axis 2 accounts for 2.46%.

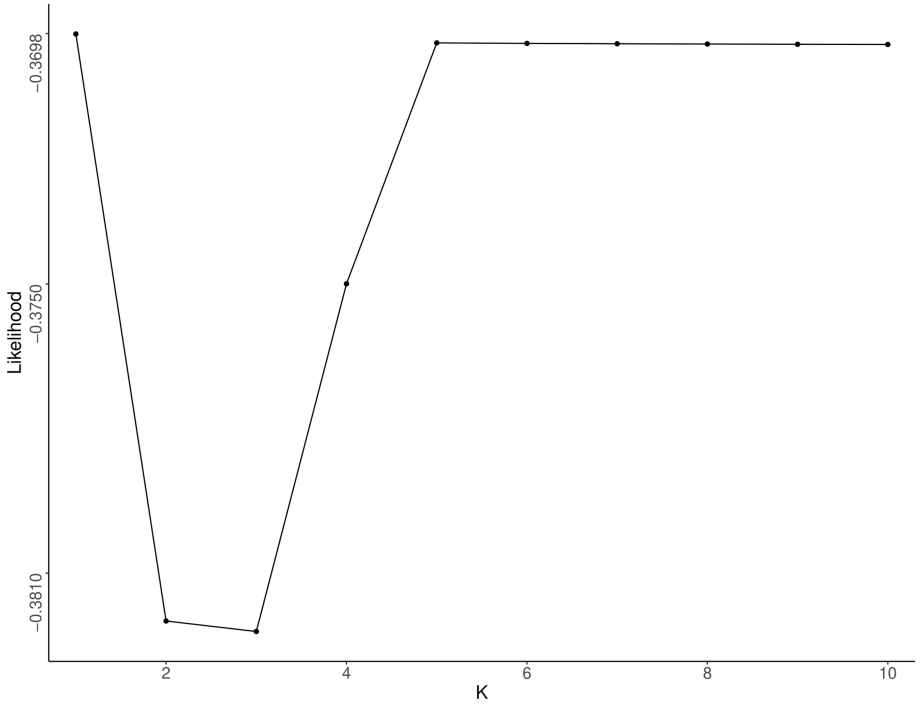

Fig. 3. FastStructure results of *Myotis septentrionalis* (Northern Long-eared Bat) for $K = 1$ through $K = 10$ genetic clusters.

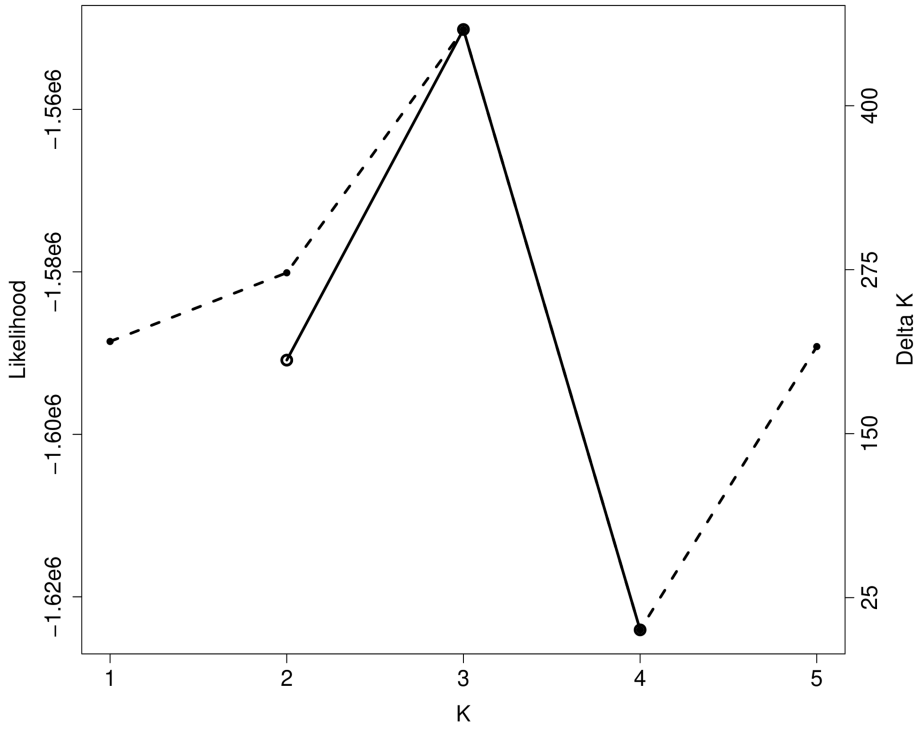

Fig. 4. STRUCTURE results for *Myotis septentrionalis* (Northern Long-eared Bat) based on relative likelihood of tested models (dashed line/closed circles; Pritchard et al. 2000) and change in likelihood from one model to the next (solid line/open circles; Evanno et al. 2005).

individuals (Kentucky and North Carolina), and 2 states having over 20 individuals (Louisiana and Oklahoma). Therefore, we combined Michigan, Minnesota, and Wisconsin into 1 northern group ($n = 8$), still somewhat small but more similar to other groups. Although this disparity could cause biases, particularly for heterozygosity and inbreeding analyses, we do not believe that it affected our overall conclusion of a lack of genetic structuring for *M. septentrionalis*. Unequal sampling can cause spurious results in microsatellite studies (Puechmaille 2016), but using a large number of SNPs (in the 1,000s) for small sample sizes can be employed for genetic studies but with caution because each individual will heavily influence results (Willing et al. 2012; Trucchi et al. 2014). Additionally, sampling was patchy across the distribution of this species but represents a collection of tissues available

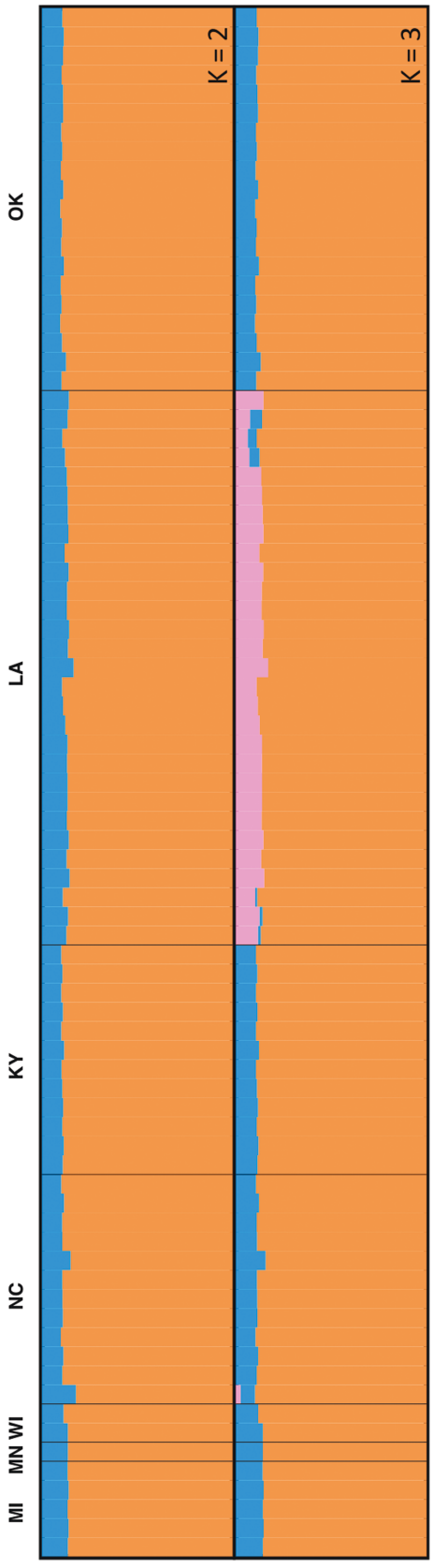

Fig. 5. Membership probabilities of *Myotis septentrionalis* (Northern Long-eared Bat) for K = 2 (top) and K = 3 (bottom) genetic clusters. Each bar represents 1 individual, and each color represents a genetic cluster.

to the authors for a threatened and rarely encountered species, especially in the upper Midwest where the population was rapidly declining after the introduction of WNS. Although a more uniform sampling scheme would have been preferable, we do not believe that it would have changed the results, especially given that the results were consistent with studies in Canada (Johnson et al. 2014) and New York and West Virginia (Johnson et al. 2015). Finally, whereas bats do not recognize state borders, humans and the government agencies responsible for conservation practices are required to do so. Therefore, we grouped bats based on state collected so that state agencies would have data on populations of interest within their state.

Our results suggest that *M. septentrionalis* experienced a rapid population expansion followed by a slower expansion approximately 340,000 years ago, corresponding with an interglacial warming period that occurred approximately 337,000 to 300,000 years ago (Lisiecki and Raymo 2005). This interglacial period was particularly warm based on oxygen isotope records (Tzedakis et al. 2009; Past Interglacials Working Group of PAGES 2016). Since that time, the population grew steadily until the introduction of WNS.

Unfortunately, few studies have examined effective population (N_e) size in bats, and none, to our knowledge, for *M. septentrionalis*. However, a 2016 study examining demographic history and N_e in 3 North American bats (*Lasiurus* [now *Aeroestes*] *cinereus*, *L. borealis*, and *Lasionycteris notivagans*) found evidence that all 3 species were experiencing consistent population growth (Sovic et al. 2016). Furthermore, Sovic et al. (2016) estimated current N_e as 800,091 individuals for *A. cinereus*, 415,311 for *Lasiurus borealis*, and 94,644 for *L. notivagans*. Given that N_e is generally smaller than census size, we can assume that the population of *M. septentrionalis* was larger than the pre-WNS N_e, estimated at over 800,000 individuals. It must be noted that N_e is affected by generation time. We note that samples were collected either prior to or during the initial WNS invasion phase (Fig. 1; Supplementary Data SD1) and do not reflect the recent >90% population decline caused by WNS in some regions of the distribution. Therefore, the current effective population size we estimated is a more accurate representation of the species pre-WNS, thus providing a pre-WNS baseline for this species.

Hibernating bat species such as *M. septentrionalis* are at increased risk of extinction and severe population declines due to WNS, climate change, and other anthropogenic factors, i.e., habitat modification (Blehert et al. 2009; Frick et al. 2010; Sherwin et al. 2013; Voigt et al. 2015; Cheng et al. 2021), all of which have likely impacted this species. Despite these adversities, we found no evidence of population genetic structure for *M. septentrionalis* among midwestern and southern regions of the United States. The mobility and dispersal capabilities of *M. septentrionalis*, along with male dispersal and random mating during pre-hibernation swarming events, may contribute to low genetic differentiation and panmixia that may provide more resilience to genetic drift found in smaller isolated populations (Frankham et al. 2014). Moreover, the long lifespan of bats may offer a buffer against effects of WNS on genetic diversity (Hailer et al. 2006; Lippé et al. 2006). The lack of strong population genetic structure is reassuring because high mortality rates in some regions may not generate serious bottleneck effects for this species (Johnson et al. 2014). Bat populations in warmer areas or with fewer caves such as Louisiana and coastal North Carolina may not experience the mass mortalities seen in the northeastern United States and Canada (Limon et al. 2019), and therefore, these populations may eventually recolonize former colonies without a severe loss of genetic diversity.

Herein, we have provided historical benchmark data on genetic diversity of *M. septentrionalis* pre-WNS. Future research will be able to determine how WNS has impacted this species years from now. Continued monitoring especially in locations affected by WNS for a

Table 3. Demographic history models for *Myotis septentrionalis* sampled from 2014 to 2018 in Kentucky, Louisiana, Michigan, Minnesota, North Carolina, Oklahoma, and Wisconsin. Model-8 indicates a model using a mutation rate of 2.5e-8, whereas model-9 indicates model with a mutation rate of 2.2e-9. *K* specifies number of parameters used in each model. DeltaL is the difference between observed and expected likelihoods. Akaike information criterion (AIC) was calculated as –2(log-likelihood) + 2*K* and DeltaAIC is the difference between the AIC of a given model and the best model.

Model	*K*	DeltaL	AIC	DeltaAIC
Bottleneck/explosion-9	5	27,774.32	10,443,593	0
Growth/decline-9	4	27,777.23	10,443,605	12
Bottleneck/explosion-8	5	27,777.90	10,443,610	17
Growth/decline-8	4	27,780.37	10,443,619	26
Constant-9	2	122,458.79	10,879,627	436,034
Constant-8	2	122,485.01	10,879,748	436,155

longer period (Barr et al. 2021) and/or where contemporary occurrence of populations are known to be isolated (Gorman et al. 2022b) is also highly encouraged.

Supplementary data

Supplementary data are available at *Journal of Mammalogy* online.

Supplementary Data SD1.—Demographic model depictions for *Myotis septentrionalis* (Northern Long-eared Bat).

Supplementary Data SD2.—Sample ID and location information for *Myotis septentrionalis* (Northern Long-eared Bat) in Kentucky (KY), Louisiana (LA), Michigan (MI), Minnesota (MN), North Carolina (NC), Oklahoma (OK), and Wisconsin (WI). Table include the county and year collected, the year white-nose syndrome (WNS) was identified in the county, and if WNS was present at time of collection.

Supplementary Data SD3.—Locus and read information for *Myotis septentrionalis* (Northern Long-eared Bat).

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Author contributions

JRG wrote original draft, reviewed and edited subsequent drafts, conducted formal analyses and methodology, and contributed to conceptualization and investigation. RDS and DAR contributed to conceptualization and investigation, reviewed and edited subsequent drafts, and provided resources and funding. DD, RP, WMF, and AS contributed to investigation and reviewed and edited subsequent drafts. CJG contributed to investigation.

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Conflict of interest

None declared.

Data availability

Scripts are available at https://github.com/jennagrimshaw/pop-genetics. Data files are available at https://github.com/jennagrimshaw/usda-myosep. Sequences were deposited under accession number: PRJNA785461 (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA785461).

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