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Exploring population genetic structure in three species of Lesser Antillean bats

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Abstract

We explore population genetic structure in phyllostomid bats (*Ardops nichollsi*, *Brachyphylla cavernarum* and *Artibeus jamaicensis*) from the northern Lesser Antilles by investigating the degree to which island populations are genetically differentiated. Our hypothesis, that the island populations are genetically distinct because of a combination of founding events, limited migration and genetic drift exacerbated by catastrophe-induced fluctuations in population size, is derived from a priori hypotheses erected in the literature. The first prediction of this hypothesis, that within each species island populations are monophyletic, was tested using a parametric bootstrap approach. Island monophyly could not be rejected in *Ardops nichollsi* ($P = 0.718$), but could be rejected in *B. cavernarum* ($P < 0.001$) and *Artibeus jamaicensis* ($P < 0.001$). A second prediction, that molecular variance is partitioned among islands, was tested using an AMOVA and was rejected in each species [*Ardops nichollsi* ($P = 0.697$); *B. cavernarum* ($P = 0.598$); *Artibeus jamaicensis* ($P = 0.763$)]. In *B. cavernarum* and *Artibeus jamaicensis*, the admixture in mitochondrial haplotypes from islands separated by > 100 km of ocean can be explained either by interisland migration or by incomplete lineage sorting of ancestral polymorphism in the source population. As an a posteriori test of lineage sorting, we used simulations of gene trees within a population tree to suggest that lineage sorting is an unlikely explanation for the observed pattern of nonmonophyly in *Artibeus jamaicensis* ($P_W < 0.01$; $P_{SE} = 0.04$), but cannot be rejected in *B. cavernarum* ($P_W = 0.81$; $P_{SE} = 0.79$). A conservative interpretation of the molecular data is that island populations of *Artibeus jamaicensis*, although isolated geographically, are not isolated genetically.

Keywords: *Ardops*, *Artibeus*, *Brachyphylla*, Lesser Antilles, population structure

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Introduction

The Caribbean has long generated interest among biogeographers. A variety of organisms have been studied in great detail including: bats (Pumo *et al*. 1988, 1996; Phillips *et al*. 1989, 1991), lizards (Roughgarden 1995; Malone *et al*. 2000), insects (Liebherr 1995; Davies & Bermingham 2002), birds (Klein & Brown 1994; Lovette *et al*. 1998) and rodents (Woods *et al*. 2001). This interest results from several factors, including the large number of islands, the varying sizes of these islands, the location between two major zoogeographical provinces, and the active tectonic history of the region. This history is most evident in the volcanic islands of the Lesser Antilles. This island chain is located on the eastern margin of the Caribbean tectonic plate, which began moving east ~70 Ma (Pindell 1994). Each of the islands included in this study were formed > 20 Ma and most have never been connected (Bender *et al*. 1979; Donnelly 1988).

Eleven species of bats have colonized islands in the northern Lesser Antilles (Novak 1994). While the establishment of a species on a volcanic island is necessarily the result of a founding event, little is known about the capacity of different bat species to move among islands. Koopman (1977) offered a general prediction for phyllostomid bats when he suggested that oceanic straits presented formidable barriers for dispersal. Similarly, Genoways (1998) proposed, based on morphological data, that migration between

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islands was unlikely in the phyllostomid genus *Sturnira*, and this led him to predict that gene flow between islands was infrequent. Owing to the volcanic origin of the Lesser Antilles, populations on a given island are probably descended from small founding populations, and this allows a number of predictions to be made. Newly founded populations are expected to have much lower haplotype diversity than source populations (Holgate 1966). Furthermore, fluctuations in population size should increase the effects of genetic drift, which is already expected to be a significant force in nonrecombining loci sampled from small populations. Both Caribbean weather patterns and the volcanic nature of the islands are likely to contribute to these fluctuations; populations of bats are vulnerable to Caribbean hurricanes, which may reduce population sizes (Pedersen 1996, 1998), and possibly blow bats off islands. Volcanic eruptions can also have a severe impact on local populations (Pedersen 1997, 2001), either directly or through the ingestion of volcanic ash by the bats during feeding and grooming (Pedersen 2001). Taken together, these factors are expected to contribute to the genetic diversification of island populations in the absence of interisland migration.

Here we investigate genetic variation in Lesser Antillean bats by sampling three phyllostomid species; the neotropical fruit bat *Artibeus jamaicensis*, the tree bat *Ardops nichollsi* and *Brachyphylla cavernarum*. All weigh between 40 and 80 g and have body lengths of 60–100 mm (Novak 1994). Ecologically they are similar; each is a dietary generalist capable of consuming fruit, insects, nectar and pollen. Although they are comparable in size, the distribution of these species differs markedly (Fig. 1). *Ardops nichollsi* occurs only in the northern Lesser Antilles, *B. cavernarum* is limited to the eastern Caribbean and *Artibeus jamaicensis* is widely distributed throughout the Caribbean, Central America and South America (Novak 1994). These distributional differences are probably related to the history of each lineage: *Brachyphylla* and *Ardops* are part of extensively Caribbean clades without close relatives on the continent, whereas *Artibeus* encompasses numerous continental species. The role of dispersal capacity, ecological limits or other extrinsic factors in shaping specific distributions in the northern Lesser Antilles is unknown.

The three species of bats examined here differ in several aspects of their life history (e.g. roosting ecology, flight capacity and fecundity). *Artibeus jamaicensis* and *Ardops nichollsi* roost primarily in trees, and as such may be more vulnerable to population declines during major storms, conversely, populations crashes should not be as frequent in *B. cavernarum*, which roosts exclusively in caves (Pedersen unpublished). Differences in fecundity may offset this vulnerability in *Artibeus jamaicensis* because it can produce as many as three pups per year. *Ardops nichollsi* or *B. cavernarum* typically produce one pup a year (and at the most two pups per year), suggesting that population levels in the these species may not have the capacity to rebound as quickly after a decline as they do in *Artibeus jamaicensis*. Radio-tracking data (Pedersen unpublished) indicate that these species also differ in their flight capacities. *B. cavernarum* is a strong flier and has been recorded flying up to 44 km round-trip circuits, including flights over small bays and along coast-lines, during the course of a single night. Radio-tracking data on Montserrat suggest that *Artibeus jamaicensis* and *Ardops nichollsi* rarely forage more than 10–12 km from a roosting site and may be less capable, or at least less inclined, to fly the longer distances noted in *B. cavernarum*. The differences in roost selection, foraging distance/behaviour and fecundity may result in different patterns of genetic variation among the three species.

Previous research on *Artibeus jamaicensis* suggests that the species has a complex history, and that we may be able to reject the hypothesis proposed by Koopman (1977). Phillips *et al*. (1989), using restriction sites, found three major genotype groups in the Antilles. One of these (labelled 'J') was prevalent on Jamaica, Puerto Rico and Barbados, while the other two genotype groups (labelled 'SV' and 'G') were found on the southern Lesser Antilles near the South American mainland. Pumo *et al*. (1988) also found evidence for genetic exchange between islands using restriction sites, and speculated that maternal lineages in these bats were not confined to limited geographical regions. Pumo *et al*.

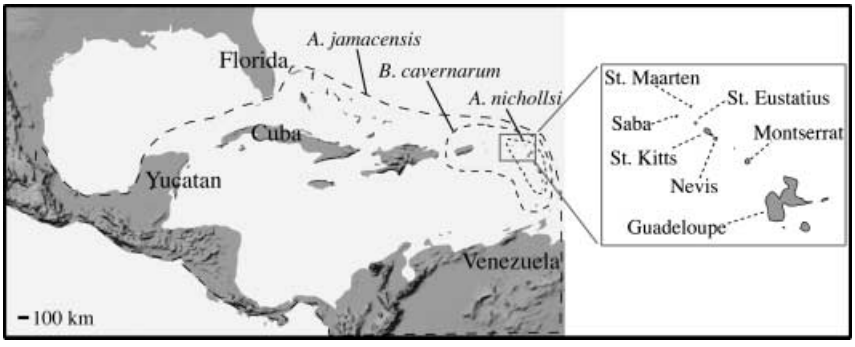

Fig. 1 Map of species ranges, following Eisenberg (1989). The range of *Ardops nichollsi*, *Brachyphylla cavernarum* and *Artibeus jamaicensis* are shown in concentric dotted lines, with *Ardops nichollsi* on the inside. The right-hand side of the figure details the locations of islands where we sampled bats.

(1996) showed that bats carrying the genotypic group 'SV' were derived from the South American mainland, and speculated that the northern Lesser Antilles would act as an intersection of described subspecies of *Artibeus jamaicensis*. There are several described subspecies from this geographical region (Leach 1821; Anderson 1906; Jones 1978), but the mtDNA partitions are not complementary to the described subspecies (Phillips *et al*. 1991).

Here, we test the hypothesis proposed by Koopman (1977) and supported by Genoways (1998), that populations on islands of the Lesser Antilles are genetically isolated. We present sequence data from the nonrecombining cytochrome *b* gene for three phyllostomid species, compare patterns of diversification in each and conduct statistical phylogenetic and coalescent-based hypothesis tests. Specifically, we test two genetic predictions of the hypothesis, that island populations are monophyletic and that genetic variance is distributed among islands rather than within the island populations.

Methods

Tissue sampling

We collected wing punches from three species of Phyllostomid bats from Montserrat, Nevis, St. Kitts, St. Eustatius, Saba and St. Maarten, islands in the Northern Lesser Antilles which are small (< 100 km^2) and separated by sufficient distance (30–100 km) to make migration difficult (Fig. 1). Forty-nine *Artibeus jamaicensis* individuals were sampled from six islands, 32 *Brachyphylla cavernarum* were sampled from five islands, and 14 *Ardops nichollsi* were sampled from three islands. Saunders *et al*. (1984) demonstrated that the probability that a sample of size n individuals contains the deepest coalescent event within a population is $P = (n - 1)/(n + 1)$, therefore we have high probability of sampling the deepest coalescent event on a given island in each species ($P_{Ardops\ nichollsi} = 0.71–0.75$; $P_{B.\ cavernarum} = 0.33–0.86$; $P_{Artibeus\ jamaicensis} = 0.6–0.87$). However, these estimates should be conservative because ancestral haplotypes are expected to be most frequent in the population (Waterson & Guess 1977; Crandall & Templeton 1996). The Saunders *et al*. (1984) equation suggests that modest sampling sizes have a good chance of capturing the basic pattern of haplotype diversity within a population, and as such may offer significant insights from a moderate investment of resources. Individuals were identified to species in the field and voucher specimens were deposited at the University of Nebraska State Museum in Lincoln.

DNA extraction, amplification and sequencing

For each sample, DNA was extracted from wing membrane sampled using a 5 mm autopsy punch (Fray Products Corp.) and wing punches were stored in a 90% ethanol solution. DNA extraction was performed with the DNeasy Tissue kit (Qiagen), following the manufacturer's instructions. The entire length, 1140 bp of the cytochrome *b* mitochondrial gene (*Cyt b*) was amplified. We chose *Cyt b* because it has been widely used in bats and has exhibited sufficient intraspecific variation in several related species (*Glossophaga soricina*, Hoffman & Baker 2001; *Lonchophylla* spp., Davalos & Jansa 2004; *Uroderma bilobatum*, Hoffman *et al*. 2003). While portions of the mitochondrial DNA (mtDNA) genome (e.g. the control region) may evolve more rapidly, the advantages of *Cyt b*, a coding gene with widely available primer and reference sequences, more than compensate for its slightly slower rate of evolution. Primers were designed using *Artibeus jamaicensis* mtDNA sequence acquired from GenBank (Accession no. AF061340; Pumo *et al*. 1998). For the *Artibeus jamaicensis* extractions, we amplified the first two-thirds of *Cyt b* with the forward primer *Aja*For1 (5′-GACATGAAAAATCACCGTTGTAT-3′) and the reverse primer *Aja*Rev1 (5′-TAGGGGTGGAATGGAATTATG-3′) and the second two-thirds of *Cyt b* with the forward primer *Aja*For2 (5′-CCAATCTCCTCTCTGCCATCC-3′) and the reverse primer *Aja*Rev2 (5′-ATTATTCCCTTTGCCGGTTTACA-3′). We used slightly modified primers for *Ardops nichollsi*; we substituted *Ani*For2 (5′-ACAAACCTCCTATCAGACATCC-3′) for *Aja*For2, AND *Ani*Rev2 (5′-TCTGATGGGATGCCTGTTGGATTGT-3′) for *Aja*Rev2. In *B. cavernarum*, we used *Aja*For1 with *Bca*Rev1 (5′-TAAGGGTGGAATGGGATTATGT-3′) to amplify the first two-thirds of *Cyt b*, and primers (5′-CAYGAAWCAGGVTCAAAYAAYCC-3′; 5′-TCTTCATTTYWGGTT-3′) from Jansa *et al*. (1999) to amplify the remainder of the gene.

Polymerase chain reaction (PCR) was used to amplify the entire gene, and amplicons were purified using polyethylene glycol precipitation. Sequencing reactions were performed with the BigDye Kit (Applied Biosystems) using 10–20 ng of PCR product in 10 μL volumes. CentriSep columns (Princeton Separations) were used to filter product from the sequencing reactions, and samples were run on an ABI 377 automated sequencer using 5% Long Ranger polyacrylamide gels. *Cyt b* was sequenced in both the 5′- and 3′-direction, and edited and aligned with SEQUENCER 3.0 (GeneCodes). Sequences were deposited in GenBank under Accession nos AY572329–AY572383.

Population genetic methods

Many analyses require a model of sequence evolution (e.g. ML estimates of genealogy and coalescent simulations) and others are improved through the use of a model-corrected distance matrix (e.g. AMOVA). We selected a model of sequence evolution using DT-MODSEL (Minin *et al*. 2003), a method that incorporates fit, a penalty for overparameterization and performance into the selection process.

In order to explore the genetic structure of each species and provide a basis for comparisons among the three species, we estimated several genetic diversity statistics. These included nucleotide diversity (π), the number of pairwise differences (k) and the number of polymorphic sites (s), which were calculated using ARLEQUIN 2.0 (Schneider *et al*. 2000), and θ ($= 2N_e\mu$), which was estimated using a genealogical sampler implemented in MIGRATE-N (Beerli 2002). Also, pairwise ML-corrected distances were computed with PAUP* (Swofford 2002), and TCS (Clement *et al*. 2001) was used to estimate a statistical parsimony network for each species.

We tested one prediction of our null hypothesis, that genetic variance is distributed among islands rather than within island populations, with an analysis of molecular variance (AMOVA; Cockerham 1969; Excoffier *et al*. 1992). The multiple allelic treatment is not appropriate for nonrecombining mtDNA and uncorrected distances do not account for multiple substitutions in *Cyt b*, so to conduct the AMOVA we used ARLEQUIN to estimate a minimum-spanning haplotype tree, and then computed a distance matrix with our chosen model of sequence evolution.

Phylogenetic analysis

Phylogenetic analysis was performed with PAUP*. For each species, we selected an out-group based on the phylogeny estimate of the Phyllostomidae, the family that contains all the species investigated here (Baker *et al*. 2000). A single *Cyt b* sequence from *Artibeus lituratus* was downloaded from GenBank (U66505; Van Den Bussche *et al*. 1996) to use as the out-group for *Ardops nichollsi*, a sequence from *Glossophaga soricina* (AF382867; Hoffman & Baker 2001) was downloaded to use as the out-group for *B. cavernarum*, and sequence from *Artibeus hartii* (U66517; Van Den Bussche *et al*. 1996) was downloaded to use as the out-group for *Artibeus jamaicensis*. In each dataset, we first excluded redundant haplotypes, and then conducted a branch and bound maximum likelihood (ML) search with TBR branch swapping under the substitution model selected above to estimate the genealogy. Well-supported nodes in the topologies were identified with ML bootstrap values computed from 200 replicates (Felsenstein 1985).

Tests of island monophyly

We tested the null hypothesis that populations of phyllostomid bats are monophyletic by island using a parametric bootstrap (Goldman 1993; Huelsenbeck *et al*. 1996; Sullivan *et al*. 2000; Carstens *et al*. 2002). This approach allows us to assess whether the optimal genealogy is significantly better than a genealogy in which islands contain monophyletic groups. We implemented the parametric bootstrap using maximum parsimony as an optimality criterion because, in doing so, we were able to include all samples from each island without the time penalties that result from branch-swapping between identical haplotypes under ML. First, we searched the data from each species for the most parsimonious phylogeny. We then designed a topological constraint that forced samples collected from each island to form monophyletic clades (Fig. 2) and searched for the most parsimonious phylogeny consistent with that constraint. Using the model of sequence evolution selected with DT-MODSEL, we optimized the branch lengths on this constrained tree, and used SEQ-GEN (Rambaut & Grassly 2001) to simulate 1000 data sets under the best model of sequence evolution on the constrained topology. PAUP* was then used to search each simulated data set for the optimal tree and the optimal tree constrained to meet the predictions of the island monophyly hypothesis. The null distribution

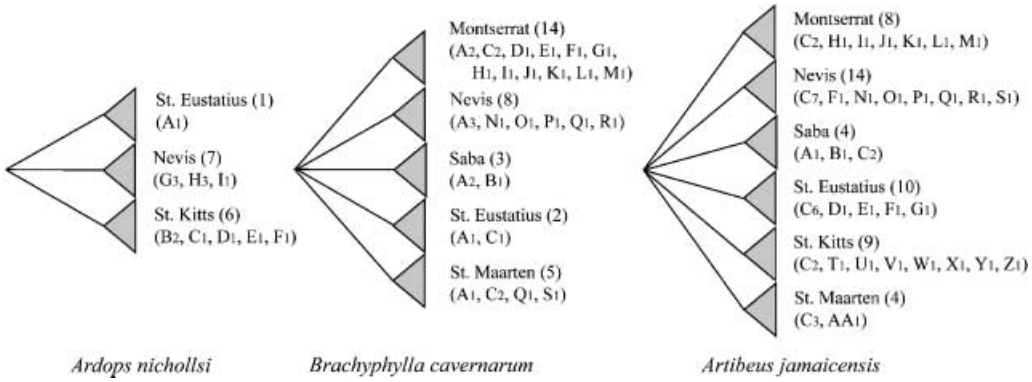

Fig. 2 Constraint trees for the island monophyly null hypothesis. We did not place any constraints on the relationships among these populations in the parametric bootstrap test of island monophyly. Shown are the islands where we sampled bats, with the number sampled per island in parentheses, as well as a list of the haplotypes that we sampled on each island, with the number of times that a particular haplotype was sampled on a given island shown as a subscript the haplotype label. From left to right: *Ardops nichollsi*, *Brachyphylla cavernarum*, *Artibeus jamaicensis*.

was formed by the difference in tree length ($TL_{constrained} - TL_{unconstrained}$) for all replicates. Significance was assessed by comparing the same difference in the actual data ($TL_{constrained} - TL_{unconstrained}$) to the null distribution.

Results

Genetic diversity

In each of the three species, the Tamura–Nei + Γ model was selected as the model with the best expected performance using DT-MODSEL. Tamura–Nei's model (Tamura & Nei 1993) allows unequal base frequencies and has substitution rates for both transition types with a single substitution rate for all transversions (e.g. *r*AC = *r*AT = *r*CG = *r*GT). Rate heterogeneity across sites was modelled with a Γ distribution with four rate categories, following Yang (1993). Base frequencies varied within each species (*Ardops nichollsi* $\pi_A = 0.297$, $\pi_C = 0.3$, $\pi_G = 0.13$, $\pi_T = 0.273$; *B. cavernarum* $\pi_A = 0.279$, $\pi_C = 0.306$, $\pi_G = 0.141$, $\pi_T = 0.274$; *Artibeus jamaicensis* $\pi_A = 0.289$, $\pi_C = 0.27$, $\pi_G = 0.13$, $\pi_T = 0.311$), as did the substitution rates for transitions (*Ardops nichollsi* *r*AG = 1.2633, *r*CT = 6.1644; *B. cavernarum* *r*AG = 2.7302, *r*CT = 5.1656; *Artibeus jamaicensis* *r*AG = 2.167, *r*CT = 6.479) and the α-shape parameter for Γ-distributed rate heterogeneity (*Ardops nichollsi* $\alpha = 0.4717$; *B. cavernarum* $\alpha = 0.3706$; *Artibeus jamaicensis* $\alpha = 0.2101$).

We identified a unique pattern of genetic diversity in each species. In *Ardops nichollsi*, we sampled 9 haplotypes in 14 samples, and no haplotype was found on more than one island. The greatest ML-corrected distance between these haplotypes was 0.00905 substitutions per site. In 32 *B. cavernarum*, we identified 18 haplotypes; 15 of which were unique (i.e. found on a single island), haplotype 'A' was found on all five islands, haplotype 'C' was found on three islands (Montserrat, St. Eustatius and St. Maarten), and haplotype 'Q' was found on two islands (Nevis and St. Maarten). The greatest ML-corrected distance between these haplotypes was 0.01120 substitutions per site, slightly higher than the greatest distance within *Ardops nichollsi*. In *Artibeus jamaicensis*, 27 haplotypes were identified in 49 samples; haplotype 'C' was sampled in 22 individuals from all six islands, haplotype 'F' was sampled in 2 individuals from different islands (Nevis and St. Eustatius) and the other 25 haplotypes were unique. The greatest ML distance was 0.07183 substitutions per site, much higher than in the other species. Regardless of the statistic used, genetic diversity was much higher in *Artibeus jamaicensis* than in the other species (Table 1).

Koopman (1977) predicted that oceanic straits were barriers to dispersal in phyllostomid bats, and this hypothesis predicts that molecular variance will be distributed among island populations. This prediction was not supported with the results of the AMOVA. In each of the three species examined, most of the genetic variance was distributed within groups (e.g. within islands). F_{ST} values among islands were not credibly larger than zero, and could not be rejected by the permutation significance tests conducted in ARLEQUIN ($F_{ST}AG_{Ardops\ nichollsi} = -0.093$, $P = 0.697$; $F_{ST}AG_{B.\ cavernarum} = -0.035$, $P = 0.598$; $F_{ST}AG_{Artibeus\ jamaicensis} = -0.044$, $P = 0.763$). Although among-population variance is effectively zero in each species, most haplotypes are restricted to a single island. This apparent anomaly results from the lack of cladistic coherence in island-restricted haplotypes.

Phylogeny estimates

The genealogy estimated for *Ardops nichollsi* had a ln *L* = –2243.42742 units, the estimate for *B. cavernarum* had a ln *L* = –2425.44490 units and the estimate for *Artibeus jamaicensis* had a ln *L* = –2658.95083 units (Fig. 3). Estimates for *Ardops nichollsi* and *B. cavernarum* were similar in that they both showed a single, well-supported (100% bootstrap) clade with very little genetic divergence or structure within the species. By contrast, haplotypes from *Artibeus jamaicensis* were deeply divergent and divided into two clades (Fig. 3). One clade contains samples from Montserrat, Nevis and St. Kitts, and is well supported (99% bs). The other clade is also well supported (100% bs), and contains the other 46 samples from six islands. Within the second clade the only relationship that is supported with relatively high bootstrap values is the sister relationship between haplotypes 'U' and 'V', which was found in 70% of the bootstrap replicates. The statistical parsimony networks are similar in *B. cavernarum* and *Artibeus jamaicensis*, in that each shows a

Table 1 Molecular diversity statistics for *Ardops nichollsi*, *Brachyphylla cavernarum* and *Artibeus jamaicensis*

Species	*n*	*P*	#haps	AG	*k*	π	θ
Ardops nichollsi	14	0.87	9	0.093	4.35 ± 2.29	0.0038 ± 0.002	0.0053
Brachyphylla cavernarum	32	0.94	19	0.035	3.62 ± 1.89	0.0032 ± 0.002	0.0048
Artibeus jamaicensis	49	0.96	27	0.036	11.58 ± 5.34	0.0101 ± 0.005	0.0179

n, number of samples collected; *P*, probability of the samples capturing the deepest coalescent event; # hap, number of haplotypes; AG, proportion of among group molecular variance; *k*, number of pairwise differences ± 1 SD; π, nucleotide diversity ± 1 SD; θ, estimated using MIGRATE-N.

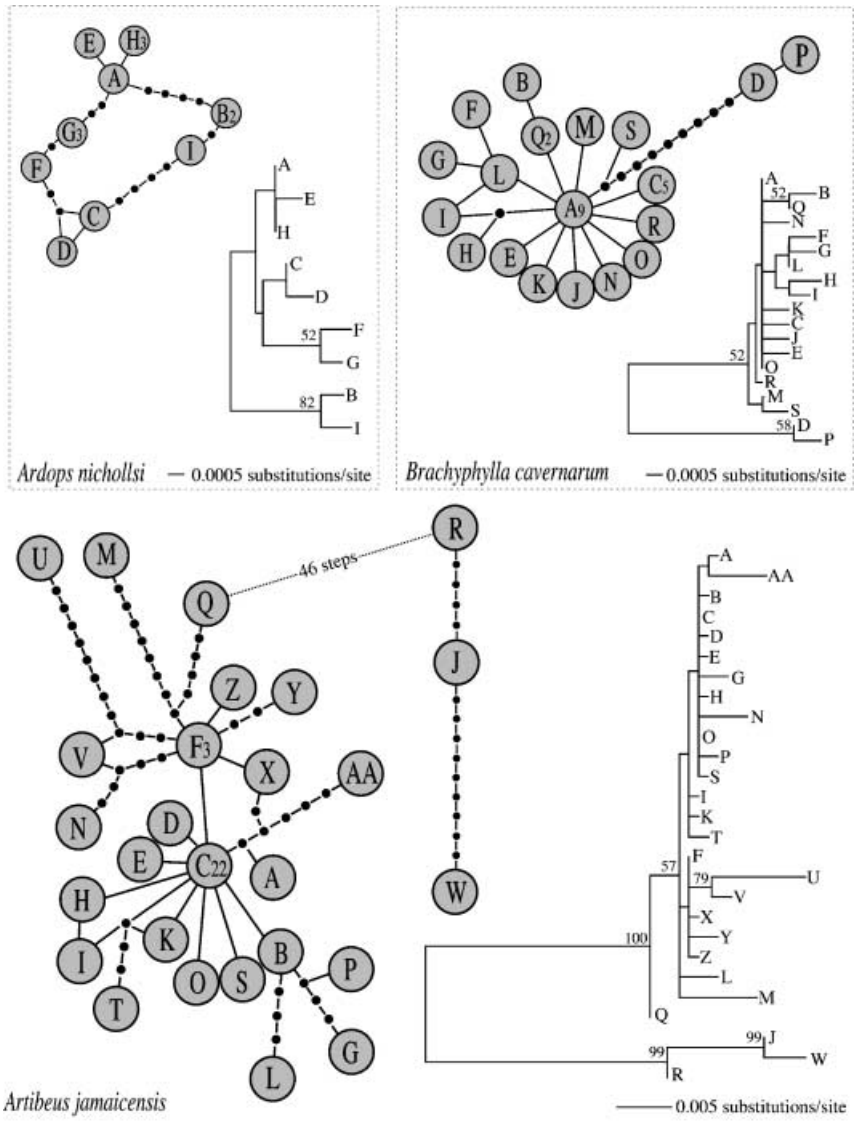

Fig. 3 Estimate of relationships among haplotypes for *Ardops nichollsi* (upper left), *Brachyphylla cavernarum* (upper right) and *Artibeus jamaicensis* (lower). For each species, we show a statistical parsimony haplotype network and a midpoint-rooted ML phylogram. In the network, 'missing' haplotypes are labelled with a solid black dot and sampled haplotypes are labelled with a letter that corresponds to Fig. 2. Haplotypes that were sampled in more than a single individual are labelled with a number after the letter that represents the number of individuals that share the haplotype. Scale for branch lengths of the ML phylogram (in units of substitutions per site) are shown below each phylogram. Numbers above nodes in the phylogram are ML bootstrap values from 200 replicates.

network that is basically star-like with a few divergent haplotypes (Fig. 3). The network for *Ardops nichollsi* reflects the similar degrees of divergence between the four clades of the genealogy, and does not have an obvious centre.

Test of island monophyly

When we forced haplotypes from each island into monophyletic clades and then searched for the optimal topology we found that constrained TL increased by 7 steps for *Ardops nichollsi*, 20 steps for *B. cavernarum* and 137 steps for *Artibeus jamaicensis*. We could not reject the island monophyly hypothesis in *Ardops nichollsi* ($P = 0.718$), however, we could reject this hypothesis for both *B. cavernarum* ($P < 0.001$) and *Artibeus jamaicensis* ($P < 0.001$).

Test of lineage sorting

Rejection of the null hypothesis of island monophyly in *B. cavernarum* and *Artibeus jamaicensis* could simply be the result of incomplete lineage sorting of ancestral polymorphism in the source population, in the absence of any interisland migration. If this is the case, it is possible that there is a history of a single colonization onto each island with no current gene flow, but the ancestral variation has not had sufficient time to sort into reciprocally monophyletic lineages. In order to investigate this possibility, we designed the following a posteriori test of lineage sorting for *B. cavernarum* and *Artibeus jamaicensis*. For the *B. cavernarum* samples, we used MESQUITE (Maddison & Maddison 2004) to simulate coalescent trees constrained within a population tree consistent with a single founding event for each island

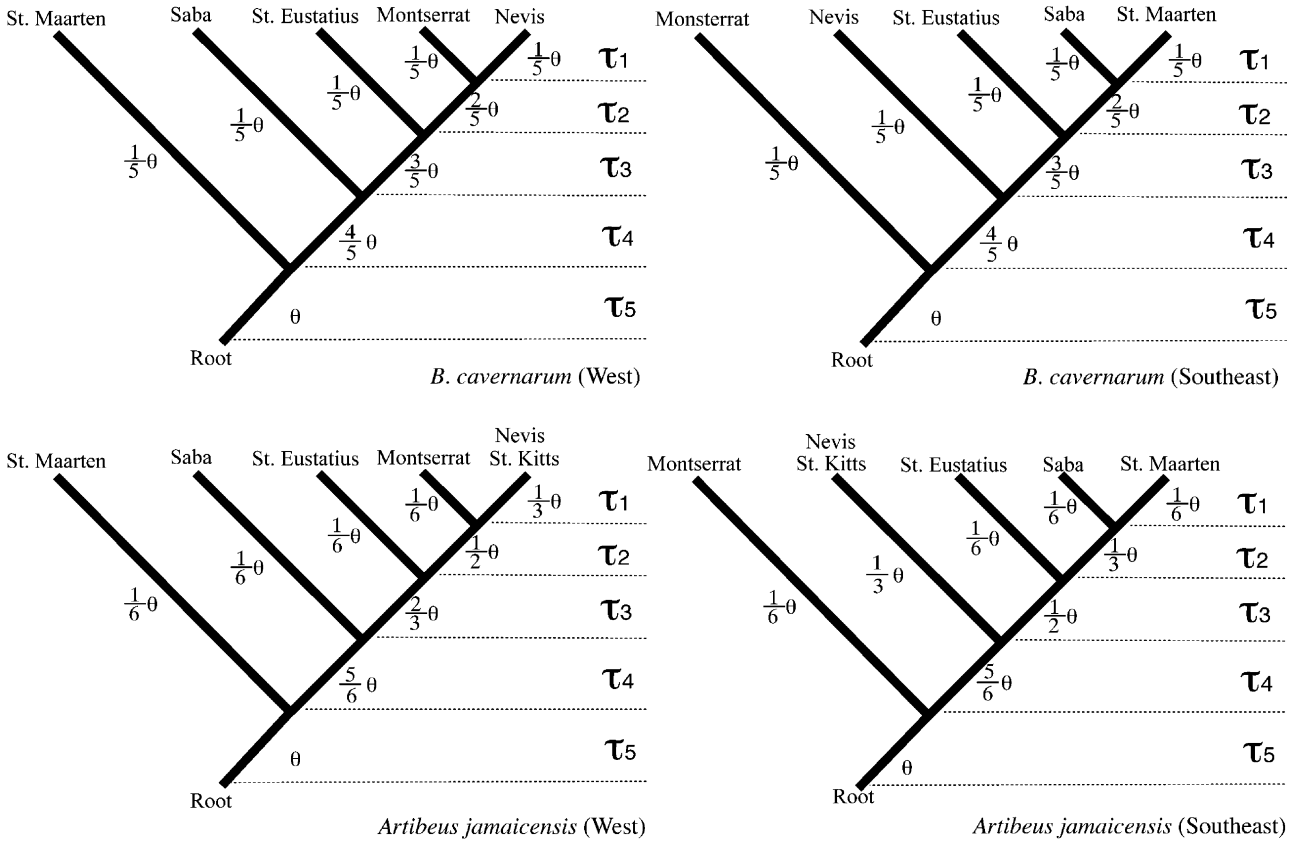

Fig. 4 Model population trees used in the MESQUITE test of lineage sorting. The intervals marked τ_1:τ_5 are equidistant and equal to τ_{Total}. The widths of each branch sum to θ at any given time period τ_i. Population trees for *Brachyphylla cavernarum* are shown in the upper half of the figure, and population trees for *Artibeus jamaicensis* are shown in the lower half of the figure. Rootings of trees correspond to colonization from the west (left) or southeast (right).

(Fig. 4). For these simulations, we used a mutation rate of 0.000001 and θ (estimated using MIGRATE-N) to calculate the N_e, and a simple model of population divergence that predicted that phylogenetic and geographical relationships would be concordant. Because we do not know if the original colonists were derived from southeastern (e.g. Venezuelan via Guadeloupe) or western (e.g. Yucatan via the Greater Antilles) populations, we conducted two tests of lineage sorting for each species. One of these used a population tree that had Montserrat (the southeastern-most island) as the basal population, and the other used a population tree with Saba and St. Maarten (the most northwesterly islands) as the basal populations. These models correspond to colonization for the southeast and west, respectively. These population trees were scaled such that there were equal periods between divergence events, and the width of the branches (representing the effective populations size at that time) summed to the empirical estimate of θ at any point in time (Fig. 4). This model is a reasonable simplification based on empirically estimated values, and should provide a context with which to evaluate the feasibility of incomplete lineage sorting as an alternative explanation to current gene flow. We compared the number of times that the most common haplotype 'A' was found in multiple populations. In this test, we were not able to reject incomplete lineage sorting as an explanation for the observed pattern of nonmonophyly in *B. cavernarum* under the assumption of colonization from either the west ($P_W = 0.81$) or southeast ($P_{SE} = 0.79$). The test we used for the *Artibeus* data was similar, with two notable exceptions. First, St. Kitts and Nevis were conjoined during periods of low sea level in the Pleistocene (Bender *et al*. 1979), so we pooled samples from these islands in our test. Second, in the actual data, a haplotype from Montserrat formed a basal clade with a haplotype from St. Kitts and Nevis (Fig. 3). We looked for this pattern when we simulated genealogies in the population phylogeny. Our test was conservative, in that we searched the set of coalescent trees to determine the number of times that a haplotype from Montserrat formed a basal clade with any haplotype from St. Kitts or Nevis. We were able to reject lineage sorting as an explanation for the pattern observed in the actual data ($P_W < 0.01$; $P_{SE} = 0.04$). Although our a posteriori test of lineage sorting does not account for

branch lengths, the low occurrence of this pattern in the simulated trees indicates that incomplete lineage sorting is not a good explanation for the estimated mtDNA gene tree of *Artibeus jamaicensis*. Furthermore, the long branch separating the clade composed of haplotypes 'J', 'W' and 'R' from the remaining samples suggests that incorporating branch lengths into the test would make the observed topology far less likely to be explainable by lineage sorting.

Discussion

Comparative patterns

At the onset of this project, based on work by Koopman (1977) and Genoways (1998), our prediction was that island populations would be genetically distinct, but only in *Ardops nichollsi* is there some evidence to support this expectation. Even in *Ardops nichollsi*, in which we could not reject the null hypothesis using a parametric bootstrap, the results of the AMOVA suggest that most of the haplotypic variance is not partitioned between islands as our null model predicts. Data from the four additional subspecies of *Ardops nichollsi* in the remainder of its range (Koopman 1994) are necessary to further test this hypothesis. Each of the other subspecies corresponds to a single-island population, whereas the individuals studied here are all assigned to a single widespread subspecies, *A. n. montserratis*, suggesting little morphological differentiation has been observed among these smaller islands.

Conversely, in the other two phyllostomid species, in which we can reject island monophyly and the AMOVA indicates that most haplotypic variance is within islands, the hypothesis of Koopman and Genoways (that there should be genetic differentiation among islands) is easily rejected. Furthermore, although these results contradict our original expectations, it appears that the lack of genetic divergence in *B. cavernarum* and *Artibeus jamaicensis* is the result of different processes. In the former, the failure to reject lineage sorting indicates that lineage sorting of ancestral polymorphism, rather than gene flow among island populations, is a plausible explanation for the observed pattern. This perhaps reflects a recent expansion of *Brachyphylla* from the western Caribbean, where its basal lineages remain. Two morphologically distinct subspecies within *B. cavernarum*, *B. c. intermedia* from Puerto Rico and *B. c. minor* from Barbados (Swanepoel & Geneways 1978), were not sampled here, and our conclusions will be improved by adding data from these species to this study. In *Artibeus jamaicensis*, however, we can reject lineage sorting, and the large amount of genetic diversity found within our sample for this species is in marked contrast to that found within the other species. This is most easily seen in the number of pairwise differences (k), nucleotide diversity (π) or estimates of θ, which are 3–4 times greater in *Artibeus jamaicensis* than they are in *Ardops nichollsi* or *B. cavernarum* (Table 1). The corrected sequence divergence in these samples, 0.07183 substitutions per site, is more than an order of magnitude higher than that found within either or the other species. The degree of differentiation in *Artibeus jamaicensis* calls for further investigation.

Artibeus jamaicensis

We are not the first researchers to identify large amounts of genetic diversity within the mitochondrial genome of *Artibeus jamaicensis* (Pumo *et al*. 1988; Phillips *et al*. 1989), and although the mtDNA divergence of our samples falls into two clades (Fig. 2) that exhibit sequence divergence of 0.07183 substitutions per site, this amount does not exceed the divergence found within other *Artibeus jamaicensis* Cyt *b* sequences. The larger of the clades identified here (which includes 28 of the 31 haplotypes), is similar to the Cyt *b* sequence from Puerto Rico (AF061340; Pumo *et al*. 1998), with ML-corrected sequence divergence of up to 0.00536 substitutions per site. The other *Artibeus jamaicensis* Cyt *b* sequences in GenBank, from Suriname and French Guiana (U66503, U66504; Van Den Bussche *et al*. 1996), are up to 0.08339 substitutions per site from AF061340. The basal clade (J + R + W) in our *Artibeus jamaicensis* genealogy (Fig. 3) is nearly as genetically close to the sequences from South America as it is to other northern Lesser Antilles sequences (0.06358–0.07184 substitutions per site from U66503 and U66504; 0.04811–0.06535 substitutions per site from AF061340), suggesting that these haplotypes may be of South American origin.

The high levels of genetic diversity in *Artibeus jamaicensis* may be a related to the location of these islands in the middle of an archipelago that stretches from the Yucatan peninsula to Venezuela (Phillips *et al*. 1989). Assuming that *Artibeus jamaicensis* was widespread throughout central and northern South America prior to its colonization of the Antilles, a series of colonization events would most likely proceed from the mainland to the closest islands, and then from the closest island to more distant islands, until all the islands were occupied (i.e. a stepping stone model). Each successive colonization would result in a loss of diversity and increased genetic differentiation of islands. If this colonization proceeded on two fronts, the pattern that we might expect where colonists derived from the Yucatan met colonists derived from Venezuela would be one of extreme genetic variation, much like that we have sampled on Montserrat, Nevis and St. Kitts. This pattern has been described as a ring species in the salamander *Ensatina eschscholtzii* (Stebbins 1949). Sequence data from Mexican populations of *Artibeus jamaicensis* is needed to test this hypothesis.

Conclusions

The genetic diversity identified in northern Lesser Antillean *Brachyphylla cavernarum* is best explained by a single

founding event and incomplete lineage sorting of ancestral polymorphism. Northern Lesser Antillean populations of *Ardops nichollsi* are also likely to result from a single founding event, but the lack of shared ancestral polymorphism is an indication that this species has inhabited these islands for a period sufficient to allow lineage sorting to proceed to completion. Genetic data in *Artibeus jamaicensis* suggests that interisland movement in this species is not uncommon. These bats appear to be capable of surviving long flights over open ocean, their roosting habits make them vulnerable to being blown off of an island and their high fecundity increases the likelihood that colonists will become established to new islands. Rather than conforming to a single pattern resulting from the isolation of island populations, the bats studied here seem constrained by individual biogeographical and life histories.

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