CHAPTER 5

Phylogeography and Genetic Structure of Three Evolutionary Lineages of West Indian Phyllostomid Bats

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Introduction

Like the Philippines and Wallacea, the West Indian archipelago has been a major center of evolution for many groups of vertebrates, including bats. This archipelago has had a complex geological history (reviewed in Buskirk 1985; Dávalos 2004b; Graham 2003; Iturralde-Vinent and MacPhee 1999; and Jones 1994, among others) and consists of two major geological units: (1) the Greater Antilles, whose islands lie on the Caribbean plate and attained their present positions and configurations beginning about 25 Ma, and (2) the Lesser Antilles, which consists of a double arc of volcanic islands along the eastern margin of the Caribbean plate that date from mid-Eocene/Oligocene (40–45 Ma; the northeast outer arc) or the Oligocene to early Miocene (20–25 Ma; the northwest inner arc; Graham 2003). Estimates of the ages of the present-day Greater Antilles, whose bats are the subject of this chapter, are shown in figure 5.1. Those data and the following synopsis are based primarily on Graham (2003).

Although Jamaica was first emergent by late to middle Eocene (49–42 Ma), it was submerged until 10 Ma and is the youngest of the major Greater Antillean islands. The ages of other Greater Antillean islands date from 15–25 Ma. Cuba is a geologically complex landform that attained its present configuration by late Miocene (19–12 Ma). Western and northern Hispaniola plus proto–Puerto Rico separated from Cuba in early to mid-Miocene (25–20 Ma); southern Hispaniola joined northern Hispaniola in about mid-Miocene (ca. 15 Ma). Puerto Rico separated from northern Hispaniola in the Oligocene/early Miocene (25–23 Ma). The Bahamas Platform occupies the southeastern margin of the North American plate and has been in place since Jurassic-Cretaceous times. For most of the Cenozoic, the Bahamas were barrier reefs or low islands. Extent of the subaerial portions of this platform has varied widely, especially during Pleistocene sea-level fluctuations. At low sea levels, the Great Bahama Bank was one of the largest islands in the Greater Antilles, although its topo-

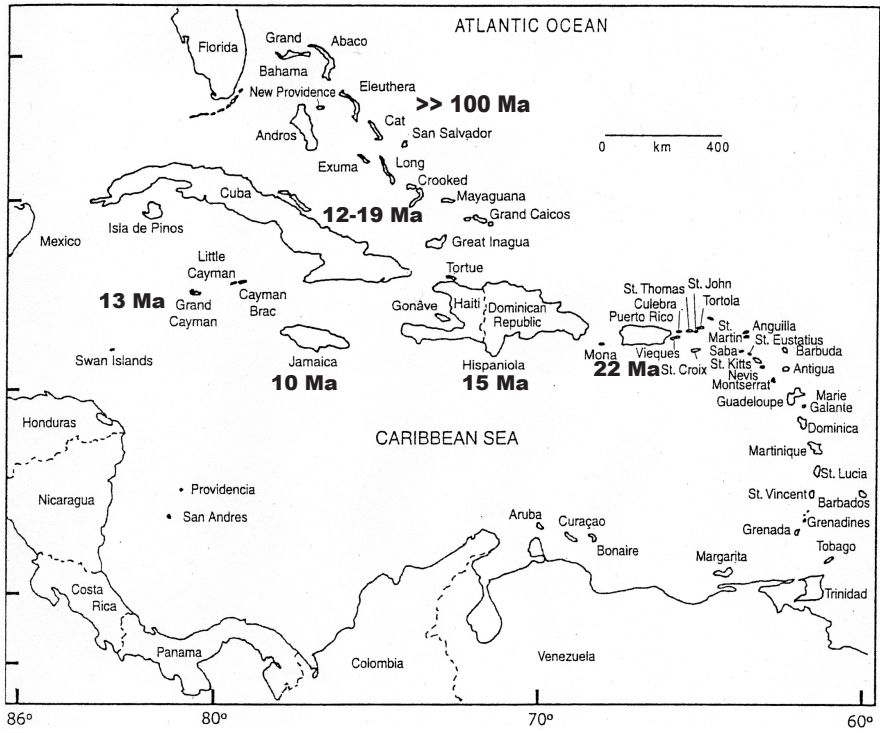


Figure 5.1. Map of the West Indies. Dates (in millions of years ago, Ma) indicate approximate time a particular island or group of islands has been in its present position and configuration based on Jones 1994 and Graham 2003. (Map reprinted with permission from Morgan 2001.)

graphic relief was much less than that of other large Antillean islands. Finally, the Cayman Islands, along with Swan Island, Jamaica, and southern Cuba, were elevated above sea level 10–15 Ma (Jones 1994). In summary, most of the contemporary Greater Antilles have been available for colonization by bats and other organisms for at least 15–20 million years.

The extant chiropteran fauna of this archipelago includes 56 species in 7 families, of which 28 species (50%) are endemic to the region (Rodríguez-Durán and Kunz 2001). For comparison, the other group of volant West Indian vertebrates—birds—contains 425 species in 49 families, of which 150 species (35%) are endemic (Hedges 2001). While no chiropteran family is endemic to the West Indies, funnel-eared bats (Natalidae) are thought to have originated there and then colonized the mainland of Central and South America (Dávalos 2005). In birds, two families are endemic to the West Indies—todies (Todidae) with one genus and five species and the monotypic palm chat (Dulidae). Most families of West Indian bats and birds, therefore, did not originate in the Caribbean. This

is also true of the West Indian flora, which includes no endemic families but over 200 endemic genera (J. Francisco-Ortega, pers. comm.).With 26 species, including 15 endemics (58%), family Phyllostomidae (New World leaf-nosed bats) is the most species-rich and ecologically diverse group of Caribbean bats. Except for a few insect-eaters, this family is represented primarily by nectar- and fruit-eating species (Genoways et al. 2005; Rodríguez-Durán and Kunz 2001). A blood-feeding phyllostomid, *Desmodus rotundus*, is known from fossils on Cuba (Morgan 2001).

The evolutionary history of the West Indian bat fauna has been widely discussed by bat biologists (e.g., Baker and Genoways 1978; Dávalos 2004a, 2004b; Genoways et al. 1998; Genoways et al. 2001; Koopman 1989; Morgan 2001). Central issues in this discussion have involved such questions as (1) Where did these bats come from? The obvious choices for bats as well as for Caribbean birds and other organisms have been North America, Mexico and Central America, or South America. (2) How did they get to these islands? The choices here are via vicariance or dispersal (Dávalos 2004b and chapter 4, this volume; Hedges 2001; Iturralde-Vinent and MacPhee 1999). (3) What were the routes of island colonization and did colonization involve a stepping-stone-like process? (4) How long have different taxa lived together on these islands? Are contemporary Caribbean bat assemblages relatively young or old (Genoways et al. 2005)? More recent discussion points stem from the use of DNA-based phylogenetic and phylogeographic approaches to address such questions as (5) Are island populations monophyletic or do they contain mixtures of lineages with different colonization histories (Carstens et al. 2004; Emerson 2002; Klein and Brown 1994)? (6) What are the current patterns and rates of migration (and gene flow) among islands and between the mainland and islands? Have most species colonized the islands only once or have they done so multiple times (e.g., Klein and Brown 1994)? Finally, since island bats (and birds) are much more prone to extinction than their mainland relatives, perhaps as a result of reduced genetic variation and inbreeding (Frankham 1997, 1998), (7) how much genetic variation do their populations contain and to what extent is this variation a function of island area (i.e., population size), length of time in the islands, and distance from mainland sources of colonization?

Answers to some of these questions are already in hand. Concerning the mode of arrival of bats in the West Indies, for example, the consensus is that dispersal has been the exclusive method (Genoways et al. 2005; Hedges 2001). According to Hedges (2001), at least 18 species of bats dispersed from Mexico/Central America, at least 14 species dispersed from northern South America, and 2 species came from North America. Based on the apparent ages of different West Indian bat lineages, Griffiths and Klingener (1988) proposed that colonization of the Greater Antilles involved a two-stage process involving two geological events: (1) "old" colonists (i.e., species belonging to endemic

subfamilies or lacking congeneric mainland relatives) used a Tertiary chain of islands leading from Central America or Mexico to colonize Cuba/Hispaniola before reaching Jamaica and Puerto Rico, and (2) "younger" colonists (i.e., species having conspecific or congeneric mainland relatives) arrived when sea levels were lower in the Pleistocene, with bats from Mexico or Central America colonizing Jamaica before reaching Cuba and Hispaniola. More recently, Dávalos (chapter 4, this volume) used molecular phylogenies and divergence analyses to determine processes involved in faunal buildup of bats in the West Indies. She tested two hypotheses: (1) West Indian bats arrived overland from South America via Gaarlandia at the Eocene/Oligocene transition (Iturralde-Vinet and MacPhee 1999). (2) They arrived by over-water dispersal from the Neotropical mainland during periods of low sea level. Results allowed her to reject hypothesis 1 and accept hypothesis 2.

In this chapter we will use DNA-based techniques to examine the phylogeography, genetic structure, and demographic history of three lineages of phyllostomid bats in the Greater Antilles. Since these lineages differ strongly in their evolutionary ages and length of residency in the West Indies, they should provide us with considerable insight into the patterns and processes of island colonization by phyllostomid bats. Using control-region mitochondrial DNA (specifically, D-loop mtDNA; Avise 2000), we will address the following questions: (1) What is the phylogeographic structure of these taxa? (2) Are island populations monophyletic? (3) How much genetic diversity resides in their populations, and how is this diversity distributed among islands? (4) What are the demographic histories of these lineages?

The three phyllostomid lineages we are studying include *Macrotus waterhousii*, *Erophylla sezekorni* and *E. bombifrons*, and *Artibeus jamaicensis*. Since these lineages differ in their evolutionary histories and general ecology, it is reasonable to expect that their genetic structure and demographic histories in the Greater Antilles are very different. One of their major differences is evolutionary age, as reflected by their positions in the phyllostomid phylogenetic tree. According to the molecular phylogeny of Baker et al. (2003), *Macrotus* is the basal genus in the family, whose age has been estimated to be 28–34 million years (Jones et al. 2005; Teeling et al. 2005). Additional genetic data (e.g., chromosome banding patterns; Baker 1979) also support the hypothesis that *Macrotus* is basal in the family. Two species of *Macrotus* are currently recognized (Simmons 2005)—*M. californicus*, which occurs in arid parts of the southwestern United States and the Mexican states of Sonora, Chihuahua, and Tamaulipas, and *M. waterhousii*, which occurs in tropical dry forest in western Mexico from southern Sonora south to Guatemala and in the Greater Antilles as far north as Abaco in the Bahamas (Koopman 1993).

The two currently recognized species of *Erophylla* (*sezekorni* and *bombifrons*) are members of the endemic West Indian subfamily Phyllonycterinae, which also includes *Phyllonycteris* with two species. *E. sezekorni* is a western clade

that occurs in the Bahamas, Cuba, Jamaica, and the Caymans; *E. bombifrons* is an eastern clade that occurs on Hispaniola and Puerto Rico (Simmons 2005). The Phyllonycterinae and another endemic West Indian subfamily, Brachyphyllinae (containing one genus, *Brachyphylla*, with two currently recognized species; Simmons 2005), fall midway in the phyllostomid molecular phylogeny of Baker et al. 2003, and both groups are thought to be about 18 million years old (Dávalos, chapter 4, this volume). According to Dávalos, *Erophylla* and *Phyllonycteris* last shared a common ancestor about 8 Ma.

Artibeus jamaicensis belongs to the highly derived subfamily Stenodermatinae and is clearly the most recent of these three lineages to have colonized the Caribbean (Genoways et al. 2005; Morgan 2001; Phillips et al. 1989; Phillips et al. 1991). This species is one of the most common phyllostomids throughout the lowland Neotropics from Mexico to northern Argentina and the West Indies. It is absent from the central and northern Bahamas.

Ecological differences between these species are summarized in table 5.1. At 55–60 g, adults of *A. jamaicensis* are substantially larger than those of the other two species, which weigh 15–20 g. Reflecting its basal position in phyllostomid phylogeny, *M. waterhousii* is an insectivore that feeds on large moths and orthopterans. It has relatively generalized roosting requirements and usually lives in small colonies near the entrances of caves and in abandoned mines and buildings (Genoways et al. 2005). Compared to the other two lineages, it appears to be more extinction-prone and is extinct on 5 of 15 islands (33%), including Puerto Rico, from which its fossils are known (Morgan 2001). *Erophylla* and *A. jamaicensis* are both plant-feeding bats and are more common than *M. waterhousii* on most Antillean islands. *Erophylla* appears to feed mostly on fruit produced by early successional shrubs and small trees; it also visits flowers for nectar and pollen and eats insects, primarily beetles (Soto-Centeno and Kurta 2006). Except in the northern Bahamas, it roosts exclusively in caves but is not restricted to "hot caves" in the Greater Antilles (see Rodríguez-Durán, chapter 9, this volume; Gannon et al. 2005); it is known to roost in abandoned buildings on Grand Bahama and Abaco (Clark and Lee 1999; THF and KLM, pers. obs.; K. Semon, pers. comm.). Compared with *M. waterhousii*, *Erophylla* bats are extinction-resistant and are not known to have become extinct on any Antillean island (Morgan 2001). *A. jamaicensis* is the most common of the three lineages where it occurs in the Greater Antilles (Gannon et al. 2005; Genoways et al. 2005). It is a frugivore that feeds mostly on fruit produced by canopy trees, especially those in the family Moraceae (figs and their relatives). Its relatively broad roosting habits include caves, hollow trees, and the foliage of canopy trees. Compared with the other two lineages, *A. jamaicensis* has a poor fossil record in the Antilles, which Morgan (2001) interpreted as indicating that it is a recent colonist in the Caribbean. Finally, although all three lineages probably have polygynous mating systems that could reduce effective population

Table 5.1. Summary of the body sizes and ecological characteristics of three phyllostomid bats

Characteristic	*Macrotus waterhousii* (15–20 g)	*Erophylla sezekorni/ bombifrons* (15–20 g)	*Artibeus jamaicensis* (55–60 g)
General distribution and abundance in Greater Antilles	Widespread and common throughout, but extinct on Puerto Rico	Widespread and usually common throughout	Widespread, but missing from most of the Bahamas; very common
Roost use	Caves, mines, abandoned buildings	Exclusively caves except in northern Bahamas; not a "hot cave" specialist	Mostly caves but also tree hallows and foliage
Colony sizes	Usually small (≤50) but up to ca. 500	1,000s to 100,000s on Puerto Rico; usually in 100s elsewhere	Usually few 100s in caves; fewer in trees
Diet	Strictly insects, esp. Lepidoptera, Orthoptera, and Odonata	A generalist that eats mostly fruit but also nectar/pollen and insects (esp. beetles); fruit tend to be from early successional shrubs/small trees	Mostly frugivorous but also nectar/ pollen and leaves; fruit tend to be from canopy trees
Reproduction and mating system	Monestrus; polygynous but specific form currently unknown	Monestrus; polygynous, probably promiscuous	Bimodal polyestrous; harem-polygynous
% islands known only as fossil [= known extinctions] (*N* islands)	33% (15)	0% (12)	0% (11)
% islands with no fossil record [= recent colonist?] (*N* islands)	0% (15)	16.7% (12)	45% (11)

Sources: Gannon et al. 2005; Genoways et al. 2005; Morgan 2001; Silva Taboada 1979; KLM and THF, unpublished data.

sizes (N_e) and increase rates of inbreeding (Frankham 1998; Storz 1999), they differ in their annual reproductive output. Females of *A. jamaicensis* are polyestrus and typically have two babies a year whereas females of the other two bats are monestrus and produce only a single baby annually (SilvaTaboada 1979).

Based on their evolutionary and ecological differences, we made the following a priori predictions about the phylogeography and genetic structure of these bats:

1. Assuming that these bats or their ancestors colonized the West Indies from Mexico or Central America, genetic diversity should decrease from west

to east across the Greater Antilles in all three lineages. It should decrease from south to north in the Bahamas in *Erophylla* and *M. waterhousii*.

2. If genetic diversity decreases with age of island residency (Frankham 1997), then diversity should be lowest in species of *Erophylla* and highest in *A. jamaicensis*. Alternatively, because it is the oldest of the three lineages, *M. waterhousii* might have the lowest genetic diversity.

3. If genetic diversity is correlated with population size and trophic position, it should be lowest in *M. waterhousii* (an insectivore) and highest in *A. jamaicensis* (a frugivore).

4. If mobility is correlated with trophic position (Fleming 1992; Levey and Stiles 1992), rates of interisland migration (gene flow) should be higher in the two plant-visiting bats than in the insectivore. Owing to its low mobility, island populations of *M. waterhousii* are more likely to be monophyletic than those of *Erophylla* and *A. jamaicensis*.

Methods

We tested these predictions using control-region mitochondrial DNA (D-loop mtDNA; Avise 2000). We collected tissue samples from the three species from islands throughout the Greater Antilles except Cuba (appendix 5.1). In addition, we analyzed tissue samples from one Mexican population of *A. jamaicensis* and *M. waterhousii* (table 5.2). Bats were captured with extendable hand nets inside of 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. This protocol was approved by the University of Miami IACUC (permit 03–119).

DNA Sequencing and Phylogenetic Analyses

Methods that we used to extract and sequence mtDNA are described in appendix 5.2. Number of haplotypes and their frequencies are shown in appendix 5.3. The evolutionary relationships among haplotypes and islands were explored using maximum likelihood (ML) analysis in PAUP 4.0b10 (Swofford 2002). Likelihood parameters from ModelTest were entered into PAUP to approximate the appropriate model of nucleotide evolution (appendix 5.4). We conducted heuristic ML searches with tree bisection and reconnection (TBR) branch swapping and tested the reliability of particular nodes by performing 100 bootstrap replicates. We used parametric bootstrapping to test the null hypothesis that island populations were monophyletic following Carstens et al. (2004).

Individuals in intraspecific studies are often too closely related to be amenable to traditional phylogenetic analyses. As an alternative, we constructed a

Table 5.2. Summary of genetic diversity data based on 334 bp of control region mtDNA

Site	N	N_h	S	Haplotype diversity (h)	Nucleotide diversity (π)
Macrotus waterhousii					
Mainland	9	2	2	0.50 ± 0.128	0.0031 ± 0.0026
Hispaniola	12	2	1	0.17 ± 0.134	0.0005 ± 0.0008
Jamaica	19	5	15	0.53 ± 0.127	0.0053 ± 0.0036
Abaco	20	1	0	0.00	0.00
Exuma	22	2	7	0.45 ± 0.078	0.0096 ± 0.0058
Grd. Cayman	10	1	0	0.00	0.00
Erophylla sezekorni/bombifrons					
Hispaniola	29	14	21	0.91 ± 0.032	0.0164 ± 0.0091
Jamaica	10	7	13	0.87 ± 0.107	0.0144 ± 0.0087
Puerto Rico	23	5	10	0.72 ± 0.058	0.0123 ± 0.0071
Abaco	16	4	5	0.66 ± 0.108	0.0056 ± 0.0038
Grd. Bahama	28	6	7	0.79 ± 0.036	0.0057 ± 0.0037
Exuma	23	4	4	0.58 ± 0.072	0.0035 ± 0.0026
Grd. Cayman	3	2	1	0.67 ± 0.314	0.0020 ± 0.0025
San Salvador	15	2	1	0.13 ± 0.112	0.0004 ± 0.0007
Cayman Brac	8	2	5	0.25 ± 0.180	0.0038 ± 0.0030
Artibeus jamaicensis					
Mainland	16	11	23	0.93 ± 0.050	0.0249 ± 0.0136
Hispaniola	18	3	2	0.22 ± 0.124	0.0007 ± 0.0009
Jamaica	17	3	2	0.23 ± 0.130	0.0007 ± 0.0010
Puerto Rico	20	5	4	0.44 ± 0.133	0.0017 ± 0.0016
Grd. Cayman	19	2	4	0.11 ± 0.092	0.0013 ± 0.0013
Cayman Brac	7	3	5	0.71 ± 0.127	0.0052 ± 0.0039

Note: Sites are listed in order of largest to smallest area within species. Data are means ±1 SE. N = number of samples; N_h = number of haplotypes ; S = number of variable sites.

minimum spanning tree (MST) for the haplotypes using Arlequin 3.01 (Excoffier et al. 2005).

Population Genetic Analyses

We used Arlequin 3.01 (Excoffier et al. 2005) to conduct standard population genetic analyses for the three species. To assess genetic diversity, we calculated the number of polymorphic sites (S), haplotype diversity (h), and nucleotide diversity (π). Diversity indices were calculated for the entire population and for each island. To examine the extent of genetic subdivision within the data sets, we computed global Φ_{ST} values for each species. In Arlequin, global Φ_{ST} values represent the correlation of random haplotypes within a population (island) relative to random haplotypes drawn from the entire data set. We also calculated pairwise Φ_{ST} values to estimate average genetic distance among island populations and mainland populations when warranted.

Islands are discrete geographic entities often separated by substantial boundaries to dispersal and gene flow. We tested the amount of genetic structure

imposed by islands using an analysis of molecular variance (AMOVA; Excoffier et al. 1992). In one set of analyses we treated each island as a separate population, but in a separate analysis we partitioned the data further. In *A. jamaicensis* and *M. waterhousii*, we partitioned the data into a mainland group and a Greater Antillean island group. In *Erophylla*, data were partitioned into two groups corresponding to the two species, *E. bombifrons* (Hispaniola and Puerto Rico) and *E. sezekorni* (Cuba, Jamaica, Cayman Islands, Bahamas). We performed Mantel tests to determine if geographic distances among populations were correlated with genetic distances (Rousset 1997). We used the How Far Is It? Web site (http://www.indo.com/distance/) to determine geographic distances among island sampling localities. All geographic distances were natural-log transformed. Genetic distances were computed in Arlequin as F_{ST} values. Finally, we estimated the per-site θ under a coalescent model implemented in Migrate-n (Beerli and Felsenstein 2001) to determine the relative effective population size of these species. Theta (for mtDNA, $\theta = 2N_{ef}\mu$ where N_{ef} is effective population size and μ is per-site mutation rate) is an important parameter because the rate at which ancestral polymorphisms sort is proportional to θ. Populations with large effective sizes will take, on average, longer to lose ancestral genetic diversity than small populations. From the standpoint of comparative phylogeography, estimates of θ provide a means to compare genetic diversity across organisms.

Demographic Analyses

We 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 (Russell et al. 2005; Von Haeseler et al. 1996). Values for S and d were calculated in Arlequin. Second, we used various neutrality tests, which in combination can indicate the presence or absence of recent population expansion. We calculated Tajima's D for each species (Tajima 1989). A significant negative Tajima's D is consistent with recent population expansion (Aris-Brosou and Excoffier 1996; Peck and Congdon 2004). We also calculated Fu's F_S and Fu and Li's D^* and F^*. A combination of a significant F_S value and nonsignificant D^* and F^* values indicates demographic expansion (Fu 1997). 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 (Rogers 1995; Rogers and Harpending 1992). We used a raggedness statistic to test goodness of fit of the observed data to a model of exponential population growth. Significance of the raggedness (rg) statistic was tested with

1,000 coalescent simulations (Harpending et al. 1993). Neutrality tests and mismatch distribution analyses were conducted in DnaSP 4.0 (Rozas et al. 2003).

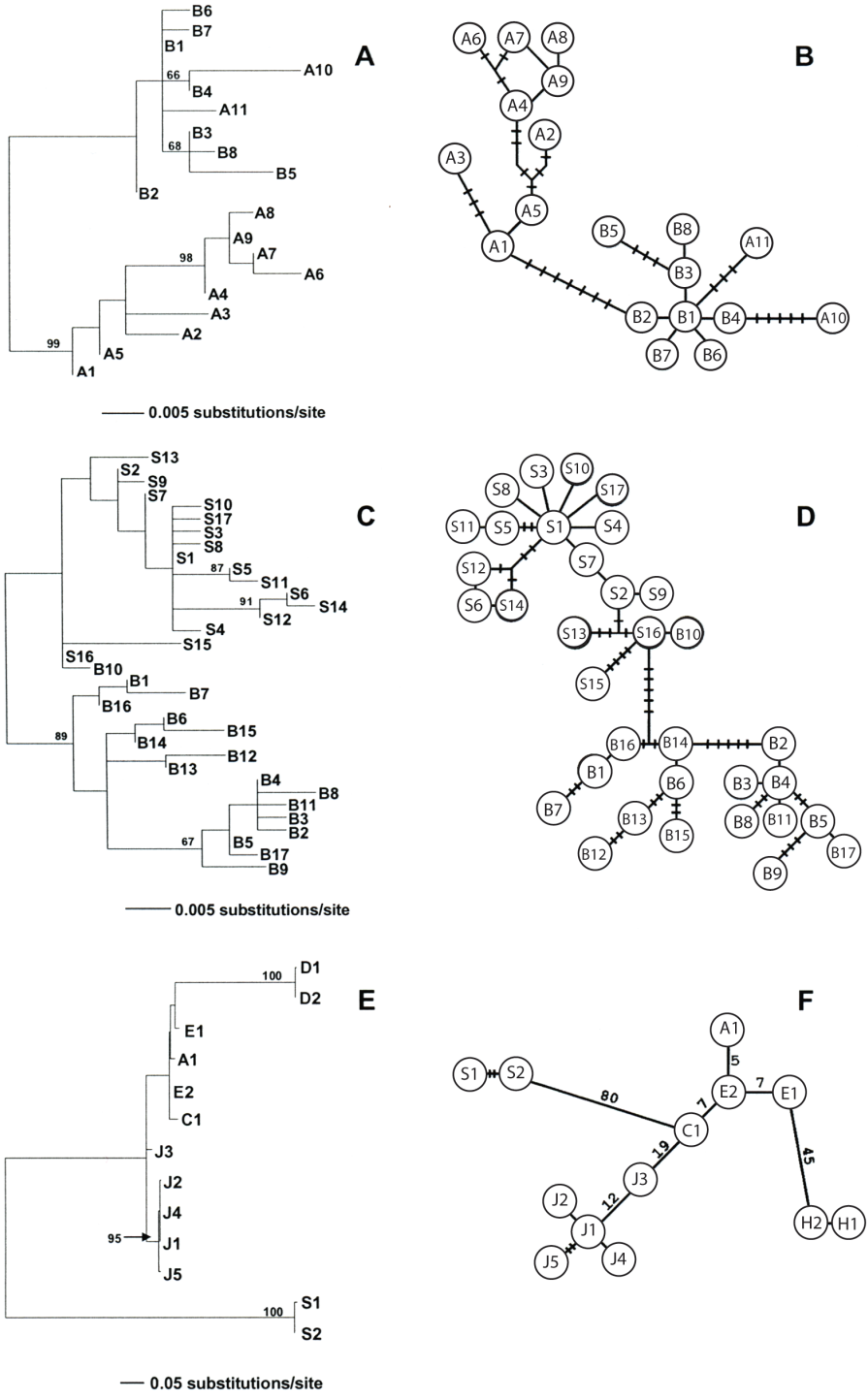
Results

For *Artibeus jamaicensis* we sequenced a total of 97 individuals from six geographic areas (five islands). There were 19 haplotypes (overall $h = 0.52$). The most common haplotype B1 (67 of 97 individuals) was shared among all five sampled Greater Antillean islands, and three haplotypes (B2, B3, and B5) were shared among at least two islands (fig. 5.2A). There were no shared haplotypes between island and mainland samples. We sequenced 155 individuals of *Erophylla sezekorni/bombifrons* from nine Greater Antillean islands and found 34 haplotypes (overall $h = 0.89$). Haplotypes were shared extensively between islands within the *E. bombifrons* and *E. sezekorni*, but not between them (fig. 5.2C). We sequenced 92 individuals of *Macrotus waterhousii* from six geographic areas (five islands) and found 13 haplotypes (overall $h = 0.88$). In contrast to the other species, there were no shared haplotypes among islands in *M. waterhousii* (fig. 5.2E).

Phylogenetic and Phylogeographic Analyses

Traditional phylogenetic analyses (e.g., maximum likelihood) were hindered by several factors. As with most intraspecific analyses, there were often too few polymorphic sites to provide any resolution among haplotypes. Both *A. jamaicensis* and *Erophylla* phylogenies suffered from this problem. In *M. waterhousii*, sequences were actually too divergent. Samples from Sonora, Mexico, and Hispaniola presented significant alignment problems due to their dissimilarity to other geographic areas. Most importantly, the absence of Cuba from the data set tempered all of our phylogenetic (and population genetic) interpretations. We will discuss phylogenetic relationships within our taxa using a larger database elsewhere (KLM and THF, unpublished data).

ML and MST analyses based on sequence evolution models summarized in appendix 5.4 indicated that phylogeographic structure differs strongly in the three species. In *A. jamaicensis*, both the ML tree and the MST showed a clear split between mainland and Greater Antillean haplotypes (fig. 5.2A, B). There was strong bootstrap support (99%) for the mainland clade, and the MST showed seven mutational steps among island and mainland haplotypes. Interestingly, in both trees, two mainland haplotypes (A10 and A11) were nested within the Greater Antillean group, a strong indication that recolonization of the mainland by island populations has occurred in this species. In *Erophylla*, there was good support for the two clades corresponding to specific designations: *bombifrons* (Puerto Rico and Hispaniola) and *sezekorni* (Cuba, Jamaica, Caymans, Bahamas; fig. 5.2C, D). However, as seen in the MST, two intermediate haplotypes (B10 and S16) blurred the boundary between the two clades (fig.


A
B6
B7
B1
A10
66
B4
A11
B3
68
B8
B5
B2
A8
A9
98
A7
A6
A4
A3
A2
99
A5
A1
0.005 substitutions/site
B
A6
A7
A8
A9
A4
A2
A3
A5
A1
B5
B8
A11
B3
B2
B1
B4
A10
B7
B6
C
S13
S2
S9
S7
S10
S17
S3
S8
S1
87
S5
S11
91
S6
S14
S12
S4
S15
S16
B10
B1
B7
B16
B6
89
B15
B14
B12
B13
B4
B8
B11
B3
B2
67
B5
B17
B9
0.005 substitutions/site
D
S3
S10
S8
S17
S11
S5
S1
S4
S12
S7
S6
S14
S2
S9
S13
S16
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S15
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B2
B1
B6
B3
B4
B7
B13
B8
B11
B5
B15
B12
B17
B9
E
100
D1
D2
E1
A1
E2
C1
J3
J2
J4
95
J1
J5
100
S1
S2
0.05 substitutions/site
F
A1
5
S1
S2
80
7
E2
7
E1
C1
19
45
J3
12
J2
H2
H1
J1
J5
J4

5.2D). Finally, data for *M. waterhousii* revealed four main groups: group 1 from Sonora, Mexico; group 2 from Grand Cayman, Exuma, and Abaco (and presumably Cuba); group 3 from Jamaica; and group 4 from Hispaniola (fig. 5.2E, F). As mentioned above, groups 1 and 4 were extremely divergent, and there are clear distinctions between all groups (fig. 5.2F). These data strongly support the hypothesis that *M. waterhousii* is a polytypic taxon in the West Indies. A formal taxonomic analysis of *Macrotus* is needed to delimit species boundaries.

For both *A. jamaicensis* and *E. sezekorni/bombifrons*, we rejected the null hypothesis of island monophyly, which indicates either that these bats likely fly across ocean gaps regularly or that ancestral polymorphisms have not yet fully sorted ($\delta TL_{A.\ jamaicensis} = 28$, $p < 0.01$; $\delta TL_{E.\ sezekorni/bombifrons} = 28$, $p < 0.01$). Given the long residence time of *Erophylla* in the Caribbean, the former explanation is more likely than the latter for those species. For *M. waterhousii*, we were unable to reject the null hypothesis of island monophyly ($\delta TL_{M.\ waterhousii} = 1$, $p = 0.43$). Because of the absence of shared haplotypes among islands and the high level of divergence revealed in the ML tree and MST (fig. 5.2.E, F), this result was not unexpected. *M. waterhousii* appears to be a much less vagile bat than the other two species.

Population Genetic Analyses

Molecular diversity varied substantially within and between species (table 5.2). The mainland population of *A. jamaicensis* had the highest haplotype and nucleotide diversity of any population in this study. In contrast, molecular diversity was generally low in island populations of *A. jamaicensis* and was not correlated with island area (fig. 5.3A), latitude, or longitude (data not shown). In *Erophylla*, there was a clear trend of high molecular diversity on large islands and low genetic diversity on small islands. The regression equation for nucleotide diversity (fig. 5.3B) is $Y = -0.008 + 0.005 \log \text{Area}$ ($r^2 = 0.83$, $p << 0.001$). Nucleotide diversity, but not haplotype diversity, was also correlated with latitude (but not with longitude) in *Erophylla*. Controlling for island area in a multiple regression analysis, nucleotide diversity decreased with increasing latitude ($p = 0.049$; fig. 5.4). In general, molecular diversity values in the Greater Antilles were higher in *Erophylla* than in *Artibeus* and *Macrotus* (table 5.2). Molecular diversity in *M. waterhousii* was low in both mainland and island populations (table 5.2). Although h and π were very high for all *M. waterhousii*

Figure 5.2. Relationships among haplotypes for *Artibeus jamaicensis* (*A*, *B*), *Erophylla sezekorni/bombifrons* (*C*, *D*), and *Macrotus waterhousii* (*E*, *F*). Panels A, C, and E are midpoint-rooted ML phylograms; panels B, D, and F are statistical parsimony haplotype networks. In ML phylograms, numbers above the nodes are bootstrap support values. In the networks, haplotypes are represented by *circles*, and number of mutational steps among haplotypes is represented by the number of *hatch marks* on lines between haplotypes. Haplotypes with no bars are one mutational step apart. Because haplotypes in *M. waterhousii* are very divergent, any distance greater than two mutational steps is represented numerically.

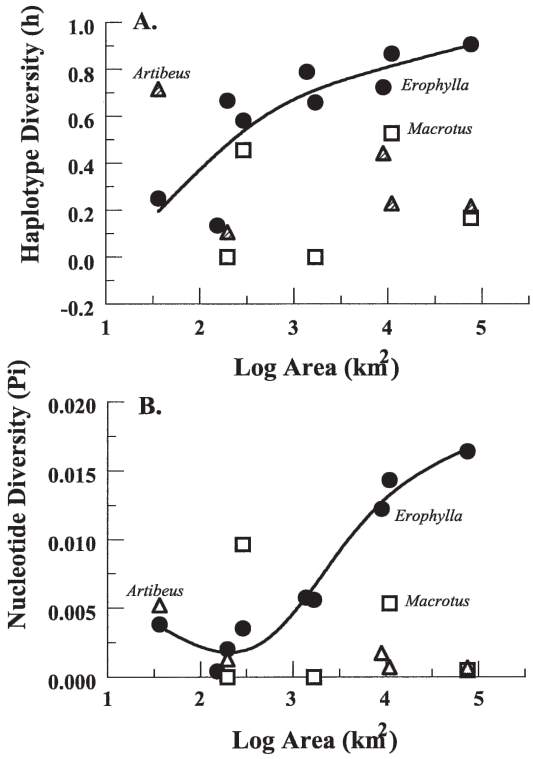

Figure 5.3. Relationship between haplotype diversity (*A*) and nucleotide diversity (*B*) and island area in three lineages of West Indian phyllostomid bats. The lines for *Erophylla sezekorni/bombifrons* represent nonlinear least-squares lines to illustrate significant trends.

populations combined, these values reflect extreme genetic divergence among populations, not genetic diversity within populations. Molecular diversity in *M. waterhousii* was not correlated with island area (fig. 5.3), latitude, or longitude (data not shown).

We used analysis of molecular variance (AMOVA) to examine the effect of ocean barriers on genetic structure (table 5.3). AMOVA revealed that in *A. jamaicensis*, 77% of genetic variation was due to differences among island and mainland populations. An analysis restricted to island populations showed that 94% of genetic variation was found within rather than between islands. Global Φ_{ST} for the island populations of *A. jamaicensis* was low but significant ($\Phi_{ST} = 0.061$, $p = 0.015$), indicating that a small amount of genetic structure exists in this species. In *Erophylla*, islands imposed substantial genetic structure but only between the two species; 65% of variation was found between *E. bombifrons* and *E. sezekorni*, while only 5% was distributed among islands within those species (table 5.3). The high global Φ_{ST} value in *Erophylla* ($\Phi_{ST} = 0.566$, $p < 0.001$) showed that island populations had substantial genetic subdi-

vision, but pairwise F_{ST} values (not shown) revealed that most of the structure occurred between the two species. Islands imposed the greatest amount of genetic structure in *M. waterhousii*. Although 67% of genetic variation was due to differences between mainland and islands, most of the remaining variation resulted from variation among islands (table 5.3). Considering only the island populations, AMOVA revealed that 95% of variation occurred among islands. As expected from these results, global Φ_{ST} was very high in this species (Φ_{ST} = 0.953, $p << 0.001$).

Genetic isolation by distance was tested for in each species using a Mantel test. There was no correlation between genetic distance (F_{ST}) and the natural log of geographic distance in *A. jamaicensis* ($r = 0.407$, $p = 0.176$). There was a significant correlation between genetic distance and the natural log of geographic distance in both *E. sezekorni/bombifrons* ($r = 0.691$, $p < 0.001$) and *M. waterhousii* ($r = 0.593$, $p = 0.001$). In both lineages, significant positive correlations indicated that genetic distance between populations increased linearly with geographic distance, as expected when populations are in migration-drift equilibrium.

Estimates of genetic diversity as measured by θ varied dramatically among the lineages. It was lowest in *A. jamaicensis* (θ = 0.006228; 95% confidence interval = 0.004347 to 0.020206), intermediate in *M. waterhousii* (θ = 0.012368; 0.01045 to 0.14791), and highest in *E. sezekorni/bombifrons* (θ = 0.022006; 0.016977 to 0.029277). Assuming that mutation rates do not differ dramatically among these species, the different effective population sizes that contribute to calculations of θ may have important biological implications for island taxa, with species with a large N_e (e.g., *Erophylla* species) requiring longer periods of isolation

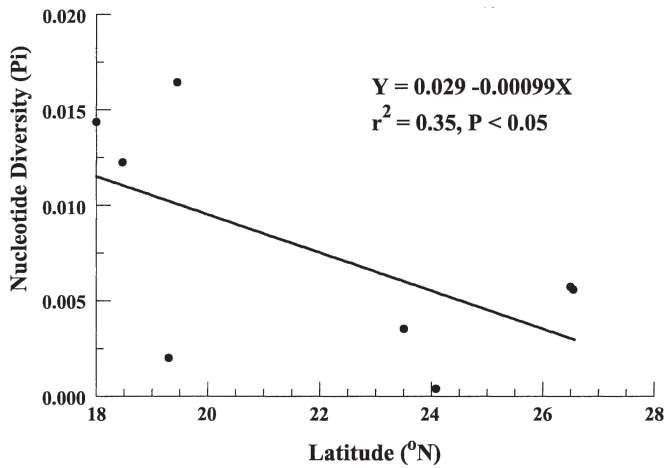

Figure 5.4. Relationship between nucleotide diversity and latitude in *Erophylla sezekorni/bombifrons*. The regression equation and statistics do not control for the effect of island area (see text for the results of a multiple regression analysis).

Table 5.3. AMOVA tables for three lineages of West Indian phyllostomid bats

Source of variation	df	Sum of squares	Variance components	Percent variation
Artibeus jamaicensis				
Among groups	1	76.660	2.851	77.21
Among islands within groups	4	1.945	−0.024	−0.65
Within islands	91	78.766	0.866	23.44
Total	96	157.371	3.693	
Erophylla sezekorni/bombifrons				
Among groups	1	218.962	3.065	65.12
Among islands within groups	7	36.065	0.240	5.09
Within islands	146	204.696	1.402	29.79
Total	154	459.723	4.707	
Macrotus waterhousii				
Among groups	1	597.689	28.655	67.21
Among islands within groups	4	870.440	13.340	31.29
Within islands	86	54.957	0.639	1.50
Total	91	1523.087	42.633	

Note: Groups in *A. jamaicensis* and *M. waterhousii* correspond to island and mainland groups.

Groups in *Erophylla* correspond to the two species (see Methods).

for island monophyly to evolve in the absence of gene flow than species with a small N_e (e.g., *A. jamaicensis*).

Demographic Analyses

We tested for the signature of recent population growth in each species using three methods—the expansion coefficient, neutrality tests, and mismatch distributions. We analyzed mainland and island populations separately in both *A. jamaicensis* and *M. waterhousii*. In *Erophylla*, we analyzed the two species separately.

The mainland population of *A. jamaicensis* showed no indication of recent population expansion. The expansion coefficient was low (table 5.4), and the mismatch distribution was multimodal (fig. 5.5A). In addition, neither Fu's F_s nor Tajima's *D* were significantly negative. Island populations of *A. jamaicensis*, on the other hand, showed a strong signature of recent population growth. The expansion coefficient was very high, and Fu's F_s was significantly negative while Fu and Li's *D** and *F** were not (table 5.4). Tajima's *D* was significantly negative, and the mismatch distribution was unimodal (fig. 5.5B). The time since population expansion was estimated by calculating τ (tau) from the mismatch distribution. Tau was 3.008, and population expansion was dated to about 45,000 BP (table 5.4).

In *Erophylla*, only the *sezekorni* clade showed signs of recent population growth (table 5.4, fig. 5.5C, D). All indices indicated population growth except Tajima's *D*. However, the nonsignificance of this value was marginal ($D = -1.39$, $p = 0.058$). Tau for this group was estimated at 2.673, and time since

Table 5.4. Summary of demographic analyses for three lineages of West Indian phyllostomid bats

	A. jamaicensis (mainland)	*A. jamaicensis* (Greater Antilles)	*E. sezekorni*	*E. bombifrons*	*M. waterhousii* (mainland)	*M. waterhousii* (Greater Antilles)
Expansion coefficient (*S/d*)	2.848	17.868	10.003	4.196	2.000	3.248
Tajima's (1989) *D*	0.294	−1.861**	−1.392†	0.065	1.235	1.555
Fu's (1997) F_s	−1.195	−5.414**	−6.131**	−2.441	2.079	24.497
Fu and Li's (1993) *D**	0.405	−0.844	−2.135	−0.360	1.063	1.619
Fu and Li's (1993) *F**	0.431	−1.409	−2.217	−0.251	1.220	1.911
Raggedness (*rg*)	0.042	0.250	0.030*	0.023	0.750	0.074
Mismatch distribution	Multimodal	Unimodal	Unimodal	Multimodal	Multimodal	Multimodal
Tau (τ)	n.a.	3.008 (0.452–4.334)	2.673 (0.491–6.018)	n.a.	n.a.	n.a.
Time since expansion	n.a.	44,900 BP	40,015 BP	n.a.	n.a.	n.a.

Note: Values in parentheses for τ are 95% confidence intervals. n.a. = no data available.

*$p < 0.005$ **$p < 0.01$ $^{\dagger}p = 0.058$

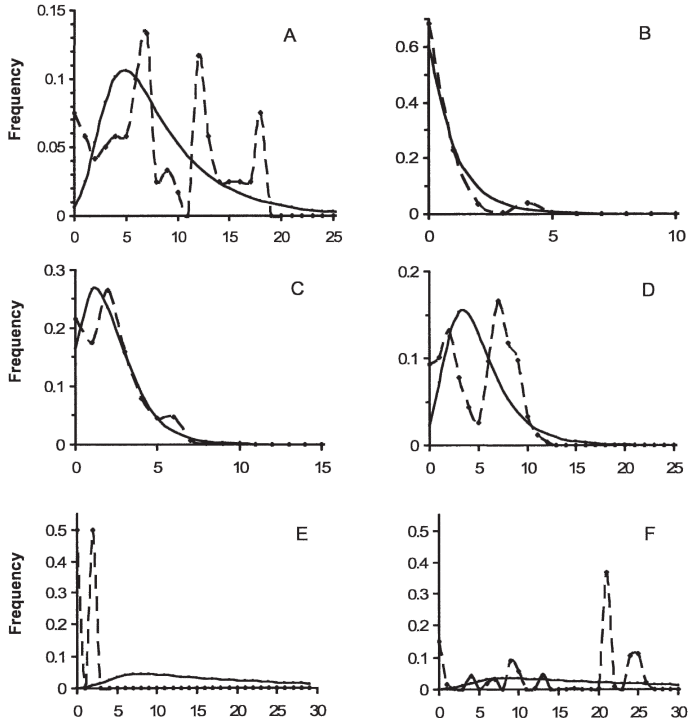

Figure 5.5. Mismatch distributions in the three lineages. *Solid lines* represent the distribution of pairwise differences under a model of exponential population growth. *Dotted lines* represent the actual distribution of pairwise nucleotide differences among haplotypes. *A*, *Artibeus jamaicensis*, mainland; *B*, *A. jamaicensis*, Greater Antilles; *C*, *Erophylla sezekorni*; *D*, *E. bombifrons*; *E*, *Macrotus waterhousii*, mainland; *F*, *M. waterhousii*, Greater Antilles.

expansion was estimated to be about 40,000 BP. The *bombifrons* clade showed no signs of population growth. Similarly, none of the population growth analyses revealed any sign of population growth in island or mainland populations of *M. waterhousii* (table 5.4, fig. 5.5 E, F).

Discussion

Greater Antillean Bats

Current data (Dávalos, chapter 4, this volume) suggest that the phyllonycterine and brachyphylline clades of phyllostomid bats have been in the Greater Antilles for over 10 million years. Island residence time of *M. waterhousii* is also likely to be long, whereas that of *A. jamaicensis* is clearly much shorter than this. By 10 Ma, most of the islands in the Greater Antilles were in their present positions and configurations, although the sizes of low-lying islands such as

the Caymans and Bahamas have fluctuated substantially with eustatic changes in sea level since then, especially during the past 2 million years. Because our D-loop mtDNA data are inadequate for rigorously testing various colonization hypotheses (e.g., those proposed by Griffiths and Klingener 1988), we do not yet know the order in which various Antillean islands were colonized by the three lineages. Nonetheless, it is highly likely that all three taxa or their ancestors colonized the northern West Indies from Mexico or Central America (i.e., from the west). Indeed, our haplotype data for *A. jamaicensis* suggest that the movement of this species has been bidirectional between the Greater Antilles and Mexico. Phillips et al. (1991) reached a similar conclusion for this species based on mtDNA restriction-site analysis.

As revealed by the ML and MST analyses, the phylogeographic patterns of the three species differ strikingly in terms of their overall genetic structure (fig. 5.2). The simplest pattern is seen in *A. jamaicensis*, in which haplotypes fall into only two groups, the mainland and the Greater Antilles. Several haplotypes are shared between islands, and the absence of island monophyly indicates that migration between islands is occurring in this species. Carstens et al. (2004) also reported the absence of monophyly in this species in the northern Lesser Antilles. Given its relatively recent entry into the northern West Indies, lineage sorting has likely not yet reached completion in *A. jamaicensis* (despite its low N_{ef}), and its level of between-island genetic subdivision is very low compared to the other two lineages. Both of these patterns support the hypothesis that interisland dispersal and gene flow is a significant part of this species' population biology. Finally, the absence of a correlation between genetic similarity and geographic distance indicates that *A. jamaicensis* has not yet reached migration-drift equilibrium in the Greater Antilles, a pattern that is seen in the two older island lineages.

Because of its island endemic status, we might expect to see a more complex phylogeographic structure in *Erophylla*, but this is not the case. As in *A. jamaicensis*, this taxon has two major groups of haplotypes that correspond to the two species—a western clade (*E. sezekorni*) and an eastern clade (*E. bombifrons*; fig. 5.2). The presence of one *bombifrons* haplotype in the *sezekorni* clade, however, indicates that these two clades are paraphyletic and that complete lineage sorting has not yet occurred between them. Our cytochrome *b* data indicate that separation between these clades is quite recent, probably within the past million years (i.e., in the Pleistocene; KLM and THF, unpublished data). Gene flow within these two clades is substantial, as indicated by AMOVA (table 5.3), as well as by shared haplotypes between the Cayman Islands and the Bahamas and between Hispaniola and Puerto Rico and the absence of island monophyly in both clades. Given the evidence for substantial north-south genetic connections over a distance of at least 600 km within *E. sezekorni*, as well as gene flow between Hispaniola and Puerto Rico, which are about 120 km apart, it is surprising that the two clades are currently separated genetically. We do

not know what prevents individuals of *Erophylla* from occasionally moving between Cuba and Hispaniola, a distance of only about 100 km presently. A similar east-west subdivision also occurs in other West Indian bats (e.g., in Natalidae [A. Tejedor, pers. comm.] and *Brachyphylla* [L. Dávalos, KLM, and THF, unpublished data]), which suggests that a barrier (perhaps a geological barrier to judge from the relatively deep water channel that separates the two islands) exists in this area that has influenced the evolution of West Indian bats.

Although it has ostensibly resided in the Greater Antilles for less time than *Erophylla* (Griffiths and Klingener 1988), *M. waterhousii* has the phylogeographic pattern expected of an old island endemic on two counts: (1) its haplotypes form four groups that are separated by large genetic distances (fig. 5.2), and (2) islands do not share haplotypes and hence are monophyletic. This bat is clearly much more sedentary than the other two taxa and has likely been isolated on different islands long enough to undergo speciation. Our genetic data suggest that the *M. waterhousii* complex probably contains at least four species, but we need data from Cuba to fully test this hypothesis. In the terminology of Griffiths and Klingener (1988), the *M. waterhousii* complex should be classified as "old island colonists" rather than "recent colonists."

As discussed by Frankham (1997, 1998), Emerson (2002), and Velland (2003), among others, colonization patterns should have predictable population genetic consequences for island species. These include (1) loss of genetic diversity each time an island is colonized owing to founder effects; when islands are colonized in stepping-stone fashion, diversity will be lost with each new colonization, so that more recently colonized islands will contain less genetic diversity than earlier-colonized islands; (2) continued loss of genetic diversity with time on islands as a result of elevated levels of inbreeding and genetic drift, especially on small islands; because of their long residence times on islands, populations of endemic species should contain less genetic diversity than those of nonendemic island species; and (3) a positive correlation between island area (i.e., population size) and genetic diversity. In addition, trophic position, which affects both population size and mobility, should also have predictable genetic consequences with (4) diversity decreasing and degree of population subdivision (e.g., as exhibited by patterns of island monophyly) increasing as trophic level increases.

We tested these predictions using regression analyses and standard population genetics analyses and found that our data support some, but not all, of them. Prediction 1 received weak support. We did not detect a significant longitudinal (west-east) effect on genetic diversity in any of the taxa, and we found a significant latitudinal effect (south-north) only in *Erophylla*. Independent of island area, nucleotide diversity decreased with latitude in this bat, a pattern we would expect if islands were colonized in a south-north fashion. Lack of support for a longitudinal pattern is surprising in *A. jamaicensis* because of its recent colonization but is less so in the other two taxa because of their longer residence times in the Greater Antilles. A pattern of rapid coloniza-

tion and population expansion (see below), coupled with relatively frequent genetic exchanges between islands, would tend to obscure longitudinal and latitudinal patterns in mobile species such as *A. jamaicensis*. Our data and those of Phillips et al. (1991) for this species, however, do support the prediction that mainland populations should contain more genetic diversity than island populations. Haplotype and nucleotide diversity in island populations of *A. jamaicensis* was substantially lower than in the mainland population we sampled (table 5.2).

Because populations of *E. sezekorni* and *E. bombifrons*, the island endemics, generally contained greater molecular diversity than those of the other two taxa on the same islands (fig. 5.3), our data do not support prediction 2. Despite its endemic status, populations of *Erophylla* contained substantial amounts of genetic diversity, more than island populations of the generally more common frugivore, *A. jamaicensis*. Data for species of *Erophylla* from nine islands (fig. 5.3) support prediction 3, but data for the other two species do not. In *A. jamaicensis*, data from Cayman Brac, a very small island, is a strong outlier; its molecular diversity is much higher than expected given the size of this island. One possible reason for this is that the Cayman Islands may be a "way station" in the movement of *A. jamaicensis* bats in both directions between Cuba and Jamaica. We need data from Cuba to test this hypothesis.

Our data provide mixed support for prediction 4. While both frugivores (*A. jamaicensis* and *E. sezekorni/bombifrons*) exhibited far less genetic subdivision than the insectivore (*M. waterhousii*) and their island populations were not monophyletic, only island populations of *Erophylla* generally contained high levels of genetic diversity (table 5.2). This is particularly true when genetic diversity is measured by θ. The rank order of species according to this parameter is *E. sezekorni/bombifrons* > *M. waterhousii* > *A. jamaicensis*, a result that likely reflects the length of island residency of these bats rather than their trophic position or current population sizes. Although it is presently one of the most common phyllostomid bats in the Greater Antilles (Gannon et al. 2005; Genoways et al. 2005), *A. jamaicensis* has apparently not been present in the islands long enough to generate a large N_{ef} and large amounts of neutral variation compared with the two older residents. Overall, our results clearly indicate that the two frugivores are substantially more mobile than the insectivore. A higher extinction rate in *M. waterhousii* (table 5.1) also supports the hypothesis that this species has had low (absolute) population sizes and very low/no rates of migration between islands.

Alternatively, perhaps *M. waterhousii* has become extinct on some Antillean islands as a result of Pleistocene and/or post-Pleistocene climatic and habitat changes. According to Pregill and Olsen (1981), xeric habitats were more extensive in the West Indies during periods of Pleistocene glacial advance when air temperatures and humidity were lower. Interglacial and post-Pleistocene increases in temperature and humidity favored expansion of more mesic habitats and contraction of xeric habitats and was likely responsible for the extinction of

a number of xeric-adapted vertebrates in the Greater Antilles. On the Mexican mainland, *M. waterhousii* currently lives in relatively xeric tropical habitats, and so the loss or reduction of similar habitats in the Greater Antilles probably caused its populations to decrease in size, thereby increasing its likelihood of extinction. If *Macrotus* bats were affected negatively by expansion of mesic habitats, frugivorous bats such as *A. jamaicensis* might have benefited from such changes. Phillips et al. (1991) postulated that this species colonized the Greater Antilles in the Pleistocene during a period of mesic habitat expansion.

Finally, the molecular genetic data indicate that the demographic histories of the three bat lineages differ significantly. Evidence for population expansion was seen in the two frugivores but not in the insectivore (fig. 5.5). A significant expansion signal was seen in island, but not in mainland, populations of *A. jamaicensis*. This pattern is what one would expect if *Artibeus* has recently colonized the Greater Antilles. Timing of the expansion appears to be late Pleistocene (ca. 45,000 BP; table 5.4), although Phillips et al. (1991) suggested that *A. jamaicensis* has been in the West Indies for about 225,000 years. A significant expansion signal was also found in the western clade of *Erophylla* but not in the eastern clade. That is, populations on the large stable islands of Hispaniola and Puerto Rico have not expanded recently, unlike those inhabiting the low-lying islands of the western Greater Antilles (e.g., the Caymans and Bahamas), whose areas increased in the late Pleistocene as sea levels fell. Since genetic diversity in *Erophylla* appears to be strongly correlated with island area (unlike the other two species), we predict that rising sea levels will cause genetic diversity in the western clade to decrease with time as low-lying islands decrease in area. We found no evidence of population expansion in either mainland or island populations of *M. waterhousii*. This is the pattern we would expect to see in a food-limited, sedentary species with low fecundity and low rates of between-population and between-island dispersal. We summarize our results with respect to the four genetic predictions in table 5.5.

Comparisons with Other Island Bats

How do the phylogeographic and genetic patterns we have documented in three lineages of West Indian phyllostomid bats compare with those found in other island bats? Specifically, how do the predictions we tested with our bats hold up for other West Indian bats and for bats in other archipelagos? Except for *A. jamaicensis* (Phillips et al. 1991; Pumo et al. 1988; Pumo et al. 1996), genetic data are very limited for other West Indian bats. In *A. jamaicensis*, Phillips et al. (1991) reported that haplotype diversity was reduced in the Greater Antilles compared to Mexico and that one island haplotype was found in Mexico—results that are concordant with ours. Carstens et al. (2004) studied the phylogeography of *A. jamaicensis* and two endemic phyllostomids, *Ardops nicholsi* and *Brachyphylla cavernarum*, on several islands in the northern Lesser Antilles using cytochrome *b* sequence data. Haplotype diversity was much higher in

Table 5.5. Comparison of four genetic predictions with results from three island archipelagos

Prediction	West Indies (4 species)	Philippines (6 species)	Wallacea (7 species)
1. Genetic diversity (GD) decreases with distance from a mainland source.	Not supported in any species; one species shows a decrease in GD with latitude.	Not tested.	Supported in 4 species; not supported in 3 species.
2. GD decreases with age of island residency (nonendemics > endemics).	Not supported; the endemic species of *Erophylla* have more GD on islands than the 2 nonendemic species.	Supported; GD > in 3 nonendemic species compared to 3 endemic species.	Supported in one comparison: GD in *Cynopterus nusatenggara* < that of *C. brachyotis*.
3. GD positively correlated with island area (population size) and negatively correlated with trophic position (herbivores > insectivores).	Not supported; only *Erophylla* had a positive correlation with island area; GD correlates better with age of island residency than with trophic position.	Not supported; only 1/6 species had a positive correlation with island area; small islands seem to retain substantial GD.	Not supported.
4. Degree of genetic subdivision inversely correlated with vagility.	Supported; subdivision much greater in the insectivore than in the 3 frugivores.	Supported; 3 "weedy" species showed less subdivision than 2 of the 3 endemic species.	Supported in the comparison between *Myotis muricola* and *Scotophilus kuhlii*; mobility of other species not described.

A. jamaicensis than in the two island endemics as predicted above, and island monophyly occurred only in *A. nicholsi*. Incomplete lineage sorting owing to recent colonization from the Greater Antilles likely accounts for the absence of monophyly in *B. cavernarum*, whereas interisland migration likely accounts for its absence in *A. jamaicensis*.

Heaney et al. (2005; Heaney and Roberts, chapter 2, this volume) and Roberts (2006a, 2006b) present allozyme and DNA data for six species of Philippine pteropodid bats from seven islands. Three species (*Cynopterus brachyotis*, *Macroglossus minimus*, and *Rousettus amplexicaudatus*) are "weedy" (i.e., early successional) species that are widely distributed throughout Southeast Asia, and three species (*Haplonycteris fischeri*, *Ptenochirus jagori*, and *Ptenochirus minor*) are Philippine endemics. All of these species are fruit eaters, but the three endemics are much more restricted to primary forest habitats than the nonendemics. As summarized in table 5.5, their data support two of the three genetic predictions they could test. Populations of the nonendemic species generally contained more genetic diversity and were less subdivided than those of the endemic species (prediction 2). In contrast, genetic diversity was correlated with island area in only one of five species (*R. amplexicaudatus*), and it was not

especially low in any species on small islands (contra prediction 3). Analysis of genetic structure indicated that subdivision was generally low in all species within islands as defined by their Pleistocene boundaries but that only the more mobile, nonendemic species evidenced gene flow between Pleistocene islands (prediction 4). They concluded that two factors, (1) mobility as reflected by habitat breadth and geographic distribution and (2) geological history, particularly Pleistocene sea-level fluctuations, have strongly influenced the genetic structure of these species.

Lincoln Schmitt and colleagues (Hisheh et al. 1998; Hisheh et al. 2004; Kitchener et al. 1993; Kitchener et al. 1997; Maharadatunkamsi et al. 2000, 2003; Schmitt et al. 1995; Schmitt et al., chapter 3, this volume) have studied the genetic structure of seven species of bats in three families (Pteropodidae, Rhinolophidae, and Vespertilionidae) in Wallacea. Like the Greater Antilles, the Lesser Sundas form a west-east chain of islands. Reflecting this topology, four of the species evidenced a significant west-east decline in heterozygosity at allozyme loci (prediction 1). No longitudinal trend was seen in two pteropodids (*Aethalops alecto*, *Dobsonia peronii*) and one vespertilionid (*Scotophilus kuhlii*). Regarding levels of genetic diversity (prediction 2), these species generally did not exhibit reduced diversity compared with other mammals, but the endemic *Cynopterus nusatenggara* had lower diversity than its nonendemic congener, *C. brachyotis* (but not the nonendemic *C. sphinx*). In general, levels of genetic diversity were not correlated with island area or trophic position (contra prediction 3); mean heterozygosity was highest in the frugivorous pteropodid *A. alecto* and lowest in the insectivorous vespertilionid *S. kuhlii*. Finally, levels of interisland genetic subdivision were relatively high in six species (F_{ST} values ranged from 0.17 to 0.40) but were notably low (0.03) in *S. kuhlii*, the only species that roosts in human structures. Genetic subdivision was correlated with vagility in the two species of vespertilionids (S. *kuhlii* and *Myotis muricola*) (prediction 4). Although the nectar-feeding pteropodid *Eonycteris spelaea* is a wide-ranging forager (e.g., Start and Marshall 1976), it apparently does not migrate regularly between islands and hence displays substantial genetic subdivision ($F_{st} = 0.12$) in Wallacea.

Data from three other island systems can also be used to test these four predictions. Prediction 1 is supported in two species of pteropodid bats (*Eidolon helvum* and *Rousettus aegyptiacus*) on a series of four islands in the Gulf of Guinea, West Africa. In both species, populations living on the two most isolated islands differ genetically and morphologically from the other islands and the mainland (Juste et al. 1996; Juste et al. 2000). Prediction 2 is generally not supported in island bats, which tend to have similar allozyme diversity compared with their mainland relatives and with other mammals (Juste et al. 2000). Like the Philippine endemic pteropodids, however, the Azorean vespertilionid *Nyctalus azoreum* has lower nucleotide (but not haplotype) diversity than its European congeners (Salguiero et al. 2004). Prediction 3 was not supported by the pteropodid studies in the Gulf of Guinea (Juste et al. 1996;

Juste et al. 2000) and in northern Melanesia (Pulvers and Colgan 2007). Finally, prediction 4 was supported in the Gulf of Guinea and Canary Island studies. Extent of genetic subdivision was higher and estimated interisland migration rates were lower in nonmigratory *R. aegyptiacus* than in migratory *E. helvum*. Similarly, *Plecotus teneriffae*, whose European relatives are highly philopatric and sedentary, exhibits greater genetic subdivision than two species of *Pipistrellus* and *Hypsugo savii* in the Canary Islands (Pestano et al. 2003a; Pestano et al. 2003b).

Conclusions

Reflecting their different ages of residency in the West Indies, the three lineages of phyllostomids that we are studying differ strongly in their phylogeography and genetic structure. The canopy frugivore *Artibeus jamaicensis* is a vagile species that colonized the northern West Indies in the late Pleistocene and has undergone population expansion since then. Its current molecular diversity, however, is low, and it has not yet attained migration-drift equilibrium in the Greater Antilles. It still likely exchanges individuals with the Mexican mainland. Belying its old endemic status, the frugivore-omnivore *Erophylla sezekorni* was nearly panmictic in the Greater Antilles until recently (i.e., 1 Ma). Separation into two monophyletic clades is now nearly complete, and its genetic diversity is strongly correlated with island area. Population expansion occurred in the late Pleistocene in the western clade (*E. sezekorni*) but not in the eastern clade (*E. bombifrons*). Despite a long residency in the West Indies, its levels of genetic diversity are still high, and genetic subdivision within the two clades is low. In contrast, the insectivore *Macrotus waterhousii* exhibits substantial genetic subdivision, and its populations contain low levels of genetic diversity. Unlike the other two taxa, populations on different islands are monophyletic, and genetic distances between islands and its mainland relatives are substantial, indicating that, like *Erophylla*, *M. waterhousii* has resided in the Greater Antilles for a substantial period of time (i.e., much longer than just the Pleistocene). Genetic isolation and low population sizes, perhaps as a result of habitat contraction, have resulted in elevated extinction risk in *M. waterhousii*. In summary, vagility and length of residency in the West Indies have had a strong effect on the genetic diversity and structure of these species and lineages.

Vagility and length of island residence are also important factors in the genetic structure of other island bats. High vagility significantly reduces extent of subdivision in pteropodid, phyllostomid, and vespertilionid bats on islands, and long island residency tends to reduce genetic diversity within populations. Recent colonization, however, can also have this effect, as exemplified by *A. jamaicensis*. Contrary to the predictions of Frankham (1997), however, populations of island bats do not generally contain less genetic variation than mainland relatives, even on small islands in some cases (Heaney et al. 2005). Perhaps this reflects the large population sizes of many bats on islands. For example,

Lloyd (2003) used mtDNA sequence data to estimate that past populations of the endemic bat *Mystacina tuberculata* on New Zealand were as large as 7.8 million females, although current population sizes are orders of magnitude smaller than this. Likewise, current population sizes of mormoopid and certain phyllostomid bats in the Greater Antilles number in the hundreds of thousands (Gannon et al. 2005). Whatever the cause, island bat populations are not necessarily genetically depauperate. As discussed by Heaney and Roberts (chapter 2, this volume) for bats and more generally by Frankham et al. (2002), this trend has important conservation implications. Low genetic diversity generally puts species at risk of extinction (e.g., *M. waterhousii* in this study) and reduces a species' ability to adapt to changing environmental conditions. Many island bats are currently at risk of extinction (Jones et al., chapter 16, this volume), but their major threat is direct human disturbance and not lack of genetic flexibility.

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Table A5.1. Sampling localities

Capture site	Location	Coordinates	*N*	Collected by
Artibeus jamaicensis				
Murciélago Cave	Yucatán, Mexico	20.15000°N, 89.21667°W	16	J. Ortega
Windsor Cave	Windsor RS, Jamaica	18.35131°N, 77.64753°W	8	THF, KLM
Dead Goat Cave	Jamaica	18.48158°N 77.53884°W	9	THF, KLM
Los Patos Cave	Domincan Rep.	17.96016°N, 71.18336°W	10	KLM, A. Tejedor
Mist net	Cano Hondo **NP**, Dominican Rep.	19.05833°N, 69.45359°W	8	KLM, A. Tejedor
Cueva Amador	Puerto Rico	18.48800°N, 66.86681°W	11	THF, A. Rodríguez-Durán
Cueva Larvas	Puerto Rico	18.41433°N, 66.72920°W	9	THF, A. Rodríguez-Durán
Mist net	Grant Cayman	19.27694°N, 81.28279°W	9	
Old Man Bay Cave	Grand Cayman	19.33717°N, 81.17647°W	10	THF, KLM
Pete's Cave	Cayman Brac	19.75383°N, 79.74130°W	7	THF, KLM
Erophylla sezekorni and *E. bombifrons*				
Ratbat Hole	Jamaica	17.87502°N, 76.48270°W	10	THF, KLM, L. Dávalos
Los Patos Cave	Dominican Republic	17.96016°N, 71.18336°W	10	KLM, A. Tejedor
Cueva de Linea	Bahia de Semana, Dominican Rep.	19.07773°N, 69.46648°W	19	KLM, A. Tejedor
Culebrones Cave	Mata de Platano RS, Puerto Rico	18.41667°N, 66.71667°W	20	C. McCain, A. Rodríguez-Durán
Cueva Larvas	Puerto Rico	18.41433°N, 66.72920°W		THF, A. Rodríguez-Durán
Mist net	Grant Cayman	19.27694°N, 81.28279°W	3	THF, KLM
Great Cave	Cayman Brac	19.73636°N, 79.73571°W	8	THF, KLM
Bahamas Cement Co.	Grand Bahama	26.52582°N, 78.77835°W	28	THF, KLM
Little Harbour Cave 1	Abaco	26.32522°N, 77.00197°W	7	THF, KLM
Long Bay Cave North	Abaco	26.14469°N, 77.18855°W	9	THF, KLM
Cabbage Hill Cave	Exuma	23.55582°N, 75.88206°W	23	THF, KLM
Lighthouse Cave	San Salvador	24.11758°N, 74.46432°W	15	THF
Macrotus waterhousii				
Aduana Mina	Sonora, Mexico	27.03900°N, 109.0170°W	9	THF, F. Molina
Portland Bay Cave 9	Jamaica	17.75326°N, 77.15795°W	19	THF, KLM, L. Dávalos
Los Patos Cave	Dominican Rep.	17.96016°N, 71.18336°W	12	KLM, A. Tejedor
Salinas Cave	Grand Cayman	19.34530°N, 81.13302°W	10	THF, KLM
Little Harbour Cave 3	Abaco	26.32653°N, 77.00171°W	20	THF, KLM
Nursery Cave	Exuma	23.57405°N, 75.90583°W	22	THF, KLM

APPENDIX 5.2.

mtDNA Sequencing and Analyses

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 µ1 of Tris-HCl, pH 8.5. We amplified fragments of approximately 350 bp of D-loop mtDNA using polymerase chain reaction (PCR). Because the traditional primers used to amplify bat control-region fragments (P and F; Wilkinson and Chapman 1991) were not reliable for our species, we used primer F1:5′-CCCCACCCT-CAACACCCAAA-3′, redesigned from the *Artibeus jamaicencis* mitochondrial genome (Pumo et al. 1988) coupled with the traditional primer F:5′-GTTGCTGGTTTCACGGA-GGTAG-3′. Total PCR volume was 10 µ1, with 1.0 µ1 Promega 10× 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 10 s, 55°C for 10 s, and 72°C for 20 s, with a final elongation step at 72°C for 5 min. Before cycle sequencing, DNA fragments were incubated with ExoSAP-IT (USB) to dephosphorylate double-stranded DNA and degrade excess primer.

Fragments were sequenced with Big Dye Terminator Cycle Sequencing Kit, version 1.1 (Applied Biosystems). Reaction volumes of 10 µ1 contained 2.5 µ1 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 µ1 of Hi-Di Formamide (Applied Biosystems) for sequencing. All samples were sequenced in both directions using an ABI 310 automated sequencer.

For each species, raw sequence data was edited in Sequencher 4.5 (Gene Codes). We used consensus sequences to determine unique haplotypes, which were then aligned in Clustal X (Thompson et al. 1994). Indels were treated as a fifth character in all analyses. ModelTest 3.7 (Posada and Crandall 1998) was used to determine the appropriate model of nucleotide evolution (appendix 5.4). We used the Akaike information criterion (AIC) test statistic in ModelTest to evaluate goodness of fit of the nucleotide evolution model to our data. The AIC has been shown to outperform the hierarchical likelihood ratio test statistic (Posada and Buckley 2004).

APPENDIX 5.3

Table A5.3. Summary of haplotypes (mtDNA control region) for three lineages of West Indian phyllostomid bats

Haplotype name	Haplotype frequency[a]	Areas of occurrence[b]
Artibeus jamaicensis, 97 samples: Yuc (16), Jam (17), His (18), PR (20), GCy (19), CyB (7)		
A1	4 (0.041)	Yuc (4)
A2	1 (0.010)	Yuc (1)
A3	1 (0.010)	Yuc (1)
A4	1 (0.010)	Yuc (1)
A5	1 (0.010)	Yuc (1)
A6	1 (0.010)	Yuc (1)
A7	1 (0.010)	Yuc (1)
A8	1 (0.010)	Yuc (1)
A9	1 (0.010)	Yuc (1)
A10	3 (0.031)	Yuc (3)
A11	1 (0.010)	Yuc (1)
B1	67 (0.691)	Jam, His, PR, GCy, CyB
B2	3 (0.031)	Jam, PR
B3	3 (0.031)	Jam, His, PR
B4	3 (0.031)	CyB
B5	2 (0.021)	GCy, CyB
B6	1 (0.010)	PR
B7	1 (0.010)	DR
B8	1 (0.010)	PR
Erophylla sezekorni and *E. bombifrons*, 155 samples: Jam (10), His (29), PR (23), GCy (3), CyB (8) GBa (28), Aba (16)		
S1	39 (0.252)	GCy (2), GBa (8), Aba (2), Exu (13), SS (14)
S2	25 (0.161)	GBa (8), Aba (9), Exu (8)
S3	10 (0.065)	GBa (7), Aba (3)
S4	8 (0.052)	GCy (1), CyB (7)
S5	5 (0.032)	GBa (3), Aba (2)
S6	5 (0.032)	Jam (4), CyB (1)
S7	1 (0.006)	SS (1)
S8	1 (0.006)	Exu (1)
S9	1 (0.006)	Exu (1)
S10	1 (0.006)	GBa (1)
S11	1 (0.006)	GBa (1)
S12	1 (0.006)	Jam (1)
S13	1 (0.006)	Jam (1)
S14	1 (0.006)	Jam (1)
S15	1 (0.006)	Jam (1)
S16	1 (0.006)	Jam (1)
S17	1 (0.006)	Jam (1)
B1	11 (0.071)	His (1), PR (11)
B2	7 (0.045)	His (1), PR (6)
B3	6 (0.039)	His (6)
B4	6 (0.039)	His (6)
B5	5 (0.032)	PR (5)
B6	4 (0.026)	His (4)
B7	2 (0.013)	His (2)

(continued on next page)

Table A5.3. *(continued)*

Haplotype name	Haplotype frequency[a]	Areas of occurrence[b]
B8	2 (0.013)	His (2)
B9	1 (0.006)	His (1)
B10	1 (0.006)	His (1)
B11	1 (0.006)	His (1)
B12	1 (0.006)	His (1)
B13	1 (0.006)	His (1)
B14	1 (0.006)	His (1)
B15	1 (0.006)	His (1)
B16	1 (0.006)	PR (1)
B17	1 (0.006)	PR (1)
Macrotus waterhousii, 92 samples: Son (9), Jam (19), His (12), GCy (10), Aba (20), Exu (22)		
S1	6 (0.065)	Son (6)
S2	3 (0.033)	Son (3)
E1	15 (0.163)	Exu (15)
E2	7 (0.076)	Exu (7)
A1	20 (0.217)	Aba (20)
C1	10 (0.109)	GCy (10)
J1	13 (0.141)	Jam (13)
J2	3 (0.033)	Jam (3)
J3	1 (0.011)	Jam (1)
J4	1 (0.011)	Jam (1)
J5	1 (0.011)	Jam (1)
H1	11 (0.120)	His (11)
H2	1 (0.011)	His (1)

Note: Aba = Abaco; CyB = Cayman Brac; DR = Dominican Republic; Exu = Exuma; GBa= Grand Bahama; GCy = Grand Cayman; His = Hispaniola; Jam = Jamaica; PR = Puerto Rico; Son = Sonora, Mexico; SS = San Salvador; Yuc = Yucatán, Mexico.

[a]Numbers in parentheses are proportions.

[b]Numbers in parentheses indicate number of individuals per geographic area with that haplotype.

APPENDIX 5.4

Table A5.4. ModelTest summary

	Artibeus jamaicensis	*Erophylla sezekorni/bombifrons*	*Macrotus waterhousii*
Substitution model	HKY + I	K81uf + I	TrN + Γ
Total bps	335	334	340
Number of indels	1	2	10
Mean indel length	1	1	2.1
Base frequencies			
A	0.3452	0.3351	0.3839
C	0.1793	0.1698	0.2002
G	0.1174	0.1405	0.1038
T	0.3582	0.3546	0.3121
% invariable sites (I)	0.8502	0.8370	0
Γ shape parameter (α)	0	0	0.2262

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