

1988

# Mitochondrial DNA Polymorphism in Three Antillean Island Populations of the Fruit Bat, *Artibeus jamaicensis*

Dorothy E. Pumo

*Hofstra University*, Dorothy.E.Pumo@hofstra.edu

Everett Z. Goldin

*Hofstra University*

Beth Elliot

*Hofstra University*

Carleton J. Phillips

*Texas Tech University*, carl.phillips@ttu.edu

Hugh H. Genoways

*University of Nebraska - Lincoln*, h.h.genoways@gmail.com

Follow this and additional works at: <http://digitalcommons.unl.edu/museummammalogy>



Part of the [Biodiversity Commons](#), [Molecular Genetics Commons](#), [Other Ecology and Evolutionary Biology Commons](#), and the [Zoology Commons](#)

Pumo, Dorothy E.; Goldin, Everett Z.; Elliot, Beth; Phillips, Carleton J.; and Genoways, Hugh H., "Mitochondrial DNA Polymorphism in Three Antillean Island Populations of the Fruit Bat, *Artibeus jamaicensis*" (1988). *Mammalogy Papers: University of Nebraska State Museum*. 262.

<http://digitalcommons.unl.edu/museummammalogy/262>

This Article is brought to you for free and open access by the Museum, University of Nebraska State at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Mammalogy Papers: University of Nebraska State Museum by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

## Mitochondrial DNA Polymorphism in Three Antillean Island Populations of the Fruit Bat, *Artibeus jamaicensis*<sup>1</sup>

Dorothy E. Pumo,\* Everett Z. Goldin,\* Beth Elliot,\*  
Carleton J. Phillips,\* and Hugh H. Genoways†

\*Department of Biology, Hofstra University; and †The University State Museum, the University of Nebraska at Lincoln

The Neotropical fruit bat, *Artibeus jamaicensis*, occurs throughout Latin America and on many islands in the Caribbean. Populations from Jamaica (in the Greater Antilles) to Barbados (in the Lesser Antilles) have been classified as a subspecies (*A. j. jamaicensis*) separate from that on the Lesser Antillean island of St. Vincent (*A. j. schwartzi*). Mitochondrial DNA (mtDNA) was isolated from 54 individuals collected on these islands, analyzed by digestion with restriction endonucleases, and the restriction sites were mapped. Three different mtDNA genotypes (16,000 ± 200 bp) were identified: J-1 (16 animals from Jamaica, one from St. Vincent, 15 from Barbados), J-2 (two animals from Jamaica), and SV-1 (18 animals from St. Vincent, two from Barbados). The J-1 and J-2 genotypes were estimated to differ from each other by only 0.4%, but the SV-1 genotype differed from J-1 and J-2 by 8.1%–10.5%. The estimated sequence divergence between SV-1 and J-1 is unusually large for mammals that are regarded as conspecific. Restriction mapping showed that the differences among the genotypes (presence or absence of particular restriction sites) were located throughout the genome. The presence of the J-1 mtDNA genotype on Jamaica and on St. Vincent and Barbados (1,400 km away) demonstrates that maternal lineages in these bats are not necessarily confined to single islands or limited geographic regions. The presence of the J-1 mtDNA genotype within the *A. j. schwartzi* population on St. Vincent and the presence of the SV-1 genotype in two specimens of *A. j. jamaicensis* from Barbados document genetic exchange between subspecific populations on these islands, which are separated by 180 km of open water.

### Introduction

The Antillean islands of the Caribbean, extending in an arc from the Yucatán Peninsula toward the northeastern coast of South America, interest biogeographers because the fauna and flora have North-, Central-, and South-American affinities (Darlington 1963). Among the native mammals, bats are the best represented in terms of the numbers of species (Koopman 1968, 1976; Jones and Phillips 1970; Baker and Genoways 1978). However, the overall distributional patterns of particular species and the presence of endemic genera and species suggest that (1) bats are not necessarily successful at colonizing every island and (2) species can become geographically isolated, even though they are capable of extended flight.

1. Key words: mitochondrial DNA, bats, zoogeography, Caribbean, *Artibeus*.

Address for correspondence and reprints: Dr. Dorothy E. Pumo, Department of Biology, Hofstra University, Hempstead, New York 11550.

Elucidation of the relationships among island populations and the possible mainland sources of species that have colonized the islands has mostly been based upon the interpretation of phenotypic data typical of conventional taxonomic studies of bats (size, coat color, and skull and dental characteristics). Although such data frequently have revealed noteworthy interisland differences and geographic trends (Koopman 1968; Jones and Phillips 1970, 1976), they are, nevertheless, limited. Other approaches, such as chromosomal analysis, protein comparisons, and ultrastructure of gene products (Straney et al. 1979; Baker et al. 1982; Koop and Baker 1983; Tandler et al. 1986), have been used successfully in chiropteran systematics, but none seems particularly well suited to the study of conspecific, island populations. One solution is to use the relatively new techniques for analyzing mitochondrial DNA (mtDNA).

The mitochondrial genome in mammals is small (~16,000 bp) in comparison with the nuclear genome and also differs by being maternally inherited and by evolving at a faster rate (Brown 1980; Lansman et al. 1983; Vawter and Brown 1986). Comparisons of mtDNA isolated from conspecific individuals can be used to investigate maternal lineages, relationships among closely related but geographically separated populations, and individual variation within populations. Restriction-endonuclease analysis of mtDNA thus is a promising way to explore intra- and interisland populations.

The Neotropical fruit bat, *Artibeus jamaicensis*, occurs from Mexico into South America and has been reported on many of the Antillean islands (Jones and Phillips 1970; Koopman 1976). Populations from Jamaica in the Greater Antilles to Barbados and St. Lucia in the Lesser Antilles have been named *A. j. jamaicensis*. They are thought to have been derived from ancestral stock in Mexico or Central America, whereas populations from Grenada, Trinidad, and Tobago are thought to have arrived in the Antilles from South America (Koopman 1968). The population of *A. jamaicensis* living on the Lesser Antillean island of St. Vincent has been described and named as an endemic subspecies, *A. j. schwartzi* (Jones and Phillips 1970; Jones 1978).

For the present investigation we used restriction-endonuclease analysis of mtDNA to (1) compare three island populations of *A. jamaicensis*, (2) determine whether populations on each island could be traced to separate female founders, (3) determine whether mtDNA lineages were confined to individual islands, and (4) test for possible genetic exchange between *A. j. jamaicensis* and *A. j. schwartzi* on Barbados and St. Vincent, respectively. The data reported here are part of a larger analysis of the distribution and evolution of *Artibeus* in the Caribbean (Phillips et al., accepted).

## Material and Methods

### Specimens

Fifty-four specimens of *Artibeus jamaicensis* from Jamaica (18 individuals), St. Vincent (19 individuals), and Barbados (17 individuals) were used in this study (table 1). Seven of the animals from Jamaica were collected in a cave roost in St. Ann's Parish; the others were netted in a fruit plantation 24 km east of the cave. All of the St. Vincent animals were captured in mist nets set in or near fruit plantations separated by 3–6 km and by a ridge 300 m in elevation. The bats from Barbados were collected in a cave roost near Bridgetown. All of the Jamaican and Barbados animals were classified as *A. j. jamaicensis*, and the animals from St. Vincent were classified as *A. j. schwartzi* on the basis of conventional taxonomy (Jones 1978). The specimens were either killed the day after capture or were transported alive (under CDC permit

to C. J. P.) to our laboratory. The liver, kidney, heart, and pectoral muscle were removed from specimens sacrificed in the field and immediately placed in cryotubes and stored in liquid nitrogen. Voucher specimens were deposited in the collections of the Carnegie Museum of Natural History and The Museum, Texas Tech University.

#### Preparation of mtDNA

Livers, and sometimes kidneys, were used for the isolation of mtDNA. The procedure was a modification of that of Wright et al. (1983). To briefly summarize, tissue was minced and homogenized in a buffer containing Tris, ethylenediaminetetraacetate (EDTA), and sucrose. Nuclei and other cellular debris were removed by differential centrifugation. The mitochondrial pellet was obtained by additional differential centrifugation and lysed by the addition of sodium dodecyl sulfate and incubation at room temperature. Further incubation in the presence of cesium chloride encourages the precipitation of cellular proteins and large DNA fragments while the smaller DNA fragments (including mtDNA) remain in solution. The mtDNA was purified by centrifugation on a cesium chloride density gradient. To maximize the yield of mtDNA, only one purification gradient was used. After centrifugation, mtDNA bands in the gradient were visualized with 300-nm light and removed by puncturing the side wall of the tube and allowing the band to drip into a collecting tube. The DNA was dialyzed to remove the ultraviolet dyes and precipitated with ethanol. Purified mtDNA was redissolved in TE (10 mM Tris, pH 7.5, 1 mM EDTA) and stored frozen.

#### Restriction-Endonuclease Analysis of mtDNA

Restriction enzymes (*Bgl*II, *Bam*HI, *Pvu*II, *Pst*I, *Hind*III, *Eco*RI, *Sal*I, *Xho*I, *Hinf*I, *Taq*I, and *Mbo*I) were obtained from New England Biolabs. Digestions were performed in the buffers recommended by either the supplier or Maniatis et al. (1982). Digestions were performed for 1–2 h or overnight with excess enzyme to verify the digestion pattern obtained.

Following enzymatic digestion, DNA fragments were end-labeled. The following labeling mixture was used to label 15 samples: 137  $\mu$ l deionized, distilled water; 16  $\mu$ l buffer (6 mM KCl, 10 mM Tris HCl, 10 mM MgCl<sub>2</sub>, 7 mM  $\beta$ -mercaptoethanol); 5  $\mu$ l DNA polymerase I; Klenow fragment (BRL); and 2  $\mu$ l [ $\alpha$ -<sup>32</sup>P]-dXTP mixture (ICN). Ten microliters of the mixture was added to the digested sample, and the mixture was then incubated for 25–35 min on ice. Yeast tRNA carrier was added to the samples, and then they were ethanol precipitated. Samples were then subjected to either 0.5%–1.25% agarose or 3.5% polyacrylamide-gel electrophoresis (Brown 1980; Maniatis et al. 1982) to determine fragment sizes. A 1-kb ladder (BRL) was included on every gel for use as a molecular-weight standard.

#### Determination of mtDNA Sequence Divergence

Restriction mapping was accomplished with the aid of the RZMAP program supplied by W. W. Ralph (Fitch et al. 1983). Estimation of sequence divergence, on the basis of analysis with the eight restriction enzymes that recognize 6-bp sequences, was undertaken only after the genotypes were mapped (fig. 1). These maps were used to determine fragment homologies because we found that in some instances (with *Hind*III in particular) fragments of approximately the same size ( $\pm 1\%$ ) were actually located in different regions of the genome and were not homologous (fig. 2). The maps themselves are tentative to the extent that the order of two of the *Hind*III fragments in each genotype could not be confirmed on the basis of double or triple digestions with the

enzymes that we used. Sequence divergence for 6-bp cutters was estimated in two ways. Equation (9) in Nei and Tajima (1983) is a conservative estimate of divergence that does not require an estimate of an  $\alpha$  value. Also, the use of this equation allows the determination of the variance by means of Nei and Tajima's (1983) equation (11). In addition, we also used Nei and Li's (1979) equation (16) and  $\alpha = 2$  to calculate sequence divergence. This equation is commonly used for analysis of vertebrate mtDNA and allows comparison with other data. Estimation of divergence, on the basis of data from the three enzymes that recognize 4-bp sequences, was undertaken separately (using the formula of Upholt [1977]) because, since these sites were not mapped, we could only assume that fragments of the same size were in fact shared.

## Results

The mitochondrial genome in *Artibeus jamaicensis* is  $16,000 \pm 200$  bp. Three different mtDNA genotypes were found in 54 animals, and these were designated J-1, J-2, and SV-1 (table 1). The restriction fragments obtained from each of the genotypes are presented in tables 2 and 3, and restriction maps are presented in figure 1.

Digestion with *Bam*HI produced two fragments in all three genotypes (table 2); double and triple digestions with other enzymes verified that all of the animals shared the same *Bam*HI restriction sites (fig. 1). *Bgl*II produced three fragments in all of our specimens, but these fragments differed in size (table 2). With restriction mapping it was found that each specimen shared one *Bgl*II site (fig. 1). *Pvu*II cleaved the SV-1 mtDNA into two fragments, of 11,849 and 4,106 bp. In the J animals the genome was cleaved into three fragments (table 2). However, with restriction mapping it was found that only a single site was conserved and that two similar-sized fragments actually were from different regions of the mtDNA genome (fig. 1). *Pst*I and *Eco*RI each cleaved the genomes in one place (fig. 1). *Sal*I cleaved the SV-1 genotype in a single place (fig. 1) but did not cleave the J mtDNA genotype. Finally, *Xho*I did not cut the mtDNA of the SV-1 lineage.

The most complex data were obtained from digestion with *Hind*III (table 2; fig. 2). The J genotype was subdivided into J-1 and J-2 because two individuals from Jamaica lacked a single restriction site present in the J-1's (fig. 1, arrow). *Hind*III produced either nine or 10 fragments in each of the three genotypes (fig. 2), including

**Table 1**  
Numbers of Specimens Examined, Geographical Localities, and Percent Occurrences of Each of the Three mtDNA Genotypes Found in Two Subspecies of *Artibeus jamaicensis*

SUBSPECIES AND LOCALITY	mtDNA GENOTYPE			TOTAL
	J-1	J-2	SV-1	
<i>A. j. jamaicensis</i> :				
Jamaica .....	16	2	0	18
Barbados .....	15	0	2	17
Total .....	31 (88.6%)	2 (5.7%)	2 (5.7%)	35
<i>A. j. schwartzi</i> :				
St. Vincent .....	1	0	18	19
Total .....	1 (5.3%)	0	18 (94.7%)	19

**Table 2**  
**Sizes of Restriction Fragments Produced by Eight**  
**Hexanucleotide Sequence Enzymes**

ENZYME	GENOTYPE		
	SV-1	J-1	J-2
<i>Bam</i> HI .....	11,151 <sup>a</sup> 4,791 <sup>a</sup>	11,151 <sup>a</sup> 4,791 <sup>a</sup>	11,151 <sup>a</sup> 4,791 <sup>a</sup>
<i>Bgl</i> II .....	6,937 5,573 3,465	8,200 <sup>b</sup> 5,428 <sup>b</sup> 2,329 <sup>b</sup>	8,200 <sup>b</sup> 5,428 <sup>b</sup> 2,329 <sup>b</sup>
<i>Pvu</i> II .....	11,849 4,106	9,596 <sup>b</sup> 4,078 <sup>b</sup> 2,315 <sup>b</sup>	9,596 <sup>b</sup> 4,078 <sup>b</sup> 2,315 <sup>b</sup>
<i>Pst</i> I .....	15,975 <sup>c</sup>	15,975 <sup>c</sup>	ND
<i>Eco</i> RI .....	15,975 <sup>c</sup>	15,975 <sup>c</sup>	ND
<i>Sal</i> I .....	15,975	NF	ND
<i>Xho</i> I .....	NF	ND	ND
<i>Hind</i> III .....	4,280 2,460 2,435 <sup>c</sup> 1,740 1,700 <sup>a</sup> 1,050 958 750 <sup>c</sup> 650 <sup>a</sup>	3,650 <sup>b</sup> 2,440 <sup>b</sup> 2,435 <sup>c</sup> 1,740 <sup>b</sup> 1,700 <sup>a</sup> 1,100 <sup>b</sup> 970 <sup>b</sup> 750 <sup>c</sup> 650 <sup>a</sup> 600 <sup>b</sup>	3,650 <sup>b</sup> 3,135 2,440 <sup>b</sup> 1,740 <sup>b</sup> 1,700 <sup>a</sup> 1,100 <sup>b</sup> 970 <sup>b</sup> 650 <sup>a</sup> 600 <sup>b</sup>

NOTE.—Data are given in base pairs. NF = no restriction fragments produced; ND = no data.

<sup>a</sup> Shared by all three morphs.

<sup>b</sup> Shared by J-1 and J-2.

<sup>c</sup> Shared by SV-1 and J-1.

two pairs of fragments (2,460–2,435 and 1,740–1,700 bp in SV and 2,440–2,435 and 1,740–1,700 bp in J; table 2) that were so close in size that they comigrated and appeared as a single dark band on agarose gels. They were separated using polyacrylamide gels. Although it appeared that all three genotypes shared most of the *Hind*III fragments, digestion of the mtDNA with *Hind*III in combination with one or two of *Bam*HI, *Pvu*II, and *Bgl*II (as double or triple combinations) revealed that many of the similar-sized fragments were not homologous (figs. 1, 2). Mapping showed that the SV-1 and J-1 genotypes shared six *Hind*III sites but that J-1 had three sites not found in SV-1 and that SV-1 had two sites not found in J-1 (fig. 2).

We also tested three restriction endonucleases that recognize 4-bp sequences. We compared 18 examples of the SV-1 genotype and 17 samples of the J-1 genotype, but there was insufficient mtDNA to test the J-2 genotype or to undertake combination digestions necessary for mapping. The considerable amount of divergence between the SV-1 and J-1 mtDNA was clearly evident (table 3). A maximum of 47% of the fragments are shared between the two genotypes if it is assumed, in the absence of a restriction map, that similar-sized ( $\pm 1\%$ ) fragments are homologous.

Sequence divergence, estimated on the basis of the restriction-site maps, showed

**Table 3**  
**Minimum Restriction Fragments Produced by Three Tetranucleotide Sequence Enzymes**

SV-1		J-1		SV-1		J-1		SV-1		J-1	
<i>MboI</i>		<i>HinfI</i>		<i>TaqI</i>							
2,150	1,700 <sup>a</sup>	1,900	2,900	4,600 <sup>a</sup>	4,600 <sup>a</sup>						
1,700 <sup>a</sup>	1,625	1,400 <sup>a</sup>	1,700	2,200	3,054						
1,600	1,450	1,370 <sup>a</sup>	1,400 <sup>a</sup>	1,700	1,020						
1,070	1,200	1,250 <sup>a</sup>	1,370 <sup>a</sup>	970 <sup>a</sup>	970 <sup>a</sup>						
1,030	1,150	850	1,250 <sup>a</sup>	950 <sup>a</sup>	950 <sup>a</sup>						
1,000	1,140	700		900 <sup>a</sup>	900 <sup>a</sup>						
750	900	660	740	700	710						
700 <sup>a</sup>	900	650	735	600	675						
650 <sup>a</sup>	700 <sup>a</sup>	640	513	540	520 <sup>a</sup>						
510 <sup>a</sup>	650 <sup>a</sup>	550	505 <sup>a</sup>	520 <sup>a</sup>	508 <sup>a</sup>						
506 <sup>a</sup>	510 <sup>a</sup>	520	500 <sup>a</sup>	515	430 <sup>a</sup>						
410 <sup>a</sup>	506 <sup>a</sup>	515	460 <sup>a</sup>	508 <sup>a</sup>	365						
360	480	505 <sup>a</sup>	450 <sup>a</sup>	430 <sup>a</sup>	235 <sup>a</sup>						
340	475	500 <sup>a</sup>	410	410	195						
335	410 <sup>a</sup>	460 <sup>a</sup>	398	235 <sup>a</sup>	110 <sup>a</sup>						
330	385	450 <sup>a</sup>	355 <sup>a</sup>	140	105						
296	305	430	350 <sup>a</sup>	110 <sup>a</sup>	80 <sup>a</sup>						
240	290	395	315 <sup>a</sup>	80 <sup>a</sup>							
225	205	355 <sup>a</sup>	285								
215	135 <sup>a</sup>	350 <sup>a</sup>	235								
158	90 <sup>a</sup>	320	180								
150	75	315 <sup>a</sup>	175 <sup>a</sup>								
140	60	215	150 <sup>a</sup>								
135 <sup>a</sup>	50 <sup>a</sup>	175 <sup>a</sup>	140 <sup>a</sup>								
120		150 <sup>a</sup>	100 <sup>a</sup>								
90 <sup>a</sup>		140 <sup>a</sup>									
70		100 <sup>a</sup>									
50 <sup>a</sup>											

NOTE.—Data are given in base pairs.

<sup>a</sup> Shared by SV-1 and J-1.

that the J-1 and J-2 mtDNA genotypes differed by only 0.4% (SD = 0.4%) (eq. [9] of Nei and Tajima [1983] or eq. [16] of Nei and Li [1979]). The SV-1 genotype differed from J-1 and J-2 by 9.2% and 10.5%, respectively (eq. [16] of Nei and Li [1979]) or by 8.1% (SD = 2.5%) and 9.1% (SD = 2.8%), respectively (eqq. [9] and [11] of Nei and Tajima [1983]). Sequence divergence estimated by means of Upholt's (1977) method and using the fragment data obtained with the unmapped 4-bp cutters showed that SV-1 differed from J-1 by  $\geq 6.5\%$ .

## Discussion

Restriction-endonuclease analysis revealed three mtDNA genotypes in 54 specimens of *Artibeus jamaicensis* from Jamaica, St. Vincent, and Barbados. The J-1 genotype was the most common (59.2% of the bats) and most widespread (all three islands; table 1), the SV-1 genotype was intermediate (33.3%, two islands), and the J-2 genotype was the least common (3.7%) and geographically most restricted (Jamaica). The J-1 and J-2 mtDNA genotypes differed only in a single *HindIII* site, whereas the

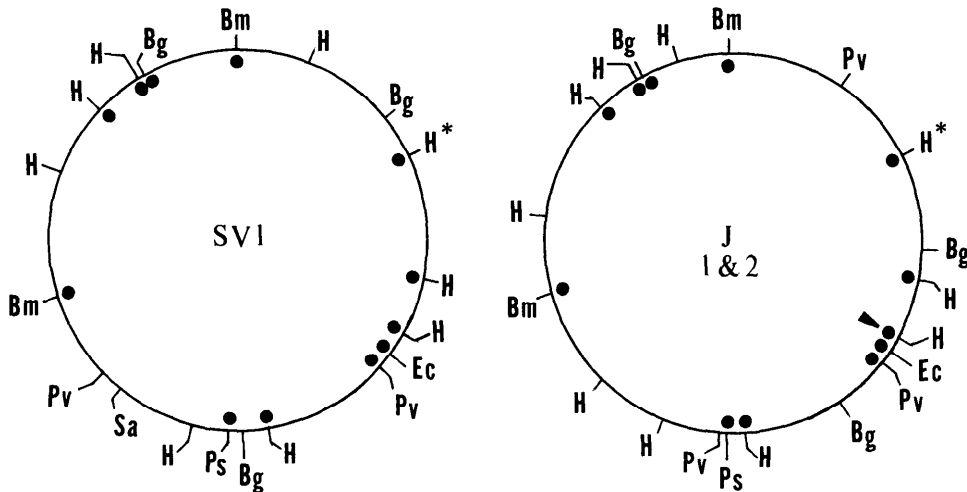


FIG. 1.—Restriction maps for the SV-1 and J mtDNA genotypes. Restriction sites thought to be conserved (within 200 bp in either direction) are marked with a black dot. The J-2 genotype differs from the J-1 in lacking the *Hind*III site marked with an arrow. The *Hind*III site marked with an asterisk is the arbitrary starting point for the linear map in fig. 2; the linear map continues clockwise from this site. Symbols for restriction enzymes are as follows: H = *Hind*III; Bm = *Bam*HI; Bg = *Bgl*II; Ec = *Eco*RI; Pv = *Pvu*II; Ps = *Pst*I; and Sa = *Sal*I.

SV-1 genotype differed from the J genotypes in 15 or 16 of 27 restriction sites (figs. 1, 2). The differences among the three genotypes did not appear to be localized in any specific region of the mitochondrial genomes (fig. 1).

The sequence divergence among the mtDNA genotypes was estimated in three ways. Two methods, using mapped data, estimate the divergence between J-1 and J-2 to be 0.4%. This is small and corresponds to the divergence seen within vertebrate species. In contrast, when the mapped SV-1 genotype is compared with the J genotypes, the numbers are much larger, ranging from 8.1% to 10.5% depending on the equation used and whether J-1 or J-2 is being compared. The SDs are small enough (2.5%–2.8%) to indicate that these large divergence values are significant. The 6.5% divergence obtained between SV-1 and J-1 by the third method (Upholt 1977) is based on unmapped fragment data and can only be considered a minimum estimate of divergence.

How does the divergence in mtDNA genotypes in *A. jamaicensis* compare with intraspecific or congeneric variation in other mammals? Estimates of mtDNA divergence within a species of deer mice, *Peromyscus*, and in a species of gopher, *Geomys*, have ranged from 2% to 4%, when Upholt's method is used (Avise et al. 1979, 1983). Recently most authors have used Nei and Li's (1979) equations (10) and (16) with  $\alpha = 2$  to estimate divergence from mapped restriction-site data. With this method, breeds of domestic horses were estimated to differ by 0.55%, whereas the Mongolian wild horse (*Equus przewalski*) and zebra (*E. zebra hartmannae*) differ by 7.5% (George and Ryder 1986). West Texas and South Carolina white-tailed deer (*Odocoileus virginianus*) differ by 1.3%, whereas California black-tailed deer (*O. hemionus*) and South Carolina white-tails differ by 6.9% (Carr et al. 1986). Finally, humans vary by ~0.32% (Brown 1980; Cann et al. 1987). The estimated amount of sequence divergence between the SV and J mtDNA genotypes in *A. jamaicensis* thus is considerably greater than that reported for other species of mammals when the same methods are used.



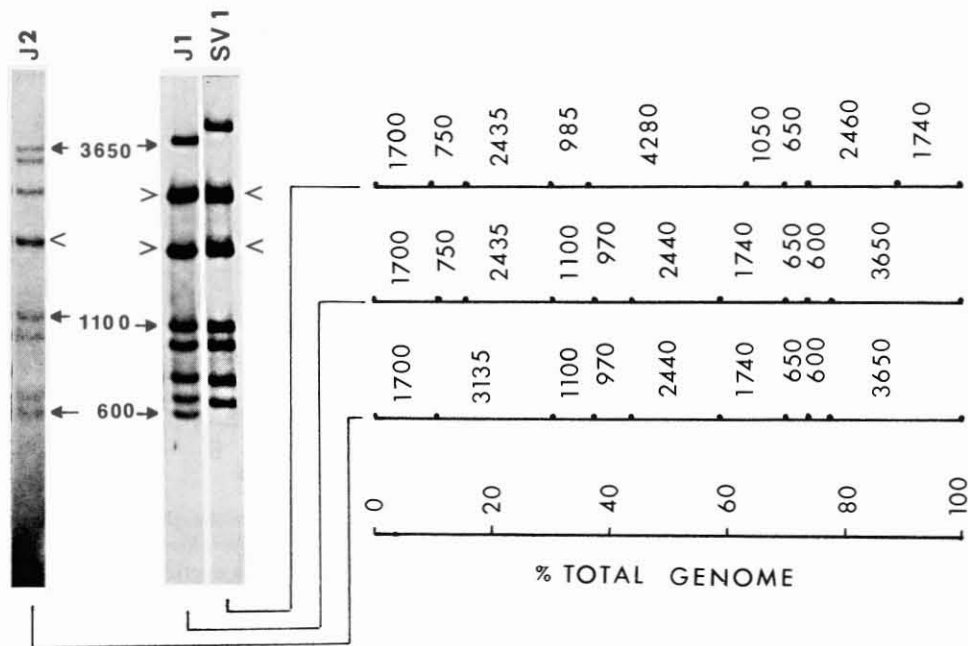


FIG. 2.—*Hind*III digestion of mtDNA from each of three maternal lineages (J-1, J-2, and SV-1) of the fruit bat, *Artibeus jamaicensis*. Open arrowheads adjacent to the gels denote bands consisting of two comigrating fragments; the middle scale is in base pairs. Relative positions of the *Hind*III recognition sites and fragment sizes are given for each of the lineages.

Brown (1980) calculated that mtDNA sequences in primates evolve at the rate of  $\sim 1\%/Myr$ . A similar rate appears to hold for deer (Carr et al. 1986). We presently have no means of independently calibrating the evolutionary rate in *Artibeus* mtDNA, but if we tentatively apply Brown's rate for primates, we estimate that the SV-1 and J genotypes in *A. jamaicensis* diverged  $>4$  Myr before the present (Mybp), whereas the J-1 and J-2 lineages on Jamaica last shared a common female ancestor  $\sim 0.2$  Mybp. It is possible that the rate in *Artibeus* is greater than those in other studied mammals. On the other hand, *A. jamaicensis* could be a very old species. Straney et al. (1979) previously applied Sarich's albumin data to their allozyme data for phyllostomid bats and concluded that the family diversified some 40 Mybp. Eventually it might be possible to calibrate the rate for bats; such data would be valuable for studies of Caribbean zoogeography because the islands have undergone dramatic changes during the past 7 Myr (Sykes et al. 1982). The Pleistocene is particularly interesting because changes in water level in the Caribbean may have made interisland movement of bats easier than at present and because numerous extinctions of bats and other mammals are documented in the fossil record (Morgan and Woods 1986).

The mtDNA data can be considered in terms of the roosting behavior of bats. Other investigators (Turner 1975; Straney et al. 1979) have speculated about the genetics of roosting groups of phyllostomid bats, and some data (including allozyme data) show that in some species roosts are temporary aggregations, whereas in other species roosts might consist of closely related individuals (see, e.g., McCracken and Bradbury 1981; Kunz 1982). On Jamaica we collected animals from two maternal lineages (J-1 and J-2) within a large cave roost on the north shore of the island. These same lineages

also were represented by bats caught at night in a fruit plantation ~24 km to the east. The estimated amount of difference (0.4%) between these genotypes is relatively small; the single difference detected in our sampling was the absence of a particular *HindIII* site in J-2 (fig. 1). mtDNA in individuals roosting together in a large limestone cave on Barbados came from more divergent sources (SV-1 and J-1), illustrating that bats in this roost are a genetic composite with mitochondrial genes traceable to two distantly related females.

The zoogeography of bats is a complex problem because, despite their ability to fly, their distribution on islands is far from uniform. To judge on the basis of commonly used phenotypic characters, it appears that they frequently become isolated to the extent that geographic variation in size and morphology are evident (Phillips 1968; Jones and Phillips 1970; Baker and Genoways 1978). *Artibeus jamaicensis* is interesting in this regard because it is a widely spread species with four named subspecies in the Antilles. *Artibeus j. jamaicensis*, which was named for Jamaica, occurs from the Greater Antilles to St. Lucia and Barbados in the Lesser Antilles. Another subspecies, *A. j. schwartzi*, occurs on the island of St. Vincent (Jones 1978); and a third subspecies occurs on Cuba, and a fourth on Trinidad and Grenada (Koopman 1968; Jones and Phillips 1970). The distinguishing taxonomic features for these subspecies are size (especially forearm length), color (variable), and the presence or absence (or incidence) of the upper third molars (Jones and Phillips 1970; Jones 1978).

In the present study we were particularly interested in the relationship between *A. j. jamaicensis* and *A. j. schwartzi*. The latter subspecies appears to be indigenous to St. Vincent and differs from *A. j. jamaicensis* by being larger and darker in color and in exhibiting variation in the presence or absence of third molars (Jones and Phillips 1970; Jones 1978). Most (88.6%) of the specimens of *A. j. jamaicensis* that we examined carried the J-1 mtDNA genotype, whereas most (94.7%) of the *A. j. schwartzi* carried the SV-1 mtDNA genotype (table 1). However, two of 17 specimens of *A. j. jamaicensis* from Barbados carried the SV-1 genotype and one of 19 specimens of *A. j. schwartzi* from St. Vincent carried the J-1 genotype (table 1). The presence of the J-1 genotype on St. Vincent and of the SV-1 on Barbados illustrates that exchange of mitochondrial genes has occurred between *A. j. schwartzi* and *A. j. jamaicensis*. This finding is interesting in view of the morphological distinctness of the St. Vincent subspecies (Jones 1978) and is the first time that hybridization between island subspecies of bats has been documented by genetic data. Moreover, the lack of intralinear, interisland variation in the assayed restriction sites in either the J-1 or the SV-1 mtDNA genotype suggests to us that this genetic exchange between St. Vincent and Barbados has occurred relatively recently. Although these islands are separated by 180 km of open water, female bats clearly have dispersed successfully between them. The flying capabilities of *A. jamaicensis* are still undefined, but available data on their foraging habits have documented 45-min flights in search of ripe fruit and distances of  $8 \pm 2$  km between day roosts and fruit trees (Fleming 1982). Movement between St. Vincent and Barbados was probably not much easier during the Pleistocene glaciers because Barbados is situated on a ridge separated from St. Vincent by the deep Tobago Basin. Even at minimum sea level the distance between the two would only have been reduced by 10 km. We are thus left with the conclusion that *A. jamaicensis* is capable of crossing a considerable water gap.

In addition to demonstrating genetic exchange between island subspecies, our data also document that mtDNA lineages are not restricted to individual islands and that "new" genetic material can be successfully introduced into an island population,

at least on an occasional basis. As shown by all of the foregoing data, it is clear that mtDNA analysis is a valuable zoogeographic tool because, on at least some occasions, it enables us to trace patterns of genetic exchange between islands. Future investigations of other islands and of mainland areas might reveal the geographic sources of Antillean Island bat populations as well as the dispersal history of these Neotropical bats.

### Acknowledgments

Financial support for this project came from Hofstra University HCLAS Executive Committee grants, National Science Foundation grant BBS-8609231 (D. E. P. and C. J. P.), Research Corporation grant C-1855 (C. J. P.), and the Mellon Institute of North American Mammal Research, Carnegie Museum of Natural History (H. H. G.). We are pleased to acknowledge a number of individuals who were of considerable help in the collection of animals, development of technique, or analysis of data: Phillip E. Ray, Robert J. Baker, Rodney Honeycutt, Wesley M. Brown, Philipa Newton, Stephen L. Williams, and Dennis Burke. Eugene Kaplan assisted us through the Hofstra University Marine Biology Laboratory in St. Ann's Bay, Jamaica. Linda Cossen, Hofstra University Special Secretarial Services, typed our manuscript.

### LITERATURE CITED

- AVISE, J. C., C. GIBLIN-DAVIDSON, J. LAERM, J. C. PATTON, and R. A. LANSMAN. 1979. Mitochondrial DNA clones and matriarchal phylogeny within and among geographic populations of the pocket gopher, *Geomys pinetis*. *Proc. Natl. Acad. Sci. USA* **76**:6694-6698.
- AVISE, J. C., J. F. SHAPIRA, S. W. DANIEL, C. F. AQUADRO, and R. A. LANSMAN. 1983. Mitochondrial DNA differentiation during the speciation process in *Peromyscus*. *Mol. Biol. Evol.* **1**:38-56.
- BAKER, R. J., and H. H. GENOWAYS. 1978. Zoogeography of Antillean bats. *Spec. Publ. Acad. Nat. Sci. Phila.* **13**:53-97.
- BAKER, R. J., M. W. HAIDUK, L. W. ROBBINS, A. CADENA, and B. F. KOOP. 1982. Chromosomal studies of South American bats and their systematic implications. Pp. 303-327 in M. A. MARES and H. H. GENOWAYS, eds. *Mammalian biology in South America*. *Spec. Publ. Pymatuning Lab. Ecol. Univ. Pittsburgh*.
- BROWN, W. M. 1980. Polymorphism in mitochondrial DNA of humans as revealed by restriction endonuclease analysis. *Proc. Natl. Acad. Sci. USA* **77**:3605-3609.
- CANN, R. L., M. STONEKING, and A. C. WILSON. 1987. Mitochondrial DNA and human evolution. *Nature* **325**:31-35.
- CARR, S. M., S. W. BALLINGER, J. N. DERR, L. H. BLANKENSHIP, and J. W. BICKHAM. 1986. Mitochondrial DNA analysis of hybridization between sympatric white-tailed deer and mule deer in west Texas. *Proc. Natl. Acad. Sci. USA* **83**:9576-9580.
- DARLINGTON, P. J., JR. 1963. *Zoogeography: the geographical distribution of animals*. John Wiley & Sons, New York.
- FITCH, W. M., T. F. SMITH, and W. W. RALPH. 1983. Mapping the order of DNA restriction fragments. *Gene* **22**:19-29.
- FLEMING, T. H. 1982. Foraging strategies of plant-visiting bats. Pp. 287-325 in T. H. KUNZ, ed. *Ecology of bats*. Plenum, New York.
- GEORGE, M., JR., and O. A. RYDER. 1986. Mitochondrial DNA evolution in the genus *Equus*. *Mol. Biol. Evol.* **3**:535-546.
- JONES, J. K., JR. 1978. A new bat of the genus *Artibeus* from the Lesser Antillean island of St. Vincent. *Occasional Pap. Mus. Tex. Tech. Univ.* **51**:1-6.
- JONES, J. K., JR., and C. J. PHILLIPS. 1970. Comments on the systematics and zoogeography

- of bats in the Lesser Antilles: studies on the fauna of Curacao and other Caribbean islands. *32*:131-145.
- . 1976. Bats of the genus *Sturnira* in the Lesser Antilles. Occasional Pap. Mus. Tex. Tech. Univ. **40**:1-16.
- KOOP, B. F., and R. J. BAKER. 1983. Electrophoretic studies of relationships of six species of *Artibeus* (Chiroptera:Phyllostomidae). Occasional Pap. Mus. Tex. Tech. Univ. **83**:1-12.
- KOOPMAN, K. F. 1968. Taxonomic and distributional notes on Lesser Antillean bats. *Am. Mus. Novitates* **2333**:1-13.
- . 1976. Zoogeography. Pp. 39-47 in R. J. BAKER, J. K. JONES, JR., and D. C. CARTER, eds. *Biology of bats of the New World family Phyllostomatidae: part I*. Texas Tech University Press, Lubbock.
- KUNZ, T. H. 1982. Roosting ecology of bats. Pp. 1-55 in T. H. KUNZ, ed. *Ecology of bats*. Plenum, New York.
- LANSMAN, R. A., J. C. AVISE, C. F. AQUADRO, J. F. SHAPIRA, and S. W. DANIEL. 1983. Extensive genetic variation in mitochondrial DNA's among geographic populations of the deer mouse, *Peromyscus maniculatus*. *Evolution* **37**:1-16.
- MCCRACKEN, G. F., and J. W. BRADBURY. 1981. Social organization and kinship in the polygynous bat, *Phyllostomus hastatus*. *Behav. Ecol. Sociobiol.* **8**:11-34.
- MANIATIS, T., E. F. FRITSCH, and J. SAMBROOK. 1982. *Molecular cloning: a laboratory manual*. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- MORGAN, G. S., and C. A. WOODS. 1986. Extinction and the zoogeography of West Indian land mammals. *Biol. J. Linnean Soc.* **28**:167-203.
- NEI, M., and W. H. LI. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA* **76**:5269-5273.
- NEI, M., and F. TAJIMA. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. *Genetics* **105**:207-217.
- PHILLIPS, C. J. 1968. Systematics of megachiropteran bats of the Solomon Islands. *Univ. Kans. Publ. Mus. Nat. Hist.* **16**:777-837.
- PHILLIPS, C. J., D. E. PUMO, H. H. GENOWAYS, and P. E. RAY. Caribbean Island zoogeography: a new approach using mitochondrial DNA to study Neotropical bats. In C. A. Woods, ed. *Caribbean Island biogeography*. E. J. Brill, Leiden (accepted).
- STRANEY, D. O., M. SMITH, I. F. GREENBAUM, and R. J. BAKER. 1979. Biochemical genetics. Pp. 157-176 in R. J. BAKER, J. K. JONES, JR., and D. C. CARTER, eds. *Biology of bats of the New World family Phyllostomatidae: part III*. Texas Tech University Press, Lubbock.
- SYKES, L. R., W. R. MCCANN, and A. L. KAFKA. 1982. Motion of the Caribbean plate during the last 7 million years and implications for earlier Cenozoic movements. *J. Geophys. Res.* **87**:10656-10676.
- TANDLER, B., T. NAGATO, and C. J. PHILLIPS. 1986. Systematic implications of comparative ultrastructure of secretory acini in the submandibular salivary gland in *Artibeus* (Chiroptera: Phyllostomidae). *J. Mammal.* **67**:81-90.
- TURNER, D. C. 1975. *The vampire bat: a field study in behavior and ecology*. The Johns Hopkins University Press, Baltimore.
- UPHOLT, W. B. 1977. Estimation of DNA sequence divergence from comparison of restriction endonuclease digests. *Nucleic Acids Res.* **4**:1257-1265.
- VAWTER, L., and W. M. BROWN. 1986. Nuclear and mitochondrial DNA comparisons reveal extreme rate variation in the molecular clock. *Science* **234**:194-196.
- WRIGHT, J. W., C. SPOLSKY, and W. M. BROWN. 1983. The origin of the parthenogenetic lizard *Cnemidophorus laredoensis* inferred from mitochondrial DNA analysis. *Herpetologica* **39**:410-416.

WESLEY M. BROWN, reviewing editor

Received March 30, 1987; revision received August 11, 1987