

Genetic structure of winter populations of the endangered Indiana bat (*Myotis sodalis*) prior to the white nose syndrome epidemic: implications for the risk of disease spread

Maarten J. Vonhof^{1,2} · Sybill K. Amelon³ · Robert R. Currie⁴ · Gary F. McCracken⁵

Received: 20 August 2015 / Accepted: 4 April 2016 / Published online: 12 April 2016
© Springer Science+Business Media Dordrecht 2016

Abstract The spread of white nose syndrome raises serious concerns about the long-term viability of affected bat species. Here we examine the geographic distribution of genetic variation, levels of population connectivity that may influence the spatial spread of WNS, and the likelihood that recent population declines in regions affected by WNS have led to the loss of unique genetic variation for the endangered Indiana bat (*Myotis sodalis*). We amplified a fragment of the mitochondrial control region for 375 individuals and genotyped 445 individuals at 10 microsatellite loci from 18 sampling sites distributed across the majority of the species' range. Analysis of mitochondrial DNA indicated the presence of at least five distinct matrilineal clusters, with the most pronounced differences between northeastern sites and those in the rest of the range. The majority of individuals in the Ozark-Central, Midwest, and Appalachian recovery units fell into a single cluster. Significant differentiation also was observed between one Appalachian and one Midwestern

site and the majority of other sites. However, using nuclear microsatellites we observed the absence of differentiation and widespread gene flow among all hibernacula, suggesting the occurrence of extensive gene flow through dispersal and mating. The absence of genetically distinct populations within the range of Indiana bats indicates a lack of barriers to WNS transmission, and it is unlikely that significant portions of the hibernating population of Indiana bats will remain disease free into the future. Further, while matrilineal gene flow was restricted among some sites and regions, we found no genetic evidence to support the division of Indiana bats into separate recovery units.

Keywords Bats · Disease · *Myotis sodalis* · Phylogeography · Units of conservation · White nose syndrome

Electronic supplementary material The online version of this article (doi:10.1007/s10592-016-0841-6) contains supplementary material, which is available to authorized users.

✉ Maarten J. Vonhof
maarten.vonhof@wmich.edu

¹ Department of Biological Sciences, Western Michigan University, Kalamazoo, MI 49008-5410, USA

² Environmental and Sustainability Studies Program, Western Michigan University, Kalamazoo, MI, USA

³ US Forest Service Northern Research Station, University of Missouri-Columbia, Columbia, MO, USA

⁴ PO Box 1357, Hendersonville, NC, USA

⁵ Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA

Introduction

The conservation and management of biodiversity requires consideration of the effects of multiple stressors on natural populations. The impacts of major anthropogenic drivers of biodiversity loss often act synergistically to reduce populations of affected species, and may leave those populations more susceptible to future impacts from additional stressors (Harvell et al. 2002; Crain et al. 2008; Laurance and Useche 2009). Emerging diseases of wildlife are a prominent stressor that can negatively impact ecosystem health and functioning and the maintenance of biodiversity (Cohen 2000; Crowl et al. 2008; Fisher et al. 2012). Emerging diseases have been linked directly with declines in wildlife populations across a wide geographic and taxonomic spectrum, and have the potential to drive threatened and

endangered species below critical thresholds for survival or recovery (Daszak et al. 2000; Pedersen et al. 2007).

White-nose syndrome (WNS) is an emerging fungal disease affecting bats in the United States and Canada. The disease is caused by a psychrophilic (cold-loving) fungus, *Pseudogymnoascus destructans*, that penetrates the epidermis of the wing membrane tissue, invades underlying connective tissues, hair follicles, and sebaceous glands, and causes severe tissue degradation (Blehert et al. 2009; Gargas et al. 2009; Meteyer et al. 2009). The disease likely causes a loss of homeostasis through the disruption of physiological processes (Cryan et al. 2010; Verant et al. 2014), with affected individuals arousing more frequently from torpor during hibernation, which depletes their stored energy reserves and eventually causes death (Reeder et al. 2012). Since its discovery in eastern New York State in the winter of 2006–2007, WNS has spread rapidly to 25 additional states and five Canadian provinces and has been confirmed to infect seven bat species as of January 2016 (US Fish and Wildlife Service 2016). Mortality rates vary widely among affected species (Turner et al. 2011; Langwig et al. 2012; Frick et al. 2015), and population models for little brown bats (*Myotis lucifugus*), one of the historically most abundant and highly-affected bat species, indicate this species could be regionally extirpated from the northeastern United States by 2026 if mortality rates remain constant (Frick et al. 2010).

The high levels of observed mortality from WNS raise serious concerns about the long-term viability of affected bat species, particularly those already of conservation concern due to other factors. The Indiana bat, *M. sodalis*, is one of two federally-endangered bat species currently affected by WNS. This small (7–10 g) insectivorous bat inhabits deciduous forests in the eastern United States where it roosts primarily in trees in the summer, and hibernates in caves and mines during the winter. It was listed as endangered in 1967, largely because over 97 % of the known population at the time was found in only four caves used as winter hibernacula and these hibernating populations were vulnerable to disturbance (Hall 1962; Humphrey 1978). Additional threats to this species identified in the most recent recovery plan include loss or degradation of critical habitats, and ongoing disturbance in both winter and summer habitats through human activity (US Fish and Wildlife Service 2007). Subsequent to its listing, efforts to identify and protect critical summer and winter roosting habitats were initiated, and global population size has since remained below historical estimates but did not continue to decline from 1983 to 2005 (Thogmartin et al. 2012a; see also Ingersoll et al. 2013). The latest recovery plan for Indiana bats (US Fish and Wildlife Service 2007) divides the species into four recovery units, including, from west to east: Ozark-Central, Midwest,

Appalachian, and Northeast. These units are a tool to ensure redundancy and resiliency of populations across identifiable units within wide-ranging species, and in the case of Indiana bats are based on combined evidence from preliminary analyses of mitochondrial DNA (mtDNA), band return data indicating different patterns of population connectivity between summer and winter habitats in different regions, and the concordance of these data with ecoregion boundaries (US Fish and Wildlife Service 2007). The observed range-wide population stationarity belied significant regional differences in population trends. The Midwestern region, which contains the bulk of the hibernating population, remained relatively constant, while westernmost populations declined (Ozark-Central experienced a median decline of −9 %), and easternmost populations increased (Appalachian and Northeastern experienced median increases of +8 and +16 %, respectively) between 1983 and 2005 (Thogmartin et al. 2012a).

With the onset of WNS, there is renewed concern about the long-term viability of Indiana bat populations. WNS is associated with high levels of mortality of Indiana bats, with a mean decline in selected monitored sites of 72 % (Turner et al. 2011), range-wide population declines of 10.3 % annually from 2006 to 2009 (Thogmartin et al. 2012a), and documented local extinction at 17 % of monitored sites (Frick et al. 2015). This decline cannot be explained wholly by WNS, as unaffected populations also declined, but populations in the Northeast where WNS was concentrated declined dramatically by 46 % during this time period (Thogmartin et al. 2012a; see also Ingersoll et al. 2013). WNS is spreading at an increasing rate among Indiana bat populations (3 % annual increase in the rate of newly affected populations), and if this pattern continues, all wintering populations may be affected by 2016 (Thogmartin et al. 2012b). This trend raises the specter of future declines in other portions of the species' range, and subsequent regional or global extinction. That said, there is variation among hibernacula in the probability of infection by *P. destructans* associated with distance from the source of the epidemic, the proximity to affected populations, and colony size (Wilder et al. 2011; Thogmartin et al. 2012b), and variation in rates of population decline associated with humidity and temperature (Hallam and Federico 2012; Langwig et al. 2012; Johnson et al. 2014). This variation suggests that different populations may experience different probabilities of infection and extinction. Bat movements and subsequent bat-to-bat transmission are likely the dominant mode of transmission of WNS to new sites (Turner et al. 2011), and thus it is vital to understand patterns of connectivity among populations to identify potential barriers to transmission, and to assess the relative degree of isolation of various hibernating populations to identify potential refuges.

Genetic studies of both host and parasite populations have demonstrated that barriers to host movement can restrict the geographic spread of pathogens (reviewed in Biek and Real 2010), and knowledge of such genetic discontinuities can be utilized to target disease control interventions or to identify low-risk regions for special conservation measures (e.g., Russell et al. 2005; Rees et al. 2009). In this paper we examine patterns of microsatellite and mitochondrial DNA variation among overwintering populations of Indiana bats prior to the onset of the WNS epidemic to assess: 1) broad patterns of population genetic structure and gene flow that may influence the spatial spread of WNS; 2) the likelihood that recent population declines in regions affected by WNS have led to the loss of unique genetic variation associated with differentiated populations; and 3) the validity of the recovery units as assessed using an expanded mtDNA data set and nuclear microsatellite markers. Demographic data on bat movement patterns are extremely difficult to collect over large scales; however, genetic tools provide the means to describe potential connectivity through analysis of dispersal patterns and gene flow. Our study provides valuable information on population connectivity across the range of Indiana bats that will inform ongoing conservation and management efforts for this endangered, WNS-affected bat species.

Methods

Sample collection

Tissue samples were obtained from 445 individuals between September and March from 1997 to 2006, during regular surveys at caves and mines used by Indiana bats as hibernacula. Sampling was conducted at six Priority 1 hibernacula (containing >10,000 individuals), seven Priority 2 hibernacula (containing 1000–10,000 individuals), and five Priority 3–4 hibernacula (containing <1000 individuals; US Fish and Wildlife Service 2007), for a total of 18 sites (Fig. 1; Table 1). In four cases, sites sampled within the same county were combined into a single sample for the purposes of our analyses (see Table 1). Bats were captured in roosts by hand during hibernation and using harp traps or mist nets at roost exits during their spring emergence from hibernation by field biologists under relevant state and federal permits. Two 3-mm biopsy punches, one from each wing, were taken from each bat and stored in 5 M NaCl with 20 % DMSO (Worthington Wilmer and Barratt 1996). The bats were released after sampling.

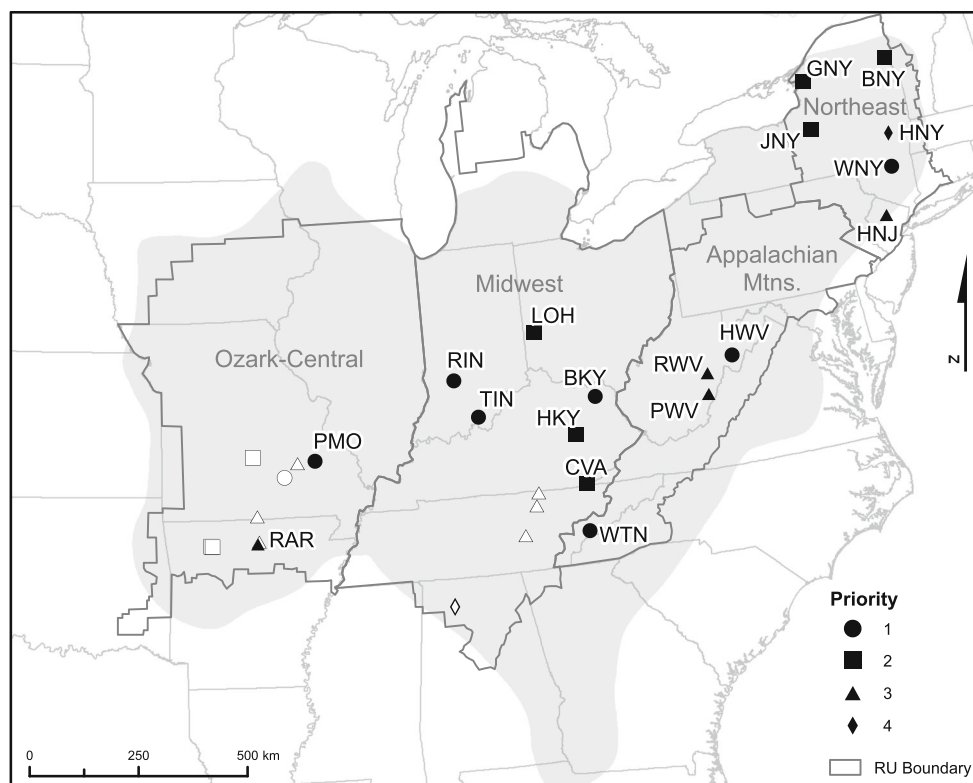
Mitochondrial DNA sequencing

Total genomic DNA was isolated using the DNeasy Tissue Kit (Qiagen). We amplified approximately 500–700 bp of the mtDNA control region hypervariable I subunit (HVI) using primers P* and F and cycling conditions outlined in Wilkinson and Chapman (1991) for a subset of the individuals captured in each hibernaculum (total number of individuals sequenced = 375; Table 1). Amplifications were carried out in 12 µl volumes containing 1.04 mM MgCl₂, 0.1 mM dNTPs, 1 unit of *Taq* DNA polymerase (Promega), 1.2 µl Promega 10× buffer, 20 ng genomic DNA, and 14 pmol of each primer. The HVI subunit in Indiana bats contains a number of repeats 82 bp in length (see Wilkinson and Chapman 1991; Wilkinson et al. 1997), and individuals differed in the number of repeat units. Therefore, we focused on the sequence 5' to the repeats, and did not include repeat sequences in our analyses. Individuals were initially sequenced in both directions, but because of differences in repeat number, determining homology of the sequences in the region containing repeats was impossible. Therefore, we sequenced individuals using the primer P*, and edited sequences using SEQUENCHER 3.0 (Gene Codes Corp.) to remove repeats, leaving the sequence from the 5' end to the start of the repeats. Sequences were aligned using the default settings in CLUSTAL W (Thompson et al. 1994). The alignment was then edited manually, and cropped to a common length. The remaining sequence variation included 10 indels, ranging in size from 1 to 37 bp. To avoid ambiguous alignments large indels (>5 bp) were edited from sequences and the remaining five indels (1–4 bp) were retained in population genetic analyses. The resulting sequences were 230–238 bp in length.

Microsatellite genotyping

We genotyped all individuals at ten highly variable microsatellite loci using primers previously developed for Indiana bats (IBat CA5, CA8, CA38, CA43, CA47, M7, and M23 from Oyler-McCance and Fike 2011; MS3D02, MS3D09, and MS3E02 from Trujillo and Amelon 2009). Single-locus amplifications were pooled into three different loads for fragment analysis. PCR and cycling conditions followed those outlined in Trujillo and Amelon (2009). Fragments were visualized on a 3730 × 1 DNA Analyzer in the DNA Core Facility at University of Missouri, Columbia and analyzed and scored using GeneMarker software (SoftGenetics LLC, State College, PA). All samples amplified at ≥8 of the 10 loci.

Fig. 1 Geographic range of Indiana bats showing the locations of sampled hibernacula and the geographic limits of the four recovery units identified in the recovery plan (US Fish and Wildlife Service 2007) for the species. *Three letter codes* for sites refer to those in Table 1. Sites included in site-level analyses are indicated by *filled symbols*. Sites with smaller sample size and included in phylogenetic analyses only are indicated by *empty symbols*



Mitochondrial DNA analysis

We utilized multiple methods to assess the presence of distinct genetic units based on mtDNA. The first was a phylogenetic approach employing maximum likelihood implemented in PHYL 3.0 (Guindon et al. 2010) to describe the presence of divergent evolutionary lineages. A sequence of *M. volans interior*, the closest sister taxon to the Indiana bat (Stadelmann et al. 2007), was included as the outgroup. We used the best fit model of sequence evolution (HKY+G) as determined using jModelTest 2 (Darriba et al. 2012), gamma distribution of variability of rates among sites calculated empirically from the data, SPR moves to explore tree space, and SH-Like procedure to assess branch supports (Guindon et al. 2010). Because relationships among haplotypes in intraspecific analyses cannot always be represented by a bifurcating tree, we also constructed an agglomerative clustering (TCS) network (Clement et al. 2000) using POPART (Leigh and Bryant 2015).

We also used several population genetic approaches to ask whether current patterns of variation are indicative of the presence of distinct genetic clusters. All population genetic analyses were performed using ARLEQUIN 3.11 (Excoffier et al. 2005). Levels of gene diversity within each site were described as haplotype (h) and nucleotide (π) diversities. Following Slatkin (1991), we calculated

pairwise F_{ST} values between sites to identify pairs that were genetically distinct and significance was assessed through 10^4 random permutations of individuals among sites. We also tested whether genetic variation was distributed among sites using an analysis of molecular variance (AMOVA; Excoffier et al. 1992).

We conducted discriminant analysis of principal components (DAPC) to identify distinct genetic clusters based on mtDNA variation. DAPC finds principal components that best summarize differences between clusters while minimizing within-cluster variation utilizing information encoded in the frequencies of single-nucleotide polymorphisms (SNPs) or microsatellite alleles (Jombart et al. 2010). DAPC relies on data transformation of large numbers of variables (allele frequencies) using principal components analysis (PCA) as a prior step to discriminant analysis (DA) to reduce the number of variables. The use of PCA ensures that variables included in the DA are perfectly uncorrelated, and that their number is less than that of analyzed individuals (a requirement of the method), while DA achieves the best discrimination of individuals into pre-defined groups (Jombart et al. 2010).

DAPC was carried out using the adegenet 1.4-2 package (Jombart 2008; Jombart and Ahmed 2011) implemented in R 3.2.3 (R Core Team 2015). We made no prior assumption of group membership and used sequential K -means clustering ($K = 1$ –18) performed on transformed data

Table 1 Sample sizes and molecular diversity statistics for mitochondrial control region sequences from sampled hibernacula

Hibernaculum	State	Code	Priority ^a	<i>N</i>	<i>N_f</i>	<i>N_m</i>	<i>N_h</i>	<i>h</i>	π
<i>Population and phylogenetic analyses</i>									
Roland + Amphitheatre Cave	Arkansas	RAR	3	21	7	14	11	0.85	0.024 ± 0.013
Pilot Knob Mine	Missouri	PMO	1	11	0	11	8	0.93	0.028 ± 0.016
Ray's Cave	Indiana	RIN	1	18	1	17	12	0.93	0.028 ± 0.016
Twin Domes Cave	Indiana	TIN	1	27	15	12	18	0.94	0.026 ± 0.014
Lewisburg Limestone Mine	Ohio	LOH	2	19	10	9	15	0.97	0.028 ± 0.015
Cave Hollow Cave	Kentucky	HKY	2	21	11	10	14	0.95	0.021 ± 0.012
Bat Cave	Kentucky	BKY	1	22	15	7	8	0.83	0.024 ± 0.013
Cumberland Gap Saltpeter Cave	Virginia	CVA	2	20	10	10	15	0.94	0.030 ± 0.016
White Oak Blowhole Cave	Tennessee	WTN	1	20	10	10	17	0.95	0.040 ± 0.021
Martha's + Snedegar's Cave	W. Virginia	PWV	3	22	22	0	15	0.96	0.035 ± 0.019
Forklick + Falling Spring + Izaak Walton Cave	W. Virginia	RWV	3/4	28	28	0	17	0.94	0.032 ± 0.017
Hellhole Cave	W. Virginia	HWV	1	19	9	10	11	0.87	0.034 ± 0.018
Jamesville Quarry Cave	New York	JNY	2	21	10	11	9	0.82	0.023 ± 0.013
Glen Park Caves	New York	GNV	2	19	10	9	2	0.28	0.001 ± 0.001
Barton Hill Mine	New York	BNV	2	21	11	10	4	0.42	0.002 ± 0.002
Haile's Cave	New York	HNV	4	18	8	10	7	0.22	0.005 ± 0.004
Walter Williams Preserve Mine	New York	WNV	1	20	10	10	4	0.57	0.019 ± 0.011
Hibernia + Mt. Hope West Mine	New Jersey	HNJ	3	28	22	6	6	0.39	0.007 ± 0.004
Overall				375	209	166	101	0.76	0.023 ± 0.012

The first letter of site codes corresponds to the hibernaculum, and the last two letters are the state code. Overall values represent sums for the number of individuals (*N*), females (*N_f*), and males (*N_m*) sequenced, the total number of haplotypes (*N_h*) across the entire sample, and means for haplotype diversity (*h*) and nucleotide diversity (π). Sites are ordered from west to east (see Fig. 1)

^a Priority refers to the priority status of hibernacula as defined by US Fish and Wildlife Service (2007): Priority 1 contain $\geq 10,000$ individuals, Priority 2 contain 1000–10,000 individuals, Priority 3 contain 50–1000 individuals, Priority 4 contain < 50 individuals

using principal components analysis (PCA) and model selection to infer the number of genetic groups. As per package instructions, the number of principal components kept in the *K*-means clustering process was determined by examining a plot of the cumulative variance explained as a function of the number of PC's retained, with the selected value chosen at the point beyond which the addition of further PC's resulted in only negligible increases in explained variance. The optimal number of clusters was determined by selecting the model (*K*) that minimized the Bayesian information criterion (BIC), or the value after which the BIC increased or decreased by a negligible amount (Jombart et al. 2010). We used 10^9 iterations and 10^3 random starting centroids for each run, both of which aid in the convergence of the algorithm.

Following the *K*-means clustering, DAPC was performed with the number of clusters set at the optimal *K*. To not overfit the DAPC, we chose the optimal number of PC's to include in the DAPC using the cross-validation approach (xval function) with a 70 % training set and a 30 % validation set. The process captures the trade-off between the power of discrimination and over-fitting the

models (using too many PC's) by measuring the ability of varying number of PC's to successfully predict cluster membership of the validation set. Following package instructions, all discriminant functions were retained, as the number of clusters was small in all cases (see “Results” section). DAPC results were visualized as scatterplots based on the retained PC's with the highest eigenvalues.

To test for isolation-by-distance (IBD), we conducted a Mantel test comparing standardized genetic distance [$F_{ST}/(1-F_{ST})$] and the natural log of geographic distance (Rousset 1997) calculated in ARC-GIS using the IBD Web Service (Jensen et al. 2005). The use of Mantel tests in spatial analysis has been criticized (Legendre et al. 2015; but see Kierepka and Latch 2015), and therefore we also applied a spatial principal components analysis (sPCA; Jombart et al. 2008) in the adegenet package. The sPCA yields scores summarizing both genetic variability and the spatial structure among individuals or sites using Moran's eigenvector maps (MEMs). In the sPCA spatial variation is first modeled through a connection network, which, in our case, was constructed using a *K*-nearest neighbors approach. A test is provided for global structure, defined as

entities being more genetically similar to their immediate neighbors than expected in a random spatial distribution, with the null hypothesis that allele frequencies are distributed at random on the connection network. The test relied on a comparison of the observed test statistic, which summarizes correlations of alleles to vectors in the matrix of global MEMs, to the distribution of test statistics obtained through a Monte Carlo randomization procedure using 10^4 permutations (Jombart et al. 2008).

Microsatellite DNA analysis

Deviations from Hardy–Weinberg equilibrium (HWE) for each locus were estimated and linkage equilibrium between loci tested using ARLEQUIN 3.11 and GENODIVE 2.0b23 software (Meirmans 2012). To test for differences among sites and regions in levels of genetic diversity, several indices of nuclear genetic diversity were estimated, including number of alleles per locus, allelic richness, and the inbreeding coefficient (F_{IS}) using FSTAT v2.9.3 (Goudet 2001), and observed and expected heterozygosity using GENODIVE. The level of genetic differentiation among pre-defined sites was determined by calculating pairwise F_{ST} values (Weir and Cockerham 1984), and tested for significance using AMOVA with 1000 permutations using ARLEQUIN 3.11.

We applied four different approaches to determine the most likely number of distinct genetic clusters independent of original sampling locations. First, we utilized the model-based Bayesian clustering approach in STRUCTURE 2.3.3 software (Pritchard et al. 2000; Falush et al. 2003) with and without population membership as a prior (Hubisz et al. 2009). To determine the optimal number of clusters (K), we ran 10 runs per K , for $K = 1$ –10, with 1 million MCMC iterations and a 20 % burn-in using the admixture model with correlated allele frequencies. The most likely number of clusters was determined using the Evanno et al. (2005) method implemented in the program STRUCTURE HARVESTER (Earl and vonHoldt 2012). The Evanno et al. (2005) method is not informative for the highest and lowest K , therefore if the highest log likelihood value was observed for $K = 1$ or 10 across all replicates, we accepted that as the K with the highest probability. Second, we applied the K means clustering approach in GENODIVE 2.0 as outlined by Meirmans (2012). This approach is based on an AMOVA framework and uses a simulated annealing algorithm to minimize the among-populations-within-groups sum of squares through maximization of F_{CT} , the variance among clusters relative to the total variance. Two summary statistics, Pseudo- F and the Bayesian information criterion (BIC), were used to determine the most likely number of clusters. Pseudo- F performs better than BIC when migration rates are high and

mating is random, but it cannot be calculated for $K = 1$ and therefore cannot be used to test whether there is any significant structure. BIC can be calculated for $K = 1$, and outperforms Pseudo- F when mating is non-random and migration rates are intermediate (Meirmans 2012). As a third approach, we applied the DAPC procedure in the adegenet package outlined above. As with mtDNA, the DAPC was based on the optimal K as determined by a K -means clustering algorithm, and results were visualized as scatterplots based on the retained PC's with the highest eigenvalues. The fourth approach was that of Duchesne and Turgeon (2012) implemented in the software FLOCK. Samples are initially randomly partitioned into K clusters (≥ 2), allele frequencies are estimated for each of the K clusters, and each genotype is then reallocated to the cluster with the highest likelihood score. Repeated reallocation based on likelihood scores (20 iterations per run) results in genetically homogeneous clusters within a run. Fifty runs were carried out for each K , and at the end of each run the software calculates the log likelihood difference (LLOD) score for each genotype (the difference between the log likelihood of the most likely cluster for the genotype and that of its second most likely cluster) and the mean LLOD over all genotypes. Strong consistency among runs (resulting in 'plateaus' of identical mean LLOD scores) is used to indicate the most likely number of clusters (Duchesne and Turgeon 2012).

Clustering methods may overestimate the number of independent genetic units if a pattern of isolation by distance (IBD) is present (Frantz et al. 2009). Therefore, and as with mtDNA, we tested whether $\ln(\text{geographic distance})$ was correlated with genetic distance ($F_{ST}/1 - F_{ST}$) using the IBD web service and performed a test of global structure in the data using a sPCA in the adegenet package.

Results

Mitochondrial DNA analyses

We observed 101 unique haplotypes among 375 Indiana bats (Genbank accession numbers KT223649–KT223749). Seventy-one haplotypes were unique to a single site, and two haplotypes were found in more than 10 sites. Overall haplotype diversity was high ($h = 0.76$), but nucleotide diversity was low ($\pi = 0.023$). Five sites, including four from New York (BNY, GNY, HNY, and WNY; see Table 1 for hibernacula abbreviations) and one from New Jersey (HNJ) had the lowest haplotype diversities, and four of the five (all except WNY) had low nucleotide diversity relative to other populations. The majority of individuals in these populations (22/28 (79 %) in HNJ, 16/21 (76 %) in BNY, 16/19 (84 %) in GNY, 16/18 (89 %) in HNY, and

12/20 (60 %) in WNY) shared a single, high frequency haplotype (Fig. 2). This same haplotype was only found in three other sites (2 individuals each in RAR, PWV and RWV).

The TCS network revealed the presence of six high-frequency, ancestral haplotypes, from which the majority of other haplotypes originated in a star-like pattern forming clusters (Fig. 2). Four of the clusters were found at high frequencies in sites in the Midwestern recovery unit and at low frequencies in sites in the Appalachian recovery unit. One cluster occurred at high frequency in Northeastern sites, and was also found at low frequency in Midwestern sites. The final cluster, which differed from all other clusters by a relatively large number of mutational steps, was found in sites in the Midwestern, Appalachian, and Northeastern recovery units. The phylogeny based on PhyML 3.0 with *Myotis volans interior* as the outgroup revealed little segregation of Indiana bat populations into distinct clades (Online Resource 1). Rather, the phylogeny was largely comb-like with short branches (Online Resource 1), and there were no well-supported clades within the phylogeny.

Pairwise F_{ST} values between sites varied widely (Table 2), with little differentiation between some site pairs, and high levels of differentiation between other pairs. Five sites in the Northeast recovery unit (GNY, BNY, HNY, WNY, and HNJ) had high (0.27–0.69) and

significant pairwise F_{ST} values with all sites in other recovery units as well as the JNY site. However, WNY was also significantly differentiated (F_{ST} : 0.16–0.24) from the other northeastern sites. Although it fell within the Northeast recovery unit, the JNY site was undifferentiated from the majority of sites in the other recovery units. Although some sites were significantly differentiated, consistently high and significant F_{ST} values were not observed among sites falling in the Ozark-Central, Midwestern, and Appalachian recovery units. One exception was WTN, which had moderate to high F_{ST} values (>0.14) with all Ozark-Central and Midwestern sites, and was only undifferentiated from the three other Appalachian recovery unit sites (HWV, PWV, and RWV). The other exception was HKY, which had moderate to high F_{ST} values (>0.1) with all sites except RIN, TIN, and LOH. An AMOVA with all sites forming a single group indicated significant variation among (22.6 %) and within (77.4 %) sites.

We applied K -means clustering and DAPC analysis to the mtDNA data to distinguish distinct genetic clusters. In the K -means clustering process we retained 30 PC's, and BIC values declined steadily until $K = 5$, after which the rate of decline slowed (Online Resource 2). For $K = 5$ three of the inferred clusters included individuals from sites situated in the Ozark-Central, Midwest, and Appalachian recovery units (Fig. 3a). The dominant sites in Cluster 1 included sites situated on the Cumberland Plateau (BKY

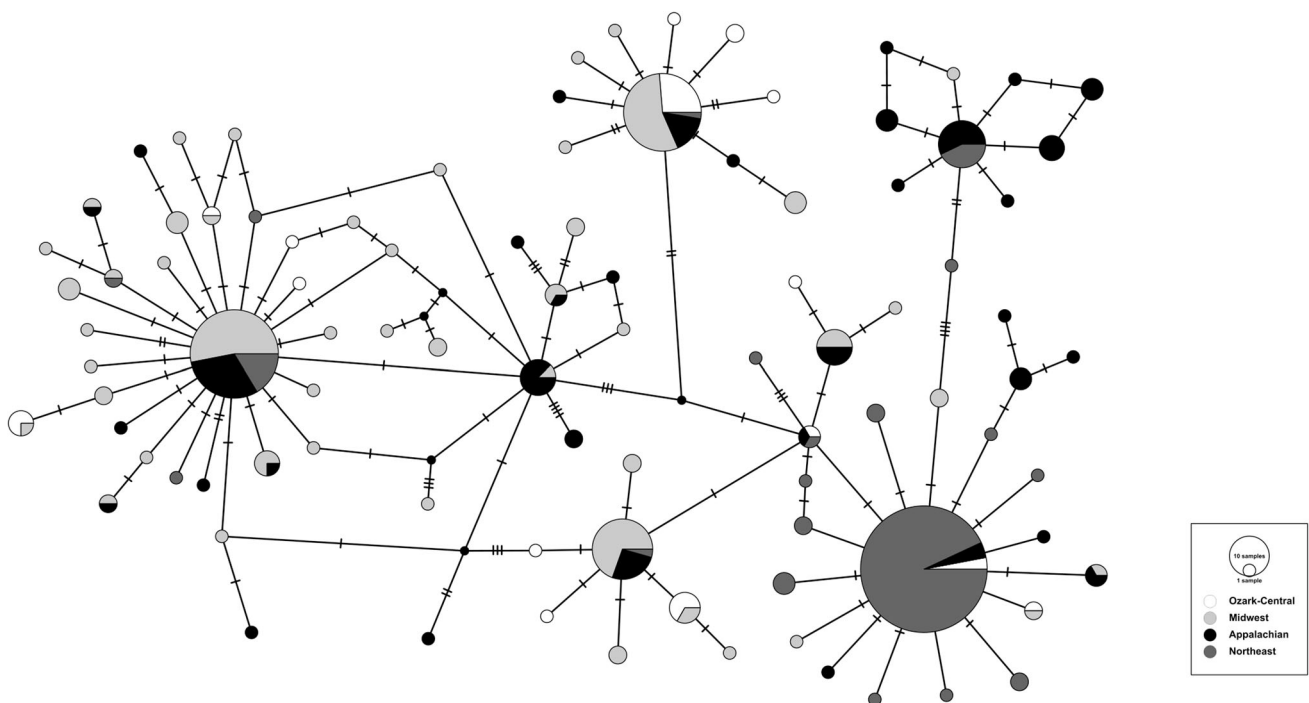


Fig. 2 Estimates of relationships among haplotypes for Indiana bats based on an agglomerative clustering (TCS) network analysis. The size of the circle corresponds to the frequency of the haplotype, and

different shadings represents sites in different recovery units. 'Missing' haplotypes are denoted by black hash marks

Table 2 Pairwise F_{ST} values for microsatellite (lower diagonal) and mtDNA (upper diagonal) between sites

	RAR	PMO	RIN	TIN	LOH	HKY	BKY	CVA	WTN	PWV	RWV	HWV	JNY	GNY	BNY	HNY	WNY	HNJ
RAR	–	0.007	0.030	0.089	0.126	0.231	0.021	0.028	0.247	0.088	0.094	0.180	0.133	0.494	0.499	0.415	0.363	0.450
PMO	0.001	–	0.031	0.124	0.114	0.261	0.001	0.028	0.177	0.043	0.046	0.138	0.051	0.403	0.407	0.290	0.242	0.327
RIN	0.000	0.001	–	0.015	0.015	0.055	0.026	0.021	0.165	0.038	0.013	0.063	0.040	0.509	0.515	0.423	0.353	0.463
TIN	0.004	0.000	0.001	–	0.013	0.010	0.036	0.049	0.186	0.072	0.044	0.069	0.090	0.545	0.550	0.474	0.409	0.509
LOH	0.003	0.002	0.001	0.002	–	0.005	0.028	0.030	0.159	0.062	0.022	0.052	0.062	0.559	0.564	0.478	0.396	0.515
HKY	0.002	0.001	0.003	0.006	0.001	–	0.137	0.142	0.242	0.158	0.115	0.109	0.172	0.690	0.692	0.618	0.523	0.644
BKY	0.009	0.002	0.004	0.005	0.001	0.002	–	0.033	0.193	0.047	0.028	0.110	0.057	0.484	0.489	0.400	0.344	0.435
CVA	0.002	0.003	0.005	0.005	0.000	0.001	0.001	–	0.153	0.025	0.008	0.083	0.014	0.382	0.388	0.298	0.265	0.338
WTN	0.003	0.000	0.002	0.002	0.002	0.004	0.007	0.001	–	0.018	0.050	0.002	0.140	0.409	0.417	0.343	0.149	0.374
PWV	0.006	0.005	0.006	0.006	0.003	0.004	0.010	0.013	0.004	–	0.024	0.006	0.047	0.354	0.361	0.283	0.158	0.314
RWV	0.007	0.001	0.003	0.004	0.001	0.000	0.005	0.002	0.006	0.010	–	0.002	0.010	0.356	0.362	0.284	0.202	0.316
HWV	0.010	0.003	0.001	0.006	0.001	0.002	0.000	0.000	0.005	0.012	0.006	–	0.064	0.467	0.474	0.388	0.235	0.423
JNY	0.000	0.005	0.003	0.004	0.002	0.002	0.004	0.004	0.002	0.001	0.006	0.006	–	0.367	0.372	0.268	0.243	0.304
GNY	0.002	0.004	0.007	0.008	0.007	0.009	0.010	0.001	0.001	0.010	0.010	0.012	0.004	–	0.067	0.025	0.237	0.020
BNY	0.001	0.001	0.001	0.004	0.001	0.004	0.006	0.002	0.004	0.004	0.006	0.007	0.003	0.006	–	0.015	0.239	0.016
HNY	0.010	0.003	0.002	0.002	0.001	0.004	0.001	0.003	0.006	0.015	0.005	0.001	0.004	0.009	0.010	–	0.171	0.034
WNY	0.008	0.007	0.002	0.006	0.003	0.004	0.009	0.003	0.008	0.011	0.011	0.007	0.007	0.014	0.006	0.003	–	0.158
HNJ	0.003	0.001	0.001	0.004	0.002	0.004	0.003	0.001	0.004	0.011	0.005	0.003	0.004	0.005	0.003	0.008	0.003	–

Site codes refer to those in Table 1. Statistical significance (indicated with italics) was determined using permutations of individuals among populations and controlling for multiple comparisons

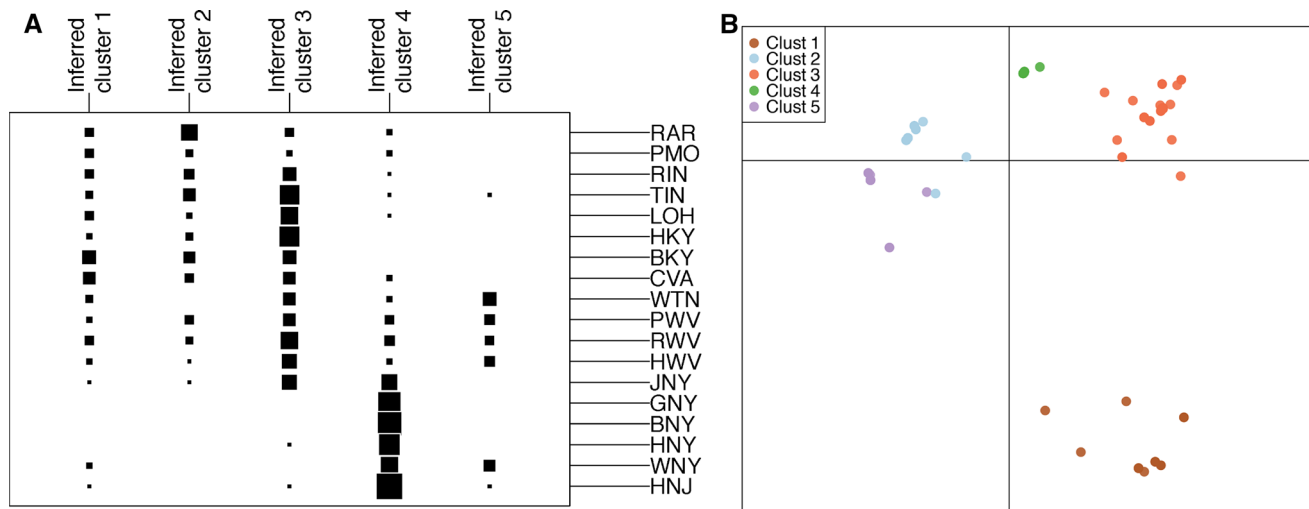


Fig. 3 Clustering of sites based on mtDNA in the discriminant analysis of principal components (DAPC) analysis. **a** Number of clusters inferred based on *K*-means clustering of mtDNA (tested

K = 1–18). **b** Projection of clusters in discriminant space using the first two principal components (variance explained: PC1: 44.7 %, PC2: 19.2 %)

and CVA), Cluster 2 had a higher contribution from western sites (RAR, RIN, and TIN, but also BKY), and Cluster 3 contained the majority of individuals from these recovery units plus site JNY and the lowest contribution from the two Ozark-Central sites. Cluster 4 consisted primarily of individuals from sites in the Northeast recovery unit, including site JNY, while Cluster 5 included individuals primarily from Appalachian sites and site WNY (Fig. 3a). In the DAPC analysis 5 principal components and 4 discriminant functions were retained. Clusters were clearly differentiated in discriminant space and results were highly consistent with those of the *K*-means clustering (Fig. 3b).

Based on BIC values additional information could be obtained by delineating larger numbers of clusters (Online Resource 2), however inspection of plots of assignments of individuals from sampled sites to inferred clusters for *K* > 5 revealed that additional clusters included individuals primarily from the Ozark-Central, Midwest, and Appalachian recovery units and consisted of relatively small numbers of individuals relative to other clusters (see Online Resource 3 for *K* = 6–8 to illustrate). In addition, the cluster consisting primarily of individuals from Northeast sites (Cluster 4 in Fig. 3), the cluster consisting of the majority of individuals from most Midwest, and Appalachian sites (Cluster 3), and the cluster consisting of individuals from Appalachian sites plus WNY (Cluster 5) remained consistent for the majority of *K*.

Pairwise genetic distance ($F_{ST}/(1-F_{ST})$) and the natural log of geographic distance were correlated ($r = 0.44$, $P = 0.0014$; Fig. 4a) across all sites, indicating IBD. The test for global structure using Moran's eigenvector maps in

the sPCA also indicated a high degree of spatial structure in the mtDNA data ($P < 0.001$; Fig. 4b).

Microsatellite analyses

The microsatellite loci were highly polymorphic with alleles per locus ranging from 21 to 32 with a mean N_a of 26.44. The overall observed heterozygosity (H_o) was 0.863 and ranged from 0.722 to 0.916 for each locus. There was no deviation from HWE and no evidence for the presence of high frequency null alleles or linkage disequilibrium. Diversity was high in all sites, and none of the diversity measures varied substantially between sites (Table 3).

F_{ST} values among sites were uniformly low and ranged from 0 to 0.015 (Table 2). AMOVA analysis revealed that almost all variation was accounted for at the level of the site (99.6 %), with only 0.4 % of variation among sites. No isolation by distance was observed among sites based on Mantel tests ($r = -0.03$, $P = 0.397$; Fig. 4c), and the test for global structure in the sPCA indicated no significant global structure in the microsatellite data ($P > 0.05$; Fig. 4d). The four methods we used to delineate distinct genetic clusters returned concordant results. *K* = 1 had the highest Ln(prob) in STRUCTURE analyses, and no plateaus were reached for any $K \geq 2$ using FLOCK, indicating that *K* = 1 best fits the data. The *K*-means clustering approach in GENODIVE returned *K* = 2 as the most likely number of clusters; however, inspection of the individual assignments to clusters revealed that individual assignments to clusters did not differ from chance ($\chi^2 = 24.48$, $P > 0.1$), and there was no geographic pattern associated with the defined clusters. Similarly, although *K* = 3 was

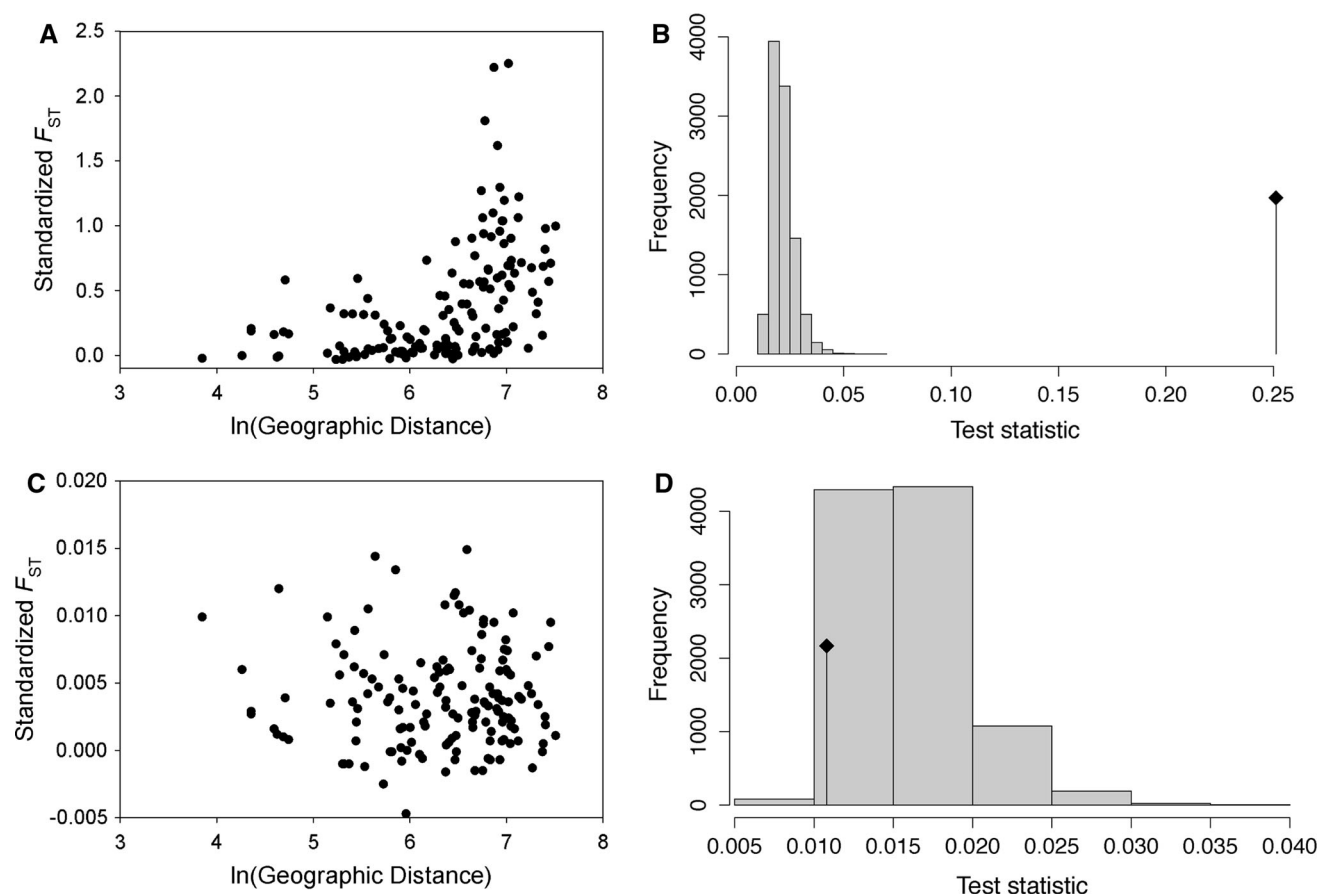


Fig. 4 Spatial structure in mtDNA (**a** and **b**) and microsatellite (**c** and **d**) data. **a** and **c** shows the comparison of standardized genetic distance [$F_{ST}/(1 - F_{ST})$] and the natural logarithm of geographic distance and significance was assessed using Mantel tests (see text). **b** and **d** shows the results of a test of global structure using Moran's

eigenvector maps (MEMs) in a spatial principle components analysis, and are based on comparison of the observed test statistic summarizing the correlation of alleles to vectors of global MEMs to a distribution of test statistics based on 10^4 randomizations. Observed values are indicated by the *diamond symbol*

the most likely number of clusters in the DAPC analysis (Online Resource 4), there was no clear pattern by site or region in individual assignments to clusters, and cluster assignments did not differ from chance ($\chi^2 = 126$, $P > 0.3$; see Online Resource 5 for K -means clustering and DAPC results). Therefore, we concluded that $K = 1$ was the most likely the true number of clusters.

Discussion

Describing patterns of gene flow and genetic structure before the onset of WNS contributes to our understanding of current patterns of disease spread and how populations in different regions may respond following introduction of the disease. Analyses of mtDNA identified a number of distinct genetic clusters, with observed high pairwise F_{ST} values (>0.2 , and up to 0.69) among many sites and significant IBD and global structure indicating limited exchange of females among hibernacula in different

regions. However, despite regional differences in patterns of female dispersal among hibernacula, the absence of differentiation among all hibernacula shown by nuclear microsatellites suggests the occurrence of extensive gene flow through dispersal and mating between individuals originating from different regions. The widespread nuclear gene flow across the range of Indiana bats indicates a lack of barriers to WNS transmission. Further, we found no genetic evidence to support the division of Indiana bats into separate recovery units.

Pre-WNS genetic structure

The clusters identified based on mtDNA differed in geographic extent, observed number of hibernacula, and proportion of the total census population size they encompassed. Patterns of gene flow between sites within the Ozark-Central, Midwest, and Appalachian recovery units were complex, as indicated by low pairwise F_{ST} values among some sites and high values among others,

Table 3 Sample sizes and diversity statistics (means across the 10 loci genotyped) for microsatellite genotypes from sampled hibernacula

Hibernaculum	Code	<i>N</i>	<i>N_A</i>	<i>AR</i>	<i>H_O</i>	<i>H_E</i>	<i>F_{IS}</i>
Roland + Amphitheatre Cave	RAR	21	13.4	12.7	0.79	0.89	0.116
Pilot Knob Mine	PMO	20	12.8	12.4	0.84	0.90	0.066
Ray's Cave	RIN	18	13.5	13.5	0.87	0.91	0.043
Twin Domes Cave	TIN	29	14.6	12.7	0.88	0.90	0.014
Lewisburg Limestone Mine	LOH	30	16.0	13.8	0.83	0.91	0.092
Cave Hollow Cave	HKY	29	14.8	12.9	0.84	0.90	0.066
Bat Cave	BKY	36	14.8	12.4	0.86	0.89	0.034
Cumberland Gap Saltpeter Cave	CVA	25	14.9	13.4	0.87	0.90	0.028
White Oak Blowhole Cave	WTN	24	14.7	13.1	0.83	0.89	0.068
Martha's + Snedegar's Cave	PWV	22	14.4	13.4	0.85	0.90	0.064
Forklick + Falling Spring + Izaak Walton Cave	RWV	27	14.0	12.4	0.90	0.89	−0.011
Hellhole Cave	HWV	28	15.2	13.5	0.88	0.90	0.031
Jamesville Quarry Cave	JNY	21	13.0	12.4	0.83	0.90	0.080
Glen Park Caves	GNV	19	12.2	12.0	0.84	0.89	0.052
Barton Hill Mine	BNV	30	16.0	13.4	0.85	0.91	0.058
Haile's Cave	HNV	18	12.8	12.8	0.88	0.90	0.013
Walter Williams Preserve Mine	WNV	20	12.7	12.3	0.88	0.89	0.006
Hibernia + Mt. Hope West Mine	HNV	28	14.1	12.6	0.89	0.90	0.013
Overall		445	14.1	13.2	0.86	0.90	0.046

N number of individuals genotyped, *N_A* number of alleles, *AR* allelic richness, *H_O* observed heterozygosity, *H_E* expected heterozygosity, *F_{IS}* inbreeding coefficient

and the lack of a single, optimal solution in the *K*-means clustering analysis. The majority of sampled hibernacula, encompassing much of the range of Indiana bats, and the Priority I hibernacula known to contain the largest numbers of individuals, fell into three genetic clusters encompassing individuals from many of the same sites. One of these clusters (inferred Cluster 3; see Fig. 3) contained the majority of sampled individuals in the Midwest, and Appalachian recovery units, while the other two clusters contained primarily individuals from western sites (RAR and PMO in Ozark-Central and RIN and TIN in Midwest; Cluster 2) and individuals from sites on the Cumberland Plateau (BKY and CVA; Cluster 1), respectively. What is clear from the mtDNA data is that movement of females occurs among some sites but not others, resulting in spatial structure across the range, while the nuclear microsatellite data indicate widespread gene flow across the entire range of the Indiana bat. Band returns and isotopic data indicate that summer populations in northern parts of the Midwest primarily make use of hibernacula in the southern portion of the range (Gardner and Cook 2002; Kurta and Murray 2002; Britzke et al. 2012), and that individuals may utilize different hibernacula in different years (Kurta and Murray 2002). Hence, there may be regular opportunities for individuals from different summer and winter populations to interact during the autumn and winter when mating takes

place, resulting in wide and overlapping catchment areas for hibernacula across the majority of the range.

The two other clusters identified in the mtDNA analysis grouped individuals primarily from sites in the Northeast recovery unit (Cluster 4) or individuals from sites in the Appalachian recovery unit plus the WNV site in the Northeast recovery unit (Cluster 5). Movements between summer and winter habitats occur over relatively short distances in the Northeast and Appalachian recovery units (Harvey 2002; Hicks et al. 2005; Britzke et al. 2006), suggesting that the scale of movements may be lower, and hence the catchment areas smaller, in these regions as compared to the Midwest and Ozark-Central recovery units. The five hibernacula in New York that we sampled represented >70 % of the wintering population identified in the region, and only 14 active hibernacula have been identified in this region (Hicks and Novak 2002). Thus, there appear to be a limited number of sites among which individuals may move relative to other parts of the range, and limited opportunity and/or need for long distance dispersal may result in restricted levels of maternal gene flow between some northeastern sites. However, none of the northeastern sites were far apart (maximum straight line distance was 360 km), and it is unclear why the WNV site was differentiated from other northeastern populations. The straight line distance from WNV to the two closest sites (HNV and

HNJ) was only ~80 km, and other northeastern sites that were further apart and had similar or larger topographic features separating them experienced higher matrilineal gene flow. Movements between summer and winter habitats are also restricted in the Appalachian recovery unit (Harvey 2002), and although one identified cluster consisted primarily of individuals from this recovery unit, only one site (WTN) was consistently differentiated from sites in other recovery units based on pairwise F_{ST} values.

The lack of population differentiation at nuclear loci suggests widespread movement of males and exchange of genes between individuals originating in different regions. Extensive gene flow in spite of high levels of female philopatry to breeding sites is common in temperate zone bats (Burland et al. 1999; Kerth et al. 2000, 2002; Castella et al. 2001; Petit et al. 2001; Vonhof et al. 2008; Dixon 2011; Vonhof et al. 2015) because many different breeding groups come together at swarming or hibernation sites where mating takes place (Kerth et al. 2003; Veith et al. 2004). Clearly there is sufficient overlap among the identified mtDNA clusters during mating to erase any signature of restricted movements by either sex. Similar patterns of complex mtDNA structure (differentiation among some sites but not others) combined with widespread gene flow and uniformly weak differentiation based on nuclear markers has been documented for several other bat species in eastern North America (Vonhof et al. 2008; Burns et al. 2014; Johnson et al. 2015; McLeod et al. 2015; Vonhof et al. 2015; Wilder et al. 2015), suggesting that the lack of major topographic barriers east of the Rocky Mountains facilitates genetic panmixia across wide areas in Indiana bats and other bat species.

Relevance to post-WNS conservation

The geographic extent of WNS now encompasses virtually the entire range of the Indiana bat, and it is likely that all wintering populations of this species will be affected by 2016 (Thogmartin et al. 2012b). Although we found matrilineal differentiation among subsets of winter populations, and such restricted gene flow has been shown to delay the spread of WNS (Miller-Butterworth et al. 2014), there are few barriers to gene flow across the range of Indiana bats to limit the exchange of individuals among winter populations. Thus, the evidence for widespread gene flow and an almost complete absence of barriers to gene flow among winter populations of Indiana bats supports the potential for widespread transmission of WNS through individual contacts associated with dispersal and mating.

Contact patterns inferred from population genetic analyses of hosts may overestimate the opportunities for spatial spread if the infectious period of the disease is short relative to the timescale of dispersal (Cross et al. 2005).

Conversely, measures of gene flow may underestimate disease spread if contact rates among host individuals are higher than the probability of successfully immigrating and reproducing. Because WNS is spread by fungal conidia that can survive for extended periods with a potentially long infectious period, and because bats may have interactions during migration and swarming in addition to those associated with mating, the potential for intraspecific transmission and geographic spread of WNS is likely high. In addition, multiple bat species are affected by the disease, and transmission to new sites may be driven simultaneously by the dispersal and mating dynamics of multiple bat species. The introduction of *P. destructans* to novel sites by one host species may be followed by subsequent environment-to-host transmission to other host species, particularly if there is amplification of fungal populations within the environment after its initial introduction (Hoyt et al. 2014; Langwig et al. 2015; Reynolds et al. 2015). While intraspecific disease progression within sites is unaffected by the population sizes of other bat species present (Langwig et al. 2012), suggesting that within-site contacts among different host species leading to disease transmission are limited, initial transmission from host to environment at new sites by the suite of affected bat species may act synergistically with patterns of intraspecific contacts to drive the geographic spread of the disease. These combined effects and the clear lack of barriers to dispersal observed in this study make it unlikely that significant portions of the hibernating population of Indiana bats will remain disease free into the future.

WNS has now been detected in all states with significant hibernating populations of Indiana bats, and evidence indicates that in WNS-affected areas mortality rates of Indiana bats are high (>70 %; Turner et al. 2011), with affected populations trending to extinction within a short timeframe (Langwig et al. 2012). As more and more populations become affected each year, the prognosis for the long-term survival of Indiana bats is not promising (Thogmartin et al. 2012b), and it is likely that the disease is placing strong selective pressure on hosts for the evolution of resistance. High levels of genetic variation exist in all populations and regions at the neutral microsatellite markers we employed, suggesting no long-term effect of pre-WNS population declines on overall neutral diversity. This is not surprising, as even at its lowest point, range-wide population estimates were sufficient (>300,000 individuals) to prevent substantial loss of variation via genetic drift. In WNS-affected areas however, populations are currently going through massive population declines and potentially severe bottlenecks that may result in substantial decay in genetic diversity. How this will affect the evolutionary trajectory of surviving populations of Indiana bats is unclear, as neutral and adaptive loci can exhibit very

different patterns of change over time in response to bottlenecks (Dlugosch and Parker 2008; Funk et al. 2012). For example, distributions of quantitative variation in polygenic traits are relatively insensitive to the loss of rare alleles (Lande 1980; Barton and Charlesworth 1984), and additive variance may actually increase after bottlenecks due to frequency shifts at loci involved in non-additive gene interactions such as epistasis or dominance (Willi et al. 2006; Taft and Roff 2012). Conversely, variation in adaptive loci may decrease in concert with neutral variation during population declines, or decrease because strong selection drives rapid fixation of favorable variants and the loss of variation at linked loci (Cohen 2002; Radwan et al. 2010). Based on our results using neutral markers, there were no sites that contained unique genetic variation pre-WNS. However, to assess the future evolutionary potential of Indiana bats, research is needed to assess standing patterns of variation at immunogenetic and other potentially adaptive loci, and how levels of variation and expression patterns (Field et al. 2015) may change over time in affected populations.

Our analyses are based on samples collected before the onset of WNS, and our inferences regarding gene flow across the range are relevant only to that time period. It is important to recognize that as population density decreases there may be consequent changes in patterns of dispersal and social and mating behavior that may dramatically alter levels of population connectivity and the distribution of genetic variation in space (Lachish et al. 2011; Pärn et al. 2011). Genetic differentiation reaches a new equilibrium state much faster than within-population diversity in recently fragmented populations (Crow and Aiko 1984; Varvio et al. 1986), and large population declines in formerly genetically panmictic populations can result in increased levels of genetic differentiation within a surprisingly small number of generations (e.g., Kekkonen et al. 2011; Lachish et al. 2011). With decreased densities, bats in affected regions may travel longer distances or congregate in a smaller number of sites to maintain social relationships or find mates, or alternatively they may be selected to reduce contact with conspecifics (see Langwig et al. 2012) or disperse more widely in a greater number of roosts to avoid infection, particularly given that infection risk increases linearly with colony size (Thogmartin et al. 2012b). Data on fine-scale movements and colonial behavior will be needed to track changes in social and dispersal behavior, but regardless of the exact dynamics, it is likely that the large declines in population size associated with WNS are likely to result in dramatic changes in the distribution of genetic variation across the landscape for affected bat species.

Implications for the management of recovery units

The identification of discrete genetic units is important for the conservation of endangered species (Crandall et al. 2000; Fraser and Bernatchez 2001). The four recovery units identified in the current recovery plan for Indiana bats were based on preliminary mtDNA evidence, different population trends, and broad differences in habitat and land use across regions (US Fish and Wildlife Service 2007). As such, they should be supported by genetic or demographic data that confirms regional differentiation into discrete units, but our analysis indicates that from a genetic perspective the division of the Indiana bat populations into recovery units is not supported by the high levels of genetic connectivity we observed. While we found differentiation into discrete clusters based on mtDNA, there was substantial overlap among regions and recovery units in the distribution of clusters, and there was extensive gene flow across the entire range based on analyses of nuclear microsatellites. Two of the recovery units are particularly questionable, as the Ozark-Central recovery unit was not differentiated on the basis of either marker, and only one site in the Appalachian recovery unit (WTN) was consistently differentiated from sites in the other recovery units. Our results further highlight the need to utilize multiple molecular markers when defining units of conservation, as designations using single loci can be misleading (Palsbøll et al. 2007; Fallon 2007).

From a post-WNS perspective, recovery units may still be warranted if there is evidence of regional differences in demographic trends or threats to population persistence, including the incidence of WNS. Recent data and models indicate that Indiana bat populations in affected areas, particularly in the Northeast, have declined dramatically (Turner et al. 2011; Thogmartin et al. 2012a), raising questions about regional extinction. Under the ESA, recovery units represent portions of a listed species range that are created to establish recovery goals or carry out management actions, and are designated when deemed essential to recovering the species (US Fish and Wildlife Service and National Marine Fisheries Service 1998). Since WNS has now been found in all recovery units and across the entire range of Indiana bats, the designation of separate units may no longer be an effective management tool, as all populations are highly connected and are likely to experience similar population trends in the near future. Our focus needs to shift to monitoring demographic trends, spatial distribution, and potentially adaptive genetic variation of survivors to assess future genetic health, connectivity, and evolutionary potential of a much-reduced range-wide Indiana bat population into the future.

Acknowledgments Funding for this study was provided by the US Fish and Wildlife Service, the US Forest Service, and the University of Missouri. We thank V. Brack, Jr., E. Britzke, R. Clawson, M. Craddock, M. Harvey, A. Hicks, S. Pruitt, K. Womack, and C. Stihler for collecting samples, L. Eggert, A. Isabelle and L. Saidak for help in the lab, and J. Ryan and J. Glatz for help producing the range map. S. Gill provided valuable comments on earlier versions of this manuscript.

Funding This study was funded by the US Fish and Wildlife Service, the US Forest Service, and the University of Missouri.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Barton NH, Charlesworth B (1984) Genetic revolutions, founder effects, and speciation. *Ann Rev Ecol Syst* 15:133–164
- Biek R, Real LA (2010) The landscape genetics of infectious disease emergence and spread. *Mol Ecol* 19:3515–3531
- Blehert DS, Hicks AC, Behr M et al (2009) Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227
- Britzke ER, Hicks AC, Von Oettingen SL, Darling SR (2006) Description of spring roost trees used by female Indiana bats (*Myotis sodalis*) in the Lake Champlain Valley of Vermont and New York. *Am Midl Nat* 155:181–187
- Britzke ER, Loeb SC, Romanek CS, Hobson KA, Vonhof MJ (2012) Variation in catchment areas of Indiana bat (*Myotis sodalis*) hibernacula inferred from stable hydrogen ($\delta^2\text{H}$) isotope analysis. *Can J Zool* 90:1243–1250
- Burland TM, Barratt EM, Beaumont MA, Racey PA (1999) Population genetic structure and gene flow in a gleaning bat, *Plecotus auritus*. *Proc R Soc Lond B* 266:975–980
- Burns LE, Frasier TR, Broders HG (2014) Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*). *Ecol Evol* 4:4130–4149
- Castella V, Ruedi M, Excoffier L (2001) Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis*. *J Evol Biol* 14:708–720
- Clement M, Posada D, Crandall KA (2000) TCS: a computer program to estimate gene genealogies. *Mol Ecol* 9:1657–1659
- Cohen ML (2000) Changing patterns of infectious disease. *Nature* 406:762–767
- Cohen S (2002) Strong positive selection and habitat-specific amino acid substitution patterns in MHC from an estuary fish under intense pollution stress. *Mol Biol Evol* 19:1870–1880
- Crain CM, Kroeker K, Halpern BS (2008) Interactive and cumulative effects of multiple human stressors in marine systems. *Ecol Lett* 11:1304–1315
- Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends Ecol Evol* 17:390–395
- Cross PC, Lloyd-Smith JO, Johnson PLF, Getz WM (2005) Duelling timescales of host movement and disease recovery determine invasion of disease in structured populations. *Ecol Lett* 8:587–595
- Crow JF, Aiko K (1984) Group selection for a polygenic behavioral trait: estimating the degree of population subdivision. *Proc Natl Acad Sci USA* 81:6073–6077
- Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE (2008) The spread of invasive species and infectious disease as drivers of ecosystem change. *Front Ecol Environ* 6:238–246
- Cryan PM, Meteyer CU, Boyles JG, Blehert DS (2010) Wing pathology of white-nose syndrome in bats suggests life-threatening disruption of physiology. *BMC Biol* 8:135
- Darriba D, Taboada GL, Doallo R, Posada D (2012) jModelTest 2: more models, new heuristics and parallel computing. *Nat Methods* 9:772
- Daszak P, Cunningham AA, Hyatt AD (2000) Emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287:443–449
- Dixon MD (2011) Population genetic structure and natal philopatry in the widespread North American bat *Myotis lucifugus*. *J Mammal* 92:1343–1351
- Dlugosch KM, Parker IM (2008) Founding events in species invasions: genetic variation, adaptive evolution, and the role of multiple introductions. *Mol Ecol* 17:431–449
- Duchesne P, Turgeon J (2012) FLOCK provides reliable solutions to the “number of populations” problem. *J Hered* 103:734–743
- Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv Genet Res* 4:359–361
- Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol Ecol* 14:2611–2620
- Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131:479–491
- Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. *Evol Bioinform Online* 1:47–50
- Fallon SM (2007) Genetic data and the listing of species under the US Endangered Species Act. *Conserv Biol* 21:1186–1195
- Falush D, Stephens M, Pritchard JK (2003) Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587
- Field KA, Johnson JS, Lilley TM, Reeder SM, Rogers EJ, Behr MJ, Reeder DM (2015) The white-nose syndrome transcriptome: activation of anti-fungal host responses in wing tissue of hibernating little brown myotis. *PLoS Path* 11:e1005168
- Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL, Gurr SJ (2012) Emerging fungal threats to animal, plant and ecosystem health. *Nature* 484:186–194
- Frantz AC, Cellina S, Krier A, Schley L, Burke T (2009) Using spatial Bayesian methods to determine the genetic structure of a continuously distributed population: clusters or isolation by distance? *J Appl Ecol* 46:493–505
- Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. *Mol Ecol* 10:2741–2752
- Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchowski CM, Kunz TH (2010) An emerging disease causes regional population collapse of a common North American bat species. *Science* 329:679–682
- Frick WF, Puechmaille SJ, Hoyt JR, Nickell BA, Langwig KE, Foster JT, Barlow KE, Bartonička T, Feller D, Haarsma A-J, Herzog C, Horáček I, van der Kooij J, Mulkens B, Petrov B, Reynolds R, Rodrigues L, Stihler CW, Turner GG, Kilpatrick AM (2015) Disease alters macroecological patterns of North American bats. *Glob Ecol Biogeogr* 24:741–749
- Funk WC, McKay JK, Hohenlohe PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. *Trends Ecol Evol* 27:489–496

- Gardner JE, Cook EA (2002) Seasonal and geographic distribution and quantification of potential summer habitat. In: Kurta A, Kennedy J (eds) The Indiana bat: biology and management of an endangered species. Bat Conservation International, Austin, pp 9–20
- Gargas A, Trest MT, Christensen M, Volk TJ, Blehert DS (2009) *Geomyces destructans* sp. nov. associated with bat white-nose syndrome. Mycotaxon 108:147–154
- Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). <http://www.unil.ch/izea/software/fstat.html>
- Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321
- Hall JS (1962) A life history and taxonomic study of the Indiana bat, *Myotis sodalis*. Read Public Mus Art Gallery Sci Publ 12:1–68
- Hallam TG, Federico P (2012) The panzootic white-nose syndrome: an environmentally constrained disease? Transbound Emerg Dis 59:269–278
- Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. Science 296:2158–2162
- Harvey MJ (2002) Status and ecology in the southern United States. In: Kurta A, Kennedy J (eds) The Indiana bat: biology and management of an endangered species. Bat Conservation Int, Austin, pp 29–34
- Hicks A, Novak PG (2002) History, status, and behavior of hibernating populations in the Northeast. In: Kurta A, Kennedy J (eds) The Indiana bat: biology and management of an endangered species. Bat Conservation International, Austin, pp 35–47
- Hicks AC, Clark M, Cooper M, Herzog C (2005) Williams complex spring emergence tracking 2004 project summary. New York State Department of Environmental Conservation, Albany
- Hoyt JR, Langwig KE, Okoniewski J, Frick WF, Stone WB, Kilpatrick AM (2014) Long-term persistence of *Pseudogymnoascus destructans*, the causative agent of white-nose syndrome, in the absence of bats. EcoHealth 12:330–333
- Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Mol Ecol Res 9:1322–1332
- Humphrey SR (1978) Status, winter habitat, and management of the endangered Indiana bat, *Myotis sodalis*. Fla Sci 41:65–76
- Ingersoll TE, Sewall BJ, Amelon SK (2013) Improved analysis of long-term monitoring data demonstrates marked regional declines of bat populations in the eastern United States. PLoS One 8:e65907
- Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BMC Genet 6:13
- Johnson JS, Reeder DM, McMichael JW, Meierhofer MB, Stern DWF, Lumadue SS, Sigler LE, Winters HD, Vodzak ME, Kurta A, Kath JA, Field KA (2014) Host, pathogen, and environmental characteristics predict white-nose syndrome mortality in captive little brown myotis (*Myotis lucifugus*). PLoS One 9:e112502
- Johnson LNL, McLeod BA, Burns LE, Arseneault K, Frasier TR, Broders HG (2015) Population genetic structure within and among seasonal site types in the little brown bat (*Myotis lucifugus*) and the northern long-eared bat (*M. septentrionalis*). PLoS One 10:e0126309
- Jombart T (2008) *adeigenet*: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403–1405
- Jombart T, Ahmed I (2011) *adeigenet* 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics 27:3070–3071
- Jombart T, Devillard S, Dufour A-B, Pontier D (2008) Revealing cryptic spatial patterns in genetic variability by a new multivariate method. Heredity 101:92–103
- Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genet 11:94
- Kekkonen J, Hanski IK, Jensen H, Väisänen RA, Brommer JE (2011) Increased genetic differentiation in house sparrows after a strong population decline: from panmixia towards structure in a common bird. Biol Conserv 144:2931–2940
- Kerth G, Mayer F, König B (2000) Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies. Mol Ecol 9:793–800
- Kerth G, Mayer F, Petit E (2002) Extreme sex-biased dispersal in the communally breeding, nonmigratory Bechstein's bat (*Myotis bechsteini*). Mol Ecol 11:1491–1498
- Kerth G, Kiefer A, Trappmann C, Weishaar M (2003) High gene diversity at swarming sites suggest hot spots for gene flow in the endangered Bechstein's bat. Conserv Genet 4:491–499
- Kierepka EM, Latch EK (2015) Performance of partial statistics in individual-based landscape genetics. Mol Ecol Resour 15:512–525
- Kurta A, Murray SW (2002) Philopatry and migration of banded Indiana bats (*Myotis sodalis*) and effects of radio transmitters. J Mammal 83:585–589
- Lachish S, Miller KJ, Storfer A, Goldizen AW, Jones ME (2011) Evidence that disease-induced population decline changes genetic structure and alters dispersal patterns in the Tasmanian devil. Heredity 106:172–182
- Lande R (1980) Genetic variation and phenotypic evolution during allopatric speciation. Am Nat 116:463–479
- Langwig KE, Frick WF, Bried JT, Hicks AC, Kunz TH, Kilpatrick AM (2012) Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome. Ecol Lett 15:1050–1057
- Langwig KE, Hoyt JR, Parise KL, Kath J, Kirk D, Frick WF, Foster JT, Kilpatrick AM (2015) Invasion dynamics of white-nose syndrome fungus, Midwestern United States, 2012–2014. Emerg Infect Dis 21:1023–1026
- Laurance WF, Useche DC (2009) Environmental synergisms and extinctions of tropical species. Conserv Biol 23:1427–1437
- Legendre P, Fortin M-J, Borcard D (2015) Should the Mantel test be used in spatial analysis? Methods Ecol Evol 6:1239–1247
- Leigh JW, Bryant D (2015) POPART: full-feature software for haplotype network construction. Methods Ecol Evol 6:1110–1116
- McLeod BA, Burns LE, Frasier TR, Broders HG (2015) Effect of oceanic straits on gene flow in the recently endangered little brown bat (*Myotis lucifugus*) in maritime Canada: implications for the spread of white-nose syndrome. Can J Zool 93:427–437
- Meirmans PG (2012) AMOVA-based clustering of population genetic data. J Hered 103:744–750
- Meteyer CU, Buckles EL, Blehert DS, Hicks AC, Green DE, Shearn-Bochsler V, Thomas NJ, Gargas A, Behr MJ (2009) Histopathologic criteria to confirm white-nose syndrome in bats. J Vet Diagn Investig 21:411–414
- Miller-Butterworth CM, Vonnhoff MJ, Rosenstern J, Turner GG, Russell AL (2014) Genetic structure of little brown bats (*Myotis lucifugus*) corresponds with spread of white-nose syndrome among hibernacula. J Hered 105:354–364
- Oyler-McCance SJ, Fike JA (2011) Characterization of small microsatellite loci isolated in endangered Indiana bat (*Myotis sodalis*) for use in non-invasive sampling. Conserv Genet Res 3:243–245

- Palsbøll PJ, Berube M, Allendorf FW (2007) Identification of management units using population genetic data. *Trends Ecol Evol* 22:11–16
- Pärn H, Ringsby TH, Jensen H, Saether B-E (2011) Spatial heterogeneity in the effects of climate and density-dependence on dispersal in a house sparrow metapopulation. *Proc R Soc Lond B* 279:144–152
- Pedersen AB, Jones KE, Nunn CL, Altizer S (2007) Infectious diseases and extinction risk in wild mammals. *Cons Biol* 21:1269–1279
- Petit E, Balloux F, Goudet J (2001) Sex-biased dispersal in a migratory bat: a characterization using sex-specific demographic parameters. *Evolution* 55:635–640
- Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. *Genetics* 155:945–959
- Radwan J, Biedrzycka A, Babik W (2010) Does reduced MHC diversity decrease viability of vertebrate populations? *Biol Conserv* 143:537–544
- R Core Team (2015) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. <https://www.R-project.org/>
- Reeder DM, Frank CL, Turner GG, Meteyer CU, Kurta A, Britzke ER, Vodzak ME, Darling SR, Stihler CW, Hicks AC, Jacob R, Grieneisen LE, Brownlee SA, Muller LK, Blehert DS (2012) Frequent arousal from hibernation linked to severity of infection and mortality in bats with white-nose syndrome. *PLoS One* 7:e38920
- Rees EE, Pond BA, Cullingham CI, Tinline RR, Ball D, Kyle CJ, White BN (2009) Landscape modeling spatial bottlenecks: implications for raccoon rabies disease spread. *Biol Lett* 5:387–390
- Reynolds HT, Ingersoll T, Barton HA (2015) Modeling the environmental growth of *Pseudogymnoascus destructans* and its impact on the white-nose syndrome epidemic. *J Wildl Dis* 51:318–331
- Rousset F (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* 145:1219–1228
- Russell CA, Smith DL, Childs JE, Real LA (2005) Predictive spatial dynamics and strategic planning for a raccoon rabies emergence in Ohio. *PLoS Biol* 3:e88
- Slatkin M (1991) Inbreeding coefficients and coalescence times. *Genet Res* 58:167–175
- Stadelmann B, Lin L-K, Kunz TH, Ruedi M (2007) Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes. *Mol Phylog Evol* 43:32–48
- Taft HR, Roff DA (2012) Do bottlenecks increase additive genetic variance? *Conserv Genet* 13:333–342
- Thogmartin WE, King RA, McKann PC, Szymanski JA, Pruitt L (2012a) Population-level impact of white-nose syndrome on the endangered Indiana bat. *J Mammal* 93:1086–1098
- Thogmartin WE, King RA, Szymanski JA, Pruitt L (2012b) Space-time models for a panzootic in bats, with a focus on the endangered Indiana bat. *J Wildl Dis* 48:876–887
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Trujillo RG, Amelon SK (2009) Development of microsatellite markers in *Myotis sodalis* and cross-species amplification in *M. grisescens*, *M. leibii*, *M. lucifugus*, and *M. septentrionalis*. *Conserv Genet* 10:1965–1968
- Turner GG, Reeder DM, Coleman JTH (2011) A five-year assessment of mortality and geographic spread of white-nose syndrome in North American bats and a look to the future. *Bat Res News* 52:13–27
- US Fish and Wildlife Service (2007) Indiana bat (*Myotis sodalis*) draft recovery Plan: first revision. US Fish and Wildlife Service, Fort Snelling
- US Fish and Wildlife Service (2016) White-nose syndrome.org. www.whitenosesyndrome.org. Accessed 17 Jan 2016
- US Fish and Wildlife Service, National Marine Fisheries Service (1998) Endangered species consultation handbook. US Fish and Wildlife Service, National Marine Fisheries Service, Washington, DC
- Varvio SL, Chakraborty R, Nei M (1986) Genetic variation in subdivided populations and conservation genetics. *Heredity* 57:189–198
- Veith M, Beer N, Kiefer A, Johannesen J, Seitz A (2004) The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*). *Heredity* 93:342–349
- Verant ML, Meteyer CU, Speakman JR, Cryan PM, Lorch JM, Blehert DS (2014) White-nose syndrome initiates a cascade of physiologic disturbances in the hibernating bat host. *BMC Physiol* 14:10
- Vonhof MJ, Strobeck C, Fenton MB (2008) Genetic variation and population structure in big brown bats (*Eptesicus fuscus*): is female dispersal important? *J Mammal* 89:1411–1420
- Vonhof MJ, Russell AL, Miller-Butterworth CM (2015) Range-wide genetic analysis of little brown bat (*Myotis lucifugus*) populations: estimating the risk of spread of white-nose syndrome. *PLoS One* 10:e0128713
- Weir BS, Cockerham CC (1984) Estimating *F*-statistics for the analysis of population structure. *Evolution* 38:1358–1370
- Wilder AP, Frick WF, Langwig KE, Kunz TH (2011) Risk factors associated with mortality from white-nose syndrome among hibernating bat colonies. *Biol Lett* 7:950–953
- Wilder AP, Kunz TH, Sorenson MD (2015) Population genetic structure of a common host predicts the spread of white-nose syndrome, an emerging infectious disease in bats. *Mol Ecol* 24:5495–5506
- Wilkinson GS, Chapman AM (1991) Length and sequence variation in evening bat D-loop mtDNA. *Genetics* 128:607–617
- Wilkinson GS, Mayer FM, Kerth G, Petri B (1997) Evolution of repeated sequence arrays in the D-loop region of bat mitochondrial DNA. *Genetics* 146:1035–1048
- Willi Y, Van Buskirk J, Hoffmann AA (2006) Limits to the adaptive potential of small populations. *Ann Rev Ecol Evol Syst* 37:433–458
- Worthington Wilmer J, Barratt E (1996) A non-lethal method of tissue sampling for genetic studies of chiropterans. *Bat Res News* 37:1–3