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MOLECULAR SYSTEMATICS OF THE FRUIT BAT, *ARTIBEUS JAMAICENSIS*: ORIGIN OF AN UNUSUAL ISLAND POPULATION

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DNA sequences from mitochondrial tRNA genes, the light strand replication site, and a region of the 12S rRNA gene were used to test the hypothesis that the unusual Antillean island subspecies, *Artibeus jamaicensis schwartzi,* was derived from a South American origin. Parsimony and bootstraping analyses allied the mitochondrial genome in these bats with mitochondrial DNA (mtDNA) isolated from *Artibeus planirostris* living in French Guiana rather than with mtDNA isolated from *Artibeus jamaicensis* from the Antilles or Mexico. Although the tRNA sequences differed slightly, the 12S rRNA sequences were identical in mtDNA isolated from *A. j. schwartzi* on St. Vincent and *A. planirostris* from French Guiana. It is proposed that *A. planirostris* was an early arrival to the Antilles, and that it possibly reached the Greater Antilles. Genetic analyses of these island populations may shed light on gene flow, speciation, and even extinction processes in bats. In broader interspecific comparisons, it was noted that the 12S rRNA and cytochrome-*b* genes might evolve somewhat differently among different stenodermatine species, and it was hypothesized that this could affect systematic analyses. None of the mtDNA sequence data supported the utility of the proposed genera *Dermanura* and *Koopmania.*

Key words: *Artibeus,* mitochondrial DNA, 12S rRNA, systematics, Antilles

Because bats can fly, they traditionally were excluded or down-played in analyses of vertebrate zoogeography. However, examples of geographic variation in morphometry or coat color always have begged for explanations in terms of dispersal or partial reproductive isolation (Baker and Genoways, 1978; Jones, 1989; Jones and Phillips, 1970, 1976). The advent of molecular techniques has made it possible to test independently some of the dispersal hypotheses. We previously used restriction-fragment-length-polymorphism (RFLP) analysis and restriction-site mapping of mitochondrial DNA (mtDNA) to trace dispersal of the Jamacian fruit bat, *Artibeus jamaicensis,* into the Caribbean from both Mexico and South America (Phillips et al., 1989, 1991). Although vicariance may have been important for some Caribbean vertebrates, evidence strongly suggests that *A. jamaicensis* arrived since the Pleistocene, well after the Antilles aquired their present form (Morgan, 1989; Morgan and Woods, 1986). The population of *A. jamaicensis* on the Lesser Antillean island of St. Vincent is distinctly different from *A. jamaicensis* living on nearby islands of St. Lucia, Barbados, and Grenada. In recognition of this phenomenon, Jones (1978) gave the St. Vincent population subspecific status, naming it *A. j. schwartzi.* It was difficult to imagine how an island subspecies could arise on one particular island, so Jones (1989) hypothesized a heterosis phenomenon involving gene flow to St. Vincent

from the north and south. However, Pumo et al. (1988) isolated mtDNA from specimens of *A. j. schwartzi* and reported a distinctive SV (St. Vincent) haplotype on the basis of restriction-site mapping. Thus, Jones' (1978) hypothesis did not gain support from mitochondrial data. Until now, little else was known about the unusual *A. jamaicensis* living on St. Vincent. The primary objective of the investigation reported here was to test the hypothesis that the bats on St. Vincent originated somewhere other than St. Vincent. This was tested by sequencing mtDNA of SV and comparing the sequences to mtDNA sequences from other haplotypes associated with *A. jamaicensis,* as well as mtDNA from related species.

A second goal of the present investigation was to compare maternal lineages from *A. jamaicensis* and set their mitochondrial genomes in a broader context within the genus *Artibeus.* For this purpose, we obtained mtDNA sequence data from *A. lituratus, A.* (*Koopmania*) *concolor, A. obscurus,* and *A. planirostris,* as well as the so-called small-sized species, *A.* (*Dermanura*) *phaeotis, A. cinereus,* and *A. gnomus.* Owen (1987, 1991) recently argued in favor of using the name *Dermanura* for the small-sized species of *Artibeus* and proposed the name *Koopmania* for the species *concolor.* The appropriateness of generic status for these species has been questioned directly (Lim, 1993), or indirectly by not using Owen's nomenclature (Lim and Wilson, 1993; Van Den Bussche et al., 1993; Wilson and Reeder, 1993), and we have taken the position that all of these species belong in the genus *Artibeus.*

Materials and Methods

Collecting localities and deposition of voucher specimens are given in Appendix I. DNA was prepared from tissue by the proteinase K method (Kocher et al., 1989). After extraction by phenol-chloroform-isoamyl alcohol, samples were subjected to the polymerase chain reaction (PCR) using the 12S primers described by Kocher et al. (1989). Primers were designed for sequencing the region containing the genes for tRNA-alanine, tRNA-asparagine, tRNA-cysteine, tRNA-tyrosine, and the origin of light strand replication (O_L). The 5′ primer was RNATRIPBOV, 5′-AAGAGCCTTCAAAGCC and the 3′ primer was RNACO1AJJ, 5′-CAACGGGAAATGAACAT. Amplifications were performed using *Taq* polymerase (Perkin Elmer Cetus) according to the manufacturer's instructions with both positive and negative controls. Excess primers and nucleotides were removed from the PCR product by using GeneClean (Bio 101). Purified, amplified fragments were sequenced using Sequenase v.2.0 (United States Biochemical) and [^{35}S]dATP (Amersham). The GenBank accession numbers for sequences are U26275-U26296.

DNA sequence was analyzed using MacVector (v.4.0, IBI), MALIGN (Wheeler and Gladstein, 1993), and PAUP v.3.1.1 (Swofford, 1993). Alignment of DNA sequences from 12S rDNA is difficult because the amplified sequences encode both loop and stem regions of rRNA. One way to resolve the alignment problem is to ignore all sequences from regions that are most difficult to align (Swofford and Olsen, 1990). Alternatively, algorithms can be applied to create alignments with statistical validity. In the present case, we used a combination of methods; the 3′ end of the 12S sequence, which has numerous additions and deletions, was removed from the analysis and the remaining 239 bases were aligned using MALIGN. The transition: transversion ratio was set at 1:2 for the MALIGN alignment. The DNA sequence data were treated as unordered characters in the PAUP program. Both *Brachyphylla* and *Carollia* were used as outgroups, singly and in combination. Heuristic searches were performed and reliability was tested by bootstrapping (100–500 iterations—Felsenstein, 1985). Parsimony analyses were performed using equal weighting of transitions and transversions as well as other ratios up to and including 1:10 (transitions: transversions). DNA sequence for the tRNA genes and the light-strand-replication site (O_L) were analyzed with PAUP as described. Homologous bovine and *A. lituratus* mtDNA sequences were used as an outgroup in an exhaustive search, respectively, and 100 bootstrap replications with 50% majority rule.

Results

The first phase of our analysis was based on mtDNA sequences from tRNA genes

```
                                                                                  60
                    |->tRNA^ala                                                    *
A. jamaicensis(J1)  TAAGGACTGC AAGACTTTAT CCTACAGCTG CTGAATGCAA ATCAACTACT TTAATTAAGC
A. jamaicensis(J4)  .......... .......... ......T... .......... .......... ..........
A. jamaicensis(J8)  .......... .......... ......T... .......... .......... ..........
A. jamaicensis(G)   .......... ......C... ......T..A .......... .......... ..........
A. jamaicensis(T)   .......... .......... .......... .......... .......... ..........
A. jamaicensis(SV)  .......... .......... .......... .......... .......... ..........
A. planirostris     .......... .......... ......T... .......... .........A ..........
A. lituratus        .......... ......C... ......T.. .......... .......... ..........

                                                                                 120
                          <|  |->tRNA^asn                                          *
A. jamaicensis(J1)  TAAGCCCTTA CTAGATTGAT GGGCTTCAAA CCCACGAAAT TTTAGTTAAC AGCTAAATAC
A. jamaicensis(J4)  .......... .......... .......... ....T..... .......... ..........
A. jamaicensis(J8)  .......... .......... .......... ....T..... .......... ..........
A. jamaicensis(G)   .......... .......... .......... ....T..... .......... ..........
A. jamaicensis(T)   .......... .......... .......... ....T..... .......... ..........
A. jamaicensis(SV)  .......... .......... .......... ....T..... .......... ..........
A. planirostris     .......... .......... .......... ....T..... .......... ..........
A. lituratus        .......... .......... .......... ....T..... .......... ..........

                                                                                 180
                                                                                   *
                                          <||->O_L                          <|  |
A. jamaicensis(J1)  CCTAGACAAC TGGCTTCAAT CTACTTCTCC CGCCGCGAAG AAAAAAAAGG CGGGAGAAGC
A. jamaicensis(J4)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(J8)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(G)   .......... .......... .......... .......... .......... ..........
A. jamaicensis(T)   ....A..... .......... .......... .....TA... .......... ..........
A. jamaicensis(SV)  ....A..... .......... .......... .....TA... .......... ..........
A. planirostris     ....A..... .......... .......... .....TA... .......... ..........
A. lituratus        ....A..... .......... .......... .......... .......... ..........

                                                                                 240
                    ->tRNA^cys                                                     *
A. jamaicensis(J1)  CCCGGCAGGA TTGAAGCTGC TTCTTTGAAT TTGCAATTCA ATGTGTTATA CACCACAGGG
A. jamaicensis(J4)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(J8)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(G)   .......... .......... .......... .......... .......... ..........
A. jamaicensis(T)   .......... .......... .......... .......... .......G.. ..........
A. jamaicensis(SV)  .......... .......... .......... .......... .......G.. ..........
A. planirostris     .......... .......... .......... .......... .......G.. ..........
A. lituratus        .......... .......... .......... .......... .......... ..........

                                                                                 300
                    <|->tRNA^tyr                                                   *
A. jamaicensis(J1)  CCTGGTAATA AGAGGACTTA ACCTCTGTAC TTAGATTTAC AGTCTAATAC CTACTCGGCC
A. jamaicensis(J4)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(J8)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(G)   .......... .......... .......... .......... .......... ..........
A. jamaicensis(T)   .......... ......T.C. .........T .......... .......... ..........
A. jamaicensis(SV)  .......... ......T.C. .........T .......... .......... ..........
A. planirostris     .......... ......T.C. .........T .......... .......... ......T...
A. lituratus        .......... ......T.C. .......... .......... .......... ..........
```

FIG. 1.—DNA sequences (300 bases) for four mitochondrial tRNAs and the origin of light strand replication (O_L) in six mtDNA haplotypes isolated from *Artibeus jamaicensis* and haplotypes from *A. planirostris* and *A. lituratus*. Notations above the sequences identify the tRNAs and O_L; GenBank accession numbers—U26281, U26283, U26285, U26286, U26288, U26289, U26291, U26296.

and the light-strand-replication site (O_L), which form a cluster in the mammalian mitochondrial genome. DNA sequences for $tRNA^{ala}$, $tRNA^{arg}$, $tRNA^{cys}$, $tRNA^{tyr}$, and the origin for light strand replication are compared in Fig. 1. Mutational differences distinguished each of the maternal lineages (J1, J4, G, SV) previously established on the basis of restriction-site mapping (Phillips et al., 1991; Pumo et al., 1988) of mtDNA isolated from *A. jamaicensis* from Yucatan, Mexico, and the Antilles. Most of the mutations (70%) were in loops in predicted tRNA secondary structures. A PAUP

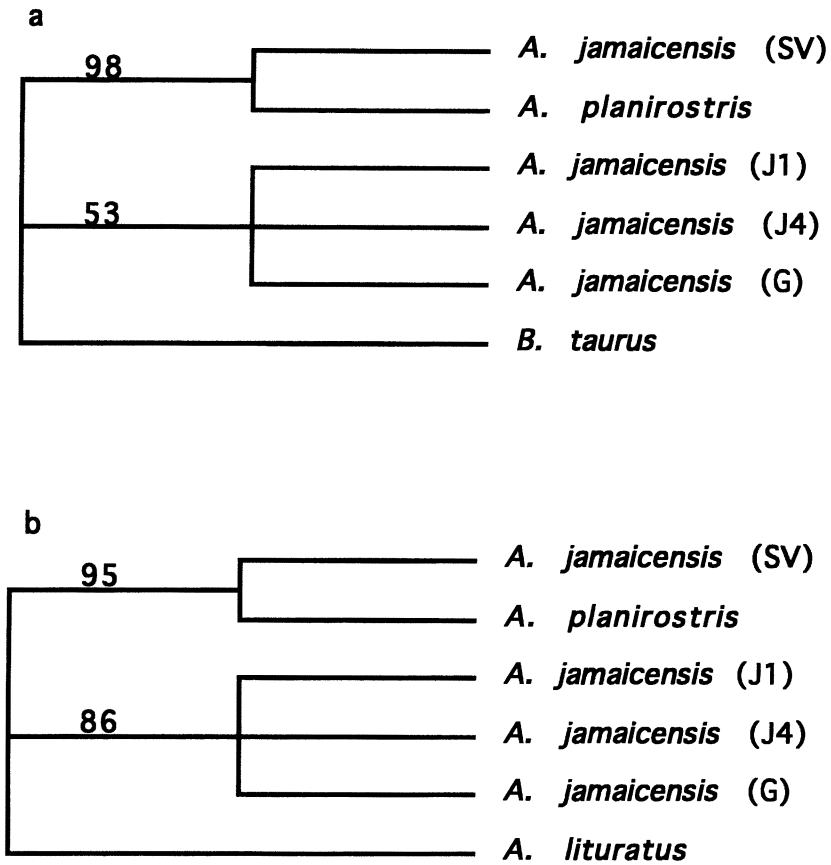

FIG. 2.—PAUP analysis of 300 bp of mitochondrial tRNA and O_L sequences using mtDNA sequence from *Bos taurus* (a) or *Artibeus lituratus* (b) as the outgroup. The topology in (a) represents the consensus tree from an exhaustive search yielding three equally most parsimonious trees each having 53 steps. The consistency index is 0.943. The retention index is 0.750. The tree in (b) also represents the results of an exhaustive search. The tree shown is the consensus of two equally most-parsimonious, 18-step trees with the consistency index equal to 0.889 and the retention index equal to 0.833. In both analyses, the St. Vincent genotype (SV) isolated from *A. jamaicensis schwartzi* living on St. Vincent is clearly allied with homologous mtDNA sequence isolated from *A. planirostris* living in French Guiana. The numbers represent bootstrap values for 100 replicates. DNA sequences from some haplotypes (Fig. 1) were identical (e.g., T = SV, J8 = J4), so only SV and J4 were used in these PAUP analyses.

analysis was used to test the hypothesis that the mtDNA lineage of SV could have been derived from a South American origin, perhaps from *A. planirostris*. The mtDNA sequence of SV paired with the mtDNA sequence isolated from *A. planirostris* obtained in French Guiana (98% bootstrap), rather than with any of the mtDNA sequences from specimens of *A. jamaicensis* or mtDNA isolated from *A. lituratus* (Fig. 2). For the second phase of our analysis, we sequenced 239 base pairs (bp) of mtDNA from the 12S rRNA gene. This phase was expanded to include *A. obscurus, A. concolor, A. phaeotis, A. cinereus,* and *A. gnomus*. Additionally, we included mtDNA sequences from *Enchisthenes hartii, Carollia perspicillata,* and *Brachyphylla cavernarum*. MALIGN software was used to align these mtDNA sequences because mutation-

al differences in this region of the 12S rRNA gene appeared to include additions and deletions as well as transition and transversion substitutions (Fig. 3).

Sequence divergence in pairwise comparisons varied widely among the species examined (Table 1). Four of the haplotype sequences that exhibited mutational differences in the tRNA/O_L region were found to be identical in the sequenced region of the 12S rRNA gene; the G haplotype was equivalent to the J1 haplotype and the SV haplotype was equivalent to the haplotype isolated from *A. planirostris.* In other comparisons within the genus *Artibeus,* sequence divergence ranged from <1% (e.g., *A. concolor* versus *A. lituratus*) to as much as 5.5% (*A. obscurus* versus *A. phaeotis*). The most divergent 12S rRNA sequence was isolated from *E. hartii*; in pairwise comparisons it differed from homologous sequences by 11.9–16.5% (Table 1).

A phylogenetic analysis was conducted with the aligned 12S rRNA sequences. Analyses run with transition: transversion weightings of 1:2 or 1:5 did not affect topology of trees, but a weighting of 1:10 resulted in a loss of resolution among *A. phaeotis, A. cinereus,* and *A. gnomus.* The results of a heuristic search with PAUP, a transition: transversion ratio of 1:2, and 100 bootstrap replications (50% majority rule), are shown in Fig. 4. The best resolution was obtained by using both *C. perspicillata* and *B. cavernarum* as outgroups. Either one alone resulted in up to nine equally parsimonious trees, whereas when 12S rRNA sequences from both species served as outgroups, a single most-parsimonious tree was obtained. In the single most-parsimonious tree, *E. hartii* was paired with *C. perspicillata* (69% of bootstrap replications) and the species of *Artibeus* were united in 97% of the replications (Fig. 4).

Two aspects of the topology of the PAUP-generated tree were particularly noteworthy. First, the three small-sized species of *Artibeus* were separated into two groups (Fig. 4). In 91% of the bootstrap replications, the 12S rRNA sequences from the two species obtained in French Guiana (*A. cinereus* and *A. gnomus*) were united with 12S rRNA sequences from species of *Artibeus* to the exclusion of the 12S rRNA sequence of *A. phaeotis* from Yucatan, Mexico. This outcome placed the small-sized species at the base of the tree, with *A. phaeotis* being derived first. Second, the remaining species of *Artibeus* were all united by a single transversion mutation so that only *A. jamaicensis, A. obscurus,* and *A. planirostris* were united in >60% of the bootstrap replications (Fig. 4).

Alternative hypotheses regarding the relationship between *E. hartii* and species of *Artibeus* were tested using MacClade version 3 (Maddison and Maddison, 1992). When both transitions and transversions were weighted equally, the placement of *Enchisthenes* at the base of the *Artibeus* clade could be accomplished only with a tree that was three steps longer than the most-parsimonious tree. The placement of *Enchisthenes* in any position within the *Artibeus* clade resulted in tree lengths ≥87 steps, at least 10 steps longer than the minimal-length tree. Placement of *Enchisthenes* closer to *Artibeus* had a similar effect when the transversion: transition ratio equaled five. With a larger ratio, the minimum number of steps changed from 153 to ≥169.

Discussion

Origin of the SV haplotype.—In previous papers we used RFLP analysis and restriction-site mapping of the mtDNA molecule to identify maternal lineages in populations of *A. jamaicensis* from Antillean islands (Phillips et al., 1989, 1991; Pumo et al., 1988). By this means we identified three groups of lineages, which we labeled J, G, and SV. Geographically, the J lineages were the most wide-spread being represented from the Yucatan Peninsula of Mexico to as far south as Grenada in the Lesser Antilles (Phillips et al., 1991). On the bases of geographic distribution (Fig. 5) and percentage of bats carrying J haplotypes at var-

```
                                                                                  60
                                                                                   *
A. jamaicensis(J1) GCCAAAGCTT AAAACTCAAG GGACTTGGCG GTGCTTCATA TCCCTCTAGA GGAGCCTGTT
A. jamaicensis(J4) .......... .......... .......... .......... .......... ..........
A. jamaicensis(G)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(SV) .......... .......... .......... .......... .......... ..........
A. planirostris    .......... .......... .......... .......... .......... ..........
A. lituratus       .......... .......... .......... .......... .......... ..........
A. obscurus        .....G.... .......... .......... ...T...... .......... ..........
A. concolor        .......... .......... .......... .......... .......... ..........
A. phaeotis        .......... .......... .......... ......T... .......... ..........
A. gnomus          .......... .......... .......... .......... .......... ..........
A. cinereus        .......... .......... .......... .......... .......... ..........
B. cavernarum      .......... .........A .......... ......T... ...TC..... ..........
C. perspicillata   ........C. .......... .......... ......T... ...T...... ..........
E. hartii          ..A.C...C. .........A .......... ......T... .......... ..........

                                                                                 120
                                                                                   *
A. jamaicensis(J1) CTATAATCGA TAAACCCCGA TCAACCTCAC CAATCCTTGC CAACTCAGCC TATATACCGC
A. jamaicensis(J4) .......... .......... .......... .......... ...T...... ..........
A. jamaicensis(G)  .......... .......... .......... .......... .......... ..........
A. jamaicensis(SV) .......... .......... .......... ...C.....T .......... ..........
A. planirostris    .......... .......... .......... ...C.....T .......... ..........
A. lituratus       .......... .......... .......... ...C...... .......... ..........
A. obscurus        .......... .......... .......... ..GC.T.... .......... ..........
A. concolor        .......... .......... .......... ...C...... .......... ..........
A. phaeotis        .......... .......... .A........ ...C...... .......... ..........
A. gnomus          .......... .......... .A........ ...C...... .......... ..........
A. cinereus        .......... .......... .A........ ...C...... .......... ..........
B. cavernarum      .......... .......... .A........ ...C...... .......... ..........
C. perspicillata   .......... .......... .A........ ...C...... .......... ..........
E. hartii          ..G....... .......... .A........ ..G....... T..TA..... ..........

                                                                                 180
                                                                                   *
A. jamaicensis(J1) CATCTTCAGC AAACCTTAAA AAAGAACTGT AGTAAGCTCA ATCACAG-TA CGCGAAAACG
A. jamaicensis(J4) .......... .......... .......... .......... .......-.. T.T.......
A. jamaicensis(G)  .......... .......... .......... .......... .......-.. ..........
A. jamaicensis(SV) .......... .......... .......... .......... ......A-.. ..........
A. planirostris    .......... .......... .......... .......... ......A-.. ..........
A. lituratus       .......... .......... ........A. .......... ......A-.. ...A......
A. obscurus        .......... .......... .......... .......... ......A-.. ..........
A. concolor        ....C..... .......... ........A. .......... ......A-.. ..TA......
A. phaeotis        ....C..... .......... .......CA. .......... ......A-.. ...A......
A. gnomus          ....C..... .......T.. ......T... .......... ......AT.. ...A......
A. cinereus        ....C..... .......T.. .........C .......... ......A-.. ..Y.......
B. cavernarum      .......... .....C.... ..G..GACAC .......... .CT...AAA. T.YA......
C. perspicillata   .......... .....C.G.. ..G..C.CAC .......... .TAATAG-.. .ATA..G...
E. hartii          .......... .....C.... ..G..G.CAC .......-CA AATCATACA. .ATA......

                                                                                 239
                                                                                   *
A. jamaicensis(J1) TTAGGTCAAG GTGTAGCCTA TGGGTTGGAA AGAAATGGGC TACATTTCC TATTACTAGG
A. jamaicensis(J4) .......... .......... .......... .......... ......... ..........
A. jamaicensis(G)  .......... .......... .......... .......... ......... ..........
A. jamaicensis(SV) .......... .......... .......... .......... ......... ...C......
A. planirostris    .......... .......... .......... .......... ......... ...C......
A. lituratus       .......... .......... .......... .......... ......... ..........
A. obscurus        .......... .......... ....C..... .......... ......... ..........
A. concolor        .......... .......... .......... .......... ......... ..........
A. phaeotis        .......... .......... .......... .......... ......... ..C..A....
A. gnomus          .......... .......... .......... .......... ......... ..........
A. cinereus        .......... .......... .......... .......... ......... ..........
B. cavernarum      .......... .......T.. .......... .......... ......... ..C.TA....
C. perspicillata   .......... .......... .......... .......... ......... ..A..T....
E. hartii          .......... ........C. ...ACC..G. .......... .......T. ..AC.-...A
```

FIG. 3.—DNA sequences (239 bases) for a region of the 12S rRNA gene from several species of *Artibeus,* four mtDNA haplotypes from *A. jamaicensis* (T = SV and J8 = J4) and species of *Brachyphylla, Carollia,* and *Enchisthenes*; GenBank accession numbers—U26275–U26280, U26282, U26284, U26287, U26290, U26292–U26295.

ious collecting localities, we concluded that these lineages reached the Caribbean from Mexico or Central America and spread southward from the Greater Antilles (Phillips et al., 1991). Only J-lineage haplotypes were found in the Greater Antilles, whereas only 1 of 28 specimens from the southern island of Grenada carried a J haplotype (Phillips et al., 1989). This geographic pattern of mitochondrial gene flow supported long-held hypotheses about the colonization of the northern Antilles (Griffiths and Klingener, 1988; Jones and Phillips, 1970; Koopman, 1968).

On Grenada, the southern-most island in the Lesser Antilles, a relatively high percentage (60%) of 28 bats carried the G-mitochondrial haplotypes. The G group also was found to the south, on Trinidad (C. J. Phillips, pers. comm.), and as far north as St. Vincent, where these haplotypes become rare (1 of 20 specimens—Phillips et al., 1989). Thus, the G haplotypes primarily were associated with bats classified in the subspecies *A. j. trinitatus,* which is hypothesized to be derived from the South American mainland (Jones, 1989; Jones and Phillips, 1970). The fact that the G group of mitochondrial haplotypes is geographically restricted to the southern Lesser Antilles (Fig. 5) suggests that bats in this lineage reached the islands relatively recently in comparison to the J lineages, which have dispersed all the way from Meso-America into the southern Caribbean.

Although the RFLP data and restriction-site mapping have helped to clarify the respective roles of the northern and southern pathways of dispersal into the Caribbean, *A. jamaicensis* living on the island of St. Vincent have remained enigmatic. This population of bats first attracted attention because of their relatively large size and dark coloration in comparison with specimens collected elsewhere in the Antilles (Jones and Phillips, 1970). Morphometrically, the bats from St. Vincent can be easily distinguished from bats on the nearby islands of St. Lucia and Barbados, and Jones (1978)

TABLE 1.—*Pairwise distances between certain phylostomid taxa based on DNA sequences of a fragment of 12S rRNA (Fig. 3). Numbers above the diagonal are mean distance values; numbers below the diagonal are absolute distance values.*

Taxon	1	2	3	4	5	6	7	8	9	10	11	12
1 *C. perspicillata*		0.076	0.067	0.084	0.076	0.084	0.088	0.105	0.092	0.076	0.076	0.119
2 *B. cavernarum*	18		0.063	0.092	0.088	0.097	0.097	0.109	0.097	0.080	0.084	0.131
3 *A. phaeotis*	16	15		0.029	0.034	0.042	0.055	0.055	0.042	0.025	0.025	0.140
4 *A. gnomus*	20	22	7		0.013	0.029	0.042	0.042	0.029	0.021	0.021	0.165
5 *A. cinereus*	18	21	8	3		0.025	0.034	0.038	0.025	0.025	0.021	0.153
6 *A. jamaicensis* (J1)	20	23	10	7	6		0.013	0.029	0.017	0.017	0.025	0.157
7 *A. jamaicensis* (J4)[a]	21	23	13	10	8	3		0.042	0.029	0.029	0.029	0.153
8 *A. obscurus*	25	26	13	10	9	7	10		0.029	0.029	0.038	0.161
9 *A. planirostris*[b]	22	23	10	7	6	4	7	7		0.017	0.025	0.157
10 *A. lituratus*	18	19	6	5	6	4	7	7	4		0.008	0.148
11 *A. concolor*	18	20	6	5	5	6	7	9	6	2		0.148
12 *E. hartii*	28	31	33	39	36	37	36	38	37	35	35	

[a] Values for *A. jamaicensis* (J8) are the same as values for (J4).

[b] Values for *A. jamaicensis* (SV) and (T) are the same as values for *A. planirostris.*

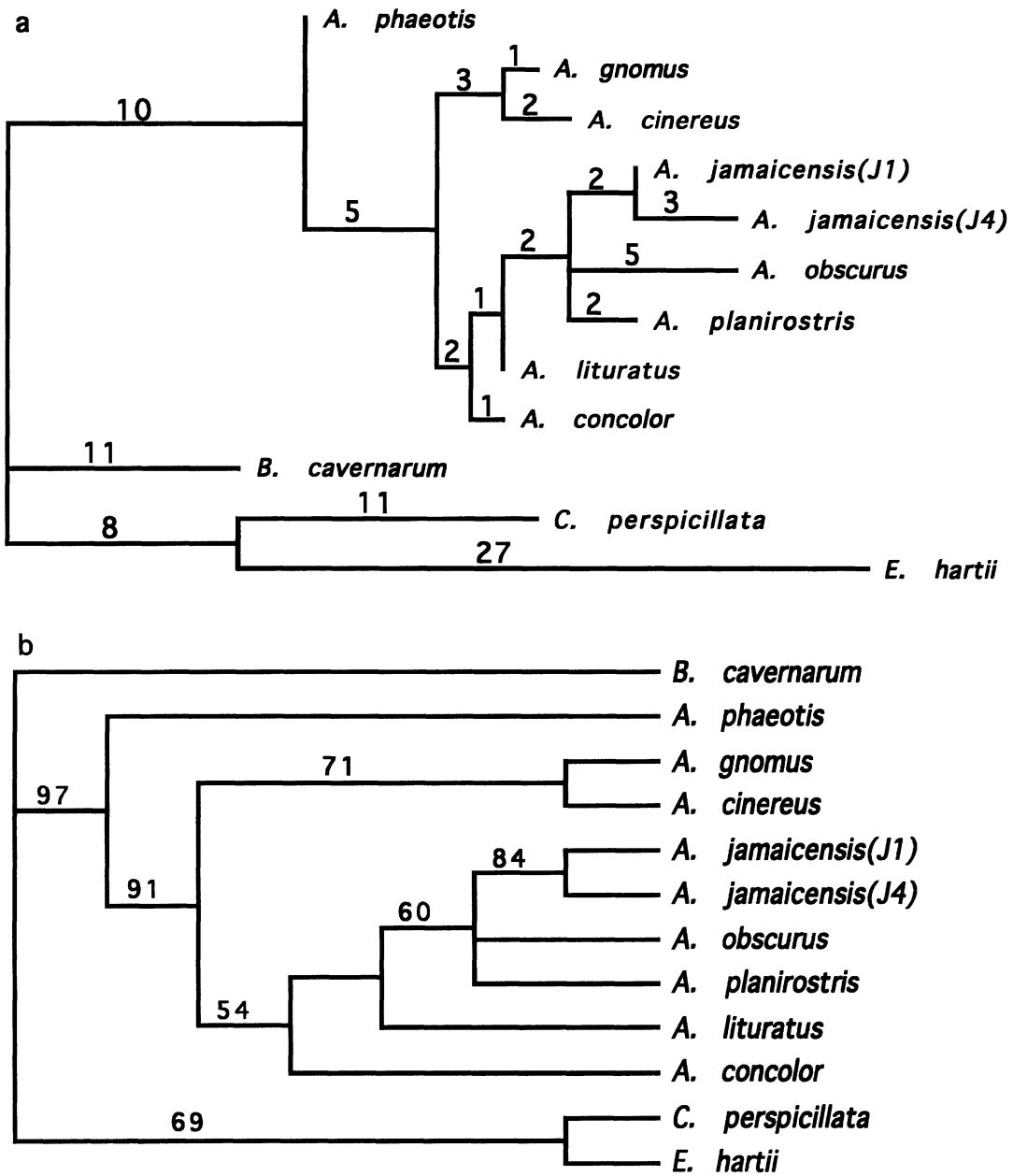

FIG. 4.—Results of a PAUP analysis of the 12S rRNA sequence data presented in Fig. 3. Because the SV 12S rRNA sequence obtained from *Artibeus jamaicensis schwartzi* was identical to 12S rRNA sequence obtained from *A. planirostris* from French Guiana, only *A. planirostris* is shown in the tree. The trees shown were obtained with transversions weighted twice as much as transitions. The single most-parsimonious tree has 96 steps: a, distance tree, numbers above the branches are distance values; b, cladogram, numbers above the branches are bootstrap values from 100 replications. The branching pattern between *A. lituratus* and *A. concolor* collapses during the bootstrap analysis. The tree topology remains the same when transitions and transversions are weighted equally; the minimum tree length is 77 steps, the consistency index is 0.792, and the retention index is 0.704. When the transversion: transition ratio is five, the tree topology is still the same, but the minimal-length tree increases to 153 steps.

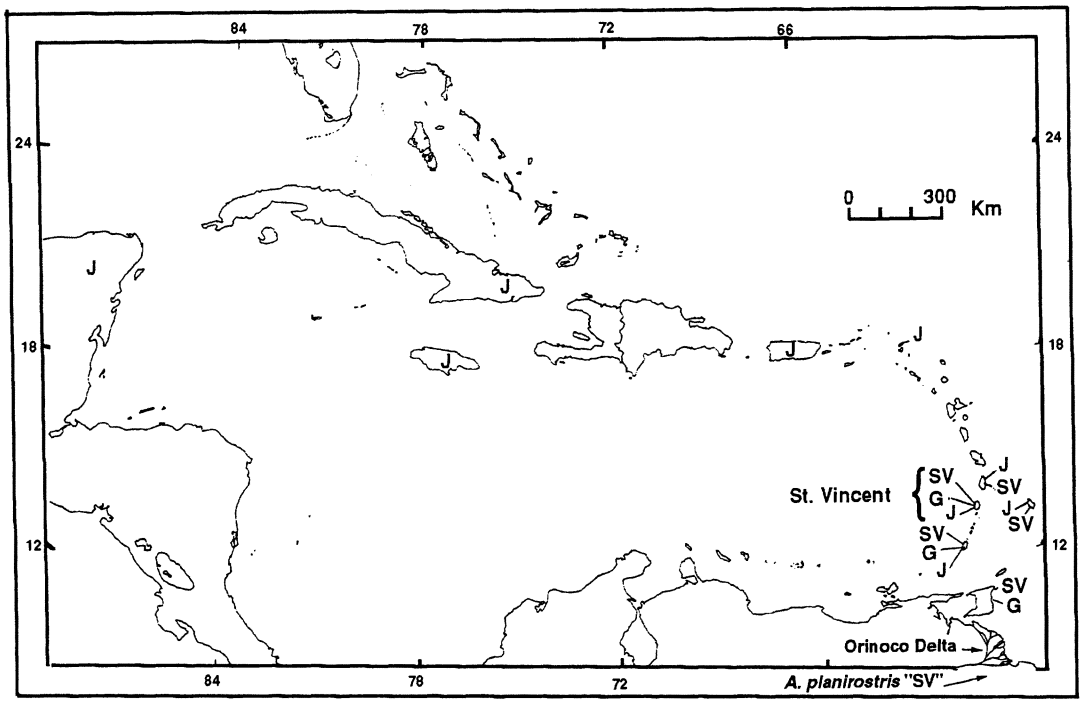

FIG. 5.—Known ranges of the J, G, and SV, mtDNA genotypes in *Artibeus jamaicensis* and *A. planirostris*. Distribution of the J genotype apparently is the consequence of gene flow into the Antilles from Meso-American or *A. jamaicensis* from Mexico, whereas the G genotype reflects gene flow into the Antilles from *A. planirostris* in northeastern South America. The Lesser Antilles island of St. Vincent represents the approximate meeting ground of these three separate pathways of gene flow.

recognized these large-sized *A. jamaicensis* as a separate subspecies, *A. j. schwartzi*. The presence of a notably different looking bat on an island that is not adjacent to a mainland raises a number of questions, including the basic question of origin. Traditionally, one might have argued that subspecies on islands result from partial reproductive isolation. However, because bats are volant and presumably can travel over water, Jones (1989) hypothesized that the large bats from St. Vincent were an example of heterosis resulting from hybridization of *A. j. jamaicensis* from the north with *A. j. trinitatus* from the south. Interestingly, St. Vincent is the island where mitochondrial gene flow from the north (the J lineages) and south (the G lineages) meet (Fig. 5). However, these two haplotypes are rare on St. Vincent; most (93%) of the 27 individuals of *A. j. schwartzi* examined from St. Vincent and nearby Bequia (18 km south) carried a distinctively different mitochondrial haplotype labeled SV by Pumo et al. (1988).

The SV haplotype also was found in *A. jamaicensis* living on St. Lucia (79%), Barbados (12%), Grenada (36%), and Trinidad (1 of 2 examined), but not in any bats from the northern islands of Aguilla, Puerto Rico, Jamaica, or Cuba (Phillips et al., 1989, 1991; Pumo et al., 1988). In summary, the morphologically based subspecies *A. j. schwartzi* is associated with a distinctive SV mitochondrial haplotype and the geographic distribution of this haplotype suggested to us that it represented mitochondrial gene flow into the Lesser Antilles from the south rather than from the northern islands or Mexico.

Bats of the genus *Artibeus* that occur in northern South America have posed something of a problem for taxonomists, and there has been some confusion and disagreement about criteria for identification of species. It is relevant to note that Handley (1987) reported that zones of integradation in northern South America linked *A. jamaicensis* of Central America with *A. planirostris* of eastern South America. However, Lim and Wilson (1993) noted that Handley (1987) presented no data to support this assertion, which he applied to his taxonomic arrangements. They re-examined museum specimens and found no evidence of integradation. Sheared principal-components analysis and discriminant analysis enabled them to clearly distinguish among five large-size species that occur in northern and northeastern South America; *A. jamaicensis, A. amplus, A. obscurus, A. planirostris,* and *A. lituratus* (Lim and Wilson, 1993). Among these species, the unusual subspecies from St. Vincent, *A. j. schwartzi,* morphologically is most similar to *A. planirostris.* Except for geographic distribution (*A. planirostris* formally is known only from south or southeast of the Orinoco River), the external appearance, cranial measurements, and length of forearm in *A. j. schwartzi* cause it to key out with *A. planirostris* rather than *A. jamaicensis* from the Antilles or Venezuela. For these reasons, we tested the hypothesis that the SV haplotype might also occur in *A. planirostris.* The PAUP analysis of mtDNA sequences from four tRNA genes and the O_L region of the mitochondrial genome strongly supports this hypothesis (Fig. 2). The hypothesis is further supported by the 12S rRNA sequences, because in this instance, no mutational differences were found in the 239-bp region sequenced from an SV haplotype isolated from a specimen of *A. j. schwartzi* obtained on St. Vincent and a specimen of *A. planirostris* obtained in French Guiana, some 1,300 km to the southeast (Fig. 3).

The SV haplotype has zoogeographic importance because it documents a genetic linkage between widely separated localities and could be interpreted as evidence of mitochondrial gene flow into the Lesser Antilles from northeastern South America. But what does it tell us about *A. jamaicensis* and *A. planirostris*? This is a more difficult biological question, but on the combined bases of morphometry and mitochondrial DNA, *A. j. schwartzi* living on St. Vincent could be assigned to *A. planirostris* rather than to any *A. jamaicensis* from the Caribbean or the Yucatan Peninsula. However, the presence of a particular mitochondrial haplotype, even one well characterized by mtDNA sequencing, may not be a suitable basis for taxonomy, even when correlated with morphometric data. Both *A. planirostris* from northeastern South America and *A. jamaicensis* from northwestern South America apparently invaded the southern Antilles. There is some circumstantial evidence that suggests *A. planirostris* even reached the Greater Antilles. *Artibeus anthonyi,* an extinct species known only from the Pleistocene in Cuba, has not been compared directly to *A. planirostris,* but bears striking morphological resemblance to *A. j. schwartzi* and, thus, *A. planirostris* as well (Phillips et al., 1989). The presence of the SV haplotype in 36% of morphologically typical *A. j. trinitatus* on Grenada and in 79% of morphologically typical *A. j. jamaicensis* on St. Lucia (Fig. 5) argues that the SV haplotype has been introduced into *A. jamaicensis* through hybridization with *A. planirostris* on these islands. But what about St. Vincent? The bats on this island have retained their morphological and mitochondrial affinity with *A. planirostris,* suggesting that they have not hybridized with *A. jamaicensis.*

Several hypotheses are possible. For instance, one might hypothesize that hybridization does not occur, or is rare on St. Vincent, and the bats living there should be recognized as a small, geographically isolated population of *A. planirostris.* Support for this hypothesis is weakened by the fact that hybridization apparently occurs on nearby

islands and there are no obvious reasons why St. Vincent might be different. Outside of the Antilles, in northern South America, there is no morphological integradation between specimens of *A. jamaicensis* and *A. planirostris* so one might conclude that the two do not hybridize there. However, it appears that the species might be allopatric in South America. B. K. Lim (pers. comm.) hypothesized that the Río Orinoco and, possibly, the arid savanna northwest of the river, combine to form a narrow, but effective, geographic barrier between the two taxa (Fig. 5). One alternative hypothesis is that *A. planirostris* was an early arrival to the Antilles and is disappearing as a morphologically distinctive bat through hybridization with *A. jamaicensis.* If extinct *A. anthonyi* does represent an early Antillean arrival of *A. planirostris,* this hypothesis would be concordant with the fact that fossil specimens of *A. anthonyi* and *A. jamaicensis* are found together in late Pleistocene cave deposits in Cuba, but the former disappears from Recent deposits (Woloszyn and Silva Toboada, 1977). This hypothesis gains further support from the fact that the J-lineage *A. jamaicensis* seem to have recently reached the Lesser Antilles from the north and the G-lineage *A. jamaicensis* has recently invaded from the south (Fig. 5), so St. Vincent, in effect, is the last meeting ground between northern and southern populations of *A. jamaicensis.*

Finally, it also should be mentioned that, in theory, the same mitochondrial haplotype could be found in two different species if the two species were derived relatively recently from a common ancestry. In fact, computer simulations demonstrate that species separated by $<0.5\ n$ generations (n = number of females in the population) are likely to share mitochondrial haplotypes (Avise et al., 1983, 1984). A variety of additional types of data, including data from the nuclear genome, will be necessary to further elucidate this subject. Meanwhile, the available data provide some insight into the dynamic nature of gene flow, speciation, and, perhaps, extinction processes in these bats. Indeed, it could be that we are observing a biological phenomenon that is fundamental to our understanding of zoogeography in volant animals.

Molecular evolution of the 12S rRNA gene.—Our data raise several questions regarding rates of molecular evolution in mitochondrial ribosomal genes. Where comparisons can be made, it sometimes appears that the 12S rRNA gene evolves slowly relative to mitochondrial protein-coding genes. For example, Van Den Bussche et al. (1993) reported 10.7% sequence difference in a pairwise comparison of cytochrome-*b* gene sequences from *A. concolor* and *A. lituratus.* Our 12S rRNA sequences from the same species differed by only 0.8% (Table 1). Among the small-sized *Artibeus,* the cytochrome-*b* gene sequences differed by 8% (Van Den Bussche et al., 1993) and the 12S rRNA sequences also were fairly divergent; *A. phaeotis* and *A. cinereus* differed by 3.4% (Table 1). If one uses these data to calculate a ratio of sequence divergences between genes (cytochrome *b*:12S rRNA), it is apparent that the relative rates of molecular evolution among genes within a mitochondrial genome might differ among species. In the present examples the 12S rRNA gene seems to be relatively more conservative in *A. concolor* and *A. lituratus* than it is in *A. phaeotis* and *A. cinereus.* Insofar as rates are concerned, the most remarkable data come from *E. hartii* because this species frequently has been classified in the genus *Artibeus.* In pairwise comparisons with this bat, both the cytochrome-*b* and 12S rRNA sequences exhibit ca. 14–18% divergence (our data and Van Den Bussche et al., 1993).

We have shown previously that different mitochondrial genes evolve quite differently and that even different protein-coding genes differ in rate and mode of evolution (Pumo et al., 1992). However, it generally is assumed that rate and mode of evolution in homologous mitochondrial genes is similar among closely related species. Our data

suggest that this assumption might be false, at least in stenodermatine bats. Why this should be is unclear, but it has implications for systematists. In the present case, conservative evolution of the 12S rRNA mitochondrial gene draws *A. concolor* into a close relationship with *A. lituratus,* whereas a fairly large number of mutations, including deletions, in the 12S rRNA gene possibly inflates the distance between *A. phaeotis* and *A. cinereus* (Fig. 4). The topology of our PAUP tree thus differs from the one based on cytochrome-*b* sequences reported by Van Den Bussche et al. (1993). In their tree, *A. concolor* falls outside the branch uniting the small-sized and large-sized *Artibeus.*

Regardless of interspecific or interlineage differences in rate or mode of evolution in particular mitochondrial genes, our data support the conclusions or comments of others (e.g., Lim, 1993; Lim and Wilson, 1993; Van Den Bussche et al., 1993; Wilson and Reeder, 1993) regarding the proposed genera *Dermanura* and *Koopmania.* Our molecular data offer no direct support for allocation of certain species into these genera instead of *Artibeus.* Moreover, our data conform to those of others (e.g., Lim's reanalysis of Owen, 1991, figure 3 in Lim, 1993; Van Den Bussche et al., 1993) with regard to *E. hartii.* Genetically, this species apparently is not closely related to species of *Artibeus,* regardless of impressive, but apparently superficial, morphological similarity that influenced the outcome of Owen's (1987, 1991) morphometric analyses.

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APPENDIX I

Specimens examined.—CJP = Carnegie Museum of Natural History; TK = The Museum, Texas Tech University. *Enchisthenes hartii* (TK 22776)—PERU: Huanuco Leoncia Prado; 9 km S, 2 km E, Tingo Maria, 9°22′S, 75°58′W. *Brachyphylla cavernarum* (CJP 4496)—ANGUILLA: The Fountain. *Carollia perspicillata* (CJP 4917)—FRENCH GUIANA: 1.75 km S, 9 km W Sinnamary. *Artibeus cinereus* (CJP 5059)—TRINIDAD: St. George Co., Asa Wright Nature Center, 8.1 km N Arima. *A. concolor* (TK 10378)—SURINAME: Commewijne, Nieuwe Grond Plantation, 5°53′N, 54°54′W. *A. gnomus* (CJP 4924)—FRENCH GUIANA: 3.5 km S, 10 km W Sinnamary. *A. jamaicensis* (G, CJP 5154)—GRENADA: St. George's Parish; 0.5 km E Vendôme. *A. jamaicensis* (J-1, CJP 4150)—JAMAICA: St. Ann's Parish; 2 km SW Priory. *A. jamaicensis* (J-4, CJP 4709 and J-8, CJP 4719)—MEXICO: Quintana Roo; 5 km SW Puerto Marelos. *A. jamaicensis* (SV, CJP 5184)—ST. VINCENT: St. George's Parish, 1 km NE Brighton Village. *A. jamaicensis* (T, CJP 5095)—TRINIDAD: St. George Co., Asa Wright Nature Center 8.1 km N Arima. *A. literatus* (CJP 5019) and *A. obscurus* (CJP 5018)—FRENCH GUIANA: 5 km S Matoury. *A. phaeotis* (CJP 4706)—MEXICO: Quintana Roo, Cancun. *A. planirostris* (CJP 4967)—FRENCH GUIANA: 1 km N Remire.