Genetic approaches to understanding the population-level impact of wind energy development on migratory bats: a case study of the eastern red bat (*Lasiurus borealis*)

Maarten Vonhof, Amy L. Russell

Documented fatalities of bats at wind turbines have raised serious concerns about the future impacts of increased wind power development on populations of migratory bat species. However, for most bat species we have no knowledge of the size of populations and their demographic trends, the degree of structuring into discrete subpopulations, and whether different subpopulations use spatially segregated migratory routes. Here, we utilize genetic data from eastern red bats (*Lasiurus borealis*), one of the species most highly affected by wind power development in North America, to (1) evaluate patterns of population structure across the landscape, (2) estimate effective population size (N_e), and (3) assess signals of growth or decline in population size. Using a large dataset of both nuclear and mitochondrial DNA variation, we demonstrate that this species forms a single, panmictic population across their range with no evidence for the historical use of divergent migratory pathways by any portion of the population. Further, using coalescent estimates we estimate that the effective size of this population is in the hundreds of thousands to millions of individuals. Our results showing high population connectivity and gene flow among populations of eastern red bats provides valuable context for understanding levels and patterns of mortality, and indicate that monitoring and management of eastern red bats must integrate information across the range of this species.

Authors:

Maarten J. Vonhof[1,2] and Amy L. Russell[3]

[1]Department of Biological Sciences, Western Michigan University, Kalamazoo, MI, USA

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

[3]Department of Biology, Grand Valley State University, Allendale, MI, USA

Corresponding author:
Maarten Vonhof
Dept. of Biological Sciences
Western Michigan University
1903 W. Michigan Avenue
Kalamazoo, MI 49008
Email: maarten.vonhof@wmich.edu
Tel: 269-387-5626
Fax: 269-387-5609

INTRODUCTION

As concerns about anthropogenic climate change and the long-term environmental impacts of the burning of fossil fuels on biological and human systems have heightened, there is increasing motivation to develop alternative sources of energy that will reduce the production of greenhouse gasses. Wind power has become an increasingly important sector of the energy industry and is one of the fastest growing sources of renewable energy (Kaldellis & Zafirakis 2011, Leung & Yang 2012). Despite the many positive aspects of wind power development, there have been environmental costs associated with turbine installation and operation (Morrison & Sinclair, 2004; Abbasi et al., 2014). In particular, fatalities of bats at wind power installations have emerged as a major environmental impact of wind power development, and large mortality events have been reported at a number of wind energy facilities in the United States and abroad (Erickson et al., 2001; Erickson, Johnson & Young, 2005; Kunz et al., 2007; Arnett et al., 2008). The bat species most affected by wind power in North America are migratory, tree-roosting species such as hoary bats (*Lasiurus cinereus*), eastern red bats (*Lasiurus borealis*), and silver-haired bats (*Lasionycteris noctivagans*), which together constitute almost three-quarters of the bat carcasses found at wind turbines (Arnett et al., 2008). Although mortalities may occur throughout April to November, most bat fatalities in North America have been reported in late summer and early autumn (reviewed by Kunz et al., 2007; Arnett et al., 2008) and appear to be concentrated during fall migration of the affected species (Cryan, 2003).

The observed high levels of mortality for these species at wind power developments raise concerns about the long-term impacts of this technology on their populations, yet we lack the necessary information to place this mortality in context with respect to baseline population estimates and demographic trends of the affected species. For most bat species we have no knowledge of the size

of populations and their demographic trends, the degree of structuring into discrete subpopulations, and whether different subpopulations use spatially segregated migratory routes. While estimates of local population sizes within particular roosts may be feasible using traditional capture-mark-recapture (CMR) methodology or survey techniques (O'Shea & Bogan, 2003; Kunz et al., 2009), only one study (Vonhof & Fenton, 2004) has estimated population size in a known area (outside of a single roost) using CMR methodology, and no reliable range-wide population estimates exist for any bat species. Traditional demographic approaches have limitations when applied to bats, as they are nocturnal, exhibit cryptic behavior, and are difficult to follow over time during extensive seasonal movements between summer breeding areas and overwintering sites (Cryan, 2003; Rivers, Butlin & Altringham, 2006). The tree-roosting migratory bat species that are killed in high numbers at wind turbines are especially inaccessible for traditional CMR studies, given their solitary nature and restriction to forested habitats (Kunz, 1982; Shump & Shump, 1982a,b). Large-scale banding studies typically experience extremely low recapture rates (e.g., Glass, 1982; reviewed in O'Shea & Bogan, 2003), and there are serious data deficiencies with respect to sex- and age-specific survival and reproductive rates that hamper our ability to widely apply demographic models to bat populations. Given these difficulties, we require other approaches to estimating population sizes and demographic trends within migratory bat populations affected by wind power development.

Genetic approaches provide an alternative to traditional demographic methods of population estimation, and allow us to estimate the degree of population structuring, demographic trends within subpopulations, and effective population size (N_e) using data on allele frequencies or the base composition of DNA sequences. Fewer individuals need to be sampled relative to CMR approaches, and individuals need only be sampled a single time for many analyses. In addition, no accessory data, such as age or sex, are required

(although such information is useful if available), and population parameters can be estimated directly from the observed patterns of genetic variation. Molecular markers can be used to examine levels of population differentiation within a species and to geographically delimit populations or groups of populations based on the observed distribution of genetic variation (Miller-Butterworth et al., 2014). Importantly, such analyses can be used to define the relevant unit for population monitoring, and highlight demographic connections among populations that may not be obvious from behavioral data alone. As mating is likely to take place during migration in bats (Dodd & Adkins, 2007; Cryan, 2008; Cryan et al., 2012; Solick et al., 2012), gene flow should occur among populations that interact during migration. Therefore it is likely that any genetically distinct populations, if they exist, will be using different migratory pathways and may be subject to different mortality rates as wind turbines are concentrated heterogeneously across the landscape. The analysis of genetic population structure is therefore highly relevant to our understanding of bat – wind turbine interactions.

While it is not possible to directly estimate adult census population size (N_c) using molecular data (although genetic markers can be used to identify individuals for traditional CMR analyses; Luikart et al., 2010), it is possible to estimate effective population size (N_e), which provides information on how quickly genetic variation is being lost, or relatedness is increasing, in a population of interest. N_e is defined as the number of individuals in an ideal Wright-Fisher population (a large, constant-sized, randomly-mating, hermaphroditic population with discrete generations) that would lose genetic variation at the same rate as the actual population (Crow & Denniston, 1988). Essentially, it estimates the number of individuals actually contributing genes to the next generation. The estimation of N_e has seen wide application in studies of threatened or isolated populations, as the magnitude of genetic drift, and hence

loss of genetic variation, is inversely proportional to N_e (Leberg, 2005; Wang, 2005; Luikart et al., 2010). Current estimates of N_e can be used to assess the 'genetic health' of populations and their capability to respond to future environmental change or anthropogenic changes via selection (Frankham, Ballou & Briscoe, 2002). Estimation of N_e is also common in phylogeographic studies interested in past changes in population sizes in relation to changing climatic conditions or vicariant events in the evolutionary history of species (Avise, 2000; Russell et al., 2011), and provides us with important insight into the demographic history of populations and species.

Here we apply population genetic approaches to assess levels of genetic differentiation among populations and provide estimates of N_e for the eastern red bat (*Lasiurus borealis*) using multiple analytic techniques. This species was chosen because it is one of the three bat species of greatest concern with regard to the biodiversity impacts of wind energy, and has the highest fatality rate at a number of wind power installations in the eastern United States (Arnett et al., 2008). Although estimates of census population size would be preferable for understanding the size of bat populations and the potential impact of fatalities at wind power installations, N_e estimates may provide us with valuable information on the size of the evolutionarily-relevant portion of the population (that portion contributing genes to the next generation), and regular monitoring of N_e might allow us to track changes in population size over time. Our data provide valuable context for understanding the population-level impacts of mortalities due to wind power for this migratory bat species.

METHODS

Sampling

Tissue samples from across the range of eastern red bats were collected by researchers capturing bats in the field or collecting carcasses at wind power developments. These researchers were required to have appropriate state and federal collecting permits. A small number of samples from one state (Michigan) were collected by one of the authors (MJV) under permit from the state of Michigan (Michigan Department of Natural Resources permit SC1257) with appropriate Institutional Animal Care and Use Committee approval (Western Michigan University protocol 05-03-01). From these samples we generated three data sets:

1) A sample of individuals from known sample sites collected in the summer months (June to mid-August when bats are likely to be resident) primarily between 2000-2006, for the purpose of assessing levels of genetic population structure and estimating N_e (Table 1, Table S1). We received tissue samples for 1 – 39 bats from any given location. We had sufficient samples ($N > 15$) for 12 localities with which to carry out population genetic analyses (Figure 1, Table 1). Unlike colonial bats roosting in buildings or trees where bats can be captured in numbers from a single site during a single sampling session, tree-roosting bats such as eastern red bats are solitary. Sampling of these bats therefore must involve the capture of foraging individuals and may encompass individuals from a wider area over a longer time scale. For six of our population samples, bats were captured within a single county or location (AR, GA, MO, ON, TX, WV-Ma), while the other six samples consisted of individuals captured in several adjacent counties within a given state (IL, MD, MI, NC, TN, WV-Pe; the site label for the latter site represents one of the two counties included). Sixteen variable microsatellite loci were genotyped for all individuals used in site-level analyses ($N = 284$). A 408 bp fragment of the hypervariable 2

portion of the mitochondrial DNA control region (hereafter HV2) was sequenced from 218 individuals used in site-level analyses (not all individuals from each location and not all locations were sequenced; Table 1), as well as 77 bats from 30 additional locations that were not included in site-level analyses, for a total of 295 individuals sequenced. We further sequenced a 651 bp fragment of the Chymase intron 1 (CHY) for a random subset of 103 individuals.

2) A sample of individuals from 2002 only ($N = 353$, including 109 individuals used in the first dataset) for estimating N_e at a single time point.

3) A sample of individuals from 2010 only ($N = 226$) for estimating N_e at a second time point. Data sets 2 and 3 were comprised of a mixture of individuals of known summer origin, as well as bats of unknown origin killed at wind power developments during fall migration. We performed microsatellite genotyping for individuals in data sets 2 and 3 but did not sequence HV2 or CHY.

Laboratory Methods

DNA was extracted from samples using a DNEasy Tissue Extraction Kit (Qiagen). All individuals in all three data sets were genotyped at 16 microsatellite loci using primers developed specifically for eastern red bats (primers Lbo-B06, C07, D08, D200, D202, D203, D204, D226, D240, D245, and D248; Eackles & King, pers. comm.), as well as primers originally developed for other bat species (MS3E10 and MS1C01, Trujillo & Amelon, 2009; IBat-Ca22 Oyler-McCance & Fike, 2011; Cora_F11_C04, Piaggio, Figueroa & Perkins, 2009a; and Coto_G12F_B11R, Piaggio et al., 2009b). Loci were multiplexed whenever possible; all PCR reactions combined 10 ng of each primer and 2 μL template DNA with an illustra PuReTaq ready-to-go PCR bead (GE Health Care) to a total volume of 25 μL. Cycling conditions followed those in Vonhof et al. (2002) with the exception of a 10s (rather than 1s)

extension step at 72°C. Multiple PCR reactions were subsequently pooled for loading on an ABI3130 Sequencer at the Vanderbilt University DNA Sequencing Facility for fragment analysis, and visualized and scored using GeneMarker software (SoftGenetics). Detailed information on locus multiplexing is available from the authors upon request.

Amplification of the 408 bp fragment of the mitochondrial HV2 was initially carried out using the reverse complement of primer F from Wilkinson & Chapman (1991; RevF: 5'-CTA CCT CCG TGA AAC CAG CAA C-3') sitting in the central conserved sequence block as the forward primer, and the primer sH651 located in the $tRNA_{Pro}$ gene (Castella, Ruedi & Excoffier, 2001) as the reverse primer. However, these primers span a region containing a large stretch of 6 bp repeats, resulting in a large amplicon of 1500-2000 bp. We therefore designed a new reverse primer (LABO-HV2R2: 5'-TCC TGT WAC CAT TAA YTA ATA TGT CCC-3') that amplified a 408 bp fragment excluding the repeats. Amplification was carried out using the above reaction conditions and the cycling conditions in Castella, Ruedi & Excoffier (2001) with a T_a of 60°C. PCR reactions were cleaned using ExoSAP-IT (PCR Product Pre-Sequencing Kit, Affymetrix) and sent to the University of Arizona Genetics Core for bi-directional sequencing. Sequences were edited using CodonCode Aligner software (CodonCode Corporation).

The nuclear chymase sequence data were generated via cloning of PCR amplicons from a randomly-selected subset of individuals. First, a region of the chymase gene spanning intron 1 was amplified through PCR using the primers Chy-F (5'-GTC CCA CCT GGG AGA ATG TG-3') and Chy-R (5'-TGG GAG ATT CGG GTG AAG-3'; Venta et al., 1996). The reaction conditions were identical to those for the microsatellite loci, except that the reaction used just 1 μL of template. The temperature profile included an initial extended denaturation of 95°C for 5 minutes, followed by 40 cycles of 95°C for 1 minute, 52°C for 1 minute and 72°C for 1.5

minutes, with a final extension step at 72°C for 4 minutes. The PCR reaction was cleaned using a PCR purification kit (Qiagen) and sent to the University of Arizona Genetics Core for bi-directional sequencing using the Chy-F and Chy-R primers. These diplotypes were edited and heterozygous sites called using Sequencher v.4.8 (GeneCodes). Individuals found to contain two or more heterozygous sites were targeted for cloning.

Cloning and transformation was performed using the TOPO TA cloning kit (Life Technologies) following manufacturer's instructions. Six to eight colonies were picked for each cloned individual. The picked colonies were each suspended in 10 µL dH_2O and heated to 95°C for 10 minutes to lyse the cells. The cell lysate was then used directly as template DNA for colony screening through PCR. The PCR reaction combined 10 ng of each primer and 10 µL cell lysate with an illustra PuReTaq ready-to-go PCR bead (GE Health Care) to a total volume of 25 µL. The temperature profile followed that described above for the initial cloned PCR. PCR reactions yielding amplicons of the expected size (~650 bp) were cleaned using ExoSAP-IT (Affymetrix) following the manufacturer's instructions. Cleaned PCR amplicons were then sent to the University of Arizona Genetics Core for bi-directional sequencing using the Chy-F and Chy-R primers.

CHY sequences were edited using Sequencher v.4.8 (GeneCodes). Heterozygous sites identified during the initial sequencing of diplotypes were resolved into phased alleles. Thirty-six individuals were experimentally phased through cloning and sequencing. All other individuals with ambiguous diplotypes were computationally phased using Phase v.2.1.1 (Stephens, Smith & Donnelly, 2001; Stephens & Donnelly, 2003) with a confidence threshold of 0.95.

Analysis of Population Structure

To describe overall levels of mtDNA diversity within sampling sites, we calculated haplotype (h) and nucleotide (π) diversities in DnaSP 5.10.1 (Librado & Rozas, 2009). We calculated pairwise F_{ST} values between sampling sites and tested for significance with 10,000 permutations in Arlequin 3.11 (Excoffier, Laval & Schneider, 2005) to identify pairs that were genetically distinct. We also performed an analysis of molecular variance (AMOVA; Excoffier, Smouse & Quattro, 1992) to describe the relative amount of genetic variation within and among locations.

Deviations from Hardy-Weinberg equilibrium (HWE) at each locus were estimated and loci were confirmed to be in linkage equilibrium using FSTAT v2.9.3 (Goudet, 1995). To test for differences among sampling sites 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, private allelic richness using HP-RARE 1.0 (Kalinowski, 2005), and observed and expected heterozygosity using GENODIVE (Meirmans, 2012). We then tested for differences among sampling locations (or groups of locations) in allelic richness, and F_{IS} in FSTAT, and expected heterozygosity in GENODIVE, using 10,000 permutations.

Different clustering algorithms can produce different solutions, and concordance among multiple techniques is suggestive of the presence of a strong genetic signal (Guillot et al., 2009). Therefore, we applied two 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, Stephens & Donnelly, 2000; Falush, Stephens & Pritchard, 2003) with 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, each with an MCMC search consisting of an initial 100,000-step burn-in followed by 400,000 steps using the admixture

model with correlated allele frequencies. The most likely number of clusters was determined using the Evanno, Regnaut & Goudet (2005) method implemented in the program STRUCTURE HARVESTER (Earl & vonHoldt, 2012). The Evanno, Regnaut & Goudet (2005) method is not informative for the highest and lowest K values; therefore, if the highest log likelihood value was observed for K = 1 or 10 across all replicates, we accepted that as the best-supported value of K.

Second, we applied the repeated allocation approach of Duchesne & Turgeon (2012) implemented in the software FLOCK. In this method, samples are initially randomly partitioned into K clusters ($K \geq 2$), allele frequencies are estimated for each of the K clusters, and each genotype is then reallocated to the cluster that maximizes the 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 calculated 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 & Turgeon, 2012).

The level of genetic differentiation among pre-defined populations was determined by calculating pairwise distance measures, including F_{ST} (Weir & Cockerham, 1984), and a measure independent of the amount of within-population diversity (Jost's D; Jost, 2008). As with mtDNA, we performed an AMOVA on microsatellite genotypes using ARLEQUIN.

Estimation of N_e

We used a number of approaches to estimate N_e for eastern red bats. Although we originally set out to estimate the short-term

variance effective population size (N_{eV}, Crandall, Posada & Vasco, 1999), it quickly became apparent that N_e was very large. This constraint precluded the use of single sample estimators based on linkage disequilibrium or summary statistics (Waples & Do, 2009; Waples & Do, 2010; Tallmon, Luikart & Beaumont, 2004; Tallmon et al., 2008), which are only effective for $N_e < 1{,}000$, or temporal methods (e.g., Jorde & Ryman, 1995), which are based on changes in allele frequencies due to genetic drift between time points (as drift is negligible with large N_e). Furthermore, the cohort-based demographic data required for the Jorde & Ryman (1995) method were simply not available for any bat species. Therefore, we focused on coalescent analyses, using three primary methods to estimate long-term inbreeding effective population size (N_{eI}, Crandall, Posada & Vasco, 1999).

1. msvar

The first approach we used was the coalescent-based software msvar v.1.3 (Beaumont, 1999), which estimates effective population size and demographic trends from microsatellite genotype data. This analysis considers a model in which a single ancestral population of size N_A experiences exponential population size change beginning at time t until the population reaches the current size N_1. Bats sampled at two different time points (2002 and 2010; datasets 2 and 3, respectively) were analyzed separately to determine whether increases in mortality over that time interval had a measurable effect on estimates of N_e. To make the analysis computationally feasible, we randomly subsampled 100 diploid individuals from each dataset. Subsampling was performed twice for each time point, yielding datasets A and B, to ensure that no bias was introduced through subsampling. Each analyzed dataset thus included 100 genotypes (= 200 chromosomes) for each of 16 autosomal microsatellite loci.

The msvar analysis requires the specification of hyperpriors for each of the four demographic parameters, N_1, N_A, t, and the

mutation rate μ. These hyperpriors describe distributions from which the locus-specific initial parameter values are drawn, and are given here as [$\log_{10}(N_1)$, $\log_{10}(N_A)$, $\log_{10}(\mu)$, $\log_{10}(t)$]. The parameter means were assumed to be normally distributed with means (7, 7, -3.5, 4.3) and standard deviations (3.5, 4, 0.5, 2). We chose these values for (1) N_1 based on estimates of N_e for eastern red bats from our own Lamarc analyses with a relatively large standard deviation to reflect our own uncertainty regarding this parameter, (2) N_A based on a null hypothesis of no change in population size with a larger standard deviation to accommodate increased uncertainty in historical parameters, (3) μ based on Storz & Beaumont's (2002) msvar analysis of microsatellite variation in *Cynopterus* fruit bats, and (4) t based on a hypothesis of population size change associated with the Last Glacial Maximum with a relatively large standard deviation to reflect our own uncertainty regarding this parameter. The parameter standard deviations were assumed to be normally distributed with means (0, 0, 0, 0) and standard deviations (0.5, 0.5, 2, 0.5). The means of the parameter standard deviations were set to 0 to start the search algorithm with no inter-locus variation; the standard deviations of the parameter standard deviations followed recommendations of Storz & Beaumont (2002). Each of the four datasets (2 time points, with 2 subsamples each) were analyzed 2-3 times, with each run lasting ~750 million to 2 billion steps, with output logged every 100,000 steps. The initial 10% of the MCMC chains from each run were excluded as a burn-in.

2. IMa2

Second, we used the coalescent-based software IMa2 (release date 27 August 2012; Hey, 2010a, b) to estimate the effective size of the panmictic eastern red bat population using dataset 1. The analysis included the CHY and HV2 sequences and 16-locus microsatellite genotypes. One hundred microsatellite genotypes (= 200 chromosomes) for each locus were subsampled at random out

of the full dataset in order to reduce the computational time of the analysis. The DNA sequence data (CHY and HV2) were edited to conform to an infinite sites model of mutation; microsatellite data were analyzed assuming a single-step model of mutation.

In the IMa2 analysis, we modified the underlying population model to consider only a single population, with a uniform prior on the size of that population varying from $\theta = 0.05$ to 99.95. We ran 40 heated chains for an initial burn-in of ~3.6 million steps, followed by an MCMC search of ~10.2 million steps. Stationarity of the search chains was validated by monitoring ESS values.

3. Lamarc

Third, we used the software package Lamarc v.2.1.8 (Kuhner, 2006) to estimate effective population size and population growth rates independently from the nuclear CHY and the mitochondrial HV2 sequence data from dataset 1. We considered a model of a single panmictic population that undergoes population size change (growth or decline) until it reaches the current population size. We implemented a Bayesian analysis in Lamarc with priors on θ ranging from 10^{-5} to 50 and on the population size change parameter (g) ranging from -500 to 2000. The data were analyzed in three independent runs, with each run consisting of an MCMC search that was 20 million steps long and sampled every 200 steps. The first 2 million steps were discarded as a burn-in. Each MCMC search was run as 3 heated chains, with relative heating temperatures of 1, 1.5, and 3, and each search was replicated three times internally within each of the independent runs. Posterior distributions for each independent run and for overall results per locus were visualized using Tracer v.1.5. Results are reported as median point estimates with 95% confidence intervals. All parameter estimates were well supported, with ESS values exceeding 100 in all cases. Effective population size was calculated from the estimated coalescent-scaled parameter θ using the equations: $\theta = N_e\mu$ for mitochondrial data and $\theta = 4N_e\mu$ for autosomal data, where N_e is the effective size of the

entire population. This software uses mutation rates in units of substitutions per site per generation; based on the relative mutation rates estimated for the same data in the IMa2 analysis, we used a mutation rate of 4.29×10^{-8} per site per generation for the HV2 dataset and 7.76×10^{-9} per site per generation for the CHY dataset.

RESULTS

Population Structure

We observed 167 unique haplotypes representing 84 segregating sites among the 295 individuals sequenced at the mitochondrial HV2 gene. The number of haplotypes per sampling site ranged from 13-23 (mean = 18.6), and haplotype diversity (h, mean = 0.986, range = 0.961 – 1) was high in all populations (Table 1). However, nucleotide diversity (π, mean = 0.011, range = 0.009 – 0.016) was relatively low in all populations (Table 1). This pattern of high haplotype diversity combined with low nucleotide diversity suggests a history of population growth in this species (Russell, Medellín & McCracken, 2005). AMOVA analysis indicated very low levels of mitochondrial differentiation among sampling sites ($F_{ST} = 0.0113$; 1.13% of the variation is explained by differences among sampling sites, and 98.87% of the variation occurs within sites). Accordingly, pairwise F_{ST} values among sites were consistently low (Table 2) and ranged from -0.03 – 0.049 (Table 2).

All microsatellite loci were unlinked and 11 of the 16 loci met HWE expectations. Four loci (MS3E10, MS1C01, D202, D226) exhibited heterozygote deficiency, but removing them from the analyses made no difference in our conclusions; therefore we present analyses with all loci included. Mean observed and expected heterozygosities were high (0.82 and 0.88, respectively), as was the mean

number of alleles per locus (= 14.77) and allelic richness (= 12.92), although private allelic richness was low (= 0.78; Table 1).

Both clustering methods [Bayesian clustering (STRUCTURE) and repeated reallocation (FLOCK)] identified $K = 1$ as the most likely number of genetic clusters, indicating a lack of population substructuring. Similarly, AMOVA analysis of microsatellite genotypes indicated an almost complete lack of structure ($F_{ST} = 0.0044$), with pairwise F_{ST} and Jost's D values between populations consistently low (Table 3; F_{ST} range: -0.005 – 0.009; Jost's D range: -0.036 – 0.068).

N_e Estimation

We used three coalescent methods to estimate N_e for eastern red bats: msvar, IMa2, and Lamarc. These methods utilize different suites of data (microsatellites only for msvar, nuclear and mitochondrial sequence data only for Lamarc, all three data types for IMa2), and therefore were expected to provide complementary estimates based on differences in the mutation rates of the markers used and differences in the underlying models assumed.

msvar

Although we found considerable variation from run to run, there were some clear patterns that emerged from these analyses. Importantly, we found no consistent difference between parameter estimates from the 2002 vs. 2010 time points (Figure 2; Figures S1-S2). We also found no consistent difference between independent subsamples of the full dataset (runs A1-A3 vs. B1 and B3 for 2002; runs A1-A3 vs. B1-B3 for 2010). For the current effective population size N_1, we recovered generally consistent estimates on the order of 10^4-10^5 (average $N_1 \approx 74{,}500$). Estimates of ancestral effective population size N_A were less consistent among runs, but did result in estimates ranging in the same order of magnitude as N_1 (average $N_A \approx 194{,}300$; Figure S1). These analyses yielded differing signals of

population growth vs. decline between runs (Table 4), although a majority of runs (8 of 11) support a model of population decline rather than growth. The time of this population size change (t) was also variable among runs, but generally was on the order of 10^3-10^4 years (average $t \approx 21{,}600$ years; Table 4, Figure S2). While the time of population size change is difficult to pinpoint with great accuracy, these analyses clearly are not informative regarding very recent population size change.

IMa2

This analysis converged on an unambiguous, unimodal posterior distribution for the single population parameter θ ($= 4N_e\mu$) for the panmictic eastern red bat population. The most probable value of θ was estimated to be 37.95 (95% CI: 32.15 – 45.55). We used Pesole et al.'s (1999) estimate of mammalian mitochondrial mutation rates ($= 2.740 \times 10^{-8}$ substitutions per site per year) to calculate locus-specific mutation rates for our data. The geometric mean of these rates ($= 8.03 \times 10^{-6}$ substitutions per locus per year $= 1.61 \times 10^{-5}$ substitutions per locus per generation; Table S2) was used to convert coalescent-scaled estimates of θ into estimates of N_e. Our analysis thus supports an effective population size of approximately 5.91×10^5 individuals (95% CI: $5.00 – 7.09 \times 10^5$; Figure 3).

Lamarc

We used coalescent-based analyses in Lamarc to provide estimates of θ and population growth independently for the nuclear CHY and mitochondrial HV2 loci. Analyses of both markers provided unambiguous, unimodal posterior probability distributions for both parameters. Utilizing the relative mutation rates estimated from IMa2, estimates of N_e across three runs in Lamarc were 5.18×10^5 (95% CI: $4.25 – 7.22 \times 10^5$; Table 5). The estimate of N_e using CHY (males and females) was significantly larger, with a mean of

1.52×10^6 (95% CI: 1.05 – 2.18×10^6; Table 5). There was a clear signal of historical population growth recovered from both loci (Table 5); however, the time scale over which this growth occurred is not estimated in the Lamarc model.

DISCUSSION

We observed extremely low levels of population structure and effective panmixia across the sampled populations of eastern red bats using both nuclear and mitochondrial DNA markers. Furthermore, there is no evidence for the historical use of different migratory pathways and no evidence for any barriers to gene flow among any of the sampled localities. Few geographic barriers to the movement of vagile organisms such as bats exist east of the Rocky Mountains, and therefore there are likely few impediments to the movement of individuals across the landscape. Phylogeographic studies of widespread bats and birds have shown low levels of genetic differentiation among eastern North American populations (however, see Miller-Butterworth et al., 2014), and, when present, genetic structure is often restricted to broad-scale differentiation between eastern and western populations on either side of the Rocky Mountains (Gibbs, Dawson & Hobson, 2000; Kimura et al., 2002; Jones et al., 2005; Turmelle, Kunz & Sorenson, 2011; Irwin, Irwin & Smith, 2011). In the case of eastern red bats, evidence from museum records indicates that they most likely migrate from northern parts of their range to the southeastern United States (Cryan, 2003) where they roost in trees during warmer periods and may hibernate beneath leaf litter for short durations during colder temperatures (Saugey et al., 1998; Moorman et al., 1999; Mormann & Robbins, 2007). However, there are summer resident populations in the southeastern United States that likely do not migrate, and it is possible that there is variation in migratory tendency across the range of eastern red bats, much like tricolored bats (*Perimyotis subflavus*;

Fraser et al., 2012). Mating likely takes place before or during migration in eastern red bats (Dodd & Adkins, 2007; Cryan, 2008; Cryan et al., 2012; Solick et al., 2012) and can take place before bats hibernate or during warm periods on the wintering grounds. Thus, the potential for mating, and hence gene flow, among individuals that spent their summers in geographically disparate areas during migration or on the wintering grounds is likely very high.

In most colonial temperate bat species, females are philopatric to natal nursery colonies or undergo short dispersal distances to nearby colonies while mating takes place during swarming and/or hibernation at distant sites that act as hotspots of gene flow between bats occupying distant roosts during the summer (Kerth et al., 2003; Veith et al., 2004; Furmankiewicz & Altringham, 2007). As a consequence, levels of mitochondrial differentiation (indicative of female movements) are often quite high among summer maternity colonies while levels of nuclear differentiation (indicative of gene flow through mating) are typically low (Castella, Ruedi & Excoffier, 2001; Bilgin et al., 2008; Kerth et al., 2008; Vonhof, Strobeck & Fenton, 2008; Bryja et al., 2009; Lack, Wilkinson & van den Bussche, 2010; Turmelle, Kunz & Sorenson, 2011). Eastern red bats and other members of the genus *Lasiurus* roost solitarily in foliage during the summer (Shump & Shump, 1982a,b), and if they exhibited philopatry it would likely occur within broader landscape units such as forest patches or stands rather than a single roost. The absence of any mitochondrial differentiation among samples of eastern red bats suggests that females may be exhibiting high levels of dispersal, and that gene flow likely takes place via female movements and mating (e.g., Russell, Medellín & McCracken, 2005; Vonhof, Strobeck & Fenton, 2008).

Estimates of N_e varied considerably (almost an order of magnitude) among the different approaches we used, ranging from 7.45×10^4 based on microsatellite genotypes only (msvar), to 1.52×10^6 for sequence data only (CHY in Lamarc), with intermediate

estimates of 5.18×10^5 for HV2 (Lamarc) and 5.91×10^5 using all markers combined (IMa2). This variation is the result of methodological differences among the approaches we used, which all utilize different aspects of the data and make varying assumptions about the underlying historical population processes that may have occurred. Further, the analyses each used different marker data, which vary in their mutation rates, and so are providing estimates across varying time scales. Nevertheless, in combination with the results of population structure analyses, our data indicate that eastern red bats form a single, large, panmictic population across their range and that minimum effective population sizes are likely in the hundreds of thousands.

The parameter most relevant to management of this species, the actual number of individuals in the population (N_c), is not obtainable from our estimates of N_e. A variety of factors may reduce N_e relative to N_c, including fluctuations in population size over time, overlapping generations, and variation among individuals in reproductive success. Attempts have been made to compare estimates of N_e to N_c, and across a wide range of organisms the average N_e / N_c ratio is 0.11 – 0.14 (Frankham, 1995; Palstra & Ruzzante, 2008); for mammals alone, the average ratio is 0.34 (Frankham, 1995). If we applied this latter mean ratio (0.34) to our point estimates of N_e, we would obtain N_c estimates of 2.19×10^5 to 4.5×10^6 individuals. However, there are a number of serious problems with the use of our coalescent estimates in this way. N_e is a theoretical concept that relates the genetic characteristics of a population to those expected of an ideal population under a Wright-Fisher model. We can estimate N_e as a measure of the evolutionary potential of populations, but there is no clear relationship between current demography and changes in genetic variation that influence coalescent estimates of N_e. Further, there are a number of methodological concerns. First, N_e has most often been estimated for very small populations of less than 1,000 individuals, and we do not know how the N_e / N_c ratio may vary with the magnitude of N_c.

Second, the majority of the ratios provided by Frankham (1995) utilize demographic, rather then genetic, estimates of N_e, and demographic estimates may differ substantially from genetic estimates even when population sizes are small (Luikart et al., 2010). Third, the majority of estimates in Frankham (1995) come from organisms with very different life histories than bats, and we do not know to what extent the N_e / N_c ratio might vary from the overall mean for bats (or most other organisms). Fourth, the calculation of N_e using coalescent-based methods requires division of estimates of θ by the mutation rate (μ), but mutation rates are extremely difficult to estimate and few good estimates exist for any gene (Ho et al., 2006; Montooth & Rand, 2008; Nabholz, Glémin & Galtier, 2009), much less for any bat species. As a result, any inaccuracy in the mutation rate estimate is amplified arithmetically in the subsequent calculation of N_e (Ovenden et al., 2007; Luikart et al., 2010). Therefore, applying a standard conversion to convert N_e to N_c is highly problematic, and it is best to use our estimates to indicate relative orders of magnitude of bat population sizes rather than to provide any specific population size estimates.

The potential value of our estimates of N_e is that they may be used as a baseline for future monitoring. Assuming fatality rates remain high and continue to grow as wind energy development continues, it is possible that regular estimates of N_e can be utilized to document population trends of affected species. That said, the loss of genetic variation from populations and declines in N_e estimates based on linkage disequilibrium are only apparent when population sizes are very small (e.g., Waples & Do, 2010), suggesting that cumulative population declines may have to be very severe before they are registered by genetic estimates. Simulation studies are required to assess the sensitivity of coalescent-based estimates of N_e to population decline, given various starting effective sizes and rates of decline, to assess the utility of this approach.

Our genetic data indicating panmixia and a lack of evidence for the use of different migratory pathways in different parts of the range highlights the need to consider the global implications of current and future fatalities associated with wind power. Regional projections of bat fatalities predict annual fatality rates numbering in the tens of thousands (Kunz et al., 2007), and the total number of fatalities is likely to continue to rise as wind power development expands. Despite this growing conservation concern, current monitoring of bat fatalities at wind power developments is performed on an ad-hoc, site-by-site basis and may vary tremendously in scope according to local regulations. While such monitoring can provide valuable insights leading to site-level mitigation strategies or changes in turbine placement in some cases, biologists lack the necessary broader context within which to assess the long term, population-level impacts of observed fatality rates and management strategies at specific sites. For instance, site-specific, per-turbine thresholds to limit fatalities through curtailment (reducing turbine blade speed and operating time on low-wind nights in summer and fall to lower fatalities; Baerwald et al., 2009; Arnett et al., 2011) ignore the fact that the demographic consequences of mortality extend well beyond any particular jurisdiction. Given that observed bat fatality rates at wind power facilities vary considerably among sites and regions (Arnett et al., 2008), our findings underscore the need for better data integration across jurisdictions and monitoring programs to adequately assess the cumulative demographic and genetic impacts of continued fatalities.

ACKNOWLEDGEMENTS

We are grateful to S. Amelon, R. Benedict, E. Britzke, D. Brown, C. Butchkoski, T. Carter, S. Castleberry, M. K. Clark, S. Darling, J. Fiedler, L. Finn, B. French, E. Gates, J. Gore, M. Gumbert, G. Johnson, J. Johnson, J. Kiser, S. Lambiase, G. Libby, K. Luzynski, A.

Miles, J. O'Keefe, E. Pannkuk, R. Perry, L. Pruitt, L. Reddy, H. Rice, L. Robbins, D. Saugey, M. Schirmacher, J. Schwierjohann, D. Sparks, C. Stihler, V. Swier, A. Tibbels, W. Tidhar, C. Willis, and L. Winhold, and to the Angelo State Natural History Collection, the Tennessee State Rabies Testing Lab, and the Carnegie Museum of Natural History for providing tissue samples to support this research. J. Glatz kindly provided GIS expertise to produce the range map. We also thank the Bats and Wind Energy Cooperative, and particularly E. Arnett, C. Hein, and W. Frick for their input and support.

REFERENCES

Abbasi T, Premalatha M, Abbasi T, Abbasi SA. 2014. Wind Energy: Increasing Deployment, Rising Environmental Concerns. *Renewable and Sustainable Energy Reviews* **31:**270-288 DOI 10.1016/j.rser.2013.11.019.

Arnett EA, Brown WK, Erickson WP, Fiedler JK, Hamilton BL, Henry TH, Jain A, Johnson GD, Kerns J, Koford RR, Nicholson CP, O'Connell TJ, Piorkowski MD, Tankersley RD. 2008. Patterns of Bat Fatalities at Wind Energy Facilities in North America. *Journal of Wildlife Management* **72:**61-78 DOI 10.2193/2007-221.

Arnett EA, Huso MMP, Schirmacher MR, Hayes JP. 2011. Altering turbine speed reduces bat mortality at wind-energy facilities. *Frontiers in Ecology and the Environment* **9:**209-214.

Avise JC. 2000. *Phylogeography: The History and Formation of Species*. Harvard University Press, Cambridge.

Baerwald EF, Edworthy J, Holder M, Barclay RMR. 2009. A large-scale mitigation experiment to reduce bat fatalities at wind energy facilities. *Journal of Wildlife Management* **73:**1077-1081.

Beaumont MA 1999. Detecting Population Expansion and Decline Using Microsatellites. *Genetics* **153:**2013-2029.

Bilgin R, Karatas A, Coraman E, Morales JC. 2008. The Mitochondrial and Nuclear Genetic Structure of *Myotis capaccinii* (Chiroptera: Vespertilionidae) in the Eurasian Transition, and Its Taxonomic Implications. *Zoologica Scripta* **37:**253-262 DOI 10.1111/j.1463-6409.2008.00326.x.

Bryja J, Kanuch P, Fornuskova A, Bartonicka T, Rehak Z. 2009. Low Population Genetic Structuring of Two Cryptic Bat Species Suggests Their Migratory Behaviour in Continental Europe. *Biological Journal of the Linnean Society* **96:**103-114 DOI 10.1111/j.1095-8312.2008.01093.x.

Castella V, Ruedi M, Excoffier L. 2001. Contrasted Patterns of Mitochondrial and Nuclear Structure Among Nursery Colonies of the Bat *Myotis myotis*. *Journal of Evolutionary Biology* **14:**708-720 DOI 10.1046/j.1420-9101.2001.00331.x.

Crandall KA, Posada D, Vasco D. 1999. Effective Population Sizes: Missing Measures and Missing Concepts. *Animal Conservation* **2:**317-319 DOI 10.1111/j.1469-1795.1999.tb00078.x.

Crow JF, Denniston C. 1988. Inbreeding and Variance Effective Population Numbers. *Evolution* **42:**482-495.

Cryan PM. 2003. Seasonal Distribution of Migratory Tree Bats (*Lasiurus* and *Lasionycteris*) in North America. *Journal of Mammalogy* **84:**579-593 DOI http://dx.doi.org/10.1644/1545-1542(2003)084<0579:SDOMTB>2.0.CO;2.

Cryan PM. 2008. Mating Behavior as a Possible Cause of Bat Fatalities at Wind Turbines. *Journal of Wildlife Management* **72:**845-849 DOI 10.2193/2007-371.

Cryan PM, Jameson JW, Baerwald EF, Willis CKR, Barclay RMR, Snider EA, Crichton EG. 2012. Evidence of Late-Summer

Mating Readiness and Early Sexual Maturation in Migratory Tree-Roosting Bats Found Dead at Wind Turbines. *PLoS ONE* **7:**e47586 DOI 10.1371/journal.pone.0047586.

Dodd LE, Adkins JK. 2007. Observations of Mating Behavior in the Eastern Red Bat (*Lasiurus borealis*). *Bat Research News* **44:**155-156.

Duchesne P, Turgeon J. 2012. FLOCK Provides Reliable Solutions to the "Number of Populations" Problem. *Journal of Heredity* **103:**734-743 DOI 10.1093/jhered/ess038.

Earl DA, vonHoldt BM. 2012. STRUCTURE HARVESTER: A Website and Program for Visualizing STRUCTURE Output and Implementing the Evanno Method. *Conservation Genetics Resources* **4:**359-361 DOI 10.1007/s12686-011-9548-7.

Erickson WP, Johnson GD, Strickland MD, Young DP, Sernka KJ, Good RE. 2001. Avian Collisions with Wind Turbines: A Summary of Existing Studies and Comparisons to Other Sources of Avian Collision Mortality in the United States. National Wind Coordinating Committee Research Document, Washington, DC.

Erickson WP, Johnson GD, Young DP. 2005. A Summary and Comparison of Bird Mortality from Anthropogenic Causes with an Emphasis on Collisions. In: Ralph CJ, Rich TD, eds. *Bird Conservation Implementation and Integration in the Americas: Proceedings of the Third International Partners in Flight Conference*. Albany, CA: USDA Forest Service Pacific Southwest Research Station, 1029-1042.

Evanno G, Regnaut S, Goudet J. 2005. Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study. *Molecular Ecology* **14:**2611-2620 DOI 10.1111/j.1365-294X.2005.02553.x.

Excoffier L, Laval G, Schneider S. 2005. Arlequin (Version 3.0): An Integrated Software Package for Population Genetics Data Analysis. *Evolutionary Bioinformatics Online* **1:**47-50.

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.

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.

Frankham R. 1995. Effective Population Size/Adult Population Size Ratios in Wildlife: A Review. *Genetical Research* **66:**95-107 DOI: http://dx.doi.org/10.1017/S0016672300034455.

Frankham R, Ballou JD, Briscoe DA. 2002. *Introduction to Conservation Genetics*. Cambridge, UK: Cambridge University Press.

Fraser EE, McGuire LP, Eger JL, Longstaffe FJ, Fenton MB. 2012. Evidence of Latitudinal Migration in Tri-Colored Bats, *Perimyotis subflavus*. *PLoS ONE* **7:**e31419 DOI 10.1371/journal.pone.0031419.

Furmankiewicz J, Altringham J. 2007. Genetic Structure in a Swarming Brown Long-Eared Bat (*Plecotus auritus*) Population: Evidence for Mating at Swarming Sites. *Conservation Genetics* **8:**913-923 DOI 10.1007/s10592-006-9246-2.

Gibbs HL, Dawson RJG, Hobson KA. 2000. Limited Differentiation in Microsatellite DNA Variation among Northern Populations of the Yellow Warbler: Evidence for Male-Biased Gene Flow? *Molecular Ecology* **9:**2137-2147 DOI 10.1046/j.1365-294X.2000.01136.x.

Glass BP. 1982. Seasonal Movements of Mexican Freetail Bats *Tadarida brasiliensis mexicana* Banded in the Great Plains.

Southwestern Naturalist **27:**127–133.

Goudet J. 1995. FSTAT (Version 1.2): A Computer Program to Calculate *F*-Statistics. *Journal of Heredity* **86:**485-486.

Guillot G, Leblois R, Coulon A, Frantz AC. 2009. Statistical Methods in Spatial Genetics. *Molecular Ecology* **18:**4734-4756 DOI 10.1111/j.1365-294X.2009.04410.x.

Hey J. 2010a. The Divergence of Chimpanzee Species and Subspecies as Revealed in Multipopulation Isolation-with-Migration Analyses. *Molecular Biology and Evolution* **27:**921-933 DOI 10.1093/molbev/msp298.

Hey J. 2010b. Isolation with Migration Models for More Than Two Populations. *Molecular Biology and Evolution* **27:**905-920 DOI 10.1093/molbev/msp296.

Ho SYW, Phillips MJ, Cooper A, Drummond AJ. 2006. Time Dependency of Molecular Rate Estimates and Systematic Overestimation of Recent Divergence Times. *Molecular Biology and Evolution* **22:**1561-1568 DOI 10.1093/molbev/msi145.

Hubisz MJ, Falush D, Stephens M, Pritchard JK. 2009. Inferring Weak Population Structure with the Assistance of Sample Group Information. *Molecular Ecology Resources* **9:**1322-1332 DOI 10.1111/j.1755-0998.2009.02591.x.

Irwin DE, Irwin JH, Smith TB. 2011. Genetic Variation and Seasonal Migratory Connectivity in Wilson's Warblers (*Wilsonia pusilla*): Species-Level Differences in Nuclear DNA Between Western and Eastern Populations. *Molecular Ecology* **20:**3102-3115 DOI 10.1111/j.1365-294X.2011.05159.x.

Jones KL, Krapu GL, Brandt DA, Ashley MV. 2005. Population Genetic Structure in Migratory Sandhill Cranes and the Role of Pleistocene Glaciations. *Molecular Ecology* **14:**2645-2657 DOI 10.1111/j.1365-294X.2005.02622.x.

Jorde PE, Ryman N. 1995. Temporal Allele Frequency Change and Estimation of Effective Size in Populations with Overlapping Generations. *Genetics* **139:**1077–1090.

Jost L. 2008. G_{ST} and Its Relatives Do Not Measure Differentiation. *Molecular Ecology* **17:**4015-4026 DOI 10.1111/j.1365-294X.2008.03887.x.

Kaldellis JK, Zafirakis D. 2011. The Wind Energy (R)evolution: A Short Review of a Long History. *Renewable Energy* **36**:1887-1901.

Kalinowski ST. 2005. HP-RARE 1.0: A Computer Program for Performing Rarefaction on Measures of Allelic Richness. *Molecular Ecology Notes* **5:**187-189 DOI 10.1111/j.1471-8286.2004.00845.x.

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. *Conservation Genetics* **4:**491-499.

Kerth G, Petrov B, Conti A, Anastasov D, Weishaar M, Gazaryan S, Jaquiéry J, König B, Perrin N, Bruyndonckx N. 2008. Communally Breeding Bechstein's Bats Have a Stable Social System That Is Independent from the Postglacial History and Location of the Populations. *Molecular Ecology* **17:**2368-2381 DOI 10.1111/j.1365-294X.2008.03768.x.

Kimura M, Clegg SM, Lovette IJ, Holder KR, Girman DJ, Milá B, Wade P, Smith TB. 2002. Phylogeographical Approaches to Assessing Demographic Connectivity Between Breeding and Overwintering Regions in a Nearctic-Neotropical Warbler (*Wilsonia pusilla*). *Molecular Ecology* **11:**1605-1616 DOI 10.1046/j.1365-294X.2002.01551.x.

Kuhner MK. 2006. LAMARC 2.0: Maximum Likelihood and Bayesian Estimation of Population Parameters. *Bioinformatics* **22:**768-

770 DOI 10.1093/bioinformatics/btk051.

Kunz TH. 1982. *Lasionycteris noctivagans*. *Mammalian Species* **172:**1-5.

Kunz TH, Arnett EB, Erickson WP, Hoar AR, Johnson GD, Larkin RP, Strickland MD, Thresher RW, Tuttle MD. 2007. Ecological Impacts of Wind Energy Development on Bats: Questions, Research Needs, and Hypotheses. *Frontiers in Ecology and the Environment* **5:**315-324 DOI http://dx.doi.org/10.1890/1540-9295(2007)5[315:EIOWED]2.0.CO;2.

Kunz TH, Betke M, Hristov N, Vonhof MJ. 2009. Methods for Assessing Abundance of Bats. In: Kunz TH, Parsons S, eds. *Behavioral and Ecological Methods for the Study of Bats, 2nd Ed*. Baltimore, MD: Johns Hopkins University Press, 133-157.

Lack JB, Wilkinson JE, van den Bussche RA. 2010. Range-Wide Population Genetic Structure of the Pallid Bat (*Antrozous pallidus*) – Incongruent Results from Nuclear and Mitochondrial DNA. *Acta Chiropterologica* **12:**401-413 DOI http://dx.doi.org/10.3161/150811010X537981.

Leberg P. 2005. Genetic Approaches for Estimating the Effective Size of Populations. *Journal of Wildlife Management* **69:**1385-1399 DOI http://dx.doi.org/10.2193/0022-541X(2005)69[1385:GAFETE]2.0.CO;2.

Leung DYC, Yang Y. 2012. Wind energy Development and its Environmental Impact: A Review. *Renewable and Sustainable Energy Reviews* **16:**1031-1039.

Librado P, Rozas J. 2009. DnaSP v5: A Software for Comprehensive Analysis of DNA Polymorphism Data. *Bioinformatics* **25:**1451-1452 DOI 10.1093/bioinformatics/btp187.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of Census and Effective Population Sizes: The

Increasing Usefulness of DNA-Based Approaches. *Conservation Genetics* **11:**355-373 DOI 10.1007/s10592-010-0050-7.

Meirmans PG. 2012. AMOVA-Based Clustering of Population Genetic Data. *Journal of Heredity* **103:**744-750 DOI 10.1093/jhered/ess047.

Miller-Butterworth CM, Vonhof 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. *Journal of Heredity* **105:**354-364 DOI 10.1093/jhered/esu012.

Montooth KL, Rand DM. 2008. The Spectrum of Mitochondrial Mutation Differs Across Species. *PLoS Biology* **6:**e213 DOI 10.1371/journal.pbio.0060213.

Moorman CE, Russell KR, Menzel MA, Lohr SM, Ellenberger JE, Van Lear DH. 1999. Bats Roosting in Deciduous Leaf Litter. *Bat Research News* **40:**74–75.

Mormann BM, Robbins LW. 2007. Winter Roosting Ecology of Eastern Red Bats in Southwest Missouri. *Journal of Wildlife Management* **71:**213-217 DOI 10.2193/2005-622.

Morrison ML, Sinclair AK. 2004. Environmental Impacts of Wind Energy Technology. *Encyclopedia of Energy* **6:**435-448.

Nabholz B, Glémin S, Galtier N. 2009. The Erratic Mitochondrial Clock: Variations of Mutation Rate, Not Population Size, Affect MtDNA Diversity Across Birds and Mammals. *BMC Evolutionary Biology* **9:**e54 DOI 10.1186/1471-2148-9-54.

O'Shea TJ, Bogan MA. 2003. *Monitoring Trends in Bat Populations of the United States and Territories: Problems and Prospects*. Springfield, VA: US Geological Survey.

Ovenden JR, Peel D, Street R, Courtney AJ, Hoyle SD, Peel SL, Podlich H. 2007. The Genetic Effective and Adult Census Size of an Australian Population of Tiger Prawns (*Penaeus esculentus*). *Molecular Ecology* **16:**127–138 DOI 10.1111/j.1365-294X.2006.03132.x.

Oyler-McCance SJ, Fike JA. 2011. Characterization of Small Microsatellite Loci Isolated in Endangered Indiana Bat (*Myotis sodalis*) for Use in Non-Invasive Sampling. *Conservation Genetics Resources* **3:**243-245 DOI 10.1007/s12686-010-9332-0.

Palstra FP, Ruzzante DE. 2008. Genetic Estimates of Contemporary Effective Population Size: What Can They Tell Us About the Importance of Genetic Stochasticity for Wild Population Persistence? *Molecular Ecology* **17:**3428-3447 DOI 10.1111/j.1365-294X.2008.03842.x.

Pesole G, Gissi C, De Chirico A, Saccone C. 1999. Nucleotide Substitution Rate of Mammalian Mitochondrial Genomes. *Journal of Molecular Evolution* **48:**427-434.

Piaggio AJ, Figueroa JA, Perkins SL. 2009a. Development and Characterization of 15 Polymorphic Microsatellite Loci Isolated from Rafinesque's Big-Eared Bat, *Corynorhinus rafinesquii*. *Molecular Ecology Resources* **9:**1191-1193 DOI 10.1111/j.1755-0998-2009-02625.x.

Piaggio AJ, Miller KEG, Matocq MD, Perkins SL. 2009b. Eight Polymorphic Microsatellite Loci Developed and Characterized from Townsend's Big-Eared Bat, *Corynorhinus townsendii*. *Molecular Ecology Resources* **9:**258-260 DOI 10.1111/j.1755-0998-2008-02243.x.

Pritchard JK, Stephens M, Donnelly P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics*

155:945-959.

Rivers NM, Butlin RK, Altringham JD. 2006. Autumn Swarming Behaviour of Natterer's Bats in the UK: Population Size, Catchment Area and Dispersal. *Biological Conservation* **127:**215-226 DOI 10.1016/j.biocon.2005.08.010.

Russell AL, Medellín RA, McCracken GF. 2005. Genetic Variation and Migration in the Mexican Free-Tailed Bat (*Tadarida brasiliensis mexicana*). *Molecular Ecology* **14:**2207-2222 DOI 10.1111/j.1365-294X.2005.02552.x.

Russell AL, Cox MP, Brown VA, McCracken GF. 2011. Population Growth of Mexican Free-Tailed Bats (*Tadarida brasiliensis mexicana*) Predates Human Agricultural Activity. *BMC Evolutionary Biology* **11:**e88 DOI 10.1186/1471-2148-11-88.

Saugey DA, Crump BG, Vaughn RL, Heidt GA. 1998. Notes on the natural history of *Lasiurus borealis* in Arkansas. *Journal of the Arkansas Academy of Science* **52:**92–98.

Shump KA, Shump AU. 1982a. *Lasiurus borealis*. *Mammalian Species* **183:**1-6.

Shump KA, Shump AU. 1982a. *Lasiurus cinereus*. *Mammalian Species* **185:**1-5.

Solick DI, Gruver JC, Clement MJ, Murray KL, Courage Z. 2012. Mating Eastern Red Bats Found Dead at a Wind-Energy Facility. *Bat Research News* **53:**15-18.

Stephens M, Donnelly P. 2003. A Comparison of Bayesian Methods for Haplotype Reconstruction. *American Journal of Human Genetics* **73:**1162-1169 DOI 10.1086/379378.

Stephens M, Smith NJ, Donnelly P. 2001. A New Statistical Method for Haplotype Reconstruction from Population Data. *American Journal of Human Genetics* **68:**978-989 DOI 10.1086/319501.

Storz JF, Beaumont MA. 2002. Testing for Genetic Evidence of Population Expansion and Contraction: An Empirical Analysis of Microsatellite DNA Variation Using a Hierarchical Bayesian Model. *Evolution* **56:**154-166 DOI 10.1111/j.0014-3820.2002.tb00857.x.

Tallmon DA, Koyuk A, Luikart G, Beaumont MA. 2008. ONeSAMP: A Program to Estimate Effective Population Size Using Approximate Bayesian Computation. *Molecular Ecology Resources* **8:**299-301 DOI 10.1111/j.1471-8286.2007.01997.x.

Tallmon DA, Luikart G, Beaumont MA. 2004. Comparative Evaluation of a New Effective Population Size Estimator Based on Approximate Bayesian Computation. *Genetics* **167:**977-988 DOI 10.1534/genetics.103.026146.

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*. *Conservation Genetics* **10:**1965-1968 DOI 10.1007/s10592-009-9869-1.

Turmelle AS, Kunz TH, Sorenson MD. 2011. A Tale of Two Genomes: Contrasting Patterns of Phylogeographic Structure in a Widely Distributed Bat. *Molecular Ecology* **20:**357-375 DOI 10.1111/j.1365-294X.2010.04947.x.

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 DOI 10.1038/sj.hdy.6800509.

Venta PJ, Brouillette JA, Yuzbasiyan-Gurkan V, Brewer GJ. 1996. Gene-Specific Universal Mammalian Sequence-Tagged Sites: Application to the Canine Genome. *Biochemical Genetics* **34:**321-341.

Vonhof MJ, Davis CS, Fenton MB, Strobeck C. 2002. Characterization of Dinucleotide Microsatellite Loci in Big Brown Bats

(*Eptesicus fuscus*), and Their Use in Other North American Vespertilionid Bats. *Molecular Ecology Notes* **2:**167-169 DOI 10.1046/j.1471-8286.2002.00189.x.

Vonhof MJ, Fenton MB. 2004. Roost Availability and Population Size of *Thyroptera tricolor*, a Leaf-Roosting Bat, in Northeastern Costa Rica. *Journal of Tropical Ecology* **20:**291-305 DOI: http://dx.doi.org/10.1017/S0266467404001403.

Vonhof MJ, Strobeck C, Fenton MB. 2008. Genetic Variation and Population Structure in Big Brown Bats (*Eptesicus fuscus*): Is Female Dispersal Important? *Journal of Mammalogy* **89:**1411-1420 DOI http://dx.doi.org/10.1644/08-MAMM-S-062.1.

Wang J. 2005. Estimation of Effective Population Sizes from Data on Genetic Markers. *Philosophical Transactions of the Royal Society B* **360:**1395-1409 DOI 10.1098/rstb.2005.1682.

Waples RS, Do C. 2009. LDNE: A Program for Estimating Effective Population Size from Data on Linkage Disequilibrium. *Molecular Ecology Resources* **8:**753-756 DOI 10.1111/j.1755-0998.2007.02061.x.

Waples RS, Do C. 2010. Linkage Disequilibrium Estimates of Contemporary N_e Using Highly Variable Genetic Markers: A Largely Untapped Resource for Applied Conservation and Evolution. *Evolutionary Applications* **3:**244-262 DOI 10.1111/j.1752-4571.2009.00104.x.

Weir BS, Cockerham CC. 1984. Estimating *F*-Statistics for the Analysis of Population Structure. *Evolution* **38:**1358-1370.

Wilkinson GS, Chapman AM. 1991. Length and Sequence Variation in Evening Bat D-Loop MtDNA. *Genetics* **128:**607-617.

Table 1. Populations sampled and diversity statistics for 16 microsatellite genotypes and mitochondrial HV2 sequences. Population labels represent two-letter state codes as in Figure 1. N_{Gen} = number of individuals genotyped, N_A = number of alleles, H_O = observed heterozygosity, H_E = expected heterozygosity, AR = allelic richness, AR_{Priv} = private allelic richness, F_{IS} = inbreeding coefficient, N_{Seq} = number of individuals sequenced at mitochondrial HV2 locus, N_H = number of haplotypes, h = haplotype diversity, π = nucleotide diversity. Overall values represent means for all measures except N_{Gen} and N_{Seq}, which represent sums.

Population	State or Province	N_{Gen}	N_A	H_O	H_E	AR	AR_{Priv}	F_{IS}	N_{Seq}	N_H	h	π
AR	Arkansas	39	18.25	0.84	0.88	13.14	0.50	0.044	25	21	0.987	0.016
GA	Georgia	31	16.75	0.81	0.87	13.12	1.16	0.064	17	13	0.963	0.009
IL	Illinois	26	15.31	0.80	0.87	12.88	0.56	0.084	26	22	0.985	0.013
MD	Maryland	21	13.31	0.81	0.86	12.19	0.80	0.057	15	15	1.000	0.012
MI	Michigan	17	12.69	0.82	0.88	12.69	0.84	0.073	16	16	1.000	0.013
MO	Missouri	27	16.25	0.84	0.89	13.20	0.80	0.056	34	21	0.961	0.009
NC	North Carolina	18	13.19	0.81	0.88	12.87	0.76	0.079				
ON	Ontario	19	14.13	0.87	0.88	13.43	1.05	0.021	19	17	0.983	0.012
TN	Tennessee	22	14.50	0.82	0.87	12.98	0.79	0.065	26	23	0.991	0.010
TX	Texas	20	14.19	0.79	0.88	13.14	0.81	0.105	21	20	0.995	0.011
WV-Pe	West Virginia	20	13.25	0.83	0.87	12.35	0.79	0.050	19	18	0.994	0.010
WV-Ma	West Virginia	25	15.44	0.85	0.88	13.02	0.45	0.036				
Overall		**285**	**14.77**	**0.82**	**0.88**	**12.92**	**0.78**	**0.061**	**218**	**18.6**	**0.986**	**0.011**

Table 2. Pairwise F_{ST} values based on mitochondrial HV2 sequence data. Significant values based on 10,000 permutations ($P > 0.05$) are denoted with an *.

Population	AR	GA	IL	MD	MI	MO	ON	TN	TX	WV-Pe
AR	-									
GA	0.012	-								
IL	-0.008	0.032	-							
MD	0.014	0.037	0.019	-						
MI	-0.006	-0.006	0.006	-0.011	-					
MO	0.024	0.021	0.037*	0.032	0.016	-				
ON	0.005	0.014	-0.005	0.008	0.000	-0.006	-			
TN	0.006	0.009	0.001	0.030	0.005	0.008	-0.004	-		
TX	0.028	0.049	0.042*	0.021	0.020	0.013	0.000	0.036	-	
WV-Pe	0.003	0.015	-0.001	0.014	0.005	-0.009	-0.030	-0.016	0.009	-

Table 3. Pairwise F_{ST} (below diagonal) and Jost's D (above diagonal) values based on 16-locus microsatellite genotypes. No pairwise F_{ST} values were significant based on 10,000 permutations.

	AR	GA	IL	MD	MI	MO	NC	ON	TN	TX	WV-Pe	WV-Ma
AR	-	0.027	0.02	0.001	0.013	0.018	0.001	-0.025	0.025	0.033	0.025	0.001
GA	0.004	-	0.041	0.02	0.039	0.068	0.011	0.027	0.029	0.047	0.054	0.029
IL	0.003	0.006	-	0.026	0.046	0.037	0.011	0.007	0.018	0.055	0.037	0.026
MD	0	0.003	0.004	-	0.012	0.022	0.035	0.009	0.012	0.041	0.02	-0.022
MI	0.002	0.006	0.007	0.002	-	0.001	-0.033	0.012	0.015	0.012	0.021	0.026
MO	0.003	0.009	0.005	0.003	0	-	0.006	0.015	0.052	0.049	0.013	0.006
NC	0	0.002	0.002	0.005	-0.004	0.001	-	-0.017	0.02	-0.002	0.018	0.003
ON	-0.003	0.004	0.001	0.001	0.002	0.002	-0.002	-	0.026	-0.036	0.024	0.023
TN	0.004	0.004	0.003	0.002	0.002	0.007	0.003	0.004	-	0.031	0.029	0.026
TX	0.005	0.007	0.008	0.006	0.002	0.006	0	-0.005	0.004	-	0.021	0.039
WV-Pe	0.004	0.008	0.005	0.003	0.003	0.002	0.002	0.003	0.004	0.003	-	0.011
WV-Ma	0	0.004	0.004	-0.003	0.004	0.001	0.001	0.003	0.004	0.006	0.002	-

Table 4. Results of the msvar analyses.

Year	Current N_e (mode ± variance)	Ancestral N_e (mode ± variance)	Time of growth (mode ± variance)	Trend
2002_A1	125,786 ± 4.9	24,191 ± 4.5	5,353 ± 1.9	Growth
2002_A2	21,120 ± 6.2	57,497 ± 3.1	6,924 ± 1.9	Decline
2002_A3	106,925 ± 3.3	22,460 ± 6.0	27,256 ± 5.8	Growth
2002_B1	137,848 ± 6.1	14,626 ± 4.6	11,710 ± 3.3	Growth
2002_B3	195,164 ± 4.4	651,754 ± 3.1	21,915 ± 1.1	Decline
2010_A1	46,279 ± 3.5	59,872 ± 2.6	88,776 ± 1.9	Decline
2010_A2	36,766 ± 5.6	427,688 ± 3.8	16,088 ± 4.3	Decline
2010_A3	24,733 ± 2.4	44,036 ± 5.5	32,866 ± 1.6	Decline
2010_B1	22,845 ± 3.8	81,332 ± 7.6	5,161 ± 4.2	Decline
2010_B2	12,670 ± 5.4	29,191 ± 3.8	9,978 ± 1.3	Decline
2010_B3	89,050 ± 10.0	724,656 ± 2.8	11,552 ± 7.2	Decline

Table 5. Results of Lamarc analysis.

	θ (95% CI)	N_e (95% CI)	g (95% CI)
HV2			
Run 1	0.022 (0.018, 0.031)	5.0 x 10^5 (4.16 – 7.26 x 10^5)	964.25 (361.03, 1007.18)
Run 2	0.024 (0.019, 0.029)	5.52 x 10^5 (4.33 – 6.78 x 10^5)	965.75 (358.34, 1007.50)
Run 3	0.022 (0.018, 0.033)	5.0 x 10^5 (4.25 – 7.61 x 10^5)	965.95 (382.04, 1006.35)
Overall	0.022 (0.018, 0.031)	5.18 x 10^5 (4.25 – 7.22 x 10^5)	965.32 (367.14, 1007.01)
CHY			
Run 1	0.048 (0.033, 0.067)	1.54 x 10^6 (1.07 – 2.15 x 10^6)	958.85 (496.01, 1002.27)
Run 2	0.046 (0.032, 0.067)	1.50 x 10^6 (1.03 – 2.17 x 10^6)	957.10 (486.19, 1002.01)
Run 3	0.047 (0.033, 0.069)	1.52 x 10^6 (1.06 – 2.21 x 10^6)	952.73 (479.76, 1001.04)
Overall	0.047 (0.033, 0.068)	1.52 x 10^6 (1.05 – 2.18 x 10^6)	956.23 (487.32, 1001.77)

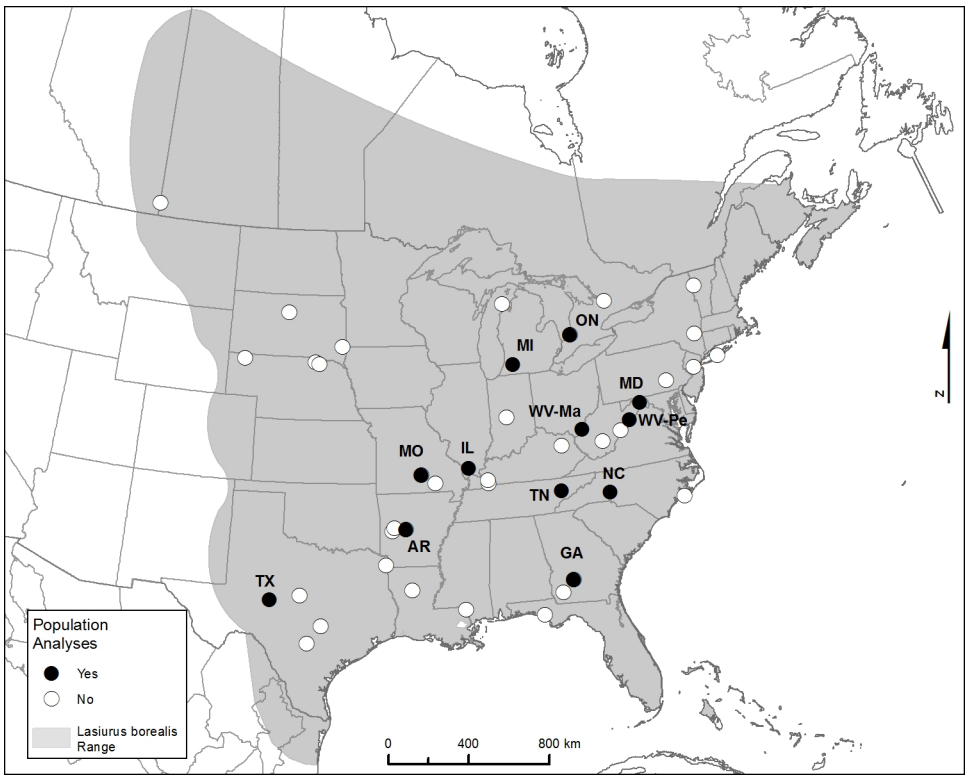

Figure 1. Map showing the range of eastern red bats and all sampling locations. Only labeled locations (black dots) had sufficient sample sizes to be included in population-level analyses, and labels reflect two-letter state or province codes (two sampling locations within West Virginia are further labeled with the first two letters of the county to distinguish them).

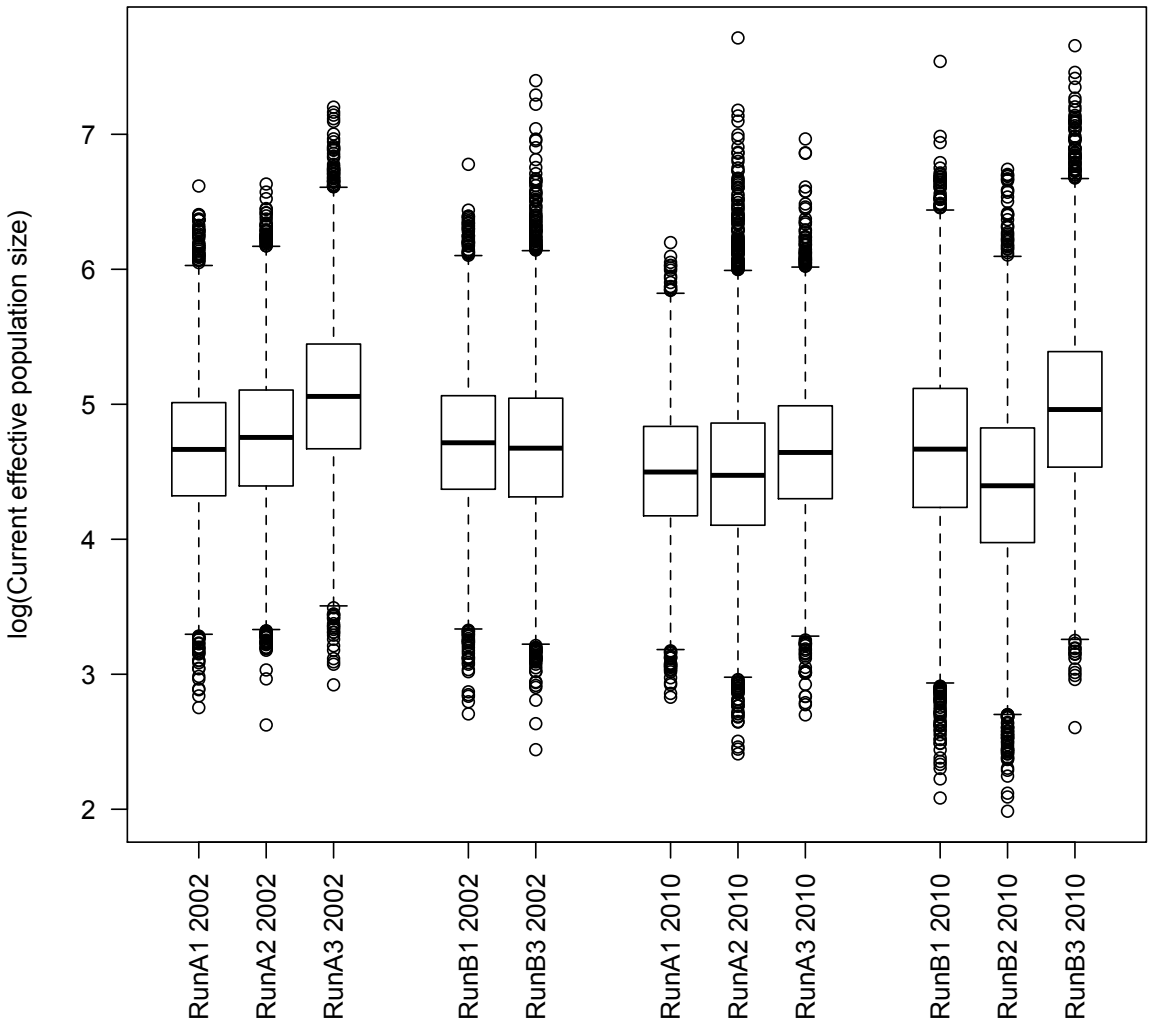

Figure 2. Tukey boxplot of current N_e from msvar analyses. Estimates are given on the $\log_{10}$ scale. Datasets A and B represent different subsamples of the full dataset from each respective year.

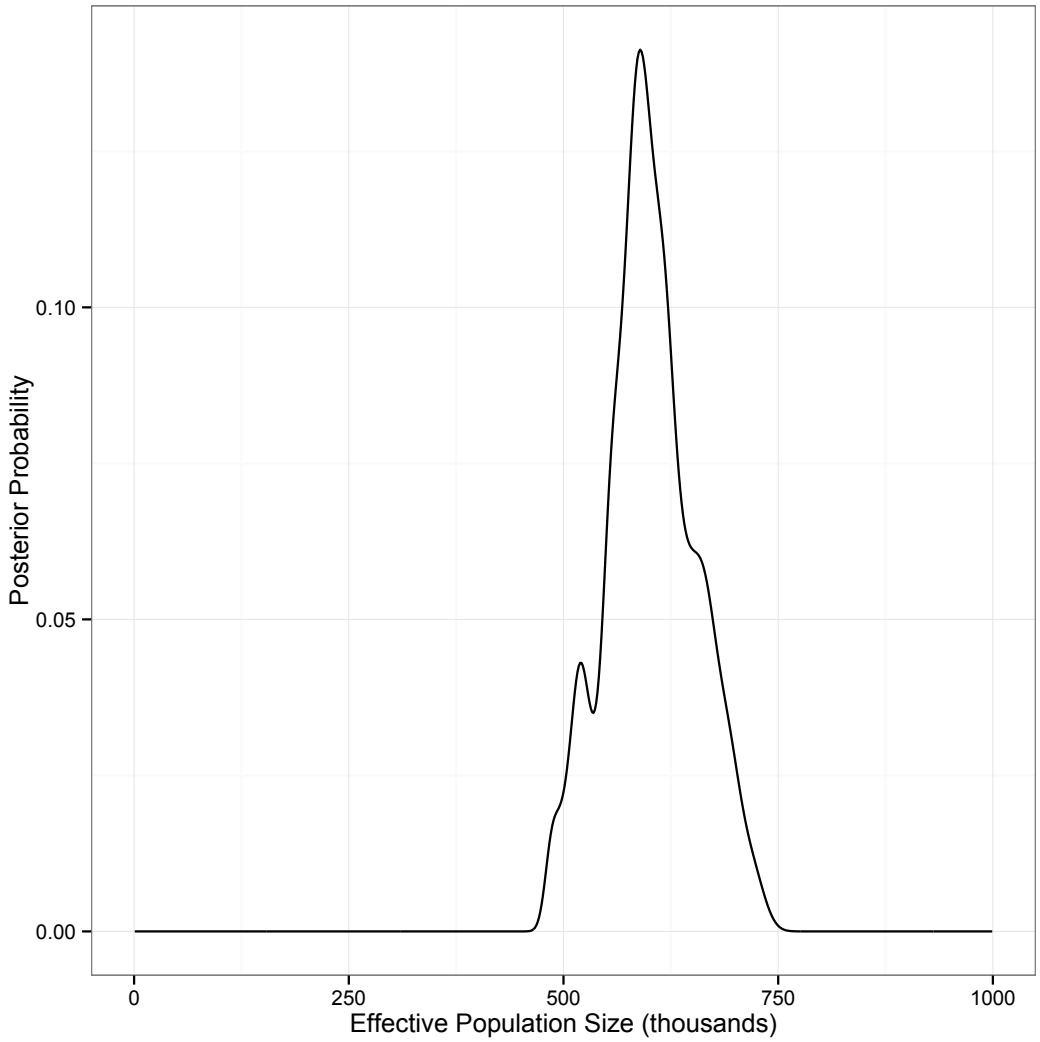

Figure 3. Posterior probability of N_e for eastern red bats, estimated using IMa2. The analysis includes autosomal DNA sequence data, mitochondrial DNA sequence data, and autosomal microsatellite genotype data.