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The Genetic Structure and Mating System of the Buffy Flower Bat (Erophylla sezekorni)

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UNIVERSITY OF MIAMI

THE GENETIC STRUCTURE AND MATING SYSTEM OF THE BUFFY FLOWER BAT (*EROPHYLLA SEZEKORNI*)

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Kevin Lager Murray

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The buffy flower bat (*Erophylla sezekorni*) is a neotropical leaf-nosed bat (Phyllostomidae) that is endemic to the Greater Antilles. Although this species is one of the most common and abundant species of mammals in the West Indies, very little is known about its ecology and evolution. To address this deficiency, I studied the genetic structure and mating system of the buffy flower bat on several islands throughout its range, focusing a more intensive study on the island of Exuma, Bahamas. I first studied the effects of ocean barriers on genetic diversification within *Erophylla* and two related endemic genera of endemic West Indian bats, *Brachphylla*, *Phyllonycteris* (Chapter II). I found evidence that ocean barriers inhibit gene flow and promote speciation within these genera. Focusing on genus *Erophylla* (Chapter III), I found that ocean channels usually act as barriers to gene flow among island populations within species. However, relatively shallow and narrow ocean channels formed semi-permeable barriers allowing gene flow between some island populations. Within the buffy flower bat, *Erophylla sezekorni* (Chapter IV), genetic diversity of mitochondrial DNA fragments was positively correlated with island size, with small islands having reduced genetic diversity. However, genetic diversity at several nuclear microsatellite loci was not correlated with island area and levels of genetic diversity were high for most island populations. In addition, island

populations within the Great Bahamas Bank and Little Bahamas Bank showed high levels of gene flow between island and showed no evidence of genetic bottlenecks. Populations of *E. sezekorni* on Exuma (Chapters IV and V) exhibited a polygynous mating system that included vigorous visual, acoustic, and olfactory male display behaviors. However, the social structure that I observed had a negligible effect on genetic diversity and genetic structure within these populations. Overall, the buffy flower bat exhibits very few of the genetic symptoms of island life, such as reduced genetic diversity and increased genetic isolation, and is evolutionarily adapted to persist on small oceanic islands.

DEDICATION

To Lynford Kelsay, Anita Lewis, Lois Pierson, Harold Lager, Edward Murray, Joshua Stratman, and Don Davis.

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CHAPTER I

GENERAL INTRODUCTION

> *Many of the seemingly contradictory conclusions or assertions to be found in the island literature appear to me to be resolvable once scale has been brought in as an organizing principle, and once it is recognized that particular island effects or theories hold relevance within only a limited portion of the time-space array…*
>
> Robert J. Whittaker (2000)

Spatial and temporal scales are critical factors in island biogeography (MacArthur and Wilson 1967, Haila 1990, Lomolino 2000, Whittaker 2000). The definition of an island itself is dependent upon the vagility of species. Thus, to wide-ranging or migratory species, even relatively large, isolated islands are not really islands (Haila 1990). In bats, for example, several studies have shown that genetic boundaries within species correspond to island boundaries, whereas this is not the case in other species (Carstens et al. 2004, Heaney et al. 2005, Fleming et al. *in press*). Temporal scale adds an additional layer of complexity to island systems. The fundamental processes of island biogeography, immigration, extinction, and evolution (Lomolino 2000), often occur over long time periods during which climates and even the islands themselves may change substantially. As an organizing principle, we can categorize spatial and temporal variability of island species into four ecological scales: 1) individual, 2) population dynamics, 3) population differentiation, and 4) evolutionary (Haila 1990). The individual scale encompasses the area in which an organism performs all vital ecological functions (e.g. foraging, reproduction, dispersal, etc.). At the population dynamics scale, populations may or may not function as independent units. They will be independent if

they are closed to immigration; otherwise, they are not. As spatial and temporal scales increase, populations will begin to differentiate, and at the evolutionary scale, speciation occurs (Haila 1990, Whittaker 2000).

This multi-scale approach has been used very successfully for several species of bats, such as *Cynopterus sphinx* (Storz et al. 2001a,b, Campbell et al. 2006), *Myotis bechsteinii* (Kerth et al 2000, Kerth et al. 2002a,b), *Myotis myotis* (Castella et al. 2000, 2001, Ruedi and Castella 2003, Ruedi et al. 2008), and *Rhinolophus ferrumequinum* (Rossiter et al. 2000a,b, 2006). However, no studies have looked at an island bat species at multiple taxonomic, spatial, and temporal scales. Bats in the genus *Erophylla* are common and widespread throughout the Greater Antilles. There are two species of *Erophylla*, the brown flower bat (*E. bombifrons*) which occurs on Hispaniola and Puerto Rico and the buffy flower bat (*E. sezekorni*) which occurs on Cuba, Jamaica, the Cayman Islands, and the Bahamas. These bats offer an excellent opportunity to study the genetic structure of an endemic island species at multiple scales.

I begin my dissertation at the evolutionary scale in chapter II by examining molecular differentiation between species within *Erophylla* and its two most closely related genera, *Phyllonycteris* and *Brachyphylla*. Each of these genera is endemic to the West Indies. The current taxonomy of these bats is based on morphology, and the number of species within each genus as well as the relationships between those species is not well-resolved (Morgan 2001, Timm and Genoways 2003, Davalos 2004). In addition, there is strong potential for cryptic species within these genera due to the isolating effect of islands. I used mitochondrial genes (cytochrome-*b* and control region) to investigate genetic differentiation and phylogenetic relationships within each genus. In addition, I

used nine microsatellite loci to examine more recent genetic divergence between species of *Erophylla*.

In chapter III, I focus on the population differentiation scale, examining the phylogeography of both *Erophylla bombifrons* and *E. sezekorni*. Most phylogeographic studies of island bats employ molecular markers from the mitochondrial genome and essentially focus on an historical timescale (Carstens et al. 2004, Salgueiro et al 2004, Roberts 2006). While this scale is crucial for understanding the evolutionary history of a species, contemporary processes should not be ignored. I applied molecular dating techniques to establish a rough timescale of divergence within each species of *Erophylla*. I then applied a suite of molecular markers, including mitochondrial DNA and nuclear microsatellites, to examine both historical and contemporary patterns of gene flow among island populations. Finally, I used demographic analyses to look for evidence of recent population growth in *E. bombifrons* and *E. sezekorni*.

In chapters IV and V, I begin at the boundary between the population differentiation and population dynamics scales, move through the population dynamics scale, and end up at the individual scale. In chapter IV, I examine the genetic consequences of island life in eleven island populations of *E. sezekorni* and use data from the mtDNA control region and 11 microsatellite markers to determine: 1) does island size affect genetic diversity?, 2) is there evidence of genetic bottlenecks in these island populations?, and 3) are these islands genetically isolated? In this chapter, I also focus on a single colony of *E. sezekorni* on Exuma. For these bats, I use 8 nuclear microsatellites to determine the genetic consequences of social structure, sex-biased dispersal, and

mating system. Finally, in Chapter V, I conclude this study at the individual scale by describing the male display behavior and mating system of *E. sezekorni* on Exuma.

Islands are widely recognized for their importance as natural laboratories for the study of evolutionary biology (MacArthur and Wilson 1967, Whittaker 2000). Their discrete nature, reduced complexity, and their ability to be replicated and manipulated in some cases, make islands ideal settings in which to study the pattern and process of evolution. The insights we gain from studies of island organisms can have far-reaching implications for continental species. In chapter VI, I briefly summarize the results of each chapter and discuss the implications of this research for both island and mainland bat species.

CHAPTER II

SPECIES ON THE EDGE: MOLECULAR DIFFERENTIATION IN THREE LINEAGES OF ENDEMIC WEST INDIAN BATS

BACKGROUND

Since the time of Linnaeus, morphological differentiation has been used as the standard criteria to describe and delineate species. It has been our most effective means of quantifying the biodiversity of the planet and has been used to describe approximately 1.7 million species (Waugh 2007). However, there are potential problems with the morphological approach. The main problem is cryptic species, organisms with distinct evolutionary histories that are morphologically indistinguishable due to convergent evolution (Lefebure et al. 2006). Numerous studies have shown that cryptic species are common in a variety of organisms (Mayer and von Helversen 2001, Bickford et al. 2006, Waugh 2007). The rate of discovery of cryptic species has dramatically increased over the past two decades, largely due to the use of molecular data (Bickford et al. 2006). A second problem is that morphological characters under selection may produce a pattern that contradicts the actual evolutionary history of a species. Examples of this include *Anolis* lizards and *Myotis* bats. Morphological similarities within these two genera are a result of convergent selective pressures for habitat type (*Anolis*) or foraging style (*Myotis*) and not common evolutionary histories (Hoofer 2003; Losos 1997; Losos 1998). For these reasons, many researchers have sought an alternative or supplementary approach to morphological taxonomy.

DNA taxonomy, the use of molecular data to describe species, has emerged over the past two decades as an alternative to morphological species delineation (Vogler and

Monaghan 2007). Molecular markers have been used successfully to detect cryptic species (Mayer and von Helversen 2001, Baker and Bradley 2006, Bickford et al. 2006) and to provide valuable information on patterns of evolution and gene flow (Ruedi and Mayer 2001, Hajibabaei et al. 2006, Giordano et al. 2007). Molecular data is not perfect, however, and suffers some significant shortcomings (Vogler and Monaghan 2007). DNA is not ideal for detecting incipient speciation because neutral gene mutations can accumulate at a slower rate than changes in morphological characters under selection (Mayer and von Helversen 2001, Rodriguez and Ammerman 2004, Hickerson et al. 2006). Also, DNA data can be just as misleading as morphological data in identifying species. Introgression, incomplete lineage sorting, and differences between the gene tree and species tree can all obscure true patterns of species divergence (Rubinoff 2006, Rubinoff et al. 2006). Despite these potential pitfalls, DNA taxonomy has been applied very successfully to bats (Order Chiroptera) in recent years (Mayer and von Helversen 2001, Baker and Bradley 2006, Clare et al. 2007). Several studies have used DNA sequence data to discover cryptic diversity within genera (Mayer and von Helversen 2001, Baker and Bradley 2006) and to clarify the phylogenetic relationships within and among various genera of bats (Hoffmann and Baker 2001, Ruedi and Mayer 2001, Hoffmann and Baker 2003, Hoffmann et al. 2003, Porter and Baker 2004, Fleming et al., *in press*).

Bats in the phyllostomid subfamilies Phyllonycterinae (*Erophylla* and *Phyllonycteris*) and Brachyphyllinae (*Brachyphylla*) represent a unique clade of bats endemic to the islands of the West Indies. The morphological taxonomy of these three related genera has been addressed in several studies (Buden 1976, Swanepoel and

Genoways 1978, Morgan 1989, Koopman 1993, Morgan 2001, Timm and Genoways 2003, Davalos 2004). However, the taxonomic arrangement of species within each genus is still unresolved, and the literature is fraught with various interpretations of the morphological data (Morgan 2001, Davalos 2004). *Brachyphylla* has been thought to contain a single species, *B. cavernarum* (Buden 1977) or two species, a smaller form, *B. nana* and a larger form, *B. cavernarum* (Swanepoel and Genoways 1978, Morgan 1989, Koopman 1993, Timm and Genoways 2003). The most recent revision, based on cytochrome-*b*, proposed three species, *B. nana*, *B. pumila* and *B. cavernarum* (Davalos 2004). *Erophylla* has been considered to contain a single species, *E. sezekorni*, throughout its range (Buden 1976, Baker et al. 1978, Gannon et al. 2005) or two species, *E. bombifrons* and *E. sezekorni* (Koopman 1993, Timm and Genoways 2003). Finally, *Phyllonycteris* has been considered to contain two species, *P. aphylla* and *P. poeyi* (Morgan 1989, Koopman 1993, Timm and Genoways 2003) or three, *P. aphylla*, *P. obtusa*, and *P. poeyi* (Morgan 2001).

We followed the process outlined by Vogler and Monaghan (2006) to investigate species relationships within these three genera. The steps are 1) compile existing taxonomic data; 2) formulate hypotheses regarding species delimitation; 3) test hypotheses with new data; and 4) revise species hypotheses. We used prior morphological and molecular research to formulate hypotheses of species relationships for *Brachyphylla*, *Erophylla*, and *Phyllonycteris*. We followed Simmons (2005) in making our species hypotheses, except for *Brachyphylla* for which new data were available (Davalos 2004). We made three initial hypotheses, 1) *Brachyphylla* is composed of three species (*B. nana*- Cuba, Grand Cayman; *B. pumila*- Hispaniola, Grand

Caicos; *B. cavernarum*- Puerto Rico, Lesser Antilles), 2) *Erophylla* is composed of two species, *E. bombifrons* (Hispaniola, Puerto Rico) and *E. sezekorni* (Jamaica, Cuba, Cayman Islands, Bahamas), and 3) *Phyllonycteris* is composed of two species, *P. aphylla* (Jamaica) and *P. poeyi* (Cuba, Hispaniola). We tested these hypotheses with new genetic data from multiple molecular markers (mitochondrial cytochrome-*b* gene and control region, and nuclear microsatellites) using a variety of techniques to quantify molecular differentiation and examine the boundaries between species within these genera.

METHODS

Sample Collection

Tissue samples were collected from throughout the geographic ranges of the three genera. Bats were captured with extendable hand nets inside caves or with mist nets set at cave entrances. We recorded age, sex, reproductive status, body mass (g), and forearm length (mm) for all captured individuals. A small piece of tissue (2-20 mg) was clipped from one wing membrane and stored in 95% ethanol until analyzed in the lab. Genomic DNA was extracted from 5 mg pieces of tissue using a standard ethanol precipitation procedure or DNeasy® DNA isolation kits (Qiagen) and stored in 50 μl of Tris-HCl, Ph 8.5.

MtDNA sequencing and analyses

We used PCR (Polymerase Chain Reaction) to amplify the entire mitochondrial cytochrome-*b* (cyt-*b*) gene with two primer sets, each covering approximately two-thirds of the gene. For *Erophylla*, we used primer set AjaFor1 and AjaRev1 to amplify the first

two-thirds of the gene and primer set EroFor2:5'-CCAACCTATTCTCTGCCATCC-3' and AjaRev2 to amplify the second two-thirds of the gene. For *Phyllonycteris*, we substituted primer PapRev1:5'-TAAGGG-TGGAAGGGAATTATG-3' for AjaRev1 and primer PapFor2:5'-CAAACCTATTATC-CGCCATTC-3' for EroFor2. Primer set AjaFor1 and BcaRev1 and primer set BnaFor2:5'-CTAACCTACTC-TCCGCCATCC-3' and BnaRev2:5'-CCCCCTTTTCTGGYTTACAAGAC-3' were used for *Brachyphylla*. Primer sequences AjaFor1, AjaRev1, AjaRev2, and BcaRev1 were taken from Carstens et al. (2004).

We also amplified fragments of approximately 350 bp of mitochondrial control region (CR) using PCR. Because traditional primers used to amplify bat control region fragments (P and F; Wilkinson and Chapman 1991) were not reliable for these genera, primer F1:5'-CCCCACCCTCAACACCCAAA-3' was redesigned from the *Artibeus jamaicensis* mitochondrial genome (Pumo et al. 1998) and coupled with traditional primer F:5'-GTTGCTGGTTTCACGGAGGTAG-3' for *Erophylla* and *Phyllonycteris*. For *Brachyphylla*, we substituted primer BN-F1:5'-TTCCTACCATCAG-CACCCAAA-3' for primer F1.

For both cyt-*b* and CR fragments, total PCR volume was 10 μl, with 1.0 μl Promega 10X buffer (1.5 Mm $MgCl_2$ added), 1 unit *Taq* DNA polymerase (Promega), 0.1 Mm dNTPs, and 14 pmol of each primer. PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72°C for 30 s, with a final elongation step at 72 °C for 5 min. Annealing temperature was lowered to 50 °C when necessary. Before cycle sequencing, DNA fragments were incubated with ExoSAP-IT (USB) to dephosphorylate double-stranded DNA and degrade excess primer.

All fragments were sequenced with Big Dye Terminator Cycle Sequencing Kit, version 1.1 (Applied Biosystems). Ten μl reaction volumes contained 2.5 μl of Big Dye reaction mix, 10-50 ng of template DNA, and 3.2 pmol of forward or reverse primer. The sequencing reaction involved an initial denaturation of 92 °C for 1 min, followed by 25 cycles of 92 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Products were run through sephadex columns (Princeton Solutions) to remove unincorporated nucleotides. Samples were then dried for 30 min with a vacuum centrifuge and resuspended in 15 μl of Hi-Di Formamide (Applied Biosystems) for sequencing. All samples were sequenced in both directions using an ABI 310 automated sequencer. For *Erophylla,* we used sequencing primers Es-int1:5'-TTCCG-ATGTTTCATGTTTCTGT-3' and Es-int2:5'-GCACCCCT-CCACATATCAARC-3' to sequence the 5' and 3'-end of the cyt-*b* gene, respectively. For *Phyllonycteris,* we used Es-int1 and Ppo-int2:5'-ATACCCCACCACATATCAAAC-3'. Raw sequence data was edited in SEQUENCHER v.4.5 (Gene Codes). We aligned all sequences for each genus in CLUSTALX (Thompson et al. 1994) and then used aligned sequence files in DNASP v.4.10 (Rozas et al. 2003) to determine unique haplotypes.

We sequenced the entire mitochondrial cyt-*b* gene (1140 bp) for 60 individuals of *Erophylla* and 11 individuals of *Phyllonycteris* and 1,089 bp of the cyt-*b* gene for 21 individuals of *Brachyphylla*. One *Erophylla* sequence from Dominican Republic (AY620438), one from Jamaica (AY620439), and one *Phyllonycteris* sequence from Jamaica (AF187033) were taken from GenBank (NCBI). Fifty-one *Brachyphylla* sequences (AY572365-AY572382, AY620440, AY620444, AY620446-AY620450, AY620453-AY620455, AY620457- AY620461, AY620463, AY620465-467) were taken from GeneBank (Carstens et al. 2004, Davalos 2004). *Brachyphylla* samples from

GenBank came from 11 islands, Anguilla (*n* = 5), Dominica (*n* = 1), Grand Cayman (*n* = 2), Guadeloupe (*n* = 4), Montserrat (*n* = 14), Nevis (*n* = 7), Puerto Rico (*n* = 3), Saba (*n* = 3), St. Eustatius (*n* = 2), St. Martin (*n* = 5), Turks and Caicos (*n* = 5).

We sequenced approximately 360 bp of the mitochondrial CR for 223 individuals of *Erophylla*, 25 individuals of *Phyllonycteris* and 27 individuals of *Brachyphylla*. Sampling locations and sample sizes for cyt-*b* and CR sequences generated in this study are summarized in Table 2.1.

Microsatellite genotyping and analyses

We obtained microsatellite genotype data from 214 individuals (Table 2.1) and 9 loci (ES6,8,22,24,27,35,38,40,46) for *Erophylla* using the techniques described in Murray et al. (2008). All samples were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Inc.) and scored with GENEMAPPER v.3.0 (Applied Biosystems, Inc.). We used FSTAT (Goudet 1995) to test for deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage equilibrium. A Bonferroni correction was used to adjust alpha and correct for multiple statistical tests when evaluating the results of HWE and linkage equilibrium tests. Genotype data were screened for null alleles, large-allele dropout, and scoring errors with MICRO-CHECKER (Van Oosterhout et al. 2004).

Molecular differentiation

Molecular differentiation was investigated by 1) calculating genetic distances for cyt-*b*, CR, and microsatellite data (*Erophylla* only) and 2) constructing phylogenetic trees for each lineage. We used the program MEGA v.4.0 (Tamura et al. 2007) to calculate

average pairwise genetic distances within and among species groups. For sequence data, we used the Kimura 2-parameter model of nucleotide substitution (Kimura 1980) to estimate all genetic distances. GENEALEX v.6 (Peakall and Smouse 2006) was used to calculate Nei's genetic distance statistic (Nei's D; Nei 1987) for microsatellite data. All genetic distance values are presented as mean ± 1 SE. Finally, Mantel tests were carried out in ARLEQUIN v.3.01 (Excoffier et al. 2005) to determine if genetic distance matrices for different molecular markers were correlated.

To compare the phylogenetic signal of cyt-*b* and control region datasets for each genus, we constructed neighbor-joining (NJ) and maximum parsimony (MP) trees separately for each dataset and lineage of bats in MEGA. K2P genetic distances were used to construct NJ trees. For MP analyses all characters were unweighted. For both NJ and MP phylogenies, statistical support for each node was assessed with 1000 bootstrap replications .

For species delimitation, Bayesian and MP phylogenetic trees were constructed using MRBAYES v.3.12 (Huelsenbeck and Ronquist 2001) and MEGA, respectively. Bayesian and MP phylogenies were constructed for the cyt-*b* gene and for a concatenated dataset of cyt-*b* and CR sequences for individuals for which we sequenced both genes. In MRBAYES, we ran four Markov chain Monte Carlo (MCMC) chains for 3.5 million generations, sampling every 100 generations. Samples from the burn-in period (350,000 generations) were discarded. We partitioned cyt-*b* datasets into three parts corresponding to codon position. Concatenated datasets in MRBAYES were also partitioned into cyt-*b* and control parts. We used the Akaike Information Criterion (AIC) test statistic in MODELTEST v.3.7 to determine the most appropriate model of nucleotide evolution (Posada and

Crandall 1998, Posada and Buckley 2004) and applied that model to each partition. Indels were ignored in all analyses. MP phylogenies were constructed in MEGA. Statistical support for each node was evaluated with Bayesian posterior probabilities (BPP) and 1000 MP bootstrap replicates.

Species delimitation using mtDNA

We used several methods to delineate species within *Brachyphylla*, *Erophylla*, and *Phyllonycteris* using mitochondrial sequence data. First, we employed the 10X rule (Hebert et al. 2004), commonly used in DNA-barcoding studies. The 10X rule states that genetic divergence between putative species must be at least 10 times the average genetic divergence within species. Second, reciprocal monophyly was used as a criterion for species delimitation (Weins and Penkroft 2002, Hickerson et al. 2006). Reciprocal monophyly has been used to define evolutionary significant units (Moritz 1994) and as part of the phylogenetic species concept (Donoghue 1985) and is defined as the condition in which all members of a putative species share more recent common ancestors than any member of another putative species. We evaluated reciprocal monophyly using Bayesian and MP phylogenetic trees.

Finally, we calculated a molecular speciation threshold for *Brachyphylla*, *Erophylla*, and *Phyllonycteris* using the method proposed by Lefebure et al. (2006). Within each bat genus, we calculated frequency distributions of intraspecific and interspecific genetic divergences (Highton 1998). We used these distributions to plot 1) the percentage of intraspecific divergences (y-axis) below a particular genetic distance value (x-axis) and 2) the percentage of interspecific divergences above a particular

genetic distance value. The point at which these plots intersect is the molecular threshold, the minimum genetic distance that allows maximum discrimination between putative species. We then used the molecular threshold and average genetic distance between putative species to make species determinations.

Species delimitation using microsatellites

For *Erophylla* microsatellite data, we used three methods to examine species boundaries. First, we used a method of species delimitation based on linear regression of intra- and interspecific genetic distances on geographic distance (Good and Wake 1992, Sites and Marshall 2003). Conspecific taxonomic units are expected to exhibit a pattern of genetic isolation by geographic distance (IBD), and the regression line should pass through the origin (i.e. at zero geographic distance populations should be genetically identical). Taxonomic units from different species are expected to show a pattern of genetic divergence independent of geographic separation, and the regression line should not pass through the origin (i.e. at zero geographic distance the species should be genetically divergent). We used linear regression to examine the relationship between average genetic distance between island populations and the geographic distance (km) between islands. *T*-tests were used to determine if regression coefficients and y-intercepts were significantly different from zero. Regression analyses were conducted in SPSS V.15.0 (SPSS Inc., Chicago, USA) and $\alpha = 0.05$.

The second method we used involved plotting a histogram of pairwise Nei's genetic distances between populations of *Erophylla* (Mallet 1996, Highton 1998). If all populations comprise a single species, the frequency distribution should be unimodal

with a mean with a low value of Nei's *D*. If sampled populations represent different species, the frequency distribution should be bimodal with smaller Nei's *D* values representing intraspecific population comparisons and larger Nei's *D* values representing interspecific population comparisons. This method assumes that divergence in multiple loci conforms to a molecular clock and is representative of reproductive isolation (Sites and Marshall 2003).

Finally, we calculated recent migration rates (i.e. gene flow) between *E. bombifrons* and *E. sezekorni* using BAYESASS v.1.3 (Wilson and Rannala 2003). Lack of gene flow between populations is considered indirect evidence of reproductive isolation. Therefore, we considered putative species with no gene flow between them to be distinct species. BAYESASS determines the proportion of non-migrants and the proportion and origin of migrants within each population using a Markov Chain Monte Carlo (MCMC) algorithm. MCMC chains were run for 5 million generations with a sampling frequency of 2000. The first one million iterations were discarded as the burn-in period. All other parameters were left at their default settings. The BAYESASS analysis was repeated three times to assess stability of estimated migration rates.

RESULTS

Molecular differentiation

Kimura 2-parameter genetic distances for cyt-*b* were always smaller within (range = 0.24-0.95%) and larger between (range = 2.10-7.83%) putative species (Table 2.2). The results were similar for CR data, but intraspecific divergences were usually larger and interspecific divergences smaller than for cyt-*b* data (Table 2.2). For microsatellite data,

average Nei's *D* was 0.26 between two *E. bombifrons* populations, 0.35 ± 0.04 among *E. sezekorni* populations, and 0.77 ± 0.02 between *E. bombifrons* and *E. sezekorni*. Mantel tests revealed that genetic distances calculated from different molecular markers were correlated. As expected, correlations between mitochondrial datasets (cyt-*b* and CR) were strongest (*Brachyphylla*: $r^2 = 0.97$, $p = 0.033$; *Erophylla*: $r^2 = 0.88$, $p = 0.013$). There were too few populations to include *Phyllonycteris* in these analyses. For *Erophylla*, Nei's *D* data was correlated with both cyt-*b* data ($r^2 = 0.68$, $p = 0.002$) and CR data ($r^2 = 0.76$, $p = 0.004$).

Neighbor-joining phylograms (Figs. 2.1 – 2.6), maximum parsimony analyses (trees not shown), and Bayesian phylogenetic analyses (Figs. 2.7 – 2.8) all showed that putative species formed well-supported monophyletic clades in *Brachyphylla* and *Phyllonycteris* but not in *Erophylla*. In *Erophylla,* a single *E. bombifrons* sample from Dominican Republic (Ebom1) was nested within the *E. sezekorni* clade. For distance (NJ) and parsimony analyses (not shown), topology was very similar between cyt-*b* and CR phylogenetic trees, but nodal support was usually lower for CR data (Figs. 2.1 – 2.6). Similarly, Bayesian tree topology was nearly identical between cyt-*b* and concatenated trees, but nodal support was higher for concatenated trees (Figs. 2.7 – 2.8). We recovered essentially the same topologies using Bayesian and MP (not shown) phylogenetic methods (Figs. 2.7 – 2.8). The major nodes (those separating putative species) were the same among all phylogenetic methods (Bayesian, MP, and NJ), with one exception. For *Phyllonycteris,* the NJ trees for the cyt-*b* gene (Fig. 2.5) differed from both Bayesian and MP trees (Fig. 2.7). In the NJ tree, *P. aphylla* (Jamaica) was ancestral to the rest of

Phyllonycteris, but on Bayesian and MP trees, *P. obtusa* (Hispaniola) was ancestral to the clade.

Species delimitation using mtDNA

We used several criteria to determine the appropriate boundaries between species within these genera. First, we applied the 10X-rule commonly used in DNA-barcoding studies (Table 2.2). According to the 10X-rule, there was sufficient interspecific relative to intraspecific divergence in the cyt-*b* gene to differentiate between three putative species groups, *B. cavernarum*-*B. nana*, *B. nana*-*B. pumila*, and *P. aphylla*-*P. obtusa*. None of the other cyt-*b* or any of the CR species comparisons conformed to the 10X rule threshold for distinguishing between species. Species delimitation based on the 10X rule would result in two species of *Brachyphylla* and a single species of both *Erophylla* and *Phyllonycteris*.

Based on reciprocal monophyly, Bayesian and MP trees (Figs. 2.7 – 2.8) supported the existence of three species each within *Brachyphylla* (*B. cavernarum*, *B. nana*, *B. pumila*) and *Phyllonycteris* (*P. aphylla*, *P. obtusa*, and *P. poeyi*), but only a single species in *Erophylla* (*E. sezekorni*). While there was evidence of strong molecular divergence between *E. bombifrons* and *E. sezekorni*, there was a single *E. bombifrons* haplotype (Ebom 1) nested within the *E. sezekorni* clade (figs. 2.3 – 2.4, 2.7 – 2.8).

Molecular threshold analysis for all three genera combined established a threshold genetic distance of 1.4% at which 97.0% of cyt-*b* samples could be assigned to the correct species group (Fig. 2.9). The molecular threshold for CR data was 2.6% genetic divergence at which 84.0% of samples could be accurately assigned to species (Fig. 2.9).

Based on these molecular thresholds and average genetic distances between putative species, our results support the existence of three species of *Brachyphylla*, two species of *Erophylla*, and three species of *Phyllonycteris* for cyt-*b* data. For the CR molecular threshold (2.6%), our results supported two species of *Brachyphylla* and *Erophylla* and at least two species of *Phyllonycteris* (data for *P. aphylla* was not available).

Species delimitation using microsatellite data

Microsatellite data from *Erophylla* conformed to some of the predictions of species delimitation based on regression of genetic distance upon geographic distance. Interspecific pairwise values of Nei's *D* were not correlated with geographic distance between populations ($r^2 = 0.02$, $df = 12$, $P = 0.67$), and the y-intercept was significantly different from zero (y-intercept = 0.79, $P < 0.01$). Intraspecific Nei's *D* values were correlated with geographic distance ($r^2 = 0.74$, $df = 19$, $P < 0.01$). However, contrary to expectation, the y-intercept was significantly different from zero (y-intercept = 0.102, $P = 0.02$) for these comparisons. We made no species determinations because these data did not conform to expectations of the model (Good and Wake 1992).

The frequency distribution of intraspecific and interspecific Nei's genetic distances was bimodal for *Erophylla* (Fig. 2.10) which is expected if sample populations come from two genetically distinct species. However, some intraspecific divergences (Fig. 2.10, asterisks) were unusually high and were similar in magnitude to interspecific divergences. Each of these high intraspecific genetic divergences involved comparisons with Jamaica. Mean Nei's genetic distance between Jamaica and other islands was 0.67 ± 0.06. Removing Jamaica from the analysis yielded a mean Nei's *D* of 0.25 ± 0.02 within

E. sezekorni and 0.75 ± 0.02 between *E. bombifrons* and *E. sezekorni*. Thus, our data support the existence of two species of *Erophylla* but also indicate that Jamaican populations have differentiated from both *E. bombifrons* and *E. sezekorni*.

Estimated recent migration rates from *E. bombifrons* populations to *E. sezekorni* populations was 0.004 ± 0.003 (1 SD) and was 0.006 ± 0.006 (1 SD) in the reverse direction; 95% confidence intervals of these estimates were 0.0001 to 0.0120 and 0.0002 to 0.0219, respectively. Migration rates were nearly identical in three runs of BAYESASS. The lack of gene flow between *E. bombifrons* and *E. sezekorni* supports the existence of two species within *Erophylla*.

DISCUSSION

Species questions

Our initial species hypotheses were: 1) *Brachyphylla* is composed of three species (*B. nana*- Cuba, Grand Cayman; *B. pumila*- Hispaniola, Grand Caicos; *B. cavernarum*- Puerto Rico, Lesser Antilles), 2) *Erophylla* is composed of two species, *E. bombifrons* (Hispaniola, Puerto Rico) and *E. sezekorni* (Jamaica, Cuba, Cayman Islands, Bahamas), and 3) *Phyllonycteris* is composed of two species, *P. aphylla* (Jamaica) and *P. poeyi* (Cuba, Hispaniola). These hypothesized species relationships were based primarily on morphological data in *Erophylla* and *Phyllonycteris* (Morgan 1989, Koopman 1993, Timm and Genoways 2003, Simmons 2005). Our initial hypothesis for *Brachyphylla* was based on cytochrome-*b* sequence data (Davalos 2004). We tested these hypotheses with new datasets from the mitochondrial control region and cytochrome-*b* gene and, in the case of *Erophylla*, from nuclear microsatellites.

Neighbor-joining (NJ), maximum Parsimony (MP), and Bayesian phylogenetic trees for cyt-*b* and concatenated datasets all showed strong nodal support for three reciprocally monophyletic clades within *Brachyphylla* (Figs. 2.1, 2.7 – 2.8). The control region dataset recovered a similar topology but provided only weak bootstrap support for the split between *B. cavernarum* and *B. pumila* (Fig. 2.2). Mean genetic distances between each putative species exceeded the molecular threshold for cytochrome-b, but not for D-loop data (Table 2.2, Fig. 2.9). In addition, cyt-*b* genetic distances among putative species of *Brachyphylla* exceeded levels of intraspecific divergence found within other species of bats ($\bar{x}$ = 1.6%; range = 0.6-2.3%) and, except for *B. pumila*, were within the range of divergences seen between sister species in bats ($\bar{x}$ = 8.3%; range = 3.3-14.7%; Baker and Bradley 2006). Mean cyt-*b* distances distinguishing *B. pumila* (Table 2.2) were slightly above mean intraspecific divergence but well below mean divergence between sister taxa compared to other bats (Baker and Bradley 2006).

Our data supported the hypothesis of three species within the genus *Brachyphylla*: *B. cavernarum* from Puerto Rico and the Lesser Antilles, *B. nana* from Cuba and Grand Cayman, and *B. pumila* from Hispaniola and the Turks and Caicos islands. Our dataset and results were similar to those of Davalos (2004). However, we included data from the mitochondrial control region and multiple individuals from Cuba (Table 2.1). Prior to Davalos (2004), taxonomy in *Brachyphylla* was based on the morphological work of Swanepoel and Genoways (1978), who recognized two species: *B. nana* (subsuming *B. pumila*) and *B. cavernarum*. Contrary to the morphological data, our results and those of Davalos (2004) indicated that *B. pumila* is more closely related to *B. cavernarum* than to *B. nana*. Although *B. pumila* formed a well-supported monophyletic clade in both

studies, taxonomic sampling from Hispaniola and the Caicos Islands was limited, and mean divergence between *B. pumila* and *B. cavernarum* was relatively small (Table 2.2). However, Morgan (2001) hinted that forms of *Brachyphylla* from Hispaniola and the Caicos Islands (*pumila*) were morphologically distinct and may warrant status as a separate species. We follow the recommendation of Davalos (2004) of three hypothesized species within *Brachyphylla*, but caution that further research including morphology, nuclear DNA and increased taxonomic sampling should be conducted to confirm the species status of *B. pumila*.

Cytochrome-*b* data supported the existence of three genetically distinct groups within *Phyllonycteris*. Neighbor-joining, Maximum Parsimony, and Bayesian phylogenetic trees all showed three distinct monophyletic clades corresponding to *P. aphylla* on Jamaica, *P. obtusa* on Hispaniola, and *P. poeyi* on Cuba (Fig. 2.5, 2.7). In addition, the average pairwise genetic distance between clades was higher than the molecular threshold (Table 2.2, Fig. 2.9). These genetic distances were also comparable to standard cyt-*b* distances between sister species of bats (Baker and Bradley 2006). Our CR and concatenated datasets did not include sequences for *P. aphylla* on Jamaica, but did support *P. obtusa* and *P. poeyi* as two distinct, well-supported clades (Figs. 2.6, 2.8). Interestingly, NJ trees based on raw genetic distances indicated that *P. obtusa* and *P. poeyi* were more closely related to each other than to *P. aphylla*. However, Bayesian trees, incorporating a more complex mutational model involving variation in mutation rates among sites and codon positions indicated that *P. aphylla* and *P. obtusa* were more closely related to each other than to *P. poeyi*. The MP analysis recovered the same

relationship as the Bayesian analysis, but with very poor support. More data is needed to resolve evolutionary relationships among these species of *Phyllonycteris*.

Our genetic data showed a clear division between Cuban (*poeyi*) and Hispaniolan (*obtusa*) samples and were contrary to current taxonomic convention, treating the forms of *Phyllonycteris* on Cuba and Hispaniola as a single species, *P. poeyi* (Morgan 1989, Koopman 1993). Though our dataset is limited, with only six samples from Hispaniola and a single sample from Jamaica, we hypothesize that there are three species in *Phyllonycteris*, *P. aphylla*, *P. obtusa*, and *P. poeyi*. We recommend that any change in species status be provisional and contingent upon more complete taxonomic sampling for this genus, a thorough review of morphology, and molecular data from the nuclear genome.

Although our data revealed two well-supported clades within *Erophylla*, corresponding to *E. bombifrons* from Hispaniola and Puerto Rico and *E. sezekorni* from the rest of the Greater Antilles, a single *E. bombifrons* haplotype was nested within the *E. sezekorni* clade (Figs. 2.3 – 2.4, 2.7 – 2.8). There are two possible interpretations of this finding. First, there may be limited gene flow between species. The other interpretation involves incomplete lineage sorting, in which ancestral haplotypes will be shared between clades, even in the absence of gene flow, until sufficient time has passed for those haplotypes to be lost stochastically via genetic drift. Our data are consistent with the incomplete lineage sorting scenario. First, our phylogenetic analyses showed deep, well-supported divergence between *bombifrons* and *sezekorni* clades (Figs. 2.3 – 2.4, 2.7 – 2.8). Second, the *E. bombifrons* haplotype was found in a basal position in NJ cyt-*b* phylogenies and MP cyt-*b* (Fig. 2.3) and MP concatenated analyses (not shown). We

would expect this pattern with incomplete lineage sorting (Avise 2000). With ongoing gene flow between groups, we would expect more haplotypes to be shared, and we would not expect those haplotypes to be basal to the lineage (Avise 2000). Finally, microsatellite data supported the incomplete lineage sorting hypothesis, as mean Nei's *D* between putative species was substantial and estimates of recent migration rates (i.e. gene flow) were essentially zero.

Mean genetic distance between *E. bombifrons* and *E. sezekorni* for the cyt-*b* gene was relatively low (Table 2.2). This value was above the molecular threshold for brachyphylline and phyllonycterine species of bats (1.4%), but was within the range of intraspecific variation found in other studies of bats ($\bar{x}$ = 1.6%; range = 0.6-2.3%; Baker and Bradley 2006). Depending on what threshold value is used, our mitochondrial data alone may be insufficient to differentiate between species of *Erophylla*. However, the preponderance of evidence, including differentiation in several morphological characters (Buden 1976, Timm and Genoways 2003), differentiation at several microsatellite loci, and lack of recent gene flow, supports the existence of two species within the genus *Erophylla*.

Molecular Markers

The molecular markers used in this study performed differently in phylogenetic analyses and species delimitation. Not surprisingly, cyt-*b* performed well at both tasks, whereas CR data did not. Cytochrome-*b* data has been shown to be an excellent molecular marker for differentiating congeneric species (Johns and Avise 1998, Baker and Bradley 2006). On the other hand, CR data performs poorly at this task because of

relatively high intraspecific divergence and potential homoplasy due to higher rates of mutation in this region of the mitochondrial genome (Avise and Walker 1999, Pesole et al. 1999). Nuclear microsatellite markers are more sensitive to recent genetic divergence than mitochondrial data and are useful in quantifying gene flow among putative species. Microsatellite data interpreted against a background of mitochondrial genes can help distinguish between the evolutionary scenarios of incomplete lineage sorting and ongoing gene flow and provide valuable information regarding nascent speciation. Finally, nuclear microsatellite markers can allay fears associated with the sole use of mitochondrial markers for species delimitation, such as maternal inheritance and lack of recombination (Rubinoff et al. 2006).

Tests of Species Delimitation

We used three methods, in conjunction with mitochondrial sequence data, to delimit species. Some methods performed well, but others were not suited to the task of differentiating congeneric and sister taxa. The 10X-rule is often used in DNA barcoding studies as a criterion to delineate confamilial species (Hebert et al. 2004, Hickerson et al. 2006). It is a criterion well-suited for this task (Hajibabaei et al. 2006) but has been shown to require large divergence times (> 2.5 million generations) between taxa to be an effective threshold value (Hickerson et al. 2006). Thus, it is a very conservative criterion and may not be an ideal method for discriminating among recently diverged taxa such as the congeneric species used in our study. Another criterion used in DNA taxonomy and DNA barcoding is reciprocal monophyly (Hickerson et al. 2006). Reciprocal monophyly is less conservative than the 10X-rule and was an effective criterion to determine species

in our study. Incomplete lineage sorting can be problematic for the reciprocal monophyly criterion. Another problem is that any particular phylogeny may have several reciprocally monophyletic, geographically isolated clades that do not conform to typical species definitions. Reciprocal monophyly is most effective when combined with other information such as the magnitude of genetic divergence among taxa. Finally, the molecular threshold method of Lefebure et al. (2006) proved to be an effective criterion for species delimitation in our study. The standard molecular threshold is simply a mean of interspecific divergences for all taxa studied to date (Johns and Avise 1998, Ferguson 2002, Baker and Bradley 2006). The molecular threshold of Lefebure et al. (2006) takes this further by incorporating both interspecific and intraspecific divergences levels into the threshold. The main problem with this approach is that it is sensitive to a priori species designations.

We also used three methods of species delimitation in conjunction with data from nine microsatellite loci. The regression method of Good and Wake (1992) provided results qualitatively similar to expectations for two distinct species of *Erophylla*. However, the specific predictions of this method and what decisions should be made when these predictions are not valid are unclear. In addition, to our knowledge this method has only been used with salamanders (genus *Rhyacotriton*) and needs to be evaluated in more taxonomic groups (Good and Wake 1992, Sites and Marshall 2003). Mismatch distributions (Mallet 1996, Highton 1998, Avise 2000) performed well in discriminating between *E. bombifrons* and *E. sezekorni*. The main problems with this technique are that it is unclear what the appropriate Nei's *D* threshold should be for microsatellite data and that unexpected genetic structure within species (e.g. Jamaican

samples within *Erophylla*; Fig. 2.11) can obscure the results. Finally, we used BAYESASS to determine if there was ongoing gene flow between putative species within *Erophylla*. We estimated recent gene flow to be near zero between these two groups. With any species delineation, it is important to establish the level of gene flow as this parameter is critical in assessing the extent of reproductive isolation. In conclusion, a broad array of quantitative techniques for species delimitation are available (Sites and Marshall 2003) and the potential for more effective techniques is being explored (Crandall et al. 2000, Little and Stevenson 2006, Nielsen and Matz 2006, Vogler and Monaghan 2006). While a single criterion for species delimitation is desirable, our study shows that a variety of methods and markers may provide the best understanding of interspecific divergence.

Conclusions

In general, our molecular data were similar to published morphological data (Buden 1976, Swanepoel and Genoways 1978, Morgan 1989, 2001, Timm and Genoways 2003) with the exception of the discovery of two cryptic species, one each within *Brachphylla* and *Phyllonycteris* (also see Davalos 2004). As some studies have shown, there is often concordance between morphological and DNA taxonomy (Avise and Walker 1999, Vogler and Monaghan 2006). These two approaches are also complimentary. Morphological data can be used to effectively detect incipient speciation, a major weakness of DNA taxonomy (Hickerson et al. 2006), and DNA taxonomy can be used to detect cryptic species, a major weakness of morphological taxonomy (Baker and Bradley 2006). All three genera of bats in our study would benefit greatly from a thorough phylogenetic analysis of morphological traits. Also, more complete taxon

sampling and additional molecular data from the nuclear genome are required to shed light on species relationships within these genera of bats.

Deep ocean passages are significant barriers to dispersal in many taxonomic groups, aiding in the processes of genetic divergence, reproductive isolation, and speciation (Losos et al. 1998, Juste et al. 2004, Carstens et al. 2004, Davalos 2007, Pulvers and Colgan 2007). It appears that even though bats are volant and highly mobile, living on the edge of ocean barriers may still have a profound affect on genetic differentiation (Juste et al. 2004, Pulvers and Colgan 2007). Each bat genus in our study showed substantial mitochondrial genetic divergences among some island populations, and *Erophylla* also showed substantial microsatellite genetic differentiation among islands. The extent to which ocean barriers are responsible for this differentiation, as well as how different bat species respond to these barriers will require further study. However, our results indicate that island bat communities may be important reservoirs of cryptic diversity and attractive targets for future taxonomic investigation.

Table 2.1. Location and number of sequences and genotypes produced in this study. Sequences taken from GenBank are listed separately in the methods. ---- = species not present on island.

	Brachyphylla		*Erophylla*			*Phyllonycteris*	
Location	Cyt-*b*	CR	Cyt-*b*	CR	Msat	Cyt-*b*	CR
Cuba	9	7	8	18	18	6	19
Dominican Republic	2	2	11	29	27	5	6
Jamaica	----	----	7	15	16	0	0
Puerto Rico	5	6	7	24	24	----	----
Abaco	----	----	3	20	20	----	----
Andros	----	----	3	0	22	----	----
Exuma	----	----	3	45	26	----	----
Grand Bahama	----	----	6	43	38	----	----
San Salvador	----	----	1	15	12	----	----
Grand Cayman	5	7	3	3	3	----	----
Cayman Brac	----	----	4	8	8	----	----
Middle Caicos	0	5	4	3	0	----	----
Total	21	27	60	223	214	11	25

Table 2.2. Average Kimura 2-parameter (Kimura 1980) genetic distances for two mitochondrial genes (cyt-*b* and CR) within and between selected taxa. The ratio of mean interspecific to mean intraspecific genetic divergence for each species comparison is listed in parentheses. The ratio should be greater than or equal to ten in species groups that adhere to the 10X rule (see methods) and these species groups are in bold.

Comparisons	Genetic Distance cytochrome-*b*	Genetic Distance control region
Brachyphylla		
Within *B. cavernarum*	0.26 ± 0.05%	0.95 ± 0.37%
Within *B. nana*	0.37 ± 0.10%	0.36 ± 0.19%
Within *B. pumila*	0.58 ± 0.15%	0.66 ± 0.24%
B. cavernarum vs. *B. nana*	6.20 ± 0.83% (**19.6**)	4.81 ± 1.13% (7.3)
B. cavernarum vs. *B. pumila*	2.60 ± 0.45% (6.2)	2.23 ± 0.65% (2.8)
B. nana vs. *B. pumila*	6.37 ± 0.81% (**13.4**)	4.91 ± 1.16% (9.6)
Erophylla		
Within *E. bombifrons*	0.60 ± 0.13%	1.51 ± 0.43%
Within *E. sezekorni*	0.24 ± 0.06%	0.66 ± 0.20%
E. bombifrons vs. *E. sezekorni*	2.10 ± 0.37% (5.0)	2.76 ± 0.73% (2.5)
Phyllonycteris		
Within *P. obtusa*	0.46 ± 0.12%	2.34 ± 0.57%
Within *P. poeyi*	0.95 ± 0.18%	1.52 ± 0.34%
P. aphylla vs. *P. obtusa*	7.83 ± 0.80% (**17.1**)	----
P. aphylla vs. *P. poeyi*	6.76 ± 0.73% (7.1)	----
P. obtusa vs. *P. poeyi*	5.25 ± 0.63% (7.5)	5.95 ± 1.13% (3.1)

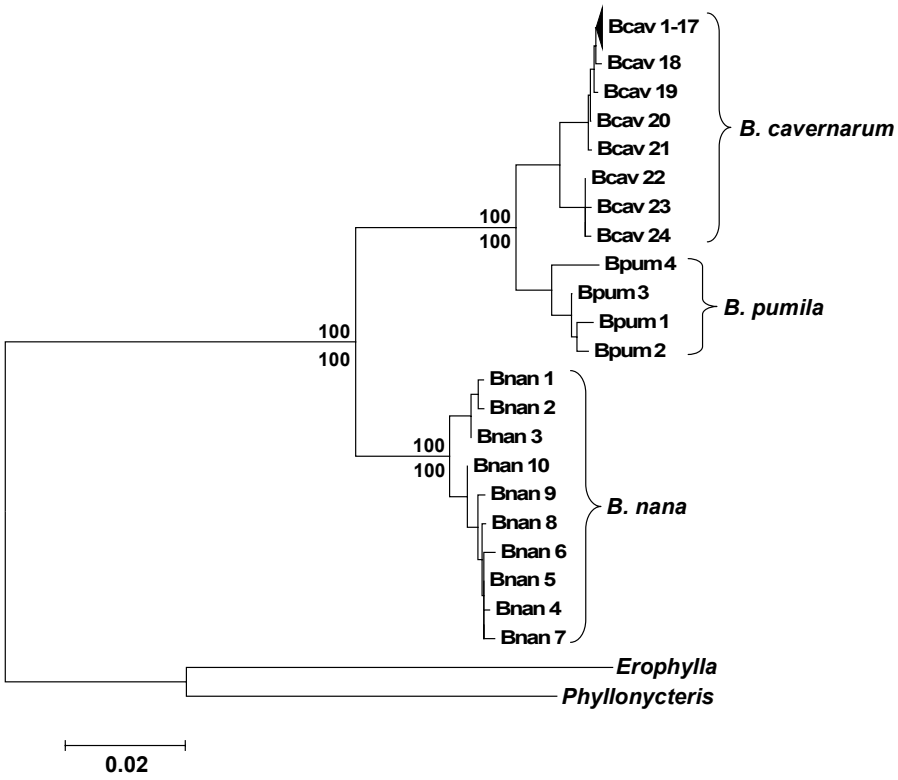

Figure 2.1. Neighbor-joining (NJ) phylogram of *Brachyphylla* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial cytochrome-b sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). The clade containing haplotypes Bcav 1-17 was collapsed to balance the tree. Erophylla and Phyllonycteris were used as outgroups. Haplotype localities are listed in Appendix I.

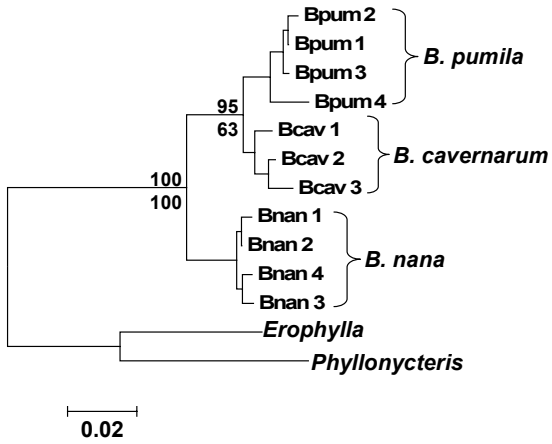

Figure 2.2. Neighbor-joining (NJ) phylogram of *Brachyphylla* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial control region sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). *Erophylla* and *Phyllonycteris* were used as outgroups. Haplotype localities are listed in Appendix I.

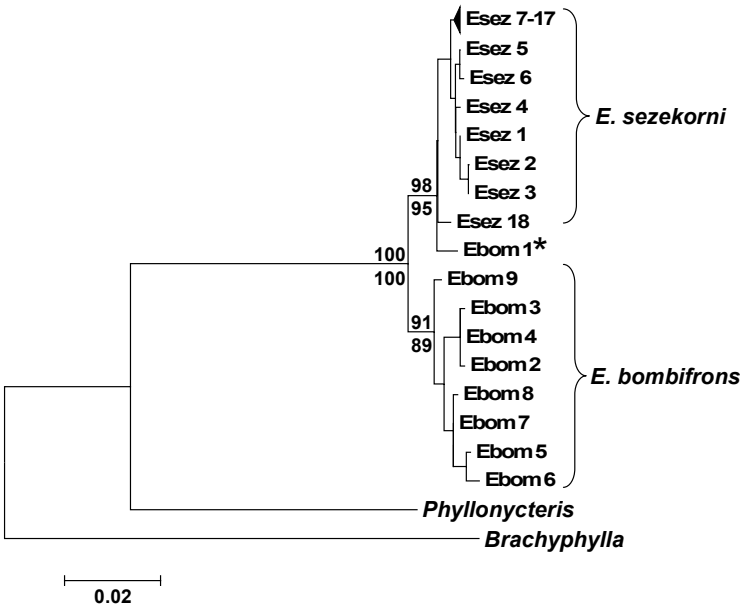

Figure 2.3. Neighbor-joining (NJ) phylogram of *Erophylla* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial cytochrome-*b* sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). The clade containing haplotypes Esez 7-17 was collapsed to balance the tree. The asterisk emphasizes that Ebom 1 is nested within the *E. sezekorni* clade. *Brachyphylla* and *Phyllonycteris* were used as outgroups. Haplotype localities are listed in Appendix I.

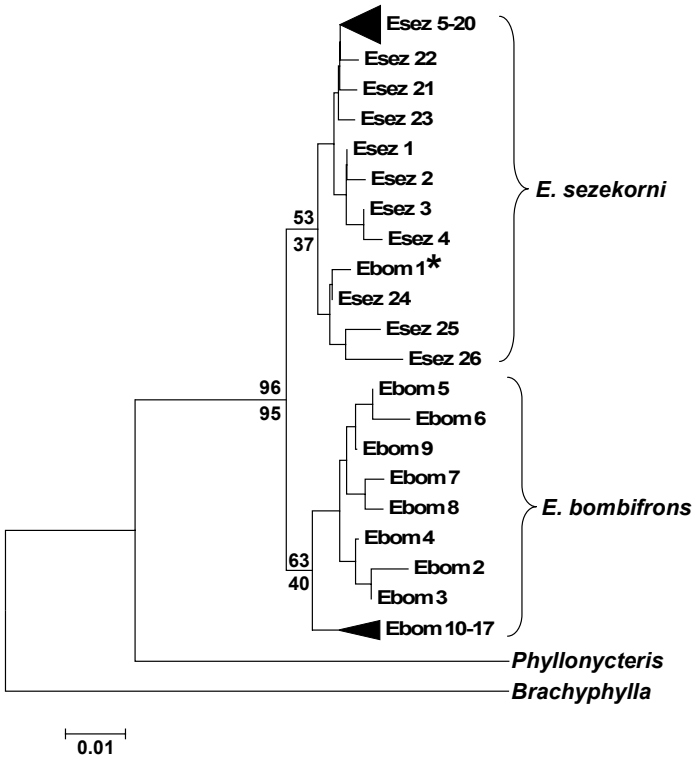

Figure 2.4. Neighbor-joining (NJ) phylogram of *Erophylla* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial control region sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). The clades containing haplotypes Esez 5-20 and Ebom 10-17 were collapsed to balance the tree. The asterisk emphasizes that Ebom 1 is nested within the *E. sezekorni* clade. *Brachyphylla* and *Phyllonycteris* were used as outgroups. Haplotype localities are listed in Appendix I.

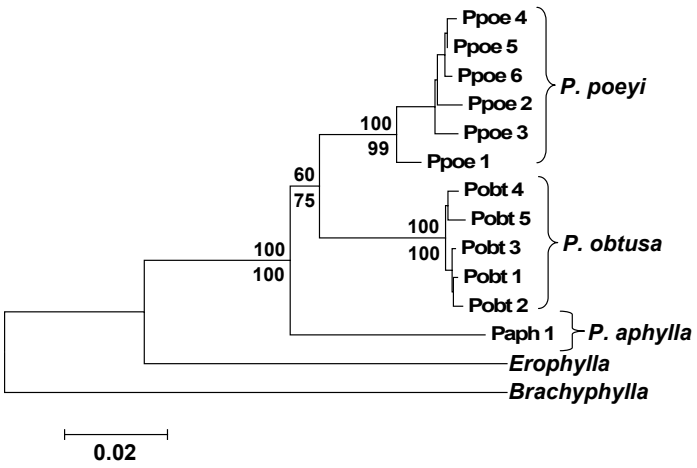

Figure 2.5. Neighbor-joining (NJ) phylogram of *Phyllonycteris* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial cytochrome-b sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). Brachyphylla and Erophylla were used as outgroups. Haplotype localities are listed in Appendix I.

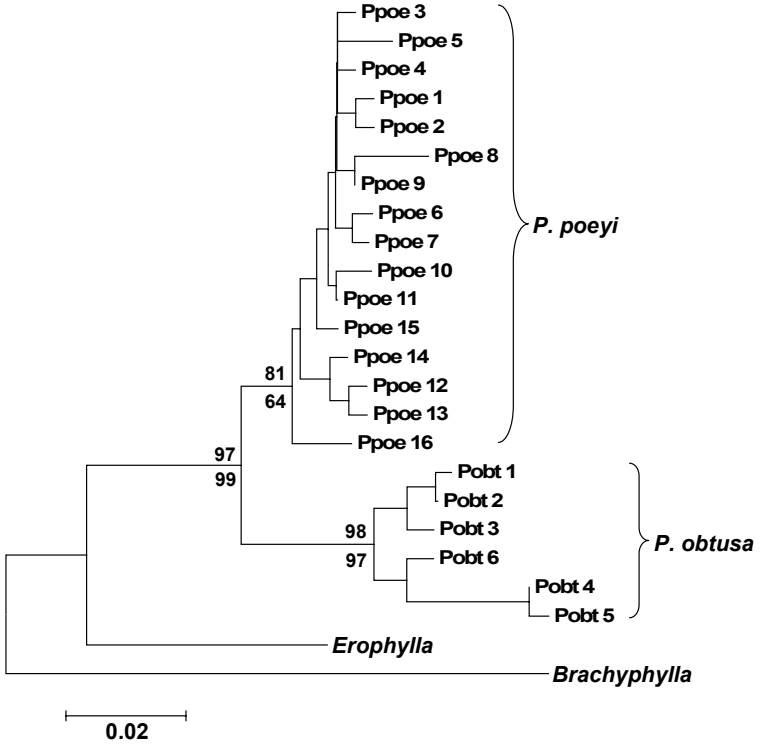

Figure 2.6. Neighbor-joining (NJ) phylogram of *Phyllonycteris* haplotypes based on Kimura 2-parameter genetic distances from mitochondrial control region sequence data. Major nodes separating species groups are labeled with NJ (above node) and maximum parsimony (below node) bootstrap support values (1000 iterations). *Brachyphylla* and *Erophylla* were used as outgroups. Haplotype localities are listed in Appendix I.

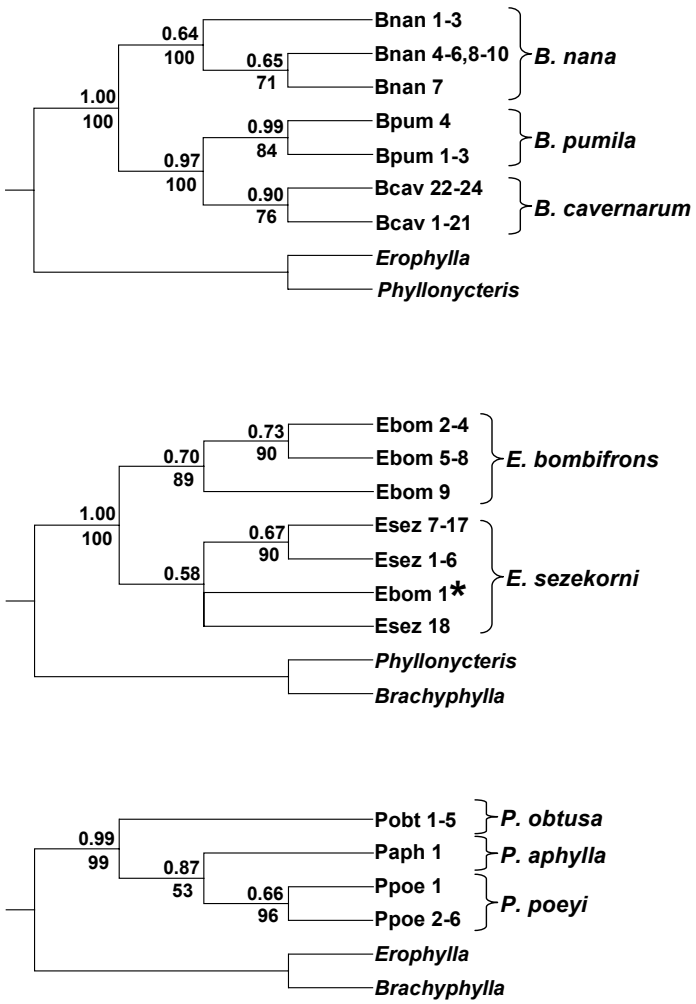

Figure 2.7. Bayesian phylogenetic trees for *Brachyphylla* (a), *Erophylla* (b) and *Phyllonycteris* (c) for mitochondrial cytochrome-*b* haplotypes. Unresolved and poorly supported clades (Bayesian posterior probability ≤ 0.50) were collapsed to simplify the tree with the exception of the *Erophylla* clade containing Ebom 1. The asterisk emphasizes that Ebom 1 is nested within the *E. sezekorni* clade. *Brachyphylla*, *Erophylla* and *Phyllonycteris* were used as outgroups. Haplotype localities are listed in Appendix I.

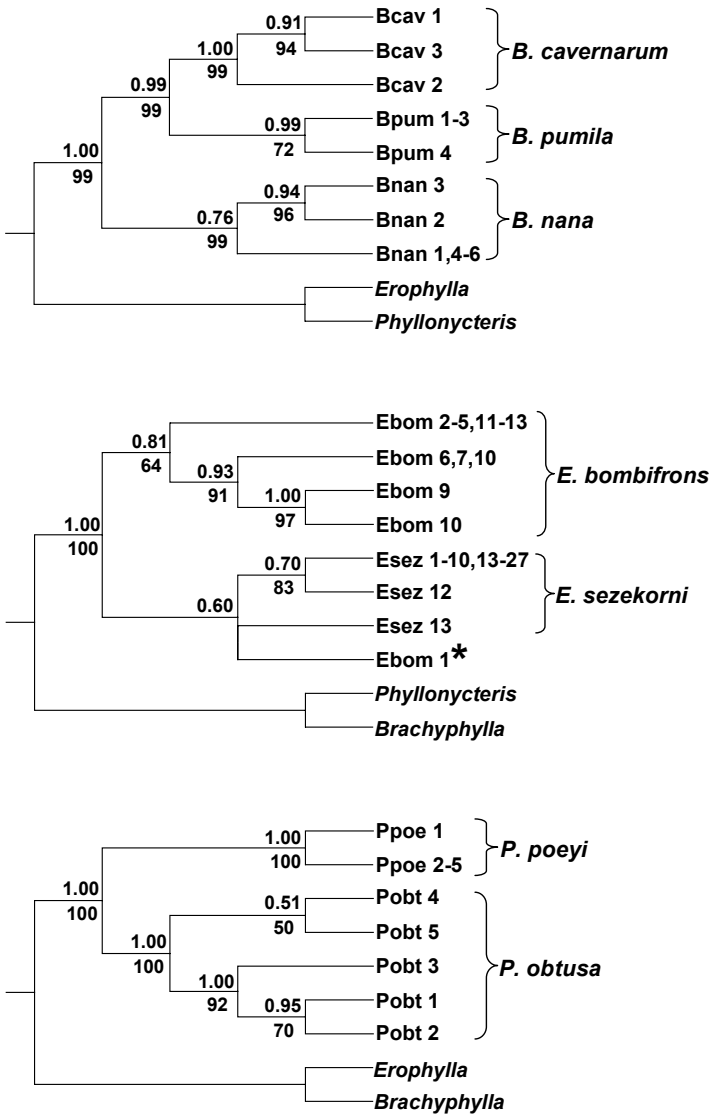

Figure 2.8. Bayesian phylogenetic trees for Brachyphylla (a), Erophylla (b) and Phyllonycteris (c) for a concatenated dataset of mitochondrial cytochrome-b and control region sequences. Unresolved and poorly supported clades (Bayesian posterior probability ≤ 0.50) were collapsed to simplify the tree with the exception of the Erophylla clade containing Ebom 1. The asterisk emphasizes that Ebom 1 is nested within the E. sezekorni clade. Brachyphylla, Erophylla and Phyllonycteris were used as outgroups. Haplotype localities are listed in Appendix I.

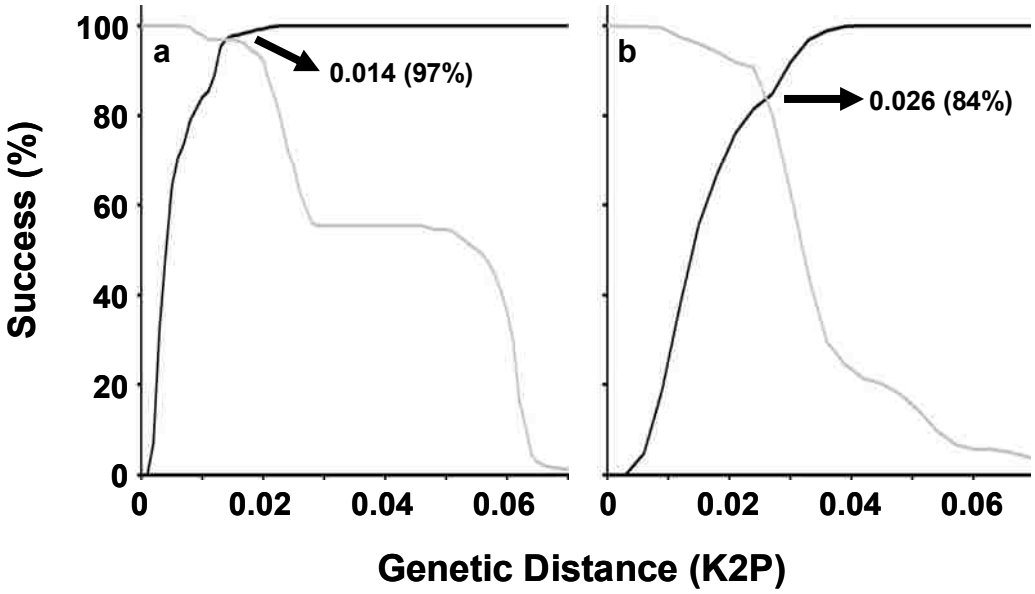

Figure 2.9. Molecular threshold of Lefebure et al. (2006) for combined data from subfamilies Brachyphyllinae and Phyllonycterinae for (a) cytochrome-b and (b) control region data. Graphs shows cumulative probability distribution of the percentage of intraspecific comparisons below a particular genetic distance value (black line) and percentage of interspecific comparisons above a particular genetic distance value (gray line). Arrows indicate the molecular threshold (intersection of white and black lines) with success rate in parentheses. Genetic distances are calculated according to the Kimura 2-parameter (K2P) mutation model.

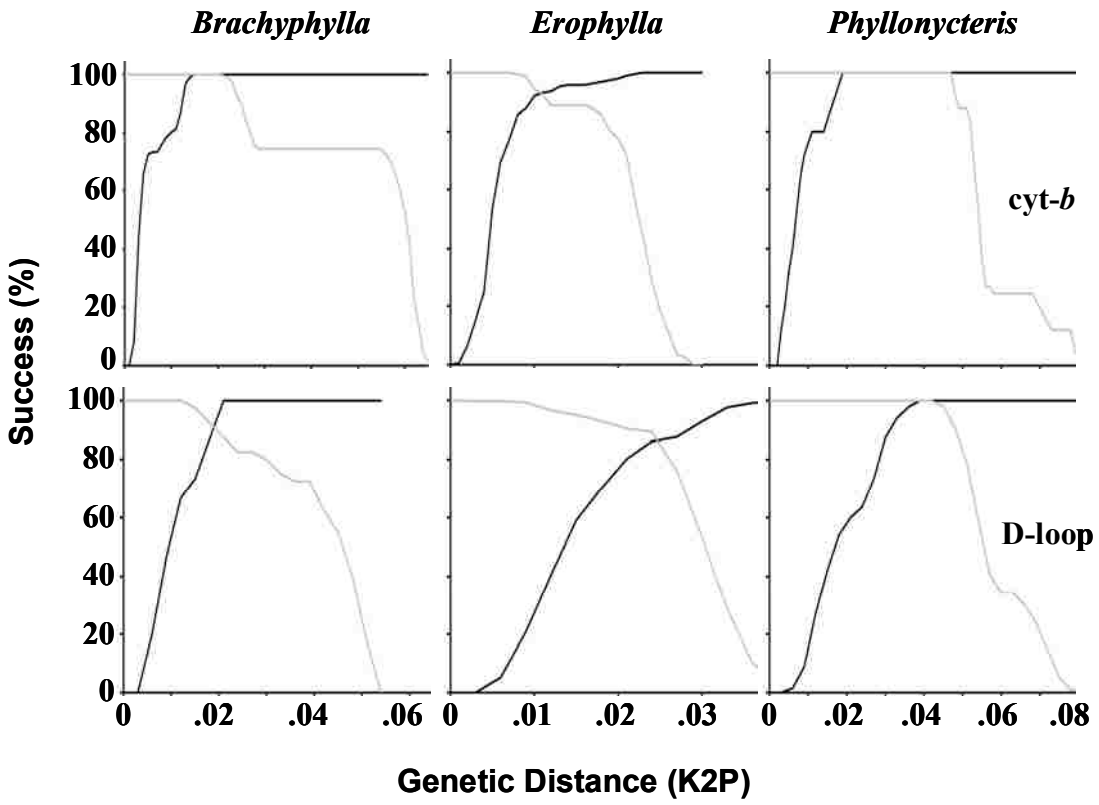

Figure 2.10. Molecular threshold graph of Lefebure et al. (2006) for three genera of phyllostomid bats, Brachyphylla, Erophylla, and Phyllonycteris for cytochrome-b (top panel) and control region (bottom panel) data. Graphs shows cumulative probability distributions of the percentage of intraspecific comparisons below a particular genetic distance value (black line) and percentage of interspecific comparisons above a particular genetic distance value (gray line). Genetic distances were calculated according to the Kimura 2-parameter (K2P) mutation model.

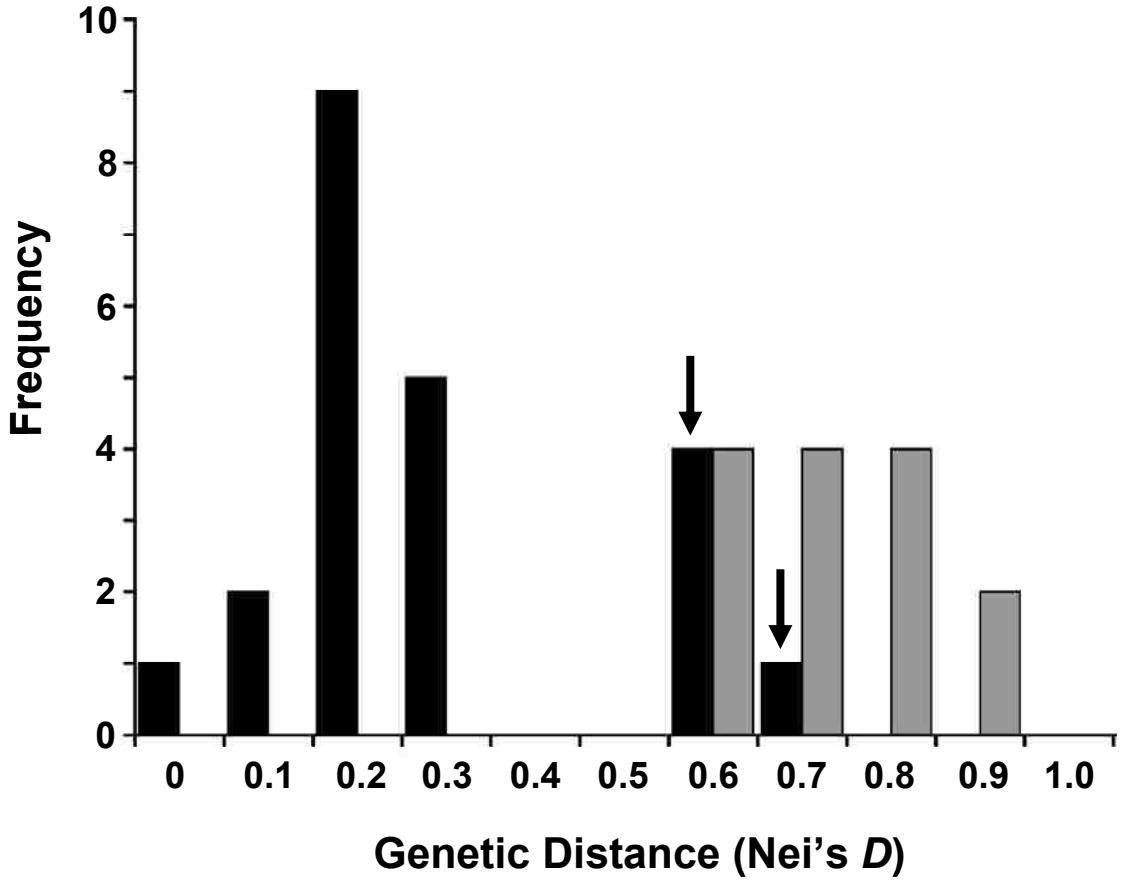

Figure 2.11. Mismatch distribution of intraspecific (black) and interspecific (gray) Nei's genetic distances (Nei's D) for Erophylla bombifrons and E. sezekorni from nine microsatellite loci. Arrows indicate genetic distance values for intraspecific comparisons involving Jamaica. A unimodal distribution indicates that E. bombifrons and E. sezekorni are conspecific and a bimodal distribution indicates that E. bombifrons and E. sezekorni are two distinct species.

CHAPTER III

PHYLOGEOGRAPHY OF THE ENDEMIC GREATER ANTILLEAN BAT GENUS *EROPHYLLA* (CHIROPTERA: PHYLLOSTOMIDAE)

BACKGROUND

The field of island biogeography is currently in the midst of not one but two paradigm shifts (Lomolino 2000, de Queiroz 2005, Cowie and Holland 2006, Heaney 2007). The first shift is away from MacArthur and Wilson's (1963, 1967) elegant equilibrium theory of island biogeography, governed by the processes of colonization and extinction (Lomolino 2000, Heaney 2007). The second paradigm shift is moving the field away from strict reliance on vicariance biogeography and reintroducing dispersal as a critical process influencing biogeographic patterns (de Queiroz 2005, Cowie and Holland 2006). The shift away from these paradigms has been driven by the steady accumulation of empirical evidence, often from phylogeographic studies, contradicting the basic tenets of equilibrium theory and vicariance biogeography (Heaney 2007). The constant species turnover predicted by the equilibrium model, for example, has been refuted by studies showing that species often have long persistence times on islands (Steppan et al. 2003, Glor et al. 2005, Jansa et al. 2006, Roberts 2006a, Davalos *in press*) and those documenting highly variable rates of gene flow (i.e. colonization) among related taxa (Carstens et al. 2004, Hisheh et al. 2004, Heaney et al. 2005, Roberts 2006b, Davalos 2007). Numerous studies, usually based on molecular dating, have also shown that the timing of vicariant events does not match species colonization or divergence times (Trewick 2000, McDowall 2002, Raxworthy et al. 2002, Yoder et al. 2003, de Queiroz 2005, Heinicke et al. 2007).

Two key aspects of phylogeographic studies have been critical in bringing about this paradigm shift: 1) patterns of divergence among taxa, which in island organisms are often associated with oceanic dispersal, and 2) evolutionary timing of divergence, which can highlight the historical circumstances the may have influenced differentiation (Heaney 2007). There are many studies demonstrating that a vast array of taxonomically diverse organisms reach islands via dispersal over water (Hedges et al. 1996, Givnish et al. 2000, Trewick 2000, Davis et al. 2002, McDowall 2002, Raxworthy et al. 2002, Briggs 2003, Yoder et al. 2003, Glor et al. 2005,de Queiroz 2005, Cowie and Holland 2006, Heaney 2007). Phylogeographic studies have also helped document examples of extremely long distance oceanic dispersal (Carranza et al. 2000, Price and Clague 2002, Mummenhoff et al. 2004, de Queiroz 2005). It is apparent from these studies that oceanic dispersal is common in 'geological' time (Heaney 2007), but ocean channels are still significant barriers to gene flow in many species (Glor et al. 2005, Fleischer et al. 2006, Pulvers and Colgan 2007, Steppan et al. 2003) Although volant organisms are considered more vagile than terrestrial organisms, ocean channels still prohibit gene flow in these taxa. In bats, ocean channels usually restrict or impede gene flow (Carstens et al. 2004, Hisheh et al. 2004, Juste et al. 2004, Heaney et al. 2005, Roberts 2006a,b, Davalos 2007, Pulvers and Colgan 2007, Schmitt et al. *in press*), but there are also examples of genetic similarity between geographically distant islands (Carstens et al. 2004, Heaney et al. 2005, Roberts 2006b, Fleming et al. *in press*). However, because most studies of island bats rely upon phylogeographic analysis of mitochondrial DNA, it is unclear to what extent genetic connectedness between islands represents current gene flow or is a signature of historic gene flow.

A second critical issue in evaluating these paradigm shifts is the dating of evolutionary events, such as splits between sister taxa or the colonization time of a particular organism. For example, the Pleistocene (1.8-0.1 mya) has often been invoked as the critical time period in the diversification of a broad array of taxa (Rand 1948, Mengel 1964, Lovette 2005). According to the Late Pleistocene Origins (LPO) paradigm, species ranges contracted to isolated and fragmented refugia during Pleistocene glaciation events, and species diverged and speciated while in isolation (Lovette 2005). However, several studies employing molecular dating techniques have shown that species divergences often pre-date the Pleistocene. For example, in North American passerine birds, Klicka and Zink (1997) and a succession of other studies (Lovette and Birmingham 1999, Cicero and Johnson 2002) showed that a majority of species divergences occurred in the Pliocene and Miocene, well before Pleistocene climatic cycles. In bats, Pleistocene climatic cycles have also been invoked to explain patterns of differentiation within archipelagos (Heaney et al. 2005, Roberts 2006a,b). Griffiths and Klingener (1988) hypothesized the low sea levels during the Pleistocene permitted the colonization of the West Indies by several species of bats. Once again, however, molecular dating has shown that the timing of colonization of several endemic bat lineages in the West Indies occurred during the Pliocene (Davalos *in press*). While the divergence times of higher level taxa of bats have been well-documented (Jones et al. 2005, Teeling et al. 2005, Davalos *in press*), a general consensus regarding the timing of speciation events and intraspecific divergences has yet to be reached.

To further the search for a new paradigm of island biogeography, we studied the phylogeography of the endemic bat genus *Erophylla* (Chiroptera: Phyllostomidae) in the

Greater Antilles. Bats are often the most diverse and common mammals on oceanic islands, making them attractive targets for island phylogeography research (Jones et al. *in press*). We sought to answer two basic questions regarding *Erophylla*, 1) to what extent are ocean channels barriers to both historic and contemporary gene flow, and 2) what effect have Pleistocene sea level changes had on genetic differentiation? To address these questions, we used a population-level approach and attempted to sample approximately 20 individuals from each of several islands throughout the range of *Erophylla*. We generated sequence data from the mitochondrial control region and genotype data from 11 microsatellite loci for these individuals. To enhance the phylogenetic signal of the dataset, we also sequenced the entire cytochrome-*b* gene for a subset of the data. This approach should allow us to elucidate both historical and contemporary phylogeographic structure within these bats.

The Caribbean has been an invaluable natural laboratory in the study of island phylogeography. It has played a central role in the dispersal-vicariance debate (Rosen 1976, 1978, Hedges 1992, 1996, 2001, Iturralde-Vinent and MacPhee 1999), has been the site of several incarnations of the taxon cycle (Ricklefs and Cox 1978, Liebherr and Hajek 1990, Miles and Dunham 1996, Ricklefs and Birmingham 1999, 2002, Cook et al. 2008), and has served as a testing ground for the effects of Pleistocene sea level change and climatic fluctuations (Pregill and Olson 1981, Griffiths and Klingener 1988, Davalos *in press*). Our study was conducted in the Greater Antilles, a geologically complex group of islands in the northern half of the Caribbean basin. The Greater Antilles include four large islands (Cuba, Hispaniola, Jamaica, and Puerto Rico), the Bahamas archipelago, and the Cayman Islands. While the positions of Greater Antillean islands have changed

substantially since their proto-Antillean origin in the Cretaceous (130 mya), they reached their present configuration approximately 10 mya, with the emergence of Jamaica (Graham 2003). Davalos (*in press*) dated the split between *Erophylla* and its sister genus, *Phyllonycteris*, to the Miocene 7.7 mya. Diversification within *Erophylla* should have post-dated this time. Sea level changes during the Pleistocene ranged from approximately 120 m below to 8-10 m above current sea level and had a profound impact on the size and distances between Greater Antillean islands (Pregill and Olson 1981, Graham 2003, Davalos *in press*). At various times during the Pleistocene, much of the present-day land area of the Bahamas and Cayman Islands (including caves) would have been inundated. Climate change during the Pleistocene is also hypothesized to have had a major impact on the fauna of the Greater Antilles (Pregill and Olson1981, Morgan and Woods 1986).

METHODS

The Study Species

The genus *Erophylla* includes two species (Koopman 1993, Timm and Genoways 2003, Simmons 2005) and is a member of the endemic West Indian phyllostomid subfamily Phyllonycterinae (Baker et al. 2003). There are no obvious continental ancestors to the Phyllonycterines in Central or South America. *Erophylla bombifrons* is restricted to Hispaniola and Puerto Rico, while *E. sezekorni* is more widely distributed on Cuba, Jamaica, the Cayman Islands, and throughout the Bahamas (Simmons 2005). Both species are omnivorous (fruits, nectar, and insects)(Soto-Centeno and Kurta 2006) and are thought to have similar life histories, although comparative studies have not been done (Baker et al. 1978, Silva Taboada 1979, Nowak 1994, Gannon et al. 2005).

Erophylla usually roosts in caves and colony sizes range from 50-650 bats (Koopman 1957, Hall et al. 1998, K. L. Murray and T. H. Fleming pers. obs.) Females are monestrous, giving birth to a single pup per year (Buden 1976, Baker et al. 1978, Silva Taboada 1979).

Sample Collection

We collected tissue samples from throughout the geographic range of *Erophylla*. Bats were captured with extendable hand nets in caves or with mist nets at cave entrances. We recorded age, sex, reproductive status, body mass (g), and forearm length (mm) for all captured individuals. A small piece of tissue (2-20 mg) was clipped from the wing and stored in 95% ethanol until analyzed in the lab. Genomic DNA was extracted from 5 mg pieces of tissue using a standard ethanol precipitation procedure or Dneasy® DNA isolation kits (Qiagen) and stored in 50 µl of Tris-HCl, Ph 8.5.

MtDNA sequencing and analyses

We amplified the entire mitochondrial cytochrome-*b* (cyt-*b*) gene with two primer sets, each covering approximately two-thirds of the gene. We used primer set AjaFor1 and AjaRev1 to amplify the first two-thirds of the gene and primer set EroFor2:5'-CCAACCTATTCTCTGCCATCC-3' and AjaRev2 to amplify the second two-thirds of the gene. Primer sequences AjaFor1, AjaRev1 and AjaRev2 were taken from Carstens et al. (2004). We also amplified fragments of approximately 350 bp of mitochondrial control region (CR) using Polymerase Chain Reaction (PCR). Traditional primers used to amplify bat control region fragments (P and F; Wilkinson and Chapman 1991) were

not reliable for *Erophylla*, so we used primer F1:5'-CCCCACCCTCAACACCCAAA-3', redesigned from the *Artibeus jamaicensis* mitochondrial genome (Pumo et al. 1998), coupled with the traditional primer F:5'-GTTGCTGGTTTCACGGAGGTAG-3'.

For both cyt-*b* and CR fragments, total PCR volume was 10 μl, with 1.0 μl Promega 10X buffer (1.5 Mm $MgCl_2$ added), 1 unit *Taq* DNA polymerase (Promega), 0.1 Mm dNTPs, and 14 pmol of each primer. PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72°C for 30 s, with a final elongation step at 72 °C for 5 min. Annealing temperature was lowered to 50 °C when necessary. Before cycle sequencing, DNA fragments were incubated with ExoSAP-IT (USB) to dephosphorylate double-stranded DNA and degrade excess primer.

All fragments were sequenced with Big Dye Terminator Cycle Sequencing Kit, version 1.1 (Applied Biosystems). 10 μl reaction volumes contained 2.5 μl of Big Dye reaction mix, 10-50 ng of template DNA, and 3.2 pmol of forward or reverse primer. The sequencing reaction involved an initial denaturation of 92 °C for 1 min, followed by 25 cycles of 92 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Products were run through sephadex columns (Princeton Solutions) to remove unincorporated nucleotides. Samples were then dried for 30 min with a vacuum centrifuge and resuspended in 15 μl of Hi-Di Formamide (Applied Biosystems) for sequencing. All samples were sequenced in both directions using an ABI 310 automated sequencer. In addition to PCR primers, we used sequencing primers Es-int1:5'-TTCCGATGTTTCATGTTTCTGT-3' and Es-int2:5'-GCACCCCTCCACATATCAARC-3' to sequence the 5' and 3'-end respectively for the cyt-*b* gene. Raw sequence data was edited in SEQUENCHER v.4.5 (Gene Codes). We

aligned all sequences in CLUSTALX (Thompson et al. 1994). We then used aligned sequence files in DNASP v.4.10 (Rozas et al. 2003) to determine unique haplotypes.

Microsatellite genotyping and analyses

We obtained genotype data and 11 microsatellite loci (ES6, 8, 17, 19, 22, 24, 27, 35, 38, 40, 46) for *Erophylla* using the techniques described in Murray et al. (2008). All samples were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Inc.) and scored with GENEMAPPER v.3.0 (Applied Biosystems, Inc.). We used FSTAT v.2.9.3 (Goudet 2001) to test for deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage equilibrium and used MICRO-CHECKER (Van Oosterhout et al. 2004) to screen each locus for null alleles, large allele dropout, and scoring errors. For all tests, we set alpha = 0.05 and used the Bonferroni-correction to account for multiple statistical tests.

Patterns of molecular differentiation

We investigated molecular differentiation by calculating average pairwise genetic distances among islands for cyt-*b*, CR, and microsatellite data. We used MEGA v.4.0 (Tamura et al. 2007) to calculate Kimura 2-parameter genetic distances (K2P; Kimura 1980) for mtDNA data (cyt-*b*, CR) and GENALEX v.6 (Peakall and Smouse 2006) to calculate Nei's genetic distance (Nei's *D*; Nei 1987) for microsatellite data. We used fixation indices to investigate genetic structure within species (Wright 1965). We calculated global Φ_{ST} for *E. bombifrons* and *E. sezekorni* for both cyt-*b* and CR data in ARLEQUIN v.3.01 (Excoffier et al. 2005).

To examine relationships among mtDNA haplotypes in *Erophylla*, we constructed phylogenies using Bayesian analysis (MRBAYES v.3.12; Huelsenbeck and Ronquist 2001) and maximum parsimony (MEGA v.4.0; Tamura et al. 2007). For these analyses, cyt-*b* and CR datasets were concatenated, yielding a final dataset of 1474 bp. In MRBAYES we ran four Markov chain Monte Carlo (MCMC) chains for 5 million generations, sampling every 100 generations. The burn-in period was 500,000 generations and these samples were discarded. Concatenated datasets were partitioned into cyt-*b* and CR parts and the cyt-*b* dataset was further partitioned into three parts corresponding to codon position. We used the Akaike Information Criterion (AIC) test statistic in MODELTEST v.3.7 to determine the most appropriate model of nucleotide evolution (Posada and Crandall 1998, Posada and Buckley 2004). We applied the appropriate nucleotide substitution model (GTR + Γ) to each partition. Indels were ignored in all analyses.

Intraspecific haplotypes are typically a mixture of closely-related ancestral and descendent lineages that are not often amenable to traditional phylogenetic analyses. As an alternative, we constructed statistical parsimony networks for both cyt-*b* and CR datasets using TCS v.1.21 (Clement et al. 2000). This technique used parsimony to construct network diagrams based on the minimum number of mutational steps between haplotypes. Two clades were separated if the number of differences between them exceeded 95% confidence intervals.

Ocean channels as barriers to dispersal

Ocean channels may be significant barriers to dispersal even in volant organisms, and we examined their importance in three ways, 1) analysis of molecular variance

(AMOVA), 2) patterns of shared mtDNA haplotypes, and 3) estimates of recent gene flow. We used AMOVA (Excoffier et al. 1992) in ARLEQUIN to quantify the relative amount of genetic variation at three hierarchical levels, 1) among species, 2) among island populations within species and 3) within island populations. If ocean channels limit dispersal, we expected most of the molecular variation to occur among island populations. Mitochondrial haplotypes shared among islands were compiled by ARLEQUIN. We expected limited haplotype sharing among islands, especially those separated by deeper, more permanent ocean channels. Finally, we estimated rates of gene flow among islands to determine the significance of ocean boundaries as barriers to dispersal. Recent migration rates among islands were estimated with BAYESASS v.1.3 (Wilson and Rannala 2003) using 11 nuclear microsatellite loci. BAYESASS determines the proportion of non-migrants and the proportion and origin of migrants within each population using a Markov Chain Monte Carlo (MCMC) algorithm. Five independent replicate analyses were conducted in BAYESASS, in which MCMC chains were run for 5 x 10^6 generations with a sampling frequency of 100. The first 1 x 10^6 generations were discarded as the burn-in period. We set the frequency of change parameter for migration rates to 0.30, but left other parameters at their default settings. To simplify the analyses, islands on the Little Bahama Bank (LBB; Abaco, Grand Bahama) and Great Bahama Bank (GBB; Andros, Cat Island, Eleuthera, Exuma, Long Island, and New Providence) were grouped together. We omitted the Cayman Islands (n = 11) and San Salvador (n = 12) from the analyses due to small sample size. The final output of BAYESASS estimated gene flow among Cuba, LBB, GBB, Jamaica, Hispaniola, and Puerto Rico. If ocean

channels are barriers to dispersal, then we expected little to no gene flow among island groups.

Effects of the Pleistocene Epoch

The Pleistocene epoch was a time of substantial environmental flux in the Caribbean including drastic changes in climate and sea levels (Pregill and Olson 1981). We first sought to establish the relevance of the Pleistocene to molecular differentiation in *Erophylla* by estimating divergence times among species and islands. If Pleistocene processes were important, then we expected molecular divergences to date to 1.8-0.1 million years ago (mya). Following Weyandt and Van Den Bussche (2007), we used four mutation rates to estimate molecular divergence times of *Erophylla*: 0.5% per million years (Myr) for transversions at the third codon position of the cyt-*b* gene (Irwin et al. 1991), 2.52% per Myr for the control region (Pesole et al. 1999), and 2% and 5% per Myr for concatenated mitochondrial sequences (Arbogast and Slowinski 1998).

Pleistocene sea level changes had several effects on the islands of the Greater Antilles. Lower sea levels during the Pleistocene increased emergent island areas and decreased distances between islands in the Greater Antilles (Pregill and Olson 1981). We expected patterns of genetic divergence among islands to reflect Pleistocene sea-crossing distances (SCD) between islands rather than current SCD. Pairwise Φ_{ST} among islands were calculated in GENALEX and used as an estimate of genetic distance. We estimated contemporary and Pleistocene SCDs using a map of the West Indies and Central America (National Geographic Society 1997). Contemporary SCDs were estimated by measuring minimum distance between current island boundaries. Because sea levels were

approximately 120 m below current levels at the last glacial maxima, Pleistocene SCDs were measured from the 120 m bathymetric contours between islands. Mantel tests were performed in ARLEQUIN to determine if geographic distance matrices were correlated with genetic distance matrices (Mantel 1967). Pairwise Φ_{ST} values were linearized (Φ_{ST} /$(1 - \Phi_{ST})$)(Slatkin 1995), and geographic distances were natural log-transformed for this analysis. We expected genetic distances to be more strongly correlated with Pleistocene sea-crossing distances than with contemporary sea-crossing distances.

Sea levels were also 8-10 m above current sea levels at various times during the Pleistocene. The rise in sea level during the Pleistocene would have inundated most of the current land area of the Bahamas (Pregill and Olson 1981), but would have had less effect on the emergent land areas of the larger, higher islands in the Caribbean (Cuba, Jamaica, Hispaniola, and Puerto Rico). As sea levels lowered, more land area became available and reduced the distance to the nearest major Greater Antillean faunal source pool (i.e., Cuba). We expected *E. sezekorni*, whose range encompasses the Bahamas, to show signs of recent population growth, but not *E. bombifrons* because Hispaniola and Puerto Rico are relatively large, stable islands. We also expected the Bahamas archipelago to have a signature of population growth, but not the larger islands in the Greater Antilles (Cuba, Jamaica, Hispaniola, and Puerto Rico).

To test these predictions, we used ARLEQUIN (Excoffier et al. 2005) and DNASP (Rozas et al. 2003) to conduct standard population genetic analyses for cyt-*b* and CR sequences. To assess genetic diversity, we calculated haplotype diversity (*h*) and nucleotide diversity (π).We then used three general methods to test our data for signatures of recent demographic expansion. First, we calculated the expansion

coefficient (S / d), where S = number of polymorphic sites and d = mean number of pairwise differences among haplotypes (Peck and Congdon 2004). High values of the expansion coefficient are consistent with recent population growth, whereas low values are indicative of stable population size (Peck and Congdon 2004, Russell et al. 2005). Values for S and d were calculated in ARLEQUIN. Second, we used two neutrality tests that can indicate the presence or absence of recent population expansion. We calculated Tajima's D (Tajima 1989) and Fu's F_S (Fu 1997) for each species. A significant negative Tajima's D or Fu's F_S is consistent with recent population expansion (Fu 1997, Peck and Congdon 2004, Russell et al. 2005). Finally, we computed mismatch distributions, plotting the observed frequencies of particular pairwise differences among haplotypes. The expectation of the exponential growth model is a unimodal distribution, whereas a population in mutation-drift equilibrium is expected to have a multimodal mismatch distribution (Slatkin and Hudson 1991, Rogers and Harpending 1992, Rogers 1995). We calculated the raggedness statistic (Rg; Harpending 1994) and Rasmos-Onsins and Rozas's R2 (Ramos-Onsins and Rozas 2002) to test goodness of fit of the observed data to a model of exponential population growth. The mutational time since expansion parameter (τ) along with its 95% confidence intervals were calculated in ARLEQUIN. The generational time since expansion (t) was estimated using the equations $\tau = 2ut$ and $u = \mu k$ where t is the time in generations since expansion, μ is the mutation rate per site per year, and k is the sequence length (Peck and Congdon 2004). We used a mutation rate (μ) of 20% per million years (Petit et al. 1999, Salgueiro et a. 2004). We then multiplied t by the generation time to get actual time since expansion. Estimated generation time for *Erophylla* was 2 years (K. L. Murray and T. H. Fleming unpub. data). Neutrality tests and

mismatch distribution analyses were conducted in DNASP 4.0 (Rozas et al. 2003). Significance of Tajima's D, Fu's F_S, Rg, and R2 were tested with 1000 coalescent simulations in DNASP.

RESULTS

We sequenced the entire mitochondrial cyt-*b* gene (1140 bp) for 60 individuals of *Erophylla* and sequenced 334 bp of the mitochondrial CR for 223 individuals (Table 3.1). One *Erophylla* cyt-*b* sequence from Dominican Republic (AY620438) and one cyt-*b* sequence from Jamaica (AY620439) were taken from GenBank (NCBI). We genotyped 320 individuals from 14 islands at 11 microsatellite loci (Fig. 3.1, Table 3.1). One locus (ES27) was not in HWE and showed evidence of null alleles in Hispaniola and Puerto Rico. We removed this locus from further analyses. The 10 loci we used in our analyses were in HWE and showed no evidence of linkage disequilibrium, null alleles, large allele dropout, or scoring errors.

Patterns of molecular differentiation

Genetic distances (K2P) among islands were greater for CR sequences than for cyt-*b* sequences, but both genes showed similar trends (Table 3.2). Genetic distances between populations of *E. bombifrons* and *E. sezekorni* were always > 2% and approached 3% in some cases. Genetic distances within species were usually < 1%, except for CR sequences from Jamaica compared to other populations of *E. sezekorni*, which exceeded 1%. Microsatellite data showed similar trends as mtDNA but with higher resolution (Table 3.3). There was substantial genetic distance (Nei's *D*) and structure

(F_{ST}) between species. Within *E. sezekorni*, populations from Cuba and the Bahamas and Cuba and Jamaica were very similar, but populations from the Caymans and Jamaica were genetically distinct from one another and from all other populations. Populations of *E. bombifrons* from Hispaniola and Puerto Rico were genetically similar. Global Φ_{ST} from CR data was 0.072 ($P = 0.034$) in *E. bombifrons* and 0.259 ($P < 0.001$) in *E. sezekorni,* showing significant genetic structure within both species. Global Φ_{ST} from cyt-*b* data was -0.057 ($P = 0.805$) in *E. bombifrons* and 0.194 ($P = 0.002$) in *E. sezekorni,* indicating significant genetic structure only within *E. sezekorni*.

Bayesian phylogenetic analysis recovered two clades within *Erophylla* corresponding to *E. sezekorni* (top clade, Fig. 3.2) and *E. bombifrons* (bottom clade, Fig. 3.2). There was very little differentiation within either clade. A single sample of *E. bombifrons* from Hispaniola was grouped within the *sezekorni* clade and was ancestral to all other samples from *E. sezekorni*. Two samples from Jamaica were also ancestral to the rest of the *sezekorni* clade. However, Bayesian posterior probabilities provided only weak to moderate support for the ancestral status of these three samples. There was limited, but well-supported, structure within the *sezekorni* clade among samples from the Bahamas, Bahamas + Cuba, Jamaica, and the Cayman Islands. There was also limited structure within the *bombifrons* clade with samples from Hispaniola and Puerto Rico mixed between lineages.

There were 43 CR haplotypes in 223 individuals and 26 cyt-*b* haplotypes in 62 individuals. Both TCS maximum parsimony networks (CR and cyt-*b*) showed a star-like arrangement of haplotypes within *E. sezekorni*, indicating recent population expansion within this species (Figs. 3.3 – 3.4). Parsimony networks for *E. bombifrons* were not star-

like. Like the Bayesian phylogeny, one haplotype from Hispaniola (B1) was grouped with the *sezekorni* clade (Figs. 3.3 – 3.4). There was a minimum of six mutational steps between *bombifrons* and *sezekorni* clades on the CR network (Fig. 3.3) and the differences between clades on the cyt-*b* network (approximately 18 mutational steps) were great enough for the program to split these two clades apart (Fig. 3.4).

Ocean channels as barriers to dispersal

AMOVA for both cyt-*b* and CR sequences showed that the majority of molecular variation was explained by between-species differences (Table 3.4). Some of the variation between species may be explained by failure of bats to disperse across the Windward Passage between Cuba and Hispaniola or by the ocean barriers separating Jamaica and the Bahamas from Hispaniola. However, species-level processes may also be influencing molecular differentiation between species. There was also a reservoir of genetic variation within island populations (Table 3.4). Counter to expectations, there was very little genetic variation among islands within species (Table 3.4).

For CR data, 10 of 43 haplotypes were shared among islands (Table 3.5). The most common haplotype (S5) was shared throughout the range of *E. sezekorni*. Several haplotypes were shared only within the Bahamas (S1, S3, S7) or between the Bahamas and Cuba (S11). Jamaica shared one haplotype exclusively with Cuba (S6) and one exclusively with Cayman Brac (S16). One haplotype was shared within the Cayman islands (S23). Hispaniola and Puerto Rico only shared haplotypes with each other. Haplotype affinities among islands were similar for CR and cyt-*b* data. Seven of 26 cyt-*b* haplotypes were shared among islands (Table 3.6). The most common haplotype (S6)

was shared by the Cuba and the Bahamas archipelago. Three other haplotypes were shared only within the Bahamas archipelago (S2, S10, S12), and two of those were restricted to the islands of the Little Bahama Bank (S2, S12). One haplotype was shared between Jamaica and Cayman Brac (S14). Again, Hispaniola and Puerto Rico only shared cyt-*b* haplotypes with each other (B4, B7). In general, Jamaica and the Cayman islands shared fewer cyt-*b* haplotypes than CR haplotypes among islands. This difference between cyt-*b* and CR for Jamaican and Cayman samples was also reflected in divergent K2P genetic distances between the two mtDNA genes (Table 3.2).

BAYESASS results showed that there was substantial gene flow between two island pairs, Cuba and the Great Bahama Bank (GBB) and Puerto Rico and Hispaniola (Table 3.7). Gene flow was unidirectional and always occurred from GBB to Cuba (5 of 5 runs) and usually occurred from Puerto Rico to Hispaniola (4 of 5 runs). However, there was little or no gene flow between most of the other island pairs (Table 3.6). These results indicated that ocean barriers can be a significant barrier to dispersal in these bats.

Effects of the Pleistocene Epoch

All estimated divergence times were 1.3 mya or later (Table 3.8), indicating that the both specific and intraspecific differentiation within the genus occurred during the Pleistocene. These data suggest that climate change and eustatic sea level change during the Pleistocene could have had an effect on molecular differentiation in *Erophylla*.

Results of Mantel tests showed that both contemporary and Pleistocene SCDs were correlated with genetic distance in *Erophylla*. For CR data, Pleistocene SCD (r = 0.59; $P < 0.001$) was more strongly correlated with genetic distance than contemporary

SCD ($r = 0.50$; $P = 0.004$). Results were similar for microsatellite data with Pleistocene SCD ($r = 0.80$; $P < 0.001$) being more strongly correlated with genetic distance than contemporary SCD ($r = 0.72$; $P < 0.001$).

Demographic analyses, expansion coefficients, neutrality tests, and mismatch distributions, supported recent demographic expansion in *E. sezekorni* but not in *E. bombifrons*. The goodness of fit statistics (Rg and R2) for the mismatch distributions were only marginally significant, $P = 0.072$ and 0.064, respectively, for the CR data. However, the results of the other demographic analyses indicated recent population growth has occurred in *E. sezekorni* (Table 3.9). There was no evidence of population growth in *E. bombifrons* (Table 3.9, Fig. 3.5).

DISCUSSION

Patterns of molecular differentiation

Our phylogenetic results and network diagrams supported the existence of two clades within *Erophylla* corresponding to the species, *E. bombifrons* and *E. sezekorni* (Figs. 3.2 – 3.4). However, there was a single haplotype of *E. bombifrons* nested within the *sezekorni* clade. This could be an example of an extremely rare dispersal event from Cuba, Jamaica, or the Bahamas to Hispaniola, but it is most likely due to incomplete lineage sorting (see Chapter 1). In other words, this one individual had an ancestral haplotype that was not removed from the population via genetic drift due to insufficient time since divergence. There was also limited evidence that Jamaican haplotypes were ancestral to the *sezekorni* clade (Fig. 3.2).

Intraspecific divergences in both *E. bombifrons* and *E. sezekorni* were small and phylogenetic relationships within each species were generally unresolved (Fig. 3.2). Genetic distances (Tables 3.2 – 3.3) and shared haplotypes (Tables 3.5 – 3.6) showed close genetic affinities among samples from the Bahamas, Bahamas + Cuba, and Hispaniola + Puerto Rico. Based upon cyt-*b* genetic distances (Table 3.2) and one shared CR haplotype (Table 3.5), Jamaica and Cuba were related historically. However, data from both the CR and microsatellite loci, which have a higher mutation rates than the cyt-*b* gene (Pesole et al. 1999), indicated more recent divergence between these islands (Tables 3.2 – 3.3). Analyses in BAYESASS showed that there is little to no recent gene flow between these two islands (Table 3.7). Unfortunately, our datasets from the Cayman Islands (n = 11) and from Middle Caicos (n = 4) were limited. Middle Caicos showed genetic affinities with each sampled population of *E. sezekorni* (Table 3.3, Figs. 3.2 – 3.4). Increased sampling and genotype data from Turks and Caicos and the southern Bahamas are needed to learn more about the evolutionary history of these populations. The Cayman Islands showed genetic affinities with both Cuba and Jamaica (Figs. 3.2 – 3.4). Genetic distance data indicated that the Cayman samples were more closely related to Cuba (Tables 3.2 – 3.3), but more data from the Cayman Islands is needed to resolve this question.

Ocean channels as barriers to dispersal

AMOVA results (Table 3.4) and shared mitochondrial haplotypes among islands several hundred kilometers apart (Tables 3.5 – 3.6), would seem to indicate that there is ongoing gene flow among islands within both species of *Erophylla*. However, there are

two potential explanations for these results, 1) ongoing gene flow, and 2) incomplete lineage sorting. Lineage sorting is the process by which ancestral haplotypes are lost from populations over time via genetic drift. Our data could reflect a historical pattern of gene flow in which ancestral haplotypes are still present in populations due to insufficient time for complete lineage sorting to occur. Our estimates of recent gene flow indicate that both explanations are possible in *Erophylla*. We found evidence of recent gene flow from the Grand Bahama Bank (GBB) to Cuba and from Puerto Rico to Hispaniola (Table 3.7). Thus, shared haplotypes between these islands could be due to ongoing gene flow. However, we found no evidence of recent gene flow among other islands in the Greater Antilles (Table 3.7). For these islands, shared haplotypes are more likely due to incomplete lineage sorting.

To our knowledge, only one other study of island bats has explored the issue of ongoing gene flow versus incomplete lineage sorting. In the Lesser Antilles, Carstens et al. (2004) found discordant patterns of phylogeographic structure among three species of phyllostomid bats. One species (*Ardops nicholsii*) showed island monophyly, haplotypes restricted to a single island and never shared between islands. The other two species (*Brachphylla cavernarum, Artibeus jamaicensis*) shared haplotypes extensively between islands. The study used effective population size and coalescent simulations to estimate the time required for complete lineage sorting. The incomplete lineage sorting hypothesis was ruled out for *A. jamaicensis*, providing evidence for recent gene flow among islands in this species, but not for *B. cavernarum*. While this issue has not been thoroughly examined in insular bats, studies of continental species have shown that incomplete

lineage sorting may be an important factor in explaining the phylogeographic structure of bats (Russell et al. 2005, Campbell et al. 2006).

When it comes to oceanic dispersal, not all ocean channels are created equal. We found that *Erophylla* will cross certain ocean channels, such as the Old Bahama Channel (Cuba to GBB) and the Mona Passage (Puerto Rico to Hispaniola), but not others. We hypothesize that the permeability of ocean barriers is influenced not only by distance across the barrier, but also by the permanence (i.e. depth) of the barrier. A few studies have shown that contemporary sea crossing distance (SCD) is correlated with genetic distances between islands (Hisheh et al. 2004, Roberts 2006b). However, Pleistocene SCD is often more strongly correlated with genetic distances than contemporary SCD (Heaney et al. 2005, Roberts 2006a, Schmitt et al. *in press*). Also, the relatively shallow and ephemeral ocean channels that are typical of Pleistocene aggregate island complexes are often ineffective barriers to dispersal in geological time. Bats from these island complexes tend to be genetically similar (Heaney et al. 2005, Roberts 2006b, Pulvers and Colgan 2007). Deep ocean channels in our study were seldom crossed by bats, regardless of the distance across them. Our results also indicated that ocean channels may be a one-way street for dispersers. The limited recent gene flow that we found was asymmetric, always occurring from Puerto Rico to Hispaniola and from the Great Bahama Bank to Cuba and not in the reverse direction. There are a number of reasons for asymmetric gene flow such as prevailing wind currents or the effective population size of dispersers. Also, hurricanes are possible agents of dispersal. There is limited evidence of hurricane-aided dispersal in bats (Fleming and Murray, in review).

Effects of the Pleistocene Epoch

The late Pleistocene and Holocene were periods of major extinctions in the Caribbean vertebrate fauna (Pregill and Olson 1981, Morgan and Woods 1986, Morgan 2001). Pregill and Olson (1981) found that a surprising number of vertebrate extinctions were of species adapted to xeric habitats (savannahs, grasslands, arid habitats). They attributed these extinctions to Pleistocene climate change, favoring a shift from xeric to mesic habitats. Morgan (2001) found that 52 of 69 local extinction events of bats in Caribbean were of obligate cave-dwelling bats. He hypothesized that high sea levels and climate change during the Pleistocene may have caused bat extinctions in two ways, 1) by inundating caves, and 2) by altering the microclimate of persistent caves. These studies show that the Pleistocene has had a profound effect on the distribution of present-day bats and other vertebrates via extinction. But, what role has the Pleistocene played in diversification and speciation of bats?

Pleistocene climate and sea level changes have been hypothesized to have played a major role in the diversification of phyllostomid bats in the Neotropics and the colonization and diversification of Caribbean bats (Griffiths and Klingener 1988, Hoffmann and Baker 2003). However, neither of these hypotheses has garnered much empirical support from phylogeographic studies. For example, species divergences within several genera of mainland phyllostomids (*Artibeus*, *Carollia*, and *Glossophaga*) and one genus of North American vespertilionid (*Corynorhinus*) date to the Late Miocene-Pliocene (2-6 mya) rather than the Pleistocene (Hoffmann and Baker 2001, Hoffmann and Baker 2003, Piaggio and Perkins 2005, Larsen et al. 2007). Similarly, Davalos (*in press*) found that several lineages of endemic bats in the Mormoopidae, Natalidae, and

Phyllostomidae colonized Caribbean islands in the Miocene and Pliocene, at least a few million years before the Pleistocene. Exceptions to this trend include: (1) bats of the genus *Uroderma*, in which three different chromosomal races (possibly distinct species) diverged during the Pleistocene (Hoffman et al. 2003); (2) bats of the genus *Erophylla* which most likely speciated during the Pleistocene (Table 3.8); and (3) *Artibeus jamaicensis*, which is thought to have colonized the Caribbean in the Pleistocene during periods of low sea level (Phillips et al. 1989, Larsen et al. 2007).

The main role of the Pleistocene in the diversification of bats may have been shaping intraspecific divergence. Several studies have shown that molecular divergence within species and among phylogroups dates to the Pleistocene (Hisheh et al. 1998, Lloyd 2003, Pestano et al. 2003a,b, Piaggio and Perkins 2005, Roberts 2006b, but see Roberts 2006a, Weyandt and Van Den Bussche 2007). For insular species of bats, there are two common intraspecific effects of the Pleistocene evident in phylogeographic patterns. First, studies of bats have often found that Pleistocene sea crossing distances (SCD) are more strongly correlated with genetic distances among islands than contemporary SCD (Heaney et al. 2005, Roberts 2006a, Schmitt et al. *in press*). Our study found the same result for *Erophylla*. It is important to note, however, that sea level changes occurred in the Pliocene as well as the Pleistocene (Haq et al. 1993). “Pleistocene” islands may correspond with Pliocene islands. For example, Roberts (2006a) found genetic similarities within Pleistocene island complexes, but found that divergences among phylogroups from these “Pleistocene” islands dated to the Pliocene. It is important that some form of molecular dating accompany these analyses to confirm that “Pleistocene” effects actually occurred during the Pleistocene.

The second common effect of the Pleistocene on intraspecific phylogeographic patterns in bats is recent population growth or demographic expansion. Many studies of bats have found signatures of recent population expansion during the Pleistocene (Hoffmann et al. 2003, Lloyd 2003, Salgueiro et al. 2004, Russell et al. 2005, Campbell et al. 2006, Roberts 2006b, Fleming et al. *in press*). We found strong evidence of population growth in *E. sezekorni*, but not *E. bombifrons* (Table 3.9, Fig. 3.5). We presume that low sea levels during glacial maxima exposed more land area for colonization, especially in the Bahamas archipelago. Also, more caves should have become available with lower sea levels. We cannot rule out the possibility, however, that population growth in *E. sezekorni* was due to habitat change associated with Pleistocene climate fluctuations. Our study and others show that the Pleistocene epoch has played an important part in the intraspecific divergence of bats.

Summary and Conclusions

Many of the phylogeographic patterns we observed in *Erophylla* are common traits of insular species and are part of the emerging paradigm of island biogeography (Heaney 2007). These traits include oceanic dispersal in geological time, highly variable rates of gene flow between islands in different taxa, marked diversification within lineages, and long persistence times on islands. Oceanic dispersal by *Erophylla* has been common on an evolutionary timescale. In addition to historical gene flow, we found evidence of limited recent gene flow among islands. Rates of both historical and recent gene flow varied substantially between island pairs in *Erophylla*. Some ocean channels formed a semi-permeable barrier to dispersal, whereas others, such as the Windward

Passage, presumably have prohibited gene flow for more than one million years. Moreover, recent gene flow has been asymmetric between islands, indicating that dispersal is not a simple process of exchange of individuals between populations. It may be influenced by a variety of factors including source-sink dynamics, prevailing wind patterns, or hurricanes. *Erophylla* is an endemic genus that has been in the Caribbean for at least 7.7 million years (Davalos *in press*), but its diversification has been limited. Speciation has occurred recently between *E. bombifrons* and *E. sezekorni* (Table 3.8). Also, within *E. sezekorni*, populations of bats from Jamaica and, perhaps, the Cayman Islands, have begun to differentiate (Tables 3.2 – 3.3). Given its long residence in the Caribbean, however, it is surprising that its diversification has occurred relatively recently during the Pleistocene.

Finally, this study shows that in order to understand the pattern and tempo of evolution in island systems, we need studies examining recent as well as historical gene flow. We also need studies that estimate the timing of dispersal and differentiation within genera and species, preferably using multiple independent nuclear and mitochondrial genes. The phylogeography of island organisms can be complex and is influenced strongly by species ecology and the geological history of the island systems involved. To understand this complexity, we need to illuminate as much of the existing phylogeographic pattern as possible.

Table 3.1. Location and number of sequences and genotypes produced in this study.

Location	cyt-*b*	CR	microsat
Cuba	8	18	18
Hispaniola	11	29	27
Jamaica	7	15	16
Puerto Rico	7	24	24
Abaco	3	20	20
Andros	3	0	22
Cat Island	0	0	26
Eleuthera	0	0	31
Exuma	3	45	27
Grand Bahama	6	43	38
Long Island	0	0	23
New Providence	0	0	25
San Salvador	1	15	12
Grand Caicos	4	3	0
Grand Cayman	3	3	3
Cayman Brac	4	8	8
Total	60	223	320

Table 3.2. Mean pairwise Kimura 2-parameter (K2P; Kimura 1980) genetic distances (%) among island populations for *Erophylla*. Control Region distances are above the diagonal and cytochrome-*b* distances are below the diagonal. All populations represent *E. sezekorni*, except for HIS and PR, which represent *E. bombifrons*.

	ABA	**AND**	**EXU**	**GBA**	**SS**	**CAI**	**CUB**	**JAM**	**GCY**	**CYB**	**HIS**	**PR**
ABA	0	----	0.47	0.60	0.45	0.56	0.87	1.44	0.56	0.88	2.83	2.69
AND	0.18	0	----	----	----	----	----	----	----	----	----	----
EXU	0.26	0.09	0	0.49	0.25	0.39	0.68	1.24	0.34	0.66	2.70	2.55
GBA	0.21	0.09	0.18	0	0.36	0.56	0.76	1.38	0.45	0.77	2.80	2.71
SS	0.44	0.26	0.18	0.35	0	0.30	0.46	1.08	0.12	0.44	2.57	2.50
CAI	0.18	0.00	0.09	0.09	0.26	0	0.74	1.34	0.40	0.72	2.87	2.79
CUB	0.22	0.10	0.19	0.16	0.36	0.10	0	1.39	0.54	0.84	2.90	2.86
JAM	0.38	0.21	0.30	0.29	0.47	0.21	0.30	0	1.16	1.29	3.34	3.16
GCY	0.31	0.24	0.32	0.27	0.50	0.24	0.25	0.43	0	0.34	2.58	2.49
CYB	0.35	0.18	0.26	0.26	0.44	0.18	0.28	0.35	0.37	0	2.76	2.63
HIS	2.16	2.04	2.13	2.10	2.31	2.04	2.07	2.02	2.16	2.18	0	1.54
PR	2.19	2.07	2.16	2.13	2.35	2.07	2.11	2.04	2.19	2.21	0.53	0

ABA = Abaco; AND = Andros; EXU = Exuma; GBA = Grand Bahama; SS = San Salvador; CAI = Grand Caicos; CUB = Cuba; JAM = Jamaica; GCY = Grand Cayman; CYB = Cayman Brac; HIS = Hispaniola; PR = Puerto Rico

Table 3.3. Mean pairwise Nei's genetic distances (Nei's *D*) genetic distances (above diagonal) and pairwise F_{ST} among island populations for *Erophylla*. All populations represent *E. sezekorni*, except for HIS and PR, which represent *E. bombifrons*.

	CUB	**ABA**	**AND**	**CAT**	**ELU**	**EXU**	**GBA**	**LNG**	**NP**	**SS**	**CAY**	**JAM**	**HIS**	**PR**
CUB	0	0.30	0.25	0.19	0.17	0.27	0.25	0.18	0.20	0.21	0.43	0.38	0.84	0.90
ABA	0.05	0	0.18	0.15	0.15	0.23	0.06	0.13	0.18	0.29	0.57	0.58	0.84	0.92
AND	0.04	0.03	0	0.10	0.10	0.17	0.14	0.15	0.10	0.22	0.52	0.61	0.75	0.93
CAT	0.03	0.03	0.02	0	0.05	0.13	0.13	0.08	0.08	0.15	0.32	0.57	0.88	0.98
ELU	0.03	0.03	0.02	0.01	0	0.13	0.13	0.08	0.06	0.14	0.37	0.47	0.79	0.93
EXU	0.04	0.04	0.02	0.02	0.02	0	0.23	0.10	0.18	0.18	0.40	0.71	0.80	0.91
GBA	0.04	0.01	0.02	0.02	0.02	0.04	0	0.13	0.13	0.24	0.55	0.57	0.91	1.01
LNG	0.03	0.02	0.03	0.01	0.01	0.02	0.02	0	0.14	0.12	0.39	0.57	0.85	0.98
NP	0.03	0.03	0.01	0.01	0.01	0.03	0.02	0.03	0	0.19	0.47	0.56	0.90	1.06
SS	0.04	0.05	0.04	0.03	0.03	0.03	0.04	0.03	0.04	0	0.35	0.63	1.00	1.02
CAY	0.10	0.12	0.11	0.08	0.09	0.09	0.11	0.09	0.10	0.09	0	0.77	1.04	1.24
JAM	0.07	0.10	0.10	0.09	0.08	0.10	0.10	0.10	0.09	0.11	0.16	0	0.95	1.03
HIS	0.11	0.12	0.10	0.12	0.11	0.10	0.12	0.12	0.11	0.14	0.18	0.14	0	0.24
PR	0.13	0.14	0.13	0.14	0.13	0.13	0.15	0.15	0.14	0.16	0.22	0.17	0.06	0

CUB = Cuba; ABA = Abaco; AND = Andros; CAT = Cat Island; ELU = Eleuthera; EXU = Exuma; GBA = Grand Bahama; LNG = Long Island; NP = New Providence; CAY = Grand Cayman + Cayman Brac; JAM = Jamaica; HIS = Hispaniola; PR = Puerto Rico

Table 3.4. Analysis of molecular variance (AMOVA) from mitochondrial cytochrome-*b* (a) and control region (b) sequence data from *Erophylla bombifrons* and *E. sezekorni*.

Source of Variation	*df*	Sum of Squares	Variance Components	Percent Variation
a) Cytochrome-b				
Among species	1	249.6	9.38	82.6%
Among islands within species	10	21.7	0.05	0.5%
Within islands	50	95.9	1.92	16.9%
Total	61	367.2	11.35	
b) Control region				
Among species	1	259.1	3.10	67.3%
Among islands within species	9	55.4	0.27	5.8%
Within islands	212	262.5	1.24	26.9%
Total	222	577.0	4.61	

Table 3.5. Control region haplotypes shared among islands in two species of *Erophylla*. Each column represents an individual haplotype. Values in columns represent number of individuals on each island with that haplotype. ---- = haplotype was not sampled on island. See Appendix 1 for explanation of each haplotype. S = *E. sezekorni*, B = *E. bombifrons*.

Island	S1	S3	S5	S6	S7	S11	S16	S23	B3	B13
Cuba	----	----	3	2	----	3	----	----	----	----
Abaco	----	10	3	----	4	3	----	----	----	----
Grand Bahama	----	10	11	----	14	6	----	----	----	----
Exuma	----	16	27	----	----	----	----	----	----	----
San Salvador	1	----	14	----	----	----	----	----	----	----
Grand Caicos	1	----	1	----	----	----	----	----	----	----
Jamaica	----	----	1	1	----	----	8	----	----	----
Grand Cayman	----	----	2	----	----	----	----	1	----	----
Cayman Brac	----	----	----	----	----	----	1	7	----	----
Hispaniola	----	----	----	----	----	----	----	----	1	1
Puerto Rico	----	----	----	----	----	----	----	----	10	7

Table 3.6. Cytochrome-*b* haplotypes shared among islands in two species of *Erophylla*. Each column represents an individual haplotype. Values in columns represent number of individuals on each island with that haplotype. ---- = haplotype was not sampled on island. S = *E. sezekorni*, B = *E. bombifrons*.

Island	S2	S6	S10	S12	S14	B4	B7
Cuba	----	5	----	----	----	----	----
Abaco	1	1	----	1	----	----	----
Grand Bahama	1	4	----	1	----	----	----
Andros	----	3	----	----	----	----	----
Exuma	----	2	1	----	----	----	----
San Salvador	----	----	1	----	----	----	----
Grand Caicos	----	4	----	----	----	----	----
Jamaica	----	----	----	----	3	----	----
Grand Cayman	----	----	----	----	----	----	----
Cayman Brac	----	----	----	----	1	----	----
Hispaniola	----	----	----	----	----	1	2
Puerto Rico	----	----	----	----	----	4	3

Table 3.7. Estimates of recent gene flow among island populations of *Erophylla* computed in BAYESASS v.1.3 (Wilson and Rannala 2003). Gene flow occurs from rows to columns. Values represent proportion of migrants between island pairs and proportion of non-migrants (bolded diagonal) within each island. Table presents the results of a single run of BAYESASS. Results were consistent between each of 5 runs with the exception of gene flow estimates between Hispaniola and Puerto Rico. In 1 of 5 runs, the directionality but not the magnitude of gene flow between these two islands was reversed.

	CUB	**LBB**	**GBB**	**JAM**	**HIS**	**PR**
CUB	**0.690**	0.001	0.001	0.006	0.005	0.003
LBB	0.018	**0.990**	0.001	0.008	0.006	0.003
GBB	0.256	0.004	**0.997**	0.016	0.005	0.003
JAM	0.018	0.001	0.001	**0.958**	0.005	0.003
HIS	0.008	0.001	0.001	0.006	**0.678**	0.003
PR	0.010	0.001	0.001	0.006	0.300	**0.987**

CUB = Cuba; LBB = Abaco + Grand Bahama; GBB = Andros + Cat Island + Eleuthera + Exuma + Long Island + New Providence; JAM = Jamaica; HIS = Hispaniola; PR = Puerto Rico

Table 3.8. Estimated divergence times (Myr) within and among species and island populations of *Erophylla*. We used 0.5% per Myr rate of divergence for transversions at the third codon position in the cytochrome-*b* gene (Irwin et al. 1991), 5% (fast) and 2% (slow) per Myr for combined mitochondrial sequences (Arbogast and Slowinski 1998), and 2.52% per Myr for the control region (Pesole et al. 1999). "Caymans" includes samples from Cayman Brac and Grand Cayman. "Bahamas" includes samples from Abaco, Andros (cyt-*b* only), Exuma, Grand Bahama, San Salvador, and Grand Caicos.

	Cytochrome-*b*	Combined mtDNA (fast)	Combined mtDNA (slow)	Control Region
Within Species				
within *bombifrons*	0.10	0.08	0.20	0.59
within *sezekorni*	0.05	0.18	0.45	0.26
Between Species				
bombifrons-sezekorni	0.50	0.44	1.10	1.07
Between Islands				
Jamaica-Cuba	0.07	0.12	0.29	0.54
Jamaica-Caymans	0.14	0.12	0.30	0.49
Jamaica-Hispaniola	0.51	0.45	1.12	1.29
Cuba-Bahamas	0.00	0.06	0.16	0.28
Cuba-Caymans	0.08	0.07	0.19	0.30
Cuba-Hispaniola	0.44	0.44	1.10	1.12
Hispaniola-Puerto Rico	0.09	0.16	0.40	0.60

Table 3.9. Results of demographic analyses to test for recent population growth in *E. bombifrons* and *E. sezekorni* in CR and cyt-*b* mitochondrial genes. Expectation column gives expected outcomes of demographic analyses if there is recent population growth.

Demographic Analysis	*E. bombifrons*		*E. sezekorni*		Expectation
	CR (n = 53)	Cyt-*b* (n = 19)	CR (n = 170)	Cyt-*b* (n = 53)	
Nucleotide diversity (π)	0.016	0.006	0.007	0.002	—
Haplotype diversity (h)	0.905	0.866	0.804	0.800	—
Expansion coefficient (S/d)	4.233	4.986	12.488	10.096	High
Tajima's (1989) D	0.372	-1.195	-1.541*	-1.927*	Significant
Fu's (1997) F_S	-2.111	0.768	-14.416**	-9.453**	Significant
Raggedness (rg)	0.021	0.067	0.031†	0.034*	Significant
Rozas's R2	0.120	0.114	0.039†	0.054*	Significant
Mismatch distribution	Multimodal	Multimodal	Unimodal	Unimodal	Unimodal
Tau (τ)	8.262	7.965	2.687	4.127	—
	(2.53-16.83)	(1.94-14.54)	(0.67-6.69)	(0.77-10.06)	
Time since expansion	—	—	40.2	18.1	
(thousands of years ago)			(10.0 – 217.7)	(3.4 – 44.1)	

† = $0.05 < P < 0.10$; * = $P < 0.05$; ** = $P < 0.01$

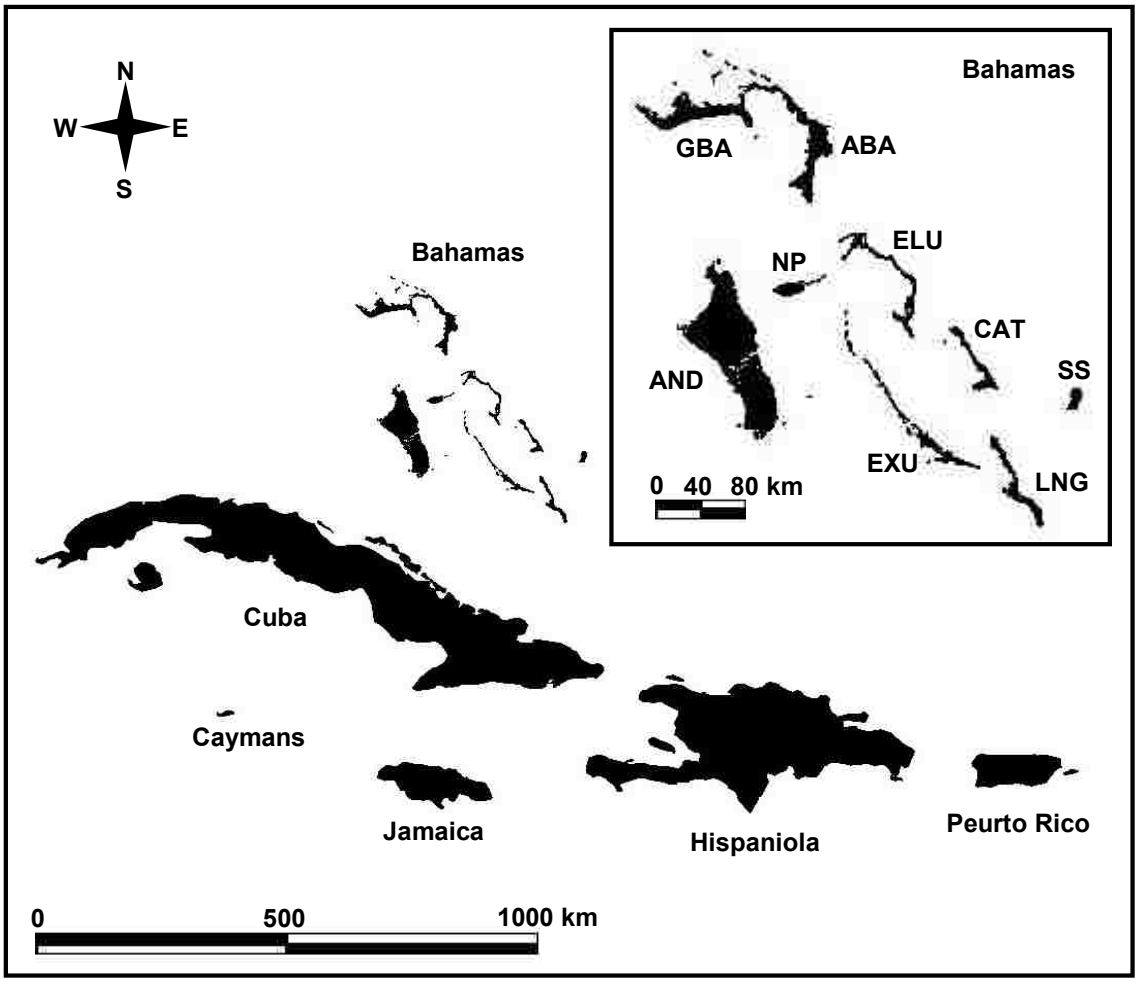

Figure 3.1. Map of the study area, the Greater Antilles and the Bahamas (inset). ABA = Abaco; AND = Andros; CAT = Cat Island; ELU = Eleuthera; EXU = Exuma; GBA = Grand Bahama; LNG = Long Island; NP = New Providence; SS = San Salvador.

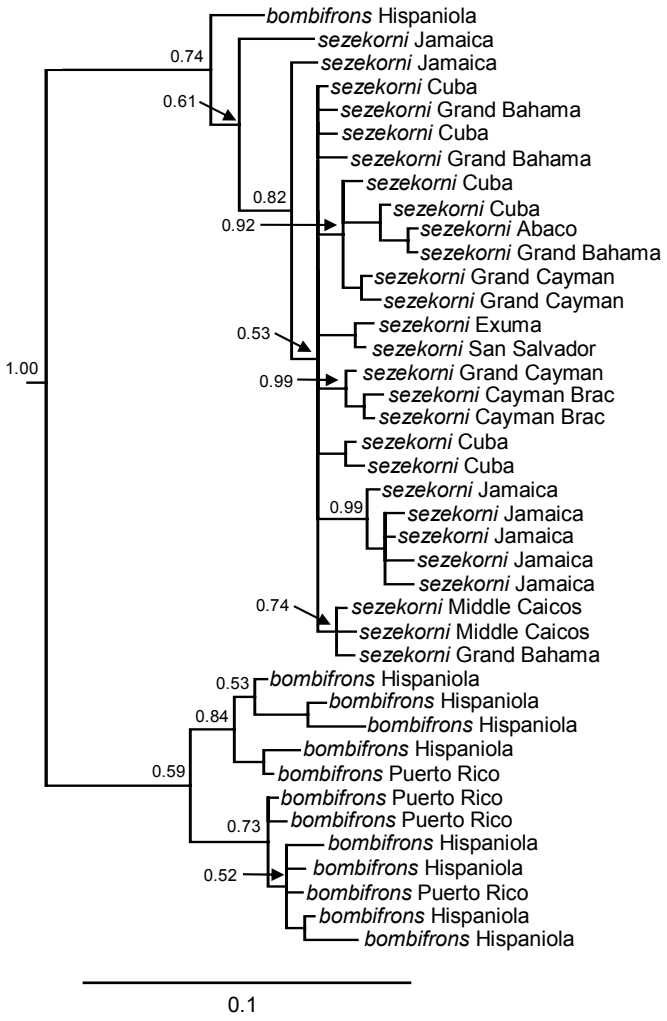

Figure 3.2. Bayesian phylogeny from 1474 bp of concatenated mitochondrial sequence data (cyt-*b* and CR) for genus *Erophylla* produced in MRBAYES v.3.12 (Huelsenbeck and Ronquist 2001). Nodes are labeled with Bayesian posterior probabilities. We ran four Markov chain Monte Carlo (MCMC) chains for 5 million generations, sampling every 100 generations. Samples from the burn-in period (500,000 generations) were discarded. Concatenated datasets were partitioned into cyt-*b* and CR parts and the cyt-*b* dataset was partitioned into codon position. We applied the GTR + Γ model of nucleotide substitution to each partition as determined by MODELTEST v.3.7 to each partition.

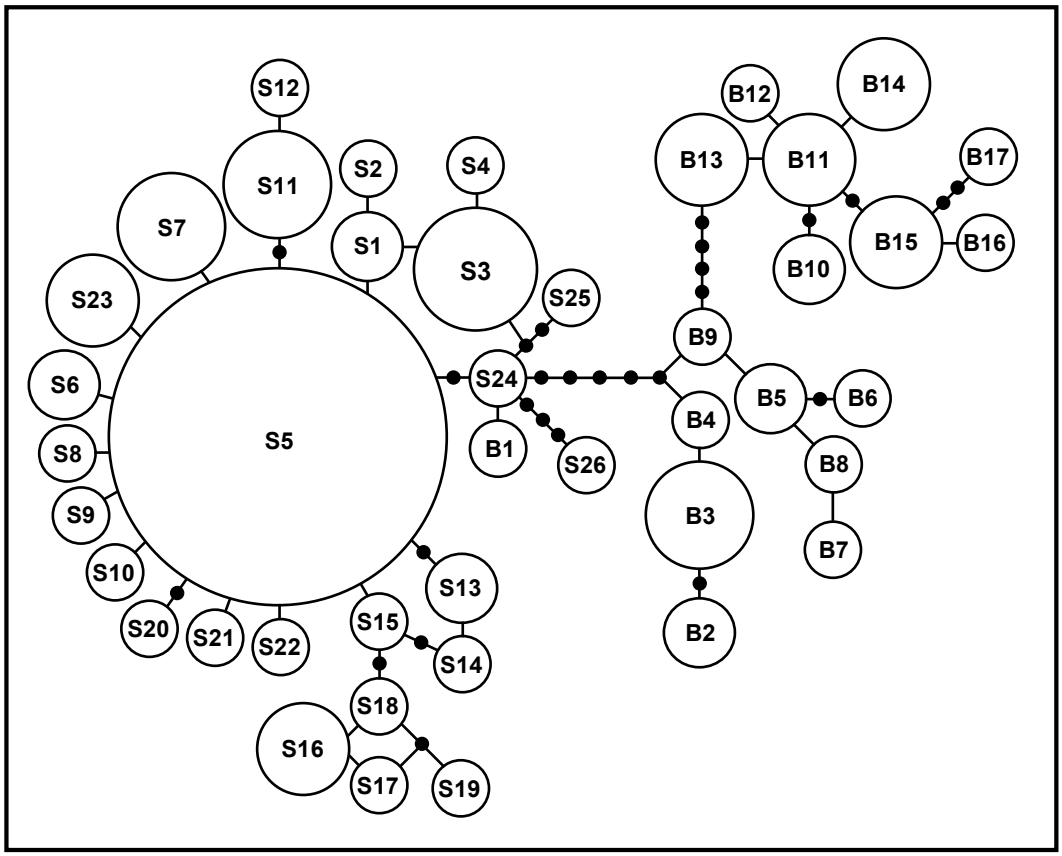

Figure 3.3. Maximum parsimony (95%) haplotype network for genus *Erophylla* produced in TCS v.1.21 (Clement et al. 2000) using mitochondrial control region (CR) sequences. Size of circles is roughly proportional to the number of sampled individuals with that haplotype. Within circles, B = *bombifrons*; S = *sezekorni*. See Appendix II for explanation of haplotypes.

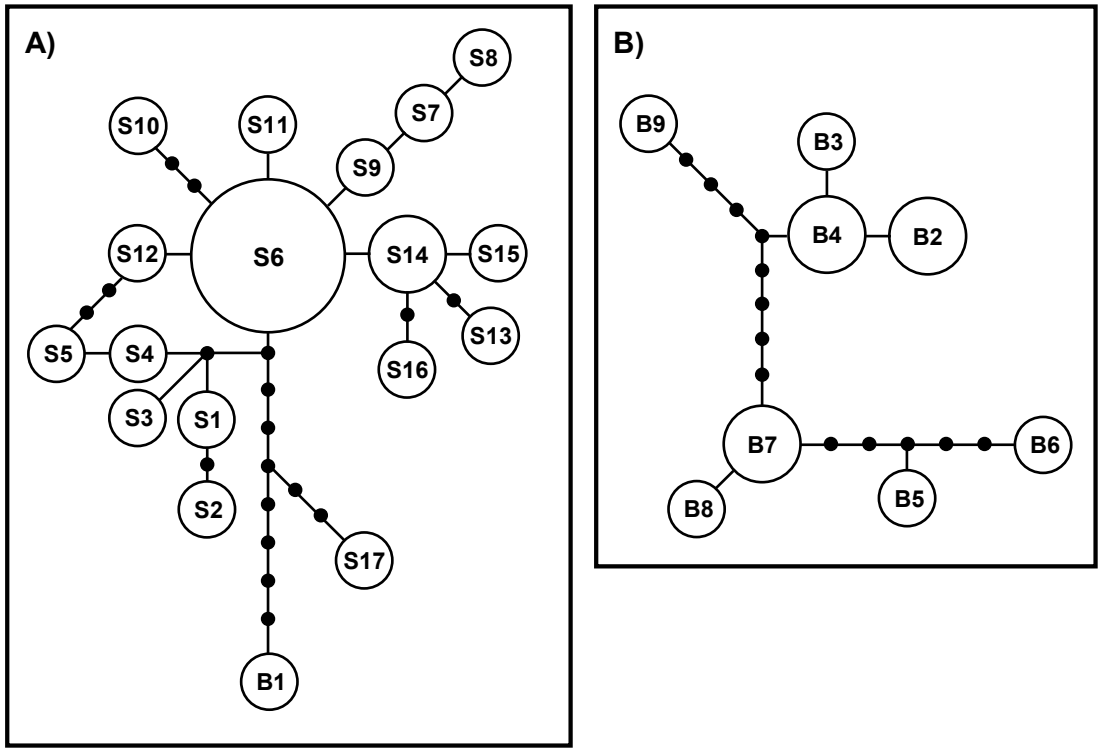

Figure 3.4. Maximum parsimony (95%) haplotype network produced in TCS v.1.21 (Clement et al. 2000) using mitochondrial cytochrome-*b* (cyt-*b*) sequences for *Erophylla sezekorni* (A) and *E.* bombifrons (B). Size of circles is roughly proportional to the number of sampled individuals with that haplotype. Within circles, B = *bombifrons*; S = *sezekorni*. See Appendix II for explanation of haplotypes.

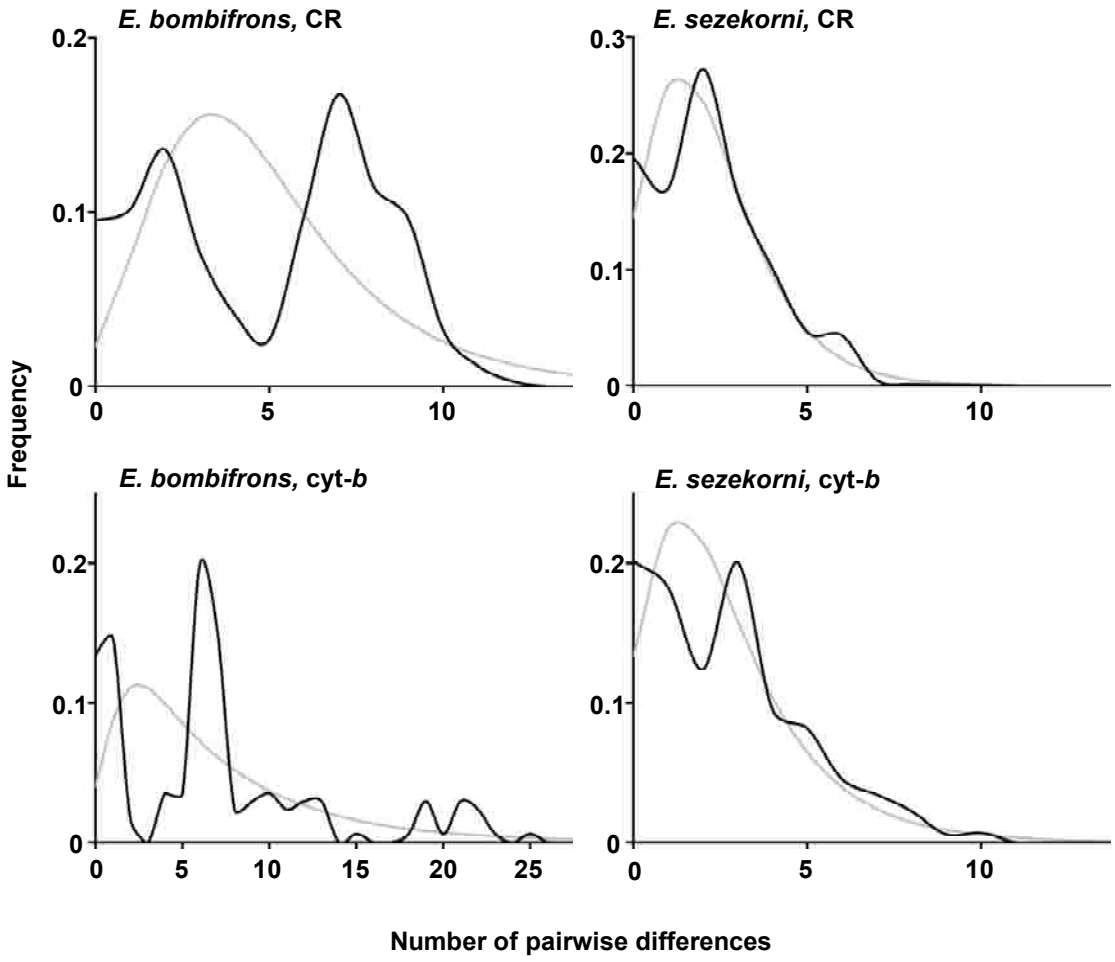

Figure 3.5. Mismatch distributions for *Erophylla bombifrons* and *E. sezekorni* from mitochondrial control region (top panels) and cytochrome-*b* sequences (bottom panels). Black line is the actual frequency distribution of number of pairwise differences between haplotypes. Gray line is the expected frequency distribution given exponential population growth.

CHAPTER IV

GENETIC CONSEQUENCES OF ISLAND LIFE AND SOCIAL STRUCTURE IN THE BUFFY FLOWER BAT, *EROPHYLLA SEZEKORNI*

BACKGROUND

Populations of vertebrates on small oceanic islands are thought to be characterized by small effective population sizes (N_e) and a high degree of isolation relative to mainland populations. In general, genetic diversity is depleted as population size decreases (Soule 1976, Frankham 1996), and these effects can be exacerbated by inbreeding, genetic drift, or reduced gene flow (Frankham 1996, Jordan and Snell 2008). Frankham (1996, 1997, 1998) demonstrated that island populations of both plants and animals had lower levels of heterozygosity and higher inbreeding coefficients than their mainland counterparts. In addition, endemic species with long residence times on islands exhibit less genetic diversity and higher levels of inbreeding than non-endemic species, indicating that the combination of small N_e and long isolation times can cause the erosion of molecular diversity via genetic drift (Frankham 1998). Small population sizes on islands result from small founding populations and may remain small due to limited habitat space or resources (Berry 1986). Within archipelagos, small islands tend to have lower heterozygosity than large islands, consistent with the prediction that populations on small islands should have reduced genetic diversity (Frankham 1996). Thus, the genetic effects of island life have been well-documented for a variety of taxonomic groups.

Mammalian populations typically exhibit these same patterns, with island forms having lower genetic diversity than mainland forms and smaller islands having less genetic diversity than larger ones (Berry 1986, Frankham 1996, 1997, Bidlack and Cook

2001, Cote et al. 2002, Alemeida et al. 2005, Jordan and Snell 2008). However, bats seem to be an exception to this rule. Most studies have found normal, rather than reduced, levels of genetic diversity in island populations (Schmitt et al. 1995, Maharadatunkamsi et al. 2000, Heaney et al. 2005) and comparable levels of genetic variation between island and mainland populations (Juste et al. 1996, 2000, Salgueiro et al. 2004, Fleming et al. *in press*). In most species studied to date, genetic diversity was not correlated with island area (Heaney et al. 2005, Fleming et al. *in press*). However, like other species of mammals, island bats do appear to be somewhat genetically isolated. Rates of gene flow among island bat populations are highly variable but are usually low (Carstens et al. 2004, Hisheh et al. 2004, Juste et al. 2004, Heaney et al. 2005, Roberts 2006a,b, Pulvers and Colgan 2007), although there are several exceptions to this trend (Carstens et al. 2004, Heaney et al. 2005, Roberts 2006b). Thus, the genetic consequences of island life observed in other mammals are usually not as marked in bats.

Although the effects of small N_e, founder effects, and reduced gene flow can all be profound, these are not the only potential factors affecting island populations. Social structure can also affect the underlying genetic structure of a species (McCracken 1987, Chesser 1991a,b, Sugg et al. 1996, Storz 1999). Various aspects of social structure contribute to the maintenance of genetic variation within populations, and this variation, in turn, fuels microevolutionary change. Specifically, social structure of a species, characterized by population sizes, population substructure, adult compositional stability, dispersal patterns, and mating system, affects several critical population genetic parameters, such as effective population size (N_e), genetic structure (F_{ST}), migration rate (Nm), levels of coancestry I, and inbreeding (F_{IS}). As a result, patterns of social

interactions among conspecifics directly influence the magnitude, rate, and direction of microevolution (Sugg et al. 1996, Storz 1999).

Several aspects of mammalian social structure such as small group size, polygyny, and female philopatry (Greenwood 1980, Clutton-Brock 1989), can reduce effective population size and potentially lead to differentiation among social groups and reduced genetic diversity and possibly inbreeding within social groups. However, empirical data indicate that this seldom occurs (Storz 1999). Instead, mammalian social groups instead exhibit low to moderate genetic structure (F_{ST}) among social groups, low relatedness I within social groups, and negative inbreeding coefficients (F_{IS}), indicating an excess of heterozygotes and no inbreeding (Storz 1999). In this instance, bats adhere to the general mammalian pattern and, with few exceptions, exhibit low to moderate genetic structure among populations and low relatedness within social groups (Burland and Worthington Wilmer 2001). There are currently no data regarding genetic structure, inbreeding levels, or relatedness among social groups of bats living on oceanic islands.

Bats are usually the most common and diverse group of mammals on oceanic islands and, therefore, they are the logical choice for a study of genetic effects of island life. The buffy flower bat (*Erophylla sezekorni*) is widespread in the Greater Antilles and is abundant on islands ranging in area from < 100 km^2 to over 100,000 km^2. This species offers a unique opportunity to study the effects of both island life and social organization on population genetic structure. To determine the genetic consequences of island life, we used data from 11 nuclear microsatellite loci and the mitochondrial control region to 1) determine the effect of island area on genetic diversity, 2) look for signatures of genetic bottlenecks, and 3) ascertain the extent to which these island populations are genetically

isolated. To examine the genetic consequences of social structure, we focused our study on the Bahamian island of Exuma. We used microsatellite data from a single colongy (2 caves) on the island to study the effects of social structure, dispersal, and mating system on genetic structure. Our overall goal was to examine the relative impacts of extrinsic (island area and degree of isolation) and intrinsic factors (social structure) on the genetic structure of the buffy flower bat, *E. sezekorni*.

METHODS

Study Species

The buffy flower bat, *Erophylla sezekorni*, is a member of the nectarivorous phyllostomid subfamily Phyllonycterinae (Baker et al. 2003, Simmons 2005). It is endemic to the Greater Antilles and is found on Cuba, Jamaica, the Cayman Islands, and the Bahamas (Simmons 2005). In the Bahamas, buffy flower bats occupy small, cool caves with varying levels of ambient light. Bats roost in mixed-sexed colonies year-round, and colony sizes range from 50-650 bats (Koopman 1957, Hall et al. 1998, Chapter 3). *Erophylla* is a generalist forager, eating fruits, insects, nectar, and pollen (Soto-Centeno and Kurta 2006). Females give birth to a single pup per year with mating occurring from late November through January (Buden 1976, Baker et al. 1978, Silva Taboada 1979). Gestation lasts 4-5 months with parturition occurring in late May or June and lactation extending through August (Buden 1976, Baker et al. 1978, Silva Taboada 1979).

Study Site

The Greater Antilles are located in the northern Caribbean Sea and are comprised of 4 large islands, Cuba, Hispaniola, Jamaica, and Puerto Rico, and 2 groups of smaller islands, the Cayman Islands, and the Bahamas. The Bahamian archipelago is a large group of islands that covers a wide geographic area, approximately 600 miles from north to south (Patterson 2002). The Bahamas were almost completely submerged during periods of high sea level, and habitat availability probably fluctuated with climatic change during the Pleistocene (Pregill and Olson 1981). In addition, Caribbean islands have been exposed frequently to the damaging effects of hurricanes (Tanner et al. 1991, Waide 1991, Gannon and Willig 1994). These factors may have caused cycles of extinction and recolonization and population bottlenecks to occur in *E. sezekorni* on Bahamian islands. To examine the genetic consequence of island life, we focused on the northern Bahamas including 2 islands on the Little Bahama Bank (LBB), Abaco and Grand Bahama, 6 islands on the Great Bahama Bank (GBB), Andros, Cat Island, Eleuthera, Exuma, Long Island, and New Providence, and 1 separate island, San Salvador (Figure 1). To study the genetic consequences of social structure we focused on the Exumas which are a group of small (≤ 290 km^2), low (≤ 40 m above sea level) limestone islands on the southeastern margin of the Great Bahama Bank in the central part of the Bahamas Archipelago. These islands are dominated by dense, broadleaf forests with a 10-15 m overstory (Patterson 2002).

There are 4 known roosts of *E. sezekorni* on Exuma, 1) Cabbage Hill Cave (CHC), Great Exuma; 2) Turnaround Bay Cave (TBC), Great Exuma; 3) Salt Pond Cave (SPC), Great Exuma; and 4) Pasture Cave (PC), Little Exuma. The 4 caves represented

three colonies on the Exumas. Bats from CHC and TBC comprised one colony, and bats from SPC and PC comprised the other two colonies. Roosts were separated by a mean distance of 25 km. For this paper we only used data from the CHC-TBC colony. Direct census counts with a Sony DCR-TRV38 digital camcorder equipped with Sony NightShot and an external IR light source (Sony Electronics, Inc., USA) were conducted from December 2004 to July 2006, and showed that there was a maximum number of 289 bats in CHC-TBC (January 2006), 350 bats in SPC (June 2005), and 149 bats in PC (December 2004). Census counts varied throughout the year, and all three subpopulations used additional, unknown caves on Exuma. Based on these data, total population size of *E. sezekorni* on the Exumas was at least 700-800 individuals.

Sample Collection

We collected tissue samples from approximately 20 individuals from throughout the geographic range of *Erophylla sezekorni*, including Cuba, Jamaica, Abaco, Grand Bahama, Andros, Cat Island, Eleuthera, Exuma, Long Island, New Providence, and San Salvador. On Exuma we attempted to sample as many adults as possible and approximately 50 offspring per year from the CHC-TBC colony. Samples were collected during the mating season (November – January) and the maternity period (June – July) in 2005 and 2006. Bats were captured with extendable hand nets in caves or with mist nets at cave entrances. We recorded age, sex, reproductive status, body mass (g), and forearm length (mm) for all captured individuals. A small piece of tissue (2-20 mg) was clipped from the wing and stored in 95% ethanol until analyzed in the lab. Genomic DNA was

extracted from 5 mg pieces of tissue using a standard ethanol precipitation procedure or Dneasy® DNA isolation kits (Qiagen) and stored in 50 μl of Tris-HCl, Ph 8.5.

MtDNA sequencing and analyses

We amplified approximately 350 bp of mitochondrial control region (CR) using Polymerase Chain Reaction (PCR). Traditional primers used to amplify bat control region fragments (P and F; Wilkinson and Chapman 1991) were not reliable for *Erophylla*, so we used primer F1:5'-CCCCACCCTCAACACCCAAA-3', redesigned from the *Artibeus jamaicensis* mitochondrial genome (Pumo et al. 1998), coupled with the traditional primer F:5'-GTTGCTGGTTTCACGGAGGTAG-3'. For CR fragments, total PCR volume was 10 μl, with 1.0 μl Promega 10X buffer (1.5 Mm $MgCl_2$ added), 1 unit *Taq* DNA polymerase (Promega), 0.1 Mm dNTPs, and 14 pmol of each primer. PCR conditions were: initial denaturation at 94 °C for 2 min, followed by 30 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72°C for 30 s, with a final elongation step at 72 °C for 5 min. Annealing temperature was lowered to 50 °C when necessary. Before cycle sequencing, DNA fragments were incubated with ExoSAP-IT (USB) to dephosphorylate double-stranded DNA and degrade excess primer.

All fragments were sequenced with Big Dye Terminator Cycle Sequencing Kit, version 1.1 (Applied Biosystems). 10 μl reaction volumes contained 2.5 μl of Big Dye reaction mix, 10-50 ng of template DNA, and 3.2 pmol of forward or reverse primer. The sequencing reaction involved an initial denaturation of 92 °C for 1 min, followed by 25 cycles of 92 °C for 10 s, 50 °C for 5 s, and 60 °C for 4 min. Products were run through sephadex columns (Princeton Solutions) to remove unincorporated nucleotides. Samples

were then dried for 30 min with a vacuum centrifuge and resuspended in 15 µl of Hi-Di Formamide (Applied Biosystems) for sequencing. All samples were sequenced in both directions using an ABI 310 automated sequencer. Raw sequence data was edited in SEQUENCHER v.4.5 (Gene Codes). We aligned all sequences in CLUSTALX (Thompson et al. 1994).

Microsatellite genotyping and analyses

We obtained genotype data from 11 microsatellite loci (ES6, 8, 17, 19, 22, 24, 27, 35, 38, 40, 46) for *Erophylla* using the techniques described in Murray et al. (2008). Because the sample from the CHC-TBC colony on Exuma was large, we only genotyped these individuals at 8 loci (ES17, 19, 22, 24, 35, 38, 40, 46). All samples were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Inc.) and scored with GENEMAPPER v.3.0 (Applied Biosystems, Inc.). We used FSTAT v.2.9.3 (Goudet 2001) to test for deviations from Hardy-Weinberg equilibrium (HWE) and genotypic linkage equilibrium and used MICRO-CHECKER (Van Oosterhout et al. 2004) to screen each locus for null alleles, large allele dropout, and scoring errors. For all tests, we set alpha = 0.05 and used the Bonferroni-correction to account for multiple statistical tests.

Effects of island life

Populations with small effective population sizes tend to have reduced genetic variation, and we expected smaller islands to have small N_e and reduced genetic diversity (Soule 1976, Frankham 1996). GENALEX v.6 (Peakall and Smouse 2006) was used to calculate three diversity indices with genotypes from 11 microsatellite loci: 1) mean

allelic richness, 2) expected heterozygosity (H_E), and 3) the inbreeding coefficient (F_{IS}). ARLEQUIN v.3.01 (Excoffier et al. 2005) was used to calculate three additional genetic diversity indices for mitochondrial control region (CR) data: 1) number of polymorphic sites (S), 2) haplotype diversity (h), and 3) nucleotide diversity (π). For the microsatellite dataset, samples from 11 islands were genotyped, and for the mtDNA CR dataset, samples from 6 islands were sequenced. Simple linear regression was used to determine if each measure of genetic diversity was correlated with island area. Island areas were taken from Lack (1976). Regression analyses were performed in SPSS V.15.0 (SPSS Inc., Chicago, USA) with $\alpha = 0.05$ for all statistical tests, unless otherwise noted.

Reduced genetic diversity on some oceanic islands is due to population bottlenecks resulting from small founding populations. Populations that have undergone recent bottlenecks will exhibit both decreased allelic richness and decreased heterozygosity. However, the reduction in number of alleles will be greater than reduction in heterozygosity. As a result, heterozygosity (H_e) will exceed heterozygosity expected at mutation-drift equilibrium (H_{eq}), and this pattern indicates a genetic bottleneck (Cornuet and Luikart 1996, Piry et al. 1999). We used microsatellite data to test for bottlenecks on 11 islands with BOTTLENECK v.1.2 (Cornuet and Luikart 1996, Piry et al. 1999). This program used a Wilcoxon's signed rank test to test the null hypothesis that H_e minus H_{eq} is equal to zero. The alternative hypothesis was that H_e minus H_{eq} > zero, indicating a genetic bottleneck. To implement BOTTLENECK, we used the two-phase mutation model incorporating both single-step and multiple-step mutations at microsatellite loci. Within this model, 95% of mutations were assumed to be single-step and 5% of mutations were multiple-step (Piry et al. 1999, Wang et al. 2005).

Islands populations are often genetically isolated due to reduced gene flow, preventing the influx of new alleles from outside sources and maintaining low levels of genetic diversity. To determine the extent to which island populations of *E. sezekorni* were genetically isolated, we estimated rates of gene flow between 9 Bahamian islands using microsatellite data. Gene flow was estimated by calculating pairwise F_{ST} values among all island pairs and then using the standard equation $F_{ST} = 1/(1 + 4Nm)$ to calculate the effective number of migrants per generation (Nm) (Slatkin and Barton 1989, Slatkin 1993, Heaney et al. 2005). In this equation, N equals the effective population size and m equals the proportion of migrants in the population. Migration rate (M) or gene flow is equivalent to the effective number of migrants per generation (Nm) (Slatkin 1993). Pairwise F_{ST} values among 11 island populations were calculated in FSTAT v.2.9.3 (Goudet 2001).

Effects of social structure

Mammalian populations typically exhibit low levels of structure among social groups and excess heterozygosity and low relatedness within social groups (Storz 1999). In accordance with this pattern, we expected *E. sezekorni* on Exuma to have low values of R within the CHC-TBC colony. To test this hypothesis, we calculated mean relatedness I within the colony for 2005 and 2006 using RELATEDNESS v.5.0.8 (Queller and Goodnight 1989). Allele frequencies for these analyses were estimated separately for each year, and 95% confidence intervals were calculated using the jackknife procedure in RELATEDNESS. Mean relatedness is a standard measure of social structure, but does not always reveal fine-scale social structure within populations (Kerth et al. 2002). In

addition to mean relatedness for each social group, we also calculated relatedness I within sex cohorts for 2006 and 2006 with RELATEDNESS. We then tested for finer-scale social structure by testing for full sibling pedigree relationships within each sex cohort and for the entire colony using KINSHIP v.1.3.1 (Goodnight and Queller 1999). In KINSHIP, we tested the primary hypothesis that alleles between pairs of individuals were identical by descent (IBD) because they were full siblings ($R_p = 0.5$, $R_m = 0.5$). The null hypothesis for all KINSHIP analyses was that alleles from pairs of individuals were not IBD ($R_p = 0$, $R_m = 0$). A likelihood ratio (primary/null) was calculated for each individual pair and the significance of the ratio was determined using the probability distribution generated from 10,000 random pair samplings ($\alpha = 0.05$).

Genetic diversity and relatedness of a population is greatly affected by patterns of dispersal. With few exceptions, dispersal in mammals is male-biased (Greenwood 1980, Clutton-Brock 1989). However, males of *E. sezekorni* held display territories and had high roost fidelity during the mating season. Thus, there was potential for a reversal in the common mammalian trend of male-biased dispersal in *E. sezekorni*. We tested for both male-biased and female-biased dispersal using 3 different measures: 1) inbreeding coefficient (F_{IS}), 2) genetic structure (F_{ST}), and 3) relatedness I. FSTAT v.2.9.3 (Goudet 2001) was used to calculate each measure for both males and females from the CHC-TBC colony for 2005 and 2006. Populations of the dispersing sex were expected to contain a mixture of 2 groups, dispersing individuals and non-dispersing individuals, whereas populations of the non-dispersing sex were expected to contain only non-dispersing individuals. Based on these expectations, F_{IS} (Wahlund effect), and R, will be higher, and F_{ST} will be lower in the dispersing sex. Significance for each test was

determined by randomizing the sex of each individual and recalculating each index of sex-biased dispersal 1000 times. The probability distribution calculated from these data was then used to determine the *P*-value for the actual data ($\alpha = 0.05$).

Mating system can also have a profound effect on social and genetic structure (Burland and Worthington Wilmer 2000, McCracken and Wilkinson 2003). We examined the genetic consequences of mating system for *E. sezekorni* in several ways. To evaluate the potential for polygyny in *E. sezekorni*, mean relatedness values for adult and offspring cohorts were calculated in RELATEDNESS. If there was substantial male reproductive skew (e.g. polygyny), then we expected mean relatedness values for offspring cohorts to be higher than for the adult population. Relatedness was considered significantly different if 95% confidence intervals for offspring and adults did not overlap. We also used KINSHIP v.1.3.1 (Goodnight and Queller 1999) to determine how many offspring in each offspring cohort were paternal halfsibs. We tested the primary hypothesis that alleles from offspring pairs were IBD because they were paternal halfsibs ($R_p = 0.5$, $R_m = 0$) against the null hypothesis that alleles from pairs of individuals were not IBD ($R_p = 0$, $R_m = 0$)..

High relatedness values between dominate and subordinate harem males of the Jamaican fruit-eating bat (*Artibeus jamaicensis*) and among lekking males of the white-bearded manakin (*Manacus manacus*) have demonstrated the potential for kin selection within social groups (Shorey et al. 2000, Ortega et al. 2003). To determine the role that kin selection played in *E. sezekorni*, we tested the hypothesis that groups of displaying males within the colony had higher relatedness than groups of non-displaying males or the population average. We calculated mean *R* for each display class (displaying and non-

displaying). We then compared these values to the mean *R* for the population. Relatedness values were considered significantly different if 95% confidence intervals did not overlap. We also compared the proportion of full-siblings in the displaying male group to the proportion of full-sibling in the non-displaying male group.

RESULTS

Effects of island life

We sequenced the mtDNA control region (CR) from 156 individuals from 6 islands (Table 4.1) and genotyped a total of 257 individuals from 11 islands in the range of *E. sezekorni* (Table 4.2). The 11 loci used in our analyses were in HWE and showed no evidence of linkage disequilibrium, null alleles, large allele dropout, or scoring errors. Linear regression showed that two measures of diversity in the mtDNA CR were significantly correlated with island area (Table 4.3). Both number of polymorphic sites (*S*) and haplotype diversity (*h*) increased as island area increased. Nucleotide diversity of CR samples was not correlated with island area, however, the *P*-value was marginal at 0.061. Unlike CR data, none of the measures of genetic diversity based on nuclear microsatellites were significantly correlated with island area. Although the *P*-value for the correlation of the inbreeding coefficient with island area was marginally significant (0.053), the slope of the relationship was positive meaning that genetic diversity was actually higher (lower F_{IS}) on smaller islands (Tables 42 – 4.3). We found no evidence for genetic bottlenecks in island populations of *E. sezekorni* (Table 4.4). Interestingly, the two largest islands, Cuba and Jamaica, both showed a significant deficiency in heterozygosity. Finally, gene flow (*Nm*) among Bahamian islands was relatively high (2.9

to 2499.8 migrants per generation) indicating that islands were not genetically isolated (Table 4.5). Gene flow was highest between adjacent islands within the Little Bahama Bank (Abaco, Grand Bahama) and the Great Bahama Bank (Andros, Cat Island, Eleuthera, Exuma, Long Island, and New Providence). It was lowest between islands on the different banks and between San Salvador and non-adjacent islands.

Effects of social structure

Relatedness values were very low for the CHC-TBC colony in both 2005 and 2006 (range: -0.019 to 0.001), and there were no significant differences in relatedness values between age and sex cohorts (Table 4.6). Full sibling pedigree relationships occurred in the population, but at a low frequency (< 10%), and the proportion of full-sibs within the population was similar among sex cohorts (Table 4.7). We found no evidence of either male-biased or female-biased dispersal in *E. sezekorni* on Exuma although the *P*-values for male F_{ST} and *R* in 2006 were low (0.067 – 0.069; Table 4.8). Mating system did not seem to have a substantial effect upon genetic structure. Relatedness of the offspring cohort was not statistically different from the adult cohort, indicating that there was not substantial reproductive skew (Table 4.6), and a low frequency of significant paternal half-sibs within the offspring cohort (ca. 4%) confirmed this conclusion (Table 4.7). Finally, relatedness of displaying males and non-displaying males were not significantly different (Table 4.6) and the proportion of full-sib relationships was actually smaller among displaying males (2.9-3.2%) than among non-displaying males (6.5-6.6%) in both 2005 and 2006.

DISCUSSION

Effects of island life

In general mammals fit the island species paradigm with reduced genetic variation, a positive correlation between island size and genetic diversity, and reduced gene flow leading to genetic isolation (Berry 1986, Frankham 1996, 1997, Bidlack and Cook 2001, Cote et al. 2002, Alemeida et al. 2005). However, bats do not fit this model and typically exhibit normal levels of genetic diversity (Schmitt et al. 1995, Juste et al. 1996, 2000, Maharadatunkamsi et al. 2000, Salgueiro et al. 2004, Roberts 2006a), no correlation between genetic diversity and island area (Heaney et al. 2005, Fleming et al. *in press*), and restricted gene flow across semi-permeable ocean barriers (Carstens et al. 2004, Heaney et al. 2005, Roberts 2006b, Pulvers and Colgan 2007, Fleming et al. *in press*). Where does *E. sezekorni* fit into this picture?

Both control region and microsatellite diversity in most island populations of *E. sezekorni* was comparable to that of mainland species (Ruedi and Castella 2003, Salgueiro et al. 2004, Campbell et al. 2006, Fleming et al. *in press*) but was not as high as some extremely diverse mainland species, such as *Artibeus jamaicensis* and *Tadarida brasiliensis* (Russell et al. 2005, Fleming et al. *in press*). For CR sequence data, we found that both number of polymorphic sites and haplotype diversity were correlated with island area (Table 4.3). However, none of our measures of nuclear microsatellite diversity were correlated with island area (Table 4.3). Different results between molecular markers may be because the effective population size of the mitochondrial genome is smaller than that of the nuclear genome (Avise 2000). Thus, founder effects may be more pronounced in mitochondrial genes than in nuclear genes or microsatellites. In addition, the mutation

rate of the mitochondrial control region is lower than that of microsatellite loci (Gibbs et al. 2000, Feulner et al. 2004). This could mean that prior to colonization, microsatellite loci had a higher reservoir of genetic diversity, reducing the impact of colonization and founder effects. An alternative explanation for this pattern could be male-biased dispersal. Post-colonization dispersal by males could have reduced founder effects in nuclear microsatellite loci via gene flow, while maternally-inherited mtDNA genes remained genetically depauperate because of the lack of female gene flow. However, this is an unlikely alternative because Muscarella (2008) showed that there is no evidence for male-biased dispersal among islands in *Erophylla* and our results showed that there is no evidence for male-biased dispersal on Exuma (Table 4.8)

A few studies have tested for evidence of genetic bottlenecks in bats (Petit et al. 1999, Ruedi and Castella 2003, Campbell et al. 2006). Each of these studies tested for genetic bottlenecks due to range contraction during Pleistocene climate change. Although there was evidence for a genetic bottleneck in *Cynopterus sphinx* in southeast Asia (Campbell et al. 2006) and in a few populations (4 of 24) of *Myotis myotis* (Ruedi and Castella 2003), there usually was no evidence for bottlenecks or reduced genetic variation due to range contractions (Petit et al. 1999, Ruedi and Castella 2003, Campbell et al. 2006). No previous studies have tested for bottlenecks in island bats, but it is logical to expect some island populations to have undergone a population bottleneck due to founder effects. Bahamian islands are very low in elevation and were mostly submerged prior to the last glacial maximum (18,000 years bp) (Pregill and Olson 1981). Thus, populations of bats on these islands may have been greatly reduced in size or extirpated. Both reduction in population size due to range contraction or founder effects upon

recolonization may have caused bottlenecks in *E. sezekorni*, but we found no evidence for this (Table 4.4). This could indicate that founding populations were large enough to prevent the loss of genetic diversity or that gene flow due to dispersal or recurrent colonization was sufficient to restore genetic diversity.

Gene flow among Bahamian islands, particularly between adjacent islands, was substantial in *E. sezekorni* (Table 4.5). Generally, one migrant per generation is considered sufficient to counter the genetic differentiation and reduction of genetic diversity caused by genetic drift (Mills and Allendorf 1996). Migration rates (*Nm*) in *E. sezekorni* were higher than this for all island pairs, even between islands on the Great Bahama Bank and Little Bahama Bank. In addition, values of *Nm* in our study were similar to those found in several species of island bats in the Philippines that exhibited high gene flow (Heaney et al. 2005). While the interpretation of *Nm* calculated from pairwise F_{ST} values may be problematic (Whitlock and McCauley 1999), these data show that either current or historical gene flow between islands has been high enough to homogenize the genetic structure of *E. sezekorni* in the Bahamas.

While most island bat populations have normal levels of genetic diversity, two studies have found that unusually small and isolated islands have populations with reduced genetic diversity (Heaney et al. 2005, Roberts 2006a). Both studies found that genetic diversity was reduced in several bat species occupying the islands of Sibuyan and Mindoro in the Philippines. These two islands are small in size and isolated from other islands by deep ocean channels. A somewhat analogous situation may be found in *E. sezekorni* on San Salvador. San Salvador is a small island (163 km^2) and is isolated from the Great Bahama Bank by a deep ocean channel. Bats on this island showed a marked

reduction in all three indices of mtDNA CR diversity (Table 4.1). The microsatellite data were equivocal but did show that San Salvador had unusually low values of expected heterozygosity and allelic richness as well as reduced levels of gene flow (Table 4.5).

Effects of social structure

The genetic structure of mammals is characterized by low to moderate levels of genetic structure among social groups, low levels of inbreeding and relatedness, and excess heterozygosity, and, in this case, bats are no exception (Storz 1999, Burland and Worthington Wilmer 2001). Bats conform to this general mammalian pattern of genetic structure, but the social structure underlying this pattern varies markedly. In temperate species of bats, males are usually solitary during the maternity period. Temperate species usually have strong female philopatry and male-biased dispersal but are often promiscuous rather than polygynous. Thus, skew in male reproductive success is low in these species (Petri et al. 1997, Rossiter et al. 2000, Burland et al. 2001). Either extreme male-biased dispersal or breeding dispersal by males and females is thought to keep levels of inbreeding and relatedness low in temperate species. A variety of forms of breeding dispersal have been demonstrated, including extra-colony copulation (Burland et al. 1999), mating during fall swarming behavior (Kerth et al. 2003), and mating along migratory routes (Petit and Mayer 1999).

In contrast, tropical species typically form mixed-sex colonies during the reproductive period. These social units may last for just the mating season or year-round (Bradbury 1977). Tropical species are usually polygynous, often with extreme levels of male reproductive skew (McCracken and Bradbury 1977, Storz et al. 2001b, Heckel and

Helversen 2002, Ortega et al. 2003), but exhibit a wide variety of dispersal patterns. For example, the greater spear-nosed bat, *Phyllostomus hastatus*, shows both male and female natal dispersal (McCracken and Bradbury 1981), while the greater white-lined bat (*Saccopteryx bilineata*) is one of the few mammalian examples of male philopatry and female-biased dispersal (Bradbury and Vehrencamp 1977). The variety of dispersal patterns shown by tropical species apparently fosters sufficient gene flow to maintain low levels of inbreeding. Ecological factors, such as high juvenile mortality or high rates of turnover among harem males, may have a similar effect, maintaining genetic variation within chiropteran social groups (Wilkinson 1985). Thus, bat species employ a variety of behavioral and life history strategies to avoid problems associated with potentially high within-colony relatedness and inbreeding depression.

Relatedness values were uniformly low in *E. sezekorni* on Exuma (Table 4.6). Kerth et al. (2002) cautioned that colony-wide estimates of relatedness may underestimate the potential for kin selection among groups of related individuals within the colony. They found a high proportion of first and second degree pedigree relationships within colonies of *Myotis bechsteinii* despite very low mean values of within-colony relatedness. This was not the case in *Erophylla sezekorni*. Only a small percentage of the colony (< 10%) had full sibling relationships (Table 4.7).

Although male-biased dispersal is the most common pattern, bats exhibit a diverse array of dispersal patterns ranging from extreme male-biased dispersal in *Myotis bechsteinii* (Kerth et al. 2002) to female-biased dispersal in *Saccopteryx bilineata* (Bradbury and Vehrencamp 1977, McCracken 1984). We found no evidence of either male-biased or female-biased dispersal for *E. sezekorni* on Exuma (Table 4.8).

Muscarella (2008) found similar results for populations of *Erophylla bombifrons* and *E. sezekorni* throughout the Greater Antilles.

Murray and Fleming (Chapter 3) used paternity analysis to document polygyny in *E. sezekorni*. However, their study found that male reproductive skew was mild. Our results were similar to those. We found that levels of relatedness within offspring cohorts for both 2005 and 2006 were comparable to relatedness within adult cohorts, indicating weak or no male reproductive skew (Table 4.6). In addition, we found only a small proportion of paternal half-siblings (< 5%) in the offspring cohorts in 2005 and 2006 (Table 4.7). This result shows that polygyny occurs in this species, but infrequently. We caution that the mild reproductive skew should not be dismissed as a potential force for sexual selection. Rossiter et al. (2006) found that slight annual male reproductive skew in *Rhinolophus ferrumequinum* accrued to significant fitness benefits for some individuals by greatly increasing lifetime reproductive success. We do not know the average lifespan of *E. sezekorni*, but we do know that some individuals survive for at least two mating seasons. Finally, a few other studies have found high relatedness values between males within social groups demonstrating the potential for kin selection (Shorey et al. 2000, Ortega et al. 2003). In the Jamaican fruit-eating bat (*Artibeus jamaicensis*), dominant and subordinate harem males are father-offspring pairs. Their inclusive fitness is increased because two males are able to maintain larger harems. Shorey et al. (2000) found that male white-bearded manakins (*Manacus manacus*) had elevated relatedness values within leks. We found no evidence for increased relatedness among displaying males of *E. sezekorni* (Tables 4.6 – 4.7), indicating that kin selection was not a factor in maintaining

groups of displaying males. A similar situation occurs in six species of manakins in lowland Ecuador (B. Loiselle, pers. comm.).

Table 4.1. Summary of island areas and mtDNA control region genetic diversity for *Erophylla sezekorni* from 6 islands in the Greater Antilles. *S* = number of polymorphic sites; *h* = haplotype diversity; π = nucleotide diversity. Island areas were taken from Lack (1976).

Island	Area (km^2)	*n*	*S*	*h*	π
Cuba	114,000	18	12	0.93 ± 0.04	0.0078 ± 0.0049
Jamaica	11,400	15	13	0.73 ± 0.12	0.0114 ± 0.0068
Grand Bahama	2,000	43	7	0.77 ± 0.03	0.0056 ± 0.0036
Abaco	1,100	20	5	0.70 ± 0.08	0.0059 ± 0.0039
Exuma	290	45	4	0.52 ± 0.05	0.0032 ± 0.0024
San Salvador	163	15	1	0.13 ± 0.11	0.0004 ± 0.0007

Table 4.2. Summary of island areas and genetic diversity at 11 microsatellite loci for *Erophylla sezekorni* from 11 islands in the Greater Antilles. Allelic ricness = number of alleles; H_e = expected heterozytosity; F_{IS} = inbreeding coefficient. Island areas were taken from Lack (1976).

Island	Area (km^2)	*n*	Allelic richness	H_e	F_{IS}
Cuba	114000	18	7.818	0.737	0.005
Jamaica	11400	15	6.364	0.639	0.035
Andros	4200	22	7.455	0.742	-0.004
Grand Bahama	2000	38	6.545	0.705	-0.029
Abaco	1100	20	6.091	0.695	-0.030
Eleuthera	420	31	7.545	0.742	-0.042
Cat Island	410	26	7.091	0.729	0.007
Long Island	340	23	7.091	0.705	-0.034
Exuma	290	27	7.182	0.759	-0.048
San Salvador	163	12	5.636	0.664	-0.089
New Providence	150	25	7.455	0.758	-0.004

Table 4.3. Summary of linear regression analyses examining the correlation between island area and genetic diversity for mtDNA control region and microsatellite datasets.

Diversity statistic	Slope	r	r^2	*P*-value	*df*
Control region					
Number of polymorphic sites (*S*)	1.777	0.923	0.851	0.009	4
Haplotype diversity (*h*)	0.095	0.832	0.693	0.040	4
Nucleotide diversity (π)	0.001	0.792	0.627	0.061	4
Microsatellite					
Allelic richness	----	0.244	0.060	0.469	9
Expected heterozygosity (H_e)	----	0.153	0.023	0.653	9
Inbreeding coefficient (F_{IS})	0.010	0.596	0.356	0.053	9

Table 4.4. Results of tests for genetic bottlenecks in 11 island populations of *E. sezekorni*. *P*-values were taken from one-tailed Wilcoxon's signed ranks tests of the hypothesis that $H_e > H_{eq}$ (heterozygosity excess) or $H_e < H_{eq}$ (heterozygosity deficiency)(see methods). A *P*-value < 0.05 for the excess heterozygosity tests indicates a genetic bottleneck.

Island	Loci with excess heterozygosity (H_e)	*P*-value for excess heterozygosity (H_e)	*P*-value for deficiency in heterozygosity (H_e)
Cuba	3 of 11	0.959	0.051
Jamaica	0 of 11	1.000	< 0.001
Andros	6 of 11	0.517	0.517
Grand Bahama	4 of 11	0.681	0.350
Abaco	6 of 11	0.416	0.618
Eleuthera	9 of 11	0.062	0.949
Cat Island	7 of 11	0.319	0.711
Long Island	5 of 11	0.711	0.319
Exuma	7 of 11	0.207	0.817
San Salvador	7 of 11	0.350	0.681
New Providence	7 of 11	0.232	0.793

Table 4.5. Pairwise F_{ST} (above diagonal; Weir and Cockerham 1984) and *Nm* (below diagonal; Slatkin 1993) values between Bahamian islands.

	ABA	**GBA**	**AND**	**NP**	**ELU**	**CAT**	**EXU**	**LNG**	**SS**
ABA	----	0.004	0.038	0.038	0.033	0.033	0.059	0.028	0.080
GBA	71.2	----	0.031	0.028	0.035	0.031	0.061	0.031	0.069
AND	6.3	7.7	----	0.008	0.015	0.012	0.033	0.028	0.045
NP	6.4	8.8	31.0	----	0.001	0.006	0.034	0.022	0.035
ELU	7.3	6.9	17.0	249.8	----	0.000	0.024	0.008	0.024
CAT	7.3	7.8	20.4	44.4	2499.8	----	0.024	0.008	0.028
EXU	4.0	3.8	7.3	7.2	10.2	10.2	----	0.013	0.036
LNG	8.6	7.7	8.6	11.0	31.0	31.0	18.4	----	0.021
SS	2.9	3.4	5.3	6.8	10.1	8.6	6.7	11.8	----

ABA = Abaco; AND = Andros; CAT = Cat Island; ELU = Eleuthera; EXU = Exuma; GBA = Grand Bahama; LNG = Long Island; NP = New Providence; SS = San Salvador. ABA and GBA are on the Little Bahama Bank; other islands except for SS are on the Great Bahama Bank.

Table 4.6. Mean relatedness values for age and sex cohorts from 2005 and 2006 from the Cabbage Hill Cave-Turnaround Bay Cave colony.

Cohort	*n*	*R*	95% confidence intervals
2005			
Adults	164	-0.008	-0.032 – 0.016
Females	48	-0.013	-0.033 – 0.007
Males	116	-0.006	-0.033 – 0.020
Displaying	30	-0.008	-0.034 – 0.018
Non-displaying	49	0.001	-0.036 – 0.037
Offspring	48	-0.019	-0.023 – -0.016
2006			
Adults	207	-0.005	-0.016 – 0.007
Females	70	-0.006	-0.018 – 0.007
Males	137	-0.006	-0.018 – 0.006
Displaying	21	-0.002	-0.013 – 0.009
Non-displaying	42	-0.006	-0.017 – 0.006
Offspring	67	-0.001	-0.012 – 0.010

Table 4.7. Summary of pairwise pedigree relationships within age and sex cohorts of *E. sezekorni* from the Cabbage Hill Cave-Turnaround Bay Cave colony in 2005 and 2006. Percentage of comparisons represents the percentage of total pairwise comparisons where the hypothesized sibling relationship was statistically significant.

Cohort	Sibship	*n*	% of comparisons	Power (1 – β)
2005				
Adults	Full	164	7.33%	0.516
Females	Full	48	6.91%	0.526
Males	Full	116	7.71%	0.526
Displaying	Full	30	3.22%	0.919
Non-displaying	Full	49	6.46%	0.919
Offspring	Half	48	3.90%	0.542
2006				
Adults	Full	207	4.89%	0.532
Females	Full	70	5.09%	0.514
Males	Full	137	4.14%	0.514
Displaying	Full	21	2.86%	0.926
Non-displaying	Full	42	6.62%	0.926
Offspring	Half	67	4.65%	0.542

Table 4.8. Summary of tests for male and female sex-biased dispersal for individuals from the Cabbage Hill Cave – Turnaround Bay Cave colony on Exuma.

Test	Males	Females	Total	Male-biased *P*-value	Female-biased *P*-value
2005					
F_{ST}	0.0015	-0.0047	-0.0002	0.940	0.150
F_{IS}	0.0009	-0.0371	-0.0093	0.140	0.910
R	0.0031	-0.0097	-0.0004	0.950	0.150
2006					
F_{ST}	0.0009	0.0079	0.0025	0.067	0.94
F_{IS}	-0.0174	-0.0047	-0.0132	0.684	0.333
R	0.0018	0.0157	0.005	0.069	0.94

CHAPTER V

SOCIAL STRUCTURE AND MATING SYSTEM OF THE BUFFY FLOWER BAT, EROPHYLLA SEZEKORNI

BACKGROUND

The vast majority of mammalian mating systems (over 90%) are polygynous (Clutton-Brock 1989). Clutton-Brock (1989) defined polygyny as multiple mating by males with the same group of females in successive mating seasons and considered promiscuity to be a form of polygyny with no prolonged bond between males and females. Monogamy is uncommon in mammals and lek-mating, involving aggregated male display areas that females visit to mate, is rare (Clutton-Brock 1989, Höglund and Alatalo 1995). Females mating with multiple males in a single mating season (polyandry) is thought to be rare in mammals but, as some authors have pointed out, female mating behavior has been poorly-studied in most species (Clutton-Brock 1989, McCracken and Wilkinson 2000).

Although a variety of factors are thought to influence mammalian mating systems, Clutton-Brock (1989) argued that they can be characterized by 4 main characteristics: 1) amount of paternal investment required, 2) defensibility of female ranges, 3) stability of female groups and 4) size of female groups. If offspring require paternal investment to survive, then a monogamous mating system is expected. If paternal investment is not required and either female ranges or female groups are defensible then polygyny is expected. Finally, if female groups are unstable, then males cannot defend either resources or females. In these cases, we expect a promiscuous mating system, often involving some form of male mating territories.

With some notable exceptions, bats species (Order Chiroptera) typically have no paternal investment (McCracken and Wilkinson 2000) and, due to high mobility and broad foraging areas (Fleming 1988), female ranges are often not defensible. Based on these common traits, we would predict that most bats are either polygynous or promiscuous, and, like other mammals, these are the most common mating systems in bats (McCracken and Wilkinson 2000). Mating systems have been investigated in only 66 of more than 1,100 bat species (6%) and fundamental knowledge regarding male and female mating behavior and genetic mating systems is available for only a small fraction of those species (McCracken and Wilkinson 2000, Burland and Worthington Wilmer 2001, Wilkinson and McCracken 2003). There are several species of monogamous bats and this mating system is thought to be more common in bats than in most other mammalian orders (McCracken and Wilkinson 2000) Only 1 species has a lek mating system, the hammer-headed fruit bat (*Hypsignathus monstrosus*, Bradbury 1977b). However, there are several species with lek-mating characteristics but that do not fit the definition of a classical lek (Wickler and Seibt 1976, Bradbury 1977a, McWilliam 1990, Berry and Brown 1995, McCracken and Wilkinson 2000).

The buffy flower bat, *Erophylla sezekorni*, belongs to family Phyllostomidae (leaf-nosed bats), a family in which the mating systems of only 11 of 147 species (7.5%) have been studied (McCracken and Wilkinson 2000). Nearly all of the species are harem polygynous, in which 1 or rarely 2 males, defend and mate with a group of females (McCracken and Wilkinson 2000). However, 1 species, *Macrotus californicus*, appears to have some lek-mating characteristics (Berry and Brown 1995). Our preliminary observations of *E. sezekorni* indicated that this species is unusual compared to other

phyllostomids, because it does not have a harem-polygynous mating system and because it roosts in multi-female/multi-male groups. In this paper we describe the social structure and mating system of *E. sezekorni* on the island of Exuma, Bahamas during two consecutive mating seasons using capture data, roost observations, and paternity analysis.

METHODS

Study Species

The buffy flower bat, *Erophylla sezekorni* is a member of the nectarivorous phyllostomid subfamily Phyllonycterinae (Baker et al. 2003, Simmons 2005). This species is endemic to the Greater Antilles and is found on Cuba, Jamaica, the Cayman Islands, and the Bahamas (Simmons 2005). In the Bahamas, buffy flower bats occupy small, relatively cool caves with varying levels of ambient light. Colony size ranges from 50-650 bats (Koopman 1957, Hall et al. 1998, pers. obs.). *Erophylla* is a generalist forager, eating fruits, insects, nectar, and pollen (Soto-Centeno and Kurta 2006). Females give birth to a single pup per year with mating occurring from late November through January (Buden 1976, Baker et al. 1978, Silva Taboada 1979). Gestation lasts 4-5 months with parturition occurring in late May or June and lactation extending through August (Buden 1976, Baker et al. 1978, Silva Taboada 1979).

Study Site

The Exumas are a group of small (≤ 290 km^2), low (≤ 40 m above sea level) limestone islands on the southeastern margin of the Great Bahama Bank in the central part of the Bahamas Archipelago. These islands are dominated by dense, broadleaf

coppice forests with a 10-15 m overstory (Patterson 2002). There are 4 known roosts of *E. sezekorni* on Exuma, 1) Cabbage Hill Cave (CHC), Great Exuma; 2) Turnaround Bay Cave (TBC), Great Exuma; 3) Salt Pond Cave (SPC), Great Exuma; and 4) Pasture Cave (PC), Little Exuma. Cabbage Hill Cave was the focal population for this study and most of our behavioral observations occurred there. We supplemented data from the CHC roost with data from TBC, SPC, and PC roosts whenever possible. Data from TBC was limited because the cave was remote and the cave ceiling was too high to identify and capture bats within social groups.

Marking and Observational Techniques

In 2 consecutive years (2004-2005 and 2005-2006) we captured and observed buffy flower bats in 4 caves (CHC, TBC, SPC, PC) on Exuma. We captured displaying males on display areas, non-displaying males in bachelor groups, and females during the mating season (November-January) using a hand net with extendable poles (BioQuip, Gardena, CA, USA). Displaying males were males that wing-displayed (see below) on display areas within the roost. Non-displaying males were defined as males that did not wing display and did not roost on display areas, but rather in clusters in other parts of the cave. Females with infant bats and males were also captured during the maternity season (June-August). All captured bats were marked with numbered aluminum alloy forearm bands (4.2 mm diameter, Porzana Limited, Icklesham, UK). Males were banded on the right forearm and females on the left forearm. Displaying males were also marked with a unique combination of plastic, metal, and reflective forearm bands, and females were marked with forearm bands covered in reflective tape (3M, St. Paul, USA). We measured

body mass (g) with a 30 g spring balance (Pesola AG, Baar, Switzerland; precision: ± 0.1 g) and forearm length (mm) with a digital caliper (Mitutoyo, Chicago, USA; precision ± 0.1 mm), and recorded sex, age, reproductive condition and display status (males only) for all captured bats (Anthony 1988, Racey 1988). All research on live animals conducted during this study followed the guidelines of the American Society of Mammalogists and was approved by the University of Miami Institutional Animal Care and Use Committee (IACUC).

We observed social structure, male display behavior, and mating behavior within roosts using a Sony DCR-TRV38 digital camcorder equipped with Sony NightShot and an external IR light source (Sony Electronics, Inc., USA) for a total of 138 hours. The population in the focal cave (CHC) was censused 33 times (0700-1800 h) during the 2005-2006 mating season (23 November 2005 to 14 January 2006) with the digital camera to monitor variation in population size and group composition. We estimated cave population size directly by counting the number of bats including numbers of displaying males, non-displaying males, and banded females. To estimate the number of females in the roost we assumed a 1 to 1 sex ratio and divided the total number of bats in the roost by one-half. When number of males in the roost exceeded one-half of the total bats, we subtracted males from total bats to estimate number of females. We used a Spearman's rank correlation to determine if estimated number of females was correlated with number of marked females (Zar 1999)

Sex ratio (number of females: number of males) was estimated for CHC with a single mist net survey (10 January 2005). We also calculated the sex ratio at birth for all caves individually and combined for both 2005 and 2006. We used a chi-square test to

determine if sex ratio deviated from 1.0 (Zar 1999) The ratio of number of displaying males to both total population size and to the number of non-displaying males was calculated from census data from the 2005-06 mating season.

We examined roost site fidelity of displaying males by observing uniquely-banded focal males on display areas. Individual sites within display areas were monitored daily throughout the 2005-06 mating season and marked individuals were recorded as present or absent on display sites. To quantify the amount of time males spent displaying, we calculated time-activity budgets of 5 displaying males, 5 non-displaying males, and 5 females for a single night (05 December 2005) at CHC. Time spent wing flapping was observed for 10-minute periods in each pre-foraging hour from 1700 hr to 2300 hr.

Ultrasonic sound recording and analysis

20 acoustic display calls were recorded from 8 display areas in the CHC roost during the 2005-06 mating season (3 different days) using a broadband bat detector with condensor microphone (UltraSoundGate 116, Avisoft Bioacoustics, Berlin, Germany) and operated using Recorder USG (Avisoft Bioacoustics, Berlin, Germany). Sampling rate was 250 kHz and format was 16 bit. Power spectra and spectrograms were formed using an FFT-length of 1024 with a resolution of 244 Hz. We used the heterodyne recording mode tuned to 45 kHz ± 10 kHz. All calls were saved directly to a laptop computer for further analysis. We analyzed calls manually in BatSoundPro Sound Analysis (vs. 3.31b; Pettersson Elektronic AB) and measured the following call parameters: duration (Dur; ms), frequency with maximum energy (FME; kHz), maximum frequency (Fmax; kHz) and minimum frequency (Fmin; kHz). Bandwidth (BW; kHz) was calculated by subtracting Fmin from Fmax. We were unable to identify the

individual males that produced display calls and, therefore, limit our analysis to a description of the display call.

Sexual dimorphism

We used body mass (g) and forearm length (mm) measurements to test for sexual size dimorphism. We compared mean body mass, forearm length, and condition index between males and females for both mating seasons. To calculate condition indices, we regressed body mass upon forearm length (body size) and then calculated standardized residual body mass for each individual (Krebs and Singleton 1993, Jakob et al. 1996, Kotiaho 1999, Kotiaho et al. 2001). Positive residuals indicate above average body condition while negative residuals indicate below average body condition. We used a one-way Analysis of Variance (ANOVA) to compare body condition of adult males and females (Zar 1999).

Reproductive condition and behavior

To monitor male testes development during the mating season, we categorized relative testes size for all captured males as 0 = undescended, 1 = small ($\bar{x}$ = 47.0 ± 3.8 mg), 2 = medium ($\bar{x}$ = 74.30 ± 3.4 mg) and 3 = large ($\bar{x}$ = 139.8 ± 6.2 mg). We also measured testes length and width at various times during the 2005-06 mating season using a digital caliper (Mitutoyo, Chicago, USA; precision ± 0.1 mm). We used these data to calculate combined testes mass for each bat (Wilkinson and McCracken 2003). Testes volume was approximated as a prolate spheroid (0.5236 X length X width^2; Myers 1977) and multiplied by 2 for combined testes volume. Testes mass and testes volume are

highly correlated in bats (Myers 1977, Hosken 1998, Wilkinson and McCracken 2003), so to be consistent with published studies, we report values as combined testes mass (CTM; mg). We used a one-way ANOVA to examine variation in CTM during the mating season (Zar 1999). In phyllostomid bats, menstruation in females is periovulatory (Rasweiler and Debonilla 1992, Rasweiler and Badwaik 2000). Therefore, menstruating females (blood on the vagina) were considered to be in estrus. Also, vaginal secretions or a distended vagina were recorded. We interpreted either vaginal secretions or a distended vagina as evidence of recent copulation but were unable to catch copulating pairs to confirm this. Copulations were observed directly or from video recording. Data on male testes development, female estrus, and female copulation behavior were compiled for 7 time periods (TP) during the mating season, TP1) November 20-30, TP2) December 1-10, TP3) Dec. 11-20, TP4) Dec. 21-31, TP5) January 1-10, TP6) Jan. 11-20, and TP7) Jan. 21-30. We used regression analysis to examine changes in mean male testes category, proportion of females in estrus and proportion of females with signs of copulatory behavior during the mating season; proportional data for females were arcsine-transformed (Zar 1999). Due to small sample size and missing data from parts of individual mating seasons, we combined data from both mating seasons for these analyses.

To examine the likelihood of polyandry and sperm competition, we regressed $\log_{10}$ CTM upon $\log_{10}$ body mass (g) for *E. sezekorni* and 1) 105 species of bats from 10 different families and 2) 25 species of phyllostomid bats (Wilkinson and McCracken 2003) and calculated standardized residuals for CTM. We used CTM from the maximal period of testicular development for these analyses. In species in which females mate

with multiple males, we expect sperm competition and greater than average testes size. Therefore, if female *E. sezekorni* mate with multiple males we expect residual testes mass to be positive and similar in magnitude to other species with female polyandry.

All statistical tests were 2-tailed with an $\alpha = 0.05$ and were performed in SPSS v.15.0 (SPSS Inc., Chicago, USA). All means were reported ± 1 SE.

Paternity analysis

We sampled genetic material from displaying males, non-displaying males, females, and infants, by clipping a small piece of tissue (5-10 mg) from the wing. We obtained microsatellite genotype data from 423 individuals and 8 loci (ES17, 19, 22, 24, 35, 38, 40, and 46) for *Erophylla* using the techniques described in Murray et al. (2008). These loci did not deviate from Hardy-Weinberg equilibrium (HWE), were in genotypic linkage equilibrium, and showed no evidence of null alleles (Murray et al. 2008). All samples were analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems, Foster City, USA) and scored with GENEMAPPER v.3.0 (Applied Biosystems, Foster City, USA).

We performed paternity analysis on 109 mother-offspring pairs, 48 from 2005 and 61 from 2006, and a total of 205 candidate males. We genotyped mothers, offspring, and candidate fathers at 8 microsatellite loci and used the software package CERVUS v.3.0 to assign paternity (Kalinowski et al. 2007). In CERVUS, Δ is the difference in LOD scores for the 2 most likely candidate fathers, and LOD scores are the likelihood of paternity of the candidate male relative to the likelihood of paternity for a random male (Marshall et al. 1998). Based on the paternity simulation analysis in CERVUS the critical values for Δ

for 90% and 95% confidence levels were 3.93 and 6.30 respectively. Census data from both mating seasons showed a maximum population size of 289 bats. For paternity simulation analysis, we estimated maximum population size of 300 bats and assumed a 1 to 1 sex ratio (150 candidate males). We therefore assumed that we had sampled 78% (117 of 150) candidate males in 2005 and 90% (135 of 150) of candidate males in 2006. Based on these estimates, we set the proportion of candidate males sampled at 80% for paternity simulation analysis for 2005 and 2006. We also assumed that 5 percent of offspring had full or half sibs in the pool of candidate males for both years.

RESULTS

Social structure

Mean population size in the CHC roost during the 2005-06 mating season was 96.4 ± 11.1 (range = 8 – 272; $n = 33$). We observed a mean of 27.0 ± 1.2 displaying males (range = 6 – 39; $n = 33$), 12.9 ± 2.1 non-displaying males (range = 0-42; $n = 33$), and 6.0 ± 1.4 banded females (range = 0 – 39; $n = 33$). Estimated female group size was large ($\bar{x} = 44.03 \pm 5.56$, range = 1 – 139; $n = 33$) and labile (Fig. 5.1). Estimated number of females was highly correlated with number of marked females ($r_S = 0.90$, $df = 30$, $P < 0.001$).

During the single mistnetting event in the 2004-2005 mating season, we found a sex ratio of 0.89 ($n = 34$) which was not significantly different than 1.0 ($\chi^2 = 0.12$, $df = 1$, $P > 0.50$). Estimated sex ratio at birth for all caves combined was 0.86 ($n = 134$) in 2005 and 1.05 ($n = 191$) in 2006. Sex ratio did not deviate significantly from 1.0 in either year (2005: $\chi^2 = 0.75$, $df = 1$, $P > 0.25$; 2006: $\chi^2 = 0.13$, $df = 1$, $P > 0.50$). Chi-square tests for

individual caves also showed no significant deviation from a sex ratio of 1.0 (data not shown). Assuming a sex ratio of 1.0, an average of 56.0% of males in the roost were displaying males (range = 28.7-85.7%). The ratio of maximum number of displaying males to maximum number of non-displaying males was 0.93.

Displaying males wing-displayed solitarily or in aggregations of 2-9 individuals in solution domes (display areas) on the cave ceiling (Table 5.1). In the 2005-2006 mating season we uniquely banded a total of 15 individual displaying males. We observed these males from 8 to 31 days depending on when forearm bands were applied. Banded displaying males were found in the exact same location on display areas for 328 of 349 (94.0%) bat days of observation. Non-displaying males roosted separately in bachelor groups usually comprised entirely of males (Table 5.1). Capture data indicated that some non-displaying males were also present on or near display areas (Table 5.1). Females typically roosted on the periphery of display areas, but also within display areas and rarely in groups of non-displaying males (Table 5.1). Not all individuals in the roost were banded, so we were not aware of the sex of every individual.

Male displays

Based on our observations in several roosts, displaying males spent a significant amount of time wing displaying either solitarily or in groups. Analysis of a single night of data (1700 hr to 2300 hr) indicated that displaying males spent 74.1 ± 5.0% of their time wing flapping (range = 58-92%). Non-displaying males spent 1.2 ± 0.5% of their time displaying (range = 0-3%) and females spent 0.7 ± 0.4% of their time displaying (range =

0-3%). Although these data are preliminary, they are consistent with the disparity that we observed many times in wing display behavior between these two classes of males.

Wing display behavior involved 3 distinct behaviors: 1) wing flapping, 2) flight display, and 3) wing buzzing. Wing flapping consisted of males flapping their wings vigorously, sometimes one wing at a time, while hanging in their display territory (Fig. 5.2A & B). The flight display was a short looping flight from one point to another on the display territory. Finally, the wing buzz involved rapid movement of both forearms in front of the body producing an audible buzz. Both flight displays and wing buzzes occurred less frequently than wing flapping. Males directed wing displays toward females on the periphery of display areas, but we never observed this behavior leading directly to a copulation. Males also directed this behavior toward male and female intruders on the display area.

In addition to wing displays, males of *E. sezekorni* used olfactory and acoustic displays. We observed that mature males (i.e. both displaying and non-displaying males) produced a garlic-scented, supraorbital secretion. These bats had a visible fluid secretion that wet the fur above the eye (Fig. 5.2C). When the secretion dried it left a yellow, powdery residue in the area of the secretion (Fig. 5.2D). Individuals producing the secretion were identified by presence of the secretion or residue and strong odor. We never observed the supraorbital secretion on immature males or females.

Displaying males produced an audible sound (tock) coupled with an ultrasonic display call (Fig. 5.3). Mean Dur was 19.3 ± 1.4 ms, FME was 46.5 ± 1.3 kHz, Fmax was 60.7 ± 0.7 kHz, Fmin was 23.3 ± 0.8 kHz, and BW was 37.5 ± 1.1 kHz. Acoustic display calls were compared to the standard orientation calls of the species in Fig. 5.3. All 3

forms of display (wing display, olfactory display, and acoustic display) were observed during the mating and maternity season.

Sexual dimorphism

Adult males and females of *E. sezekorni* were sexually dimorphic. We found that in the 2004-2005 mating season males weighed more ($\bar{x}_{male}$ = 16.68 ± 0.12 g, n = 182; $\bar{x}_{female}$ = 15.34 ± 0.12 g, n = 78; F = 42.97, df = 1, P < 0.001) and were in significantly better condition ($\bar{x}_{male}$ = 0.25 ± 0.07; $\bar{x}_{female}$ = -0.59 ± 0.08; F = 46.29, df = 1, P < 0.001) than females. Forearm length did not differ between males and females ($\bar{x}_{male}$ = 46.6 ± 0.1 mm; $\bar{x}_{female}$ = 46.5 ± 0.2 mm; F = 0.302, df = 1, P = 0.583). Results were similar for the 2005-2006 mating season. Males weighed more ($\bar{x}_{male}$ = 17.48 ± 0.12 g, n_{male} = 163; $\bar{x}_{female}$ = 15.59 ± 0.08 g, n_{female} = 166; F = 184.22, df = 1, P < 0.001) and were in significantly better condition ($\bar{x}_{male}$ = 0.60 ± 0.07; $\bar{x}_{female}$ = -0.59 ± 0.05; F = 179.12, df = 1, P < 0.001) than females. Males also had slightly but significantly longer forearms than females in 2005-06 ($\bar{x}_{male}$ = 46.2 ± 0.1 mm; $\bar{x}_{female}$ = 45.9 ± 0.1 mm; F = 4.77, df = 1, P = 0.03).

Reproductive condition and behavior

Linear regression showed that mean male testis category declined during the mating season (r = 0.86, F = 56.17, df = 1, P = 0.001; Fig. 5.4). Mean male testes category peaked early in the mating season and declined from mid-December (TP3) to late January (TP7; Fig. 5.4). There was significant variation among time periods in combined testes mass in adult males (F = 21.03, df = 3, P < 0.001). Combined testes mass averaged 142.1

± 13.6 mg (n = 10) in late November (TP1), 142.2 ± 8.0 mg (n = 21) in early December (TP2), 87.0 ± 6.1 mg (n = 11) in early January (TP5) and 68.3 ± 4.7 mg (n = 16) in mid-January (TP6). Cubic regression showed that there was a significant relationship between proportion of females in estrus and time of mating season (r = 0.99, F = 31.97, df= 3, P = 0.009; Fig. 5.4). The proportion of females in estrus peaked in mid-December (TP3) and slowly decreased for the rest of the mating season (Fig. 5.4). There was no significant relationship between proportion of females showing signs of copulation and time of mating season (r = 0.86, F = 2.70, df= 3, P = 0.218; Fig. 5.4).

During 2 mating seasons, we observed a total of only 17 copulations and 4 attempted copulations, in which a male was rebuffed by a female. Eight of 17 copulations were interrupted, 3 by the females involved, 3 by displaying males adjacent to the copulating pair, and 2 by non-territorial males adjacent to the copulating pair. All observed copulations occurred on display areas. However, our sampling effort was strongly biased toward display areas, and the display status of the copulating male was not always known. A single copulation was observed in June of 2004 during the maternity period.

We assigned paternity to 21 of 48 offspring (44%) in 2005 and 35 of 61 offspring (57%) in 2006 with an 80% level of confidence in CERVUS. Based on these results, females did not mate exclusively with displaying males (Table 5.2). However, display status of the majority of candidate fathers was unknown making any further interpretations difficult. Several males fathered more than one offspring, indicating mild reproductive skew (i.e. variance in male reproductive success). In 2005, 13 of 16 (81%) of assigned fathers had only a single offspring. Three males fathered more than 1

offspring (polygynous). One male (#547) fathered 4 offspring and 2 males (#480 and #1706) fathered 2 offspring each. Display status of males #547 and #480 was unknown, but male #1706 was a displaying male. In total, 8 of 21 (38%) offspring were fathered by polygynous males in 2005. In 2006, 22 males fathered a single offspring and 5 males fathered multiple offspring. Males #547 fathered 4 offspring, male #348 fathered 3 offspring, and males #480, #545, and #1701 fathered 2 offspring each. We knew the display status of only 1 male that fathered multiple offspring in 2006 (#1701) and this was a non-displaying male. A total of 13 of 35 (37%) offspring were fathered by polygynous males in 2006.

We assigned paternity to the offspring of 3 females (#1698, #1699, and #1957) for 2 consecutive years. Two females (#1698 and #1957) mated with a different male each year. One female (#1699) mated with the same male (#480) in consecutive years. The display status of male #480 was unknown, but this male fathered 2 offspring in each year.

We were unable to determine whether females of *E. sezekorni* mate with multiple males (polyandry) because copulations were rarely observed and because we could not identify individual females in the roost. We captured a single female with a sperm plug in January 2006. We also used residual testes mass in male *E. sezekorni* to test the potential for sperm competition and multiple mating by females. Mean combined testes mass during the period of maximal testes development for *E. sezekorni* was 142.15 ± 6.86 mg (n = 31) and was 0.79% of mean body mass. Standardized residual testes mass was 0.006 when the regression included data for all species in Wilkinson and McCracken (2003) and 0.184 when the regression included only phyllostomid bats.

DISCUSSION

The display behavior and territoriality of males of *Erophylla sezekorni* have not been described previously. Hall et al. (1998) described the wing-flapping behavior in *E. sezekorni* males on San Salvador, but only in the context of male-male aggression and not as a means of display. The olfactory and acoustic displays of males of *E. sezekorni* have never been described. Male display and mating territories are not uncommon among bats. At least 20 species of bats have some form of mating territory (McCracken and Wilkinson 2000). Numerous species (at least 15) exhibit some type of display behavior to attract females, with acoustic displays being the most common (McCracken and Wilkinson 2000, Behr and von Helversen 2004, Davidson and Wilkinson 2004). Bats use both audible and ultrasonic display calls and call from perches, roosts, or while flying. Some species have very complex acoustic display calls which have been likened to bird song (Russ and Racey 2007). Two examples are *Saccopteryx bilineata* (Bradbury and Emmons 1974, Behr and von Helversen 2004, Davidson and Wilkinson 2004) and *Pipistrellus nathusii* (Russ and Racey 2007) and both species have been shown to use acoustic display calls in female attraction. Unlike these 2 species, some bats use relatively simple display calls as does *E. sezekorni* (Sluiter and van Heerdt 1966, Wickler and Seibt 1976, Bradbury 1977b, Lundberg and Gerell 1986, French and Lollar 1998). However, we caution that our study provides only a preliminary description of the display calls of *E. sezekorni* and further research is needed to document the full vocal repertoire of this species.

Olfactory displays are also very common among bats and a number of species have sexually dimorphic gland structures, with males typically having larger or more active glands than females (Quay 1970, Voigt and von Helversen 1999, Skully et al. 2000, Altringham and Fenton 2003). Several species have been shown to use glandular secretions to scent-mark conspecifics, their territories, and themselves (McWilliam 1990, Voigt and von Helversen 1999, Rossiter et al. 2000, Keeley and Keeley 2004). The olfactory display of *S. bilineata* is very complex and is used for several functions, including scent-marking their harem territory and attracting females. This species sequesters urine and secretions from genital areas and gular glands into propatagial holding sacs. It then uses the glandular secretions in these sacs in a variety of display behaviors directed toward females. We observed the garlic-scented supraorbital secretion of *E. sezekorni* only in mature males, which is typical for bat glands and glandular secretions (Voigt and von Helversen 1999, Skully et al. 2000). We do not know whether this secretion is produced supraorbitally or sequestered there by the bat.

Aerial displays occur in a few bat species and are usually coupled with acoustic display calls in song flights (Lundberg and Gerell 1986, Leippert 1994). *Saccopteryx bilineata* males employ a hover display in which they hover in front of females and use wing beats to fan odor from glandular secretions toward females (Voigt and von Helversen 1999). Males also produce a 'whistle' call in addition to hovering flight and fanning behaviors (Behr and von Helversen 2004). Epomophorine bats (*Epomophorus wahlbergi*, *E. crypturus*, and *Epomops franqueti*), including the lekking hammer-headed bat (*Hypsignathus monstrosus*), flap their wings while calling to females from their display territories (Wickler and Seibt 1976, Bradbury 1977b). Altringham and Fenton

(2003) hypothesized that wing flapping in these species could be a visual signal or it could be used to dispense olfactory cues, like the fanning behavior of *S. bilineata*. *Erophylla sezekorni* and one other phyllostomid bat, *Macrotus californicus*, are known to use wing-flapping as a means of display (Brown and Berry 1995). Superficially, wing-flapping behavior is very similar between these two species (pers. obs.). It seems unlikely that wing-flapping functions as a visual display in *E. sezekorni* because of the absence of light in the roost (CHC). As has been hypothesized for epomophorine bats (Altringham and Fenton 2003), and shown in *S. bilineata* (Voigt and von Helversen 1999), the wing display of *E. sezekorni*, particularly the wing-buzz, may serve to fan odor from supraorbital secretions toward females.

Conspicuous sexual dimorphism, such as male ornamentation, is rare in bats (McCracken and Wilkinson 2000, Altringham and Fenton 2003). Based on the mild variance in male reproductive success that we observed, we would expect limited sexual dimorphism in *E. sezekorni*. The sexual dimorphism that we observed in the buffy flower bat was not obvious and required careful study of the species, particularly during the mating season. In addition to differences in display behavior, we observed small but consistent differences in body mass and condition between males and females. One other study has demonstrated sexual dimorphism in *E. sezekorni* (Genoways et al. 2005). They found that males had larger zygomatic breadth and mastoid breadth. They also noted that a large green-colored salivary gland was found only in sexually mature males (Genoways et al. 2005). In immature males and females this gland is very small and beige-colored. These authors found unusual secretory cells within the gland and hypothesized that it might function in pheromone production and may have “contributed to the odor

associated with these bats" (Genoways et al. 2005). The subtle sexual dimorphism that we observed may reflect low levels of sexual selection, but may also be limited by the selective constraints of flight and foraging behavior.

The aggregated male display behavior of *E. sezekorni* resembles a lek-like mating system (Höglund and Alatalo 1995). However, the standard definition of a classical lek is: 1) no male parental care, 2) mating arena is smaller than the normal home range of the species, 3) display sites contain no resources required by females, and 4) the opportunity for females to select a mate (Bradbury 1977b, 1981, McCracken and Wilkinson 2000). *Erophylla sezekorni* does not adhere to criterion 3 and therefore should not be considered a lek-mating species. This is an important point because females may need the cave to roost regardless of whether males are present. Thus, females may not be attracted to display areas solely by male display and may not visit display areas solely to mate. In addition, the results of paternity analysis indicate that females frequently mated with non-displaying males. A significant portion of matings were not with territorial displaying males. This could mean that matings occur independently of display areas and that courtship may not be the only male mating strategy or even the most successful male mating strategy in this species. Finally the lack of conspicuous sexual dimorphism in *E. sezekorni* is atypical of lekking species. Thus, the mating behavior of *E. sezekorni*, like several other bat species (Wickler and Seibt 1976, Bradbury 1977a, McWilliam 1990, Berry and Brown 1995, McCracken and Wilkinson 2000), resembled a lek but did not adhere to the classical definition.

Based on the framework established by Clutton-Brock (1989) we suggest that *E. sezekorni* has a form of promiscuous mating system, defined as multiple mating by males

or females and an absence of a pair bond between them. *Erophylla* formed multi-male/ multi-female groups and were mildly polygynous, with about 18% of fathers being polygynous in both years. Bats that roost in multi-male/ multi-female social groups are fairly common (15 of 66 studied species) and often share 1 or more of a suite of characteristics including territoriality, audible male vocalizations, aerial displays, glandular secretions, and labile female group composition (McCracken and Wilkinson 2000). *Erophylla sezekorni* exhibits all of these characteristics and fits well into this group of bats. No paternal care was observed in *E. sezekorni*. Based on ephemeral nature of its food supply, fruit, nectar and pollen (Soto-Centeno and Kurta 2006), we assume that this species is wide-ranging and that female ranges are not defensible by males. We showed that at Cabbage Hill Cave, female group size was large and labile during the mating season (Fig. 5.1). Mammal species with these characteristics tend to have promiscuous mating systems involving male displays and mating territories like those of *E. sezekorni* (McWilliam 1988, Clutton-Brock 1989, McCracken and Wilkinson 2000).

There are some significant unknowns regarding the mating system of *E. sezekorni*. We did not expect non-displaying males to father as many offspring as they did. This fact indicates that there may be successful alternative reproductive strategies in this species. However, we were not able to determine the display class of the majority of our candidate fathers and more research needs to be done to determine the relative reproductive success of displaying and non-displaying males. We know almost nothing about the potential for polyandry in *E. sezekorni*. Sperm plugs may or may not support the possibility for polyandry (Rossiter et al. 2000, Keeley and Keeley 2004), and their prevalence in *E. sezekorni* is unclear. We encountered only 1 sperm plug in 276 captured

females, but their presence could have been overlooked. Residual combined testes mass CTM for *E. sezekorni* (0.006) compared to 105 species of bats fell almost exactly on the regression line suggesting a lack of sperm competition and polyandry. Residual CTM of the buffy flower bat (0.184) was larger than 19 of 25 phyllostomid bats. Unfortunately, we know very little about multiple mating by females in these species, which makes it difficult to interpret the significance of residual CTM in *E. sezekorni* compared to other phyllostomids.

In conclusion, *E. sezekorni* formed multi-male/multi-female groups during both the mating and maternity season. Male polygyny was demonstrated by paternity analysis. Female polyandry is possible in this species, but more data is needed to determine if it occurs. Lack of parental care, large female group size, unstable female groups, and male display and mating territories indicate that promiscuity is the most likely mating system in this species. Because the social structure and characteristics that we observed in *E. sezekorni* are not at all uncommon in bats, we hypothesize that a diverse array of mating and display behaviors have yet to be discovered in this charismatic group of mammals.

Table 5.1. Summary of capture data revealing social structure in *Erophylla sezekorni*. DA = display area. NDM = non-displaying male groups; CHC = Cabbage Hill Cave; SPC = Salt Pond Cave; PC = Pasture Cave.

	Capture Location	Total Captured	Displaying Males	Other Males	Females
CHC Roost					
12/30/04	DA	8	2	1	5
01/20/05	DA	24	4	0	20
11/27/05	DA	20	6	0	14
12/14/05	DA	14	5	0	9
01/11/06	DA	8	3	0	5
12/29/04	NDM	7	0	7	0
12/30/04	NDM	6	0	6	0
01/04/05	NDM	24	0	23	1
01/20/05	NDM	7	0	5	2
11/27/05	NDM	14	0	13	1
12/09/05	NDM	5	0	5	0
12/10/05	NDM	5	0	5	0
01/11/06	NDM	11	0	8	3
SPC Roost					
01/05/05	DA	12	5	0	7
12/03/05	DA	7	4	2	1
12/14/05	DA	22	7	3	12
01/03/06	DA	24	3	0	21
01/05/05	NDM	8	0	8	0
01/16/05	NDM	9	0	9	0
12/14/05	NDM	8	0	8	0
01/03/06	NDM	9	0	8	1
PC Roost					
01/11/05	DA	17	6	0	11
01/17/05	DA	10	3	2	5
12/01/05	DA	7	4	0	3
01/11/05	NDM	20	0	20	0
01/17/05	NDM	9	0	9	0
Total					
	DA	173	52	8	113
	NDM	128	0	121	7

Table 5.2. Results of paternity analyses for 2005 and 2006. Males were referred to as unknown when we did not know display status. Proportions are in parentheses.

Display Status	Proportion of Fathers	Proportion of Offspring
2005		
Displaying	4/16 (0.25)	5/21 (0.24)
Non-displaying	5/16 (0.31)	5/21 (0.24)
Unknown	7/16 (0.44)	11/21 (0.52)
2006		
Displaying	5/27 (0.19)	5/35 (0.14)
Non-displaying	5/27 (0.19)	6/35 (0.17)
Unknown	17/27 (0.62)	24/35 (0.69)

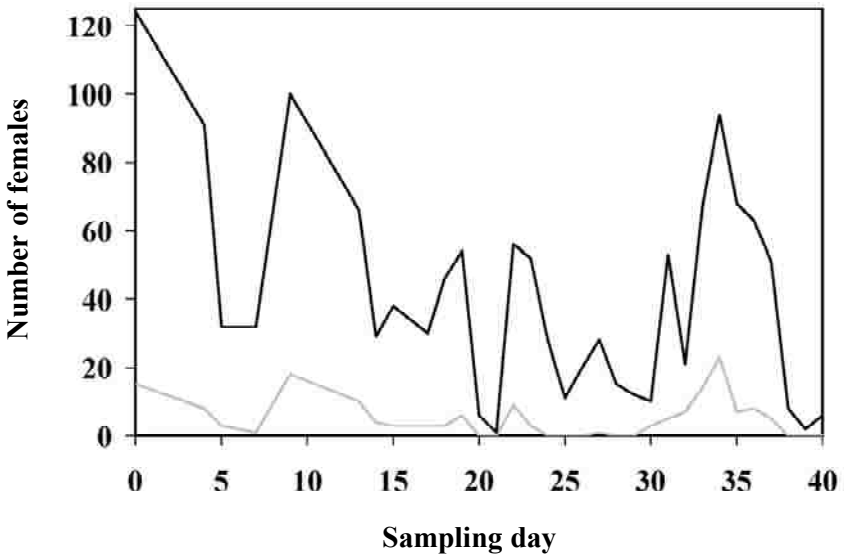

Figure 5.1. Female group lability in *Erophylla sezekorni* at Cabbage Hill Cave during the 2005-06 mating season. Black line is total number of females in the roost estimated as one-half of the total number of bats assuming a 1 to 1 sex ratio. When number of males in the roost exceeded one-half we subtracted males from total bats to calculate number of females. Gray line is number of females marked with reflective bands. Sampling day 0 = 05 December 2005 and sampling day 40 = 14 January 2006.

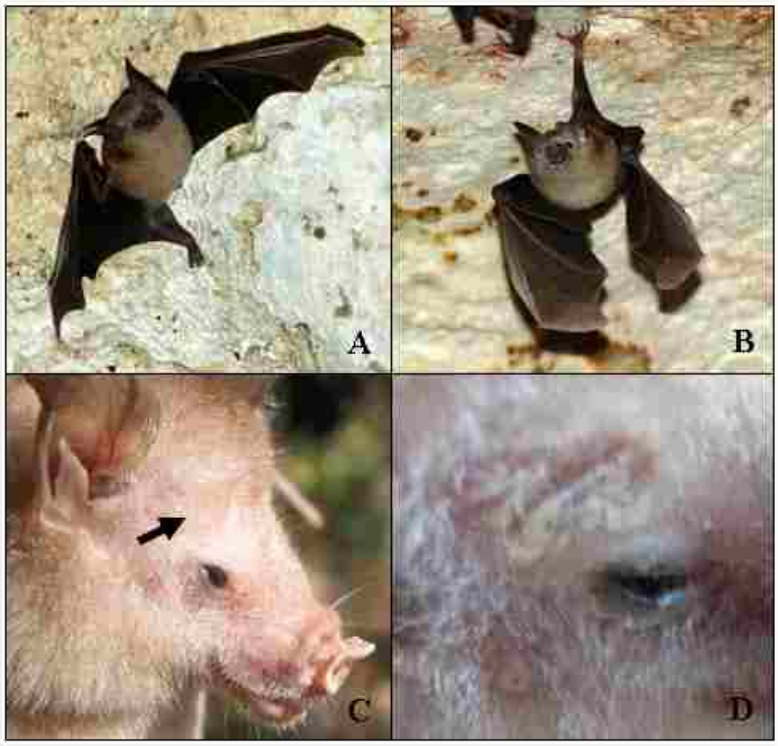

Figure 5.2. Wing display and olfactory display in male *Erophylla sezekorni*. Panels A and B show males wing displaying (see results for description). Panel C shows the supraorbital secretion (indicated by arrow) produced by mature males. Panel D shows a close-up of the yellow residue left by the secretion. Photographs A and B by THF and C and D by KLM.

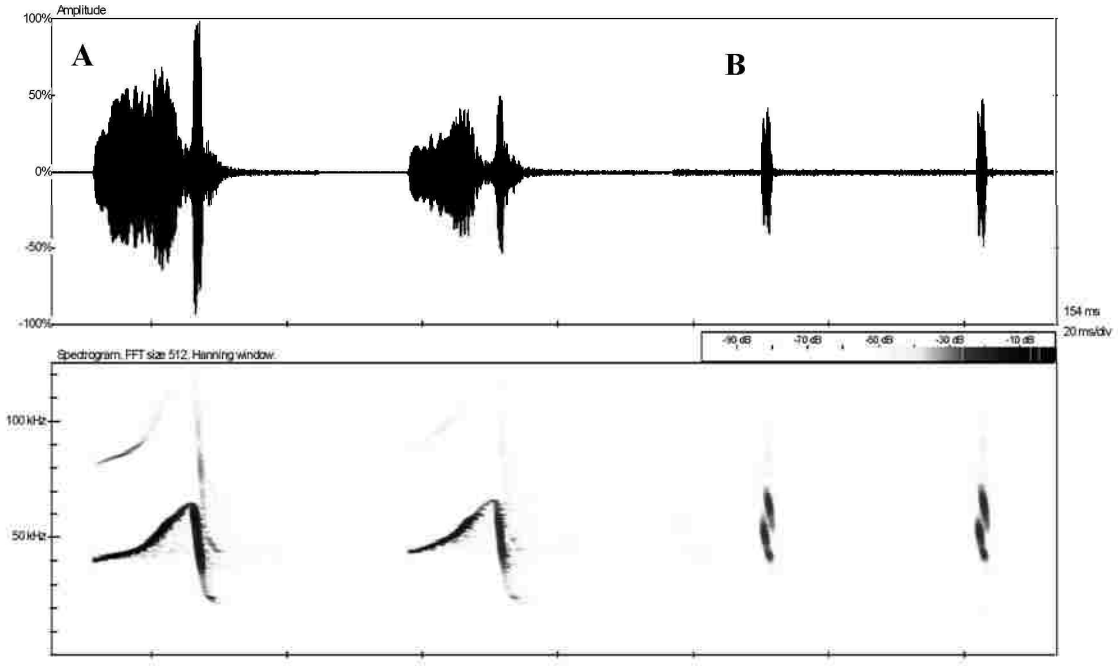

Figure 5.3. Oscillograph (top panel) and spectrograph (bottom panel) of A) 2 typical ultrasonic display calls and B) 2 typical echolocation calls of *Erophylla sezekorni*.

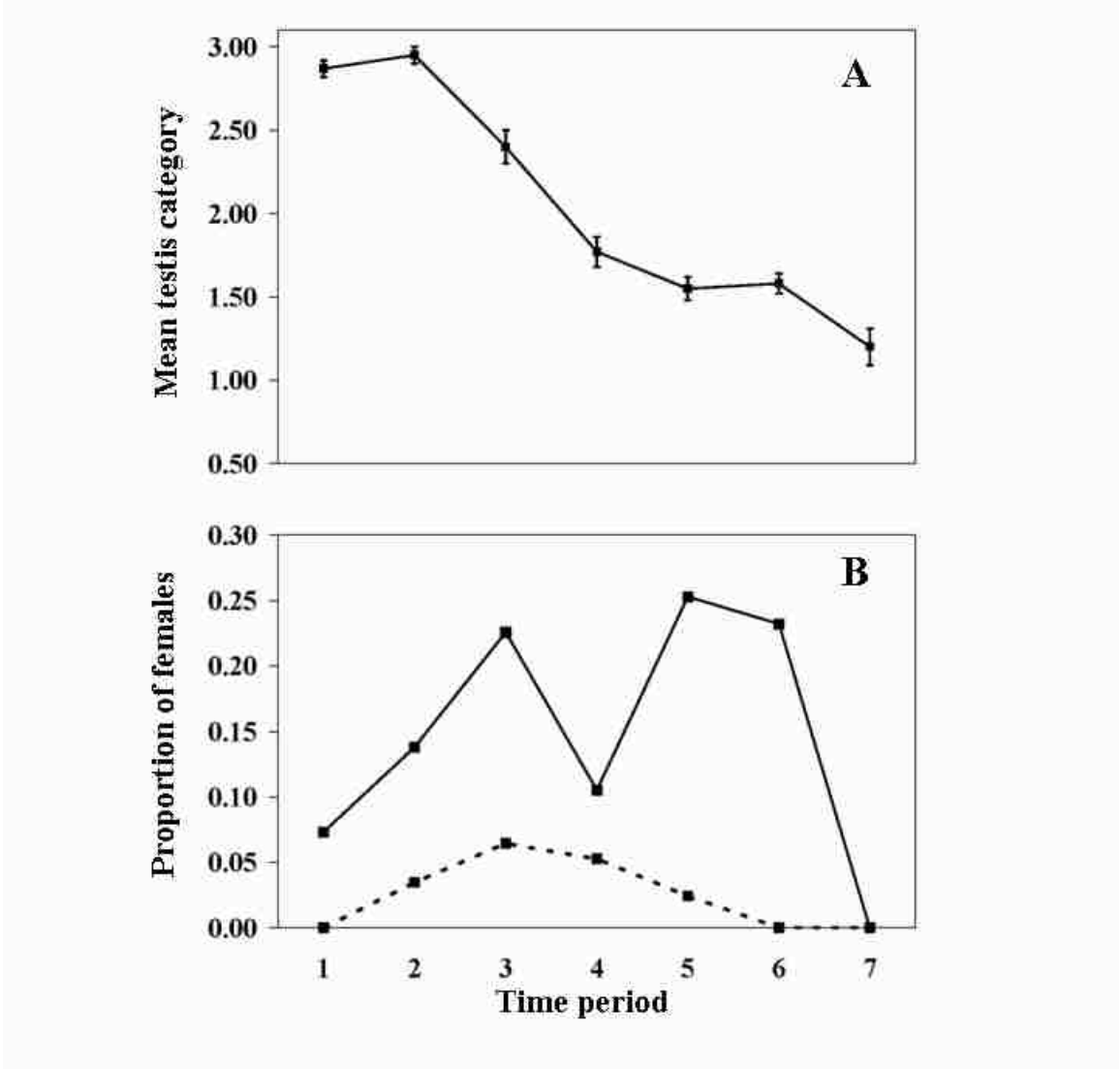

Figure 5.4. Reproductive condition and behavior in males and females of *Erophylla sezekorni*. A) mean testis category for 7 time periods during the mating season. We categorized relative testis size as undescended (0), small (1), medium (2), or large (3)(see methods). B) proportion of captured females in estrus (dotted line) and showing signs of copulation (solid line) for 7 time periods during the mating season. See methods for further description of testis size categories, female estrus, and signs of female copulation. Data from 2 mating seasons were combined for both male and females. Time periods are 1) Nov. 20-30, 2) Dec. 1-10, 3) Dec. 11-20, 4) Dec. 21-31, 5) Jan. 1-10, 6) Jan. 11-20 and 7) Jan. 21-30. Male sample sizes (*n*) for time periods 1-7 were 55, 43, 53, 48, 75, 92, and 15, respectively. Female sample sizes for time periods 1-7 were 41, 29, 31, 19, 83, 56, and 29, respectively.

CHAPTER VI

GENERAL CONCLUSION

Islands are the key to rapid progress in biogeography.

Edward O. Wilson (1995)

… it is not too much to say that when we have mastered the difficulties presented by the peculiarities of island life we shall find it comparatively easy to deal with the more complex and less clearly defined problems of continental distribution…

Alfred Russel Wallace (1902)

The islands of the West Indies have been an invaluable natural laboratory in the study of island biogeography. These islands have played a central role in the dispersal-vicariance debate (Rosen 1976, 1978, Hedges 1992, 1996, 2001, Iturralde-Vinent and MacPhee 1999), have helped shape ideas about adaptive radiation via the taxon cycle (Ricklefs and Cox 1978, Liebherr and Hajek 1990, Miles and Dunham 1996, Ricklefs and Birmingham 1999, 2002, Cook et al. 2008), and have served as testing grounds for the effects of Pleistocene sea level changes and climatic fluctuations (Pregill and Olson 1981, Griffiths and Klingener 1988, Davalos *in press*). In these brief concluding remarks, I address two basic questions: 1) how did the island laboratories of the West Indies help contribute to our understanding of the genetic structure and mating system of *Erophylla sezekorni*?, and 2) how can this understanding be applied to other insular and continental species?.

In chapter II, I explored species boundaries in three endemic West Indian bat genera. By using the West Indies as the geological stage, I was able to determine the

evolutionary histories of three related lineages over the period of several million years. For each lineage, I found that permanent ocean channels limited gene flow and facilitated genetic differentiation among island populations. Given enough time, populations on the edge of ocean barriers eventually can become new species. These results seem to indicate that only substantial boundaries, such as permanent ocean barriers, are sufficient to promote differentiation in these genera of bats. Since bats are vagile organisms, within-lineage diversification may occur only when substantial barriers to gene flow are present. In continental species, mountain ranges are common biogeographic boundaries that promote speciation. For example, the Andes in South America have helped shape genetic divergence within several lineages of phyllostomid bats (Ditchfield 2000). This chapter also showed that patterns of genetic divergence were not congruent between lineages and are consistent with the idea that the ecology of individual species may be the crucial factor in shaping divergence patterns (Heaney 2007)

In chapter III, I studied phylogeographic structure within *Erophylla*. In this case, the island laboratory allowed me to determine divergence times and rates of gene flow between individual islands. It also allowed me to test biogeographic hypotheses, such as recent demographic expansion in *E. sezekorni* due to lower sea levels during the Pleistocene and increased emergent land mass in the Bahamas. Results from this chapter highlight the importance of temporal scale in phylogeographic structuring. Several factors, such as island area, degree of isolation, and the nature of dispersal barriers, can change substantially over time. We can expect the effects of these changes to be similar in both insular and continental species. A good example of this is population expansion

from Pleistocene refugia, which I have documented in *E. sezekorni* and which has been documented in a few continental bat species (Petit et al. 1999, Ruedi et al. 2008).

In chapter IV, I took advantage of the variety of islands of different sizes in the Greater Antilles to test for a correlation between island area and genetic diversity in populations of *E. sezekorni*. I also used several island replicates to test whether or not bottlenecks and genetic isolation were common in these populations. Results show that island bats are more similar to their mainland counterparts than other mammals by having normal levels of genetic diversity, showing no evidence of bottlenecks, and exhibiting little to no genetic isolation (Berry 1986, Frankham 1997, Heaney et al. 2005, Fleming et al. *in press*). Due to vagility and high rates of gene flow, which are common in bats (Burland and Worthington Wilmer 2001), we do not expect insular or continental populations to exhibit reduced genetic diversity except in cases of extreme or prolonged reductions in effective population size (Heaney et al. 2005, Roberts 2006).

Finally, in chapters IV and V, the logistics of small islands such as Exuma in the central Bahamas made the study of genetic structure and mating system in *E. sezekorni* much more tractable. Bahamian caves are typically small, relatively comfortable, and easily accessible. On Exuma,there were at most two species of bats in a cave, and population sizes of *E. sezekorni* ranged from 50 to 350 rather than into the thousands like cave populations on larger Antillean islands or on the mainland. These traits made the study of a complex social structure and mating system possible. The data contained in Chapters IV and V provide a starting point for beginning to understand the social structure and mating systems of related species of bats such as the glossophagines, an important mainland radiation of nectar-feeding phyllostomid bats (Baker et al. 2003).

This study can also shed some light on what to expect in other species of bats that form large, mixed-sex social groups (McCracken and Wilkinson 2000). Overall, island systems provide excellent opportunities to study speciation, phylogeography, genetic structure, and mating systems in bats and other animals and plants. By posing our questions in an island setting, we may be able to accelerate the rate at which we can answer important evolutionary questions, as suggested by A. R. Wallace over 100 years ago.

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APPENDIX I

Haplotypes codes (bold), number of samples for each haplotype (superscript), and localities of samples (in parentheses) from Chapter II for cytochrome-*b*, control region, and concatenated sequences. ABA = Abaco; AND = Andros; ANG = Anguilla; CAI = Middle Caicos; CUB = Cuba; CYB = Cayman Brac; DOM = Dominica; EXU = Exuma; GBA = Grand Bahama; GCY = Grand Cayman; GUA = Guadeloupe; HIS = Hispaniola; JAM = Jamaica; MNT = Montserrat; NEV = Nevis; PR = Puerto Rico; SAB = Saba; SS = San Salvador; STM = St. Maarten; STU = St. Eustatius;

***Brachyphylla* (cytochrome-*b*)**

B. cavernarum: **Bcav1**^{3} (PR); **Bcav2**^{14} (MNT, NEV, PR, SAB, STU, STM); **Bcav3**^{1} (PR); **Bcav4**^{1} (GUA); **Bcav5**^{1} (MNT); **Bcav6**^{1} (MNT); **Bcav7**^{1} (NEV); **Bcav8**^{2} (NEV, STM); **Bcav9**^{1} (SAB); **Bcav10**^{5} (MNT, STU, STM); **Bcav11**^{1} (MNT); **Bcav12**^{4} (ANG); **Bcav13**^{1} (MNT); **Bcav14**^{1} (MNT); **Bcav15**^{2} (MNT); **Bcav16**^{1} (GUA); **Bcav17**^{1} (MNT); **Bcav18**^{1} (GUA); **Bcav19**^{1} (MNT); **Bcav20**^{2} (ANG, GUA); **Bcav21**^{1} (STM); **Bcav22**^{1} (MNT); **Bcav23**^{1} (DOM); **Bcav24**^{1} (NEV). *B. nana*: **Bnan1**^{1} (CUB); **Bnan2**^{1} (CUB) ; **Bnan3**^{1} (CUB); **Bnan4**^{7} (GCY); **Bnan5**^{1} (CUB); **Bnan6**^{1} (CUB); **Bnan7**^{1} (CUB); **Bnan8**^{1} (CUB); **Bnan9**^{1} (CUB); **Bnan10**^{1} (CUB). *B. pumila*: **Bpum1**^{2} (CAI); **Bpum2**^{3} (CAI); **Bpum3**^{1} (HIS); **Bpum4**^{1} (HIS).

***Brachphylla* (control region)**

B. cavernarum: **Bcav1**[2] (PR); **Bcav2**[3] (PR); **Bcav3**[1] (PR). *B. nana*: **Bnan1**[10] (CUB, GCY); **Bnan2**[1] (GCY); **Bnan3**[2] (CUB); **Bnan4**[1] (CUB). *B. pumila*: **Bpum1**[3] (CAI); **Bpum2**[1] (HIS); **Bpum3**[3] (CAI); **Bpum4**[1] (HIS).

***Brachphylla* (concatenated)**

B. cavernarum: **Bcav1**[2] (PR); **Bcav2**[2] (PR); **Bcav3**[1] (PR). *B. nana*: **Bnan1**[1] (CUB); **Bnan2**[1] (CUB); **Bnan3**[1] (CUB); **Bnan4**[5] (GCY); **Bnan5**[1] (CUB); **Bnan6**[1] (CUB). *B. pumila*: **Bpum1**[2] (CAI); **Bpum2**[3] (CAI); **Bpum3**[1] (HIS); **Bpum4**[1] (HIS).

***Erophylla* (control region)**

E. bombifrons: **Ebom1**[1] (HIS); **Ebom 2**[2] (HIS); **Ebom 3**[11] (HIS, PR); **Ebom 4**[1] (PR); **Ebom5**[4] (HIS); **Ebom6**[1] (HIS); **Ebom7**[1] (HIS); **Ebom8**[1] (HIS); **Ebom9**[1] (HIS); **Ebom10**[2] (HIS); **Ebom11**[6] (HIS); **Ebom12**[1] (HIS); **Ebom13**[8] (HIS, PR); **Ebom14**[6] (HIS); **Ebom15**[5] (PR); **Ebom16**[1] (PR); **Ebom17**[1] (HIS). *E. sezekorni*: **Esez1**[2] (SS, CAI); **Esez2**[1] (CAI); **Esez3**[36] (ABA, EXU, GBA); **Esez4**[1] (EXU); **Esez5**[62](ABA, CUB, EXU, GBA, GCY, JAM, SS, CAI) ; **Esez6**[3] (CUB, JAM); **Esez7**[18] (ABA, GBA); **Esez8**[1] (CUB); **Esez9**[1] (EXU); **Esez10**[1] (GBA); **Esez11**[12] (ABA, CUB, GBA); **Esez12**[1] (GBA); **Esez13**[3] (CUB); **Esez14**[1] (CUB) ; **Esez15**[1] (CUB); **Esez16**[9] (CYB, JAM); **Esez17**[1] (JAM); **Esez18**[1] (JAM); **Esez19**[1] (CUB); **Esez20**[1] (CUB); **Esez21**[1] (CUB); **Esez22**[1] (CUB); **Esez23**[8] (CYB, GCY); **Esez24**[1] (JAM); **Esez25**[1] (JAM); **Esez26**[1] (JAM).

***Erophylla* (cytochrome-*b*)**

E. bombifrons: **Ebom1**[1] (HIS); **Ebom2**[3] (HIS); **Ebom3**[1] (HIS); **Ebom4**[5] (HIS, PR); **Ebom5**[1] (HIS); **Ebom6**[1] (HIS); **Ebom7**[5] (HIS, PR); **Ebom8**[1] (HIS); **Ebom9**[1] (HIS). *E. sezekorni*: **Esez1**[2] (CUB); **Esez2**[1] (ABA) ; **Esez3**[1] (GBA) ; **Esez4**[1] (CUB); **Esez5**[1] (GCY); **Esez6**[1] (GCY); **Esez7**[19] (ABA, AND, CAI, CUB, EXU, GBA); **Esez8**[2] (CYB); **Esez9**[1] (CYB); **Esez10**[1] (GCY); **Esez11**[2] (EXU, SS); **Esez12**[1] (JAM); **Esez13**[2] (ABA, GBA); **Esez14**[1] (JAM); **Esez15**[4] (JAM, CYB); **Esez16**[1] (JAM); **Esez17**[1] (JAM); **Esez18**[1] (JAM).

***Erophylla* (concatenated)**

E. bombifrons: **Ebom1**[1] (HIS); **Ebom2**[1] (HIS); **Ebom3**[1] (HIS); **Ebom4**[1] (HIS); **Ebom5**[2] (HIS); **Ebom6**[1] (HIS); **Ebom7**[1] (HIS); **Ebom8**[1] (HIS); **Ebom9**[1] (HIS); **Ebom10**[4] (HIS, PR); **Ebom11**[2] (PR); **Ebom12**[1] (PR); **Ebom13**[1] (PR). *E. sezekorni*: **Esez1**[2] (CUB); **Esez2**[1] (CUB); **Esez3**[2] (CUB); **Esez4**[1] (CUB); **Esez5**[1]- CUB; **Esez6**[3] (CUB); **Esez7**[1] (JAM); **Esez8**[1] (JAM); **Esez9**[2] (JAM); **Esez10**[1] (JAM); **Esez11**[1] (JAM); **Esez12**[1] (JAM); **Esez13**[1] (JAM); **Esez14**[1] (GCY); **Esez15**[1] (GCY); **Esez16**[1] (GCY); **Esez17**[1] (CYB); **Esez18**[2] (CYB); **Esez19**[2] (ABA, GBA); **Esez20**[5] (ABA, GBA, EXU); **Esez21**[2] (GBA); **Esez22**[1] (GBA); **Esez23**[1] (EXU); **Esez24**[1] (SS); **Esez25**[1] (CAI); **Esez26**[1] (CAI).

***Phyllonycteris* (cytochrome-*b*)**

P. aphylla: **Paph1**[1] (JAM). *P. obtusa*: **Pobt1**[1] (HIS); **Pobt2**[1] (HIS); **Pobt3**[1] (HIS); **Pobt4**[1] (HIS); **Pobt5**[1] (HIS). *P. poeyi*: **Ppoe1**[1] (CUB); **Ppoe2**[1] (CUB); **Ppoe3**[1] (CUB); **Ppoe4**[1] (CUB); **Ppoe5**[1] (CUB); **Ppoe6**[1] (CUB).

***Phyllonycteris* (control region)**

P. obtusa: **Pobt1**[1] (HIS); **Pobt2**[1] (HIS); **Pobt3**[1] (HIS); **Pobt4**[1] (HIS); **Pobt5**[1] (HIS); **Pobt6**[1] (HIS). *P. poeyi*: **Ppoe1**[1] (CUB); **Ppoe2**[1] (CUB); **Ppoe3**[1] (CUB); **Ppoe4**[1] (CUB); **Ppoe5**[1] (CUB); **Ppoe6**[3] (CUB); **Ppoe7**[1] (CUB); **Ppoe8**[1] (CUB); **Ppoe9**[2] (CUB); **Ppoe10**[1] (CUB); **Ppoe11**[1] (CUB); **Ppoe12**[1] (CUB); **Ppoe13**[1] (CUB); **Ppoe14**[1] (CUB); **Ppoe15**[1] (CUB); **Ppoe16**[1] (CUB).

***Phyllonycteris* (concatenated)**

P. obtusa: **Pobt1** (HIS); **Pobt2** (HIS); **Pobt3** (HIS); **Pobt4** (HIS); **Pobt5** (HIS). *P. poeyi*: **Ppoe1** (CUB); **Ppoe2** (CUB); **Ppoe3** (CUB); **Ppoe4** (CUB); **Ppoe5** (CUB)

APPENDIX II

Haplotypes codes (bold), number of samples for each haplotype (superscript), and localities of samples (in parentheses) from Chapter III for both control region and cytochrome-*b* sequences. CUB = Cuba; JAM = Jamaica; GCY = Grand Cayman; CYB = Cayman Brac; ABA = Abaco; AND = Andros; CAI = Middle Caicos; EXU = Exuma; GBA = Grand Bahama; SS = San Salvador; HIS = Hispaniola; PR = Puerto Rico.

Control region haplotypes

E. bombifrons: **B1**^{1} (HIS); **B2**^{2} (HIS); **B3**^{11} (HIS, PR); **B4**^{1} (PR); **B5**^{4} (HIS); **B6**^{1} (HIS); **B7**^{1} (HIS); **B8**^{1} (HIS); **B9**^{1} (HIS); **B10**^{2} (HIS); **B11**^{6} (HIS); **B12**^{1} (HIS); **B13**^{8} (HIS, PR); **B14**^{6} (HIS); **B15**^{5} (PR); **B16**^{1} (PR); **B17**^{1} (HIS).

E. sezekorni: **S1**^{2} (SS, CAI); **S2**^{1} (CAI); **S3**^{36} (ABA, EXU, GBA); **S4**^{1} (EXU); **S5**^{62}(ABA, CUB, EXU, GBA, GCY, JAM, SS, CAI) ; **S6**^{3} (CUB, JAM); **S7**^{18} (ABA, GBA); **S8**^{1} (CUB); **S9**^{1} (EXU); **S10**^{1} (GBA); **S11**^{12} (ABA, CUB, GBA); **S12**^{1} (GBA); **S13**^{3} (CUB); **S14**^{1} (CUB) ; **S15**^{1} (CUB); **S16**^{9} (CYB, JAM); **S17**^{1} (JAM); **S18**^{1} (JAM); **S19**^{1} (CUB); **S20**^{1} (CUB); **S21**^{1} (CUB); **S22**^{1} (CUB); **S23**^{8} (CYB, GCY); **S24**^{1} (JAM); **S25**^{1} (JAM); **S26**^{1} (JAM).

Cytochrome-*b* haplotypes

E. bombifrons: **B1**^{1} (HIS); **B2**^{3} (HIS); **B3**^{1} (HIS); **B4**^{5} (PR, HIS); **B5**^{1} (HIS); **B6**^{1} (HIS); **B7**^{5} (HIS, PR); **B8**^{1} (HIS); **B9**^{1} (HIS).

E. sezekorni: **S1**^{2} (CUB); **S2**^{1} (ABA) ; **S3**^{1} (GBA) ; **S4**^{1} (CUB); **S5**^{1} (GCY); **S6**^{1} (GCY); **S7**^{19} (ABA, AND, CUB, EXU, GBA, CAI); **S8**^{2} (CYB); **S9**^{1} (CYB); **S10**^{1} (GCY); **S11**^{2} (EXU, SS); **S12**^{1} (JAM); **S13**^{2} (ABA, GBA); **S14**^{1} (JAM); **S15**^{4} (JAM, CYB); **S16**^{1} (JAM); **S17**^{1} (JAM); **S18**^{1} (JAM).