Natural hybridization generates mammalian lineage with species characteristics

Peter A. Larsen[1], María R. Marchán-Rivadeneira, and Robert J. Baker

Department of Biological Sciences and Museum, Texas Tech University, Lubbock, TX 79409

Most diploid species arise from single-species ancestors. Hybrid origins of new species are uncommon (except among polyploids) and are documented infrequently in animals. Examples of natural hybridization leading to speciation in mammals are exceedingly rare. Here, we show a Caribbean species of bat (*Artibeus schwartzi*) has a nuclear genome derived from two nonsister but congeneric species (*A. jamaicensis* and *A. planirostris*) and a mitochondrial genome that is from a third extinct or uncharacterized congener. *Artibeus schwartzi* is self-sustaining, morphologically distinct, and exists in near geographic isolation of its known parent species. Island effects (i.e., area, reduced habitat variability, and geographic isolation) likely have restricted gene flow from parental species into the Caribbean populations of this hybrid lineage, thus contributing to local adaptation and isolation of this newly produced taxon. We hypothesize differential rates of the development of reproductive isolation within the genus and estimate that 2.5 million years was an insufficient amount of time for the development of postzygotic isolation among the three species that hybridized to produce *A. schwartzi*. Reticulated evolution thus has resulted in a genomic combination from three evolutionary lineages and a transgressive phenotype that is distinct from all other known species of *Artibeus*. The data herein further demonstrate the phenomenon of speciation by hybridization in mammals is possible in nature.

Artibeus | Chiroptera | hybrid speciation | reticulate evolution | transgressive segregation

Despite empirical studies documenting the establishment of animal hybrid lineages (1–8), the evolutionary importance of speciation by natural hybridization in animals is unknown and often is considered minor because the offspring of such crosses typically are less fit than either parental species (9, 10). Nearly all reported cases of homoploid speciation events (hybrid speciation without change in chromosome number) (11) in animals are among species of insects or fish (12), and there are only a handful of suspected cases in mammals (13–17). Thus hybrid speciation appears to be especially rare in mammals, a consequence of either unfavorable conditions for hybrid speciation to occur (i.e., ecological, physiological, hybrid zone structure) or a lack of empirically based research (18, 19). Here, we describe a zone of admixture on a series of Caribbean islands within which three species of Neotropical bats have hybridized resulting in a novel lineage with species-level distinction. Our genetic and morphometric analyses have identified a unique hybrid zone among species of fruit-eating bats, leading us to the hypothesis that natural hybridization has generated a distinct lineage that exists in these insular populations. Our data also indicate that this phenotypic and genotypic combination is geographically isolated from extant parental species on the southern Lesser Antillean island of Saint Vincent.

Two species of fruit-eating bats, *Artibeus jamaicensis* and *A. planirostris*, have come into contact relatively recently (<30,000 y B.P.) in the southern Lesser Antilles (20–22) (Fig. 1). A third endemic Caribbean species (*A. schwartzi*) exhibits phenotypic and mitochondrial genetic distance values typical of those distinguishing other species within the genus (20, 23); however, its geographic distribution is limited to a few Lesser Antillean islands located at the peripheral distributions of *A. jamaicensis* and *A. planirostris* (20).

Although *Artibeus* is one of the most extensively studied genera of Neotropical bats, the origin of *A. schwartzi* is unclear and has perplexed researchers for nearly 3 decades. Indeed, several hypotheses regarding the evolutionary history of *A. schwartzi* have been proposed and include (*i*) *A. schwartzi* is a relictual population of a once widespread Caribbean species (22), (*ii*) *A. schwartzi* is conspecific with or closely related to *A. planirostris* (24), or (*iii*) *A. schwartzi* is a subspecies exhibiting heterosis originating by hybridization (25). Through a combined analysis of genetic data (nuclear and mitochondrial DNA) and morphometric variation, the present study allows a rigorous test of these hypotheses.

Results

We examined genetic and morphological variation from seven species of *Artibeus*, including all species with Caribbean distributions. We analyzed nuclear amplified fragment-length polymorphisms (AFLPs), mtDNA sequences, and/or morphological data from 73 specimens. An additional 164 individuals were identified molecularly [using cytochrome-*b* (cyt-*b*) gene sequences] in an attempt to identify the presence of mtDNA haplotypes of *A. schwartzi* on mainland South America. Specimens examined are presented in Tables S1 and S2.

Amplified Fragment Length Polymorphisms. Six primer pairs (*EcoR*I plus three bases; *Ase*I plus three bases) used for selective amplification produced 374 scorable bands. Of the 374 bands, 52 were constant, and 322 were polymorphic. Phylogenetic analysis of the AFLP data resulted in seven statistically supported clades, corresponding to seven of the eight species used in our analyses (Fig. 2). Specimens of *A. j. aequatorialis* [a subspecies distributed west of the Andes Mountains in South America (20)] formed a clade separate from the *A. jamaicensis* complex (Fig. S1). Specimens identified as *A. schwartzi* using AFLP data did not form a monophyletic clade (Fig. 2 and Fig. S1). Of 218 AFLP fragments scored among *A. jamaicensis*, *A. planirostris*, and *A. schwartzi*, 123 fragments (56.4%) varied among species. Of the variable fragments, 23 and 22 were diagnostic (either fixed or present or absent) of *A. jamaicensis* and *A. planirostris*, respectively; whereas only three fragments were unique to *A. schwartzi*. Excess homoplasy tests of the AFLP data identified a potential hybrid origin for *A. schwartzi* (Fig. 2). Inclusion of individuals collected from throughout the putative hybrid zone (St. Lucia south to Grenada) resulted in weak support for the basal nodes of *A. jamaicensis* and *A. planirostris*, whereas nodal support for all other species in the
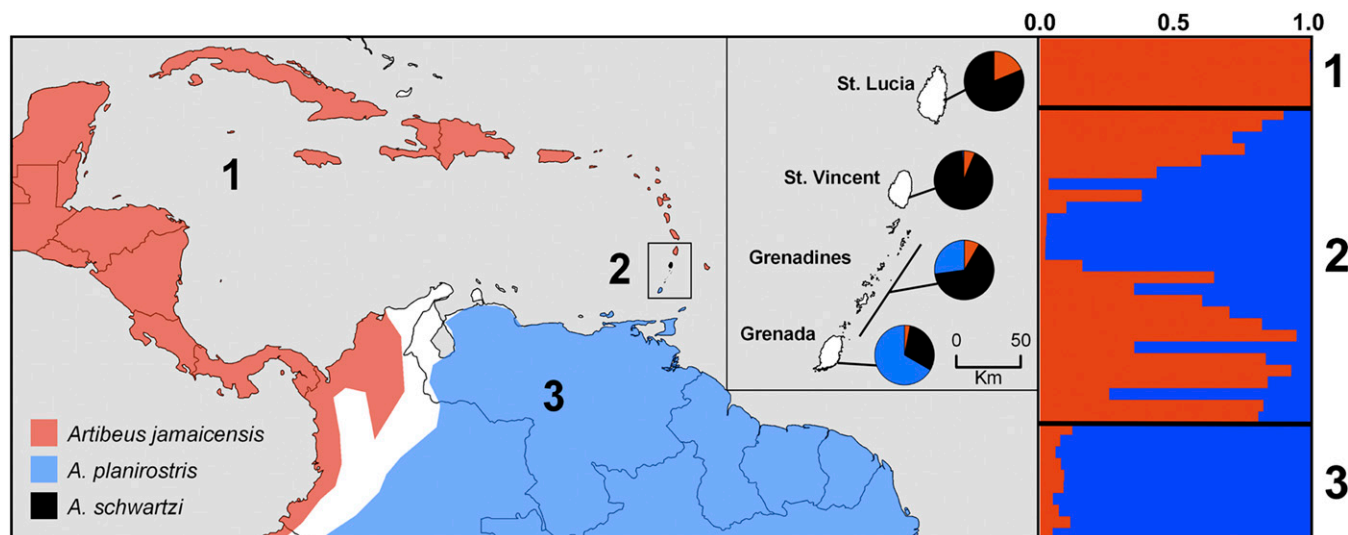
EVOLUTION

Fig. 1. Neotropical distributions and admixture among Caribbean species of *Artibeus*. (*Left*) *A. jamaicensis* is restricted to west of the Andes Mountains in South America. *A. planirostris* is distributed throughout much of South America east of the Andes Mountains. Both species recently have come into primary contact in the southern Lesser Antilles. *Inset* shows mtDNA haplotype frequencies at the region of primary contact (St. Lucia: *n* = 48; St. Vincent: *n* = 126; Grenadines: *n* = 48; Grenada: *n* = 33). (*Right*) Results of a structure analysis of 218 AFLP fragments reveals admixture between the nuclear genomes of *A. jamaicensis* and *A. planirostris* in southern Lesser Antillean populations. Sampled populations for AFLP analyses included (1) *A. jamaicensis*: Central America and Jamaica, (2) *A. jamaicensis*, *A. schwartzi*, and *A. planirostris*: St. Lucia, St. Vincent and the Grenadines, and Carriacou Island, and (3) *A. planirostris*: Grenada, Venezuela, and Ecuador.

analysis remained high (Fig. 2). Structure analyses of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* indicated genetic admixture throughout Lesser Antillean populations and that two and three populations best fit the data (Fig. 1 and Figs. S2 and S3). A principal coordinates analysis of the 218 AFLPs identified specimens of *A. schwartzi* as a cluster between *A. jamaicensis* and *A. planirostris* (Fig. S4).

Mitochondrial DNA Identifications. We compiled mtDNA identifications of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* from throughout the Neotropics using the sequence data presented here and mtDNA-based identifications previously reported or summarized (20, 22, 26, 27). *A. jamaicensis* haplotypes were distributed west of the Andes Mountains in South America (*n* = 15), throughout Central America (*n* = 22), and throughout the Greater and Lesser Antilles (*n* = 57). *A. planirostris* haplotypes were distributed east of the Andes Mountains throughout much of eastern South America (*n* = 189). A single individual with a lower genetic distance with respect to Caribbean *A. schwartzi* (~3.3% in cyt-*b* sequence) was identified in Venezuela (20); however, our analyses show the nuclear genome and cranial phenotype of this individual are typical of *A. planirostris*. Caribbean mtDNA haplotypes revealed the area of primary contact among multiple species of *Artibeus* (Fig. 1). Of 91 specimens screened from Puerto Rico (*n* = 33) and the northern Lesser Antilles (*n* = 58), *A. schwartzi* haplotypes were identified in three individuals (20). *A. schwartzi* haplotypes were most common in the southern Lesser Antilles. *A. planirostris* haplotypes comprised ~23% of the Grenada population, and a single *A. planirostris* haplotype was identified as far north as St. Vincent (22).

Morphometrics. Cranial and mandibular measurements were used to examine the morphological variation in *A. jamaicensis*, *A. planirostris*, and *A. schwartzi*. Descriptive statistics are presented in Table S3. The multivariate analysis of variance (MANOVA) test identified statistically supported differences among *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* (Wilk's lambda = 0.06; $F_{[34, 96]}$ = 8.11; $P < 0.01$). Phenotypic variation among specimens assigned to *A. jamaicensis* and *A. planirostris* showed an area of overlap

along principal component 1 (PC1), differing from specimens of *A. schwartzi* that were grouped outside either *A. jamaicensis* or *A. planirostris* (Fig. 3). The majority of the variation within our sample of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* was interpreted as skull size variation, as indicated by positive and relatively uniform loadings of PC1 (which accounted for 80.49% of the total variance; Table S4). Principal component 2 accounted for 5.36% of the variation in the sample and was interpreted as shape variation. Shape variation among *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* was highly similar. *A. schwartzi* was larger than *A. jamaicensis* and *A. planirostris* with respect to skull size proportions. Specimens of *A. schwartzi* collected from St. Vincent represented the most extreme phenotype in the sample (Fig. 3). We identified sympatric phenotypes of *A. jamaicensis* and *A. schwartzi* on two islands in the Grenadines (Carriacou and Union) as well as on St. Lucia and St. Vincent (Fig. 3).

Relaxed Molecular Clock Analyses. Divergence times were estimated using cyt-*b* sequence data from all known extant species of *Artibeus* (1,140 bp; 12 species) (*SI Materials and Methods*). Our results indicate that the diversification of *Artibeus* began during the late Miocene/early Pliocene ~5.1 million years ago (Mya) [±1.2 million years]. Time to the most recent common ancestor (TMRCA) for the clades containing *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* was estimated at 2.5 Mya (± 0.7 million years). The mean rate of evolution was 0.019 substitutions per site per million years (95% highest posterior density: 0.0154 and 0.0249), and the estimated Yule birth rate was 0.230 (95% highest posterior density: 0.122 and 0.342). A previous hypothesis regarding the timescale of diversification for *Artibeina* (*Artibeus*, *Dermanura*, and *Koopmania*) (26) was rejected based on an inordinately slow rate of evolution for the cyt-*b* gene (0.009 substitutions per site per million years) and phylogeographic incompatibilities with major paleogeographic events in the Neotropics (Fig. 4).

Discussion

Our results did not directly support any of the three hypotheses listed above for the origin of *A. schwartzi* but are similar to the third hypothesis in that the nuclear genome of *A. schwartzi* is an
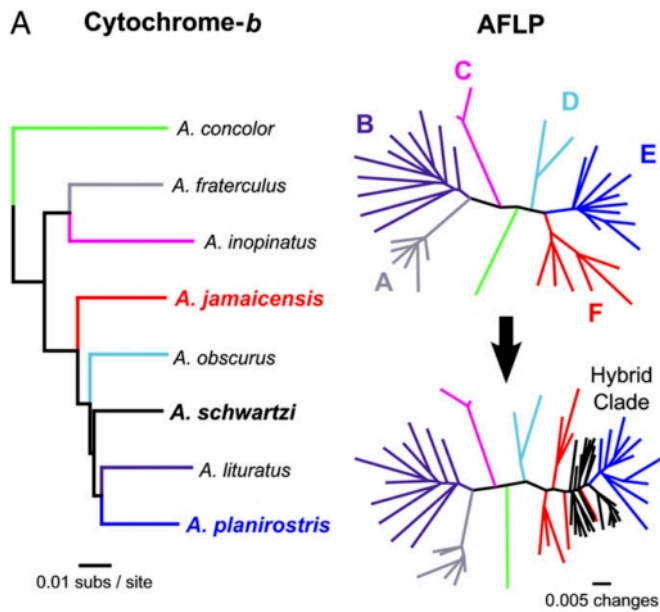
Fig. 3. Transgressive segregation by *Artibeus schwartzi*. Results of a PCA on 17 cranial and mandibular measurements of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* collected from throughout the Caribbean. Within *A. schwartzi*: ●, specimens collected from St. Vincent (SV); ○, specimens collected from St. Lucia (SL) and the Grenadines (G) (Fig. 1). Descriptive statistics and factor loadings are presented in Tables S3 and S4.

Fig. 2. Mitochondrial (cyt-*b*) and nuclear (AFLP) phylogenies of species of *Artibeus* examined herein and results of a homoplasy excess test performed on AFLP data. (*A*) Cyt-*b* and AFLP phylograms showing species-level variation within the genus. Clades A–F identify ingroup species-level clusters of the AFLP dataset. Arrow indicates the change in topology with addition of individuals from the southern Lesser Antilles. (*B*) Results of a homoplasy excess test of 374 AFLP fragments. The *y* axis identifies basal nodes for each species indicated in *A*, and the *x* axis represents bootstrap support values of 1,000 iterations. Removal of putative hybrid taxa increased bootstrap support values for *A. jamaicensis* (clade F) and *A. planirostris* (clade E) to 91% and 95%, respectively (black dots). Solid lines indicate 100% bootstrap support values for clades A and C in all analyses.

admixture of the genomes of two extant species, *A. jamaicensis* and *A. planirostris*, and the morphological variation observed throughout Lesser Antillean populations of *A. schwartzi* indicates a hybrid origin (Figs. 1–3 and Fig. S1) (23). Our nuclear AFLP dataset identif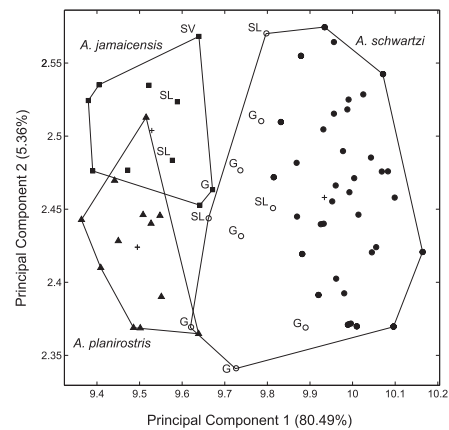ied seven statistically supported species-level clades corresponding to seven species of *Artibeus* examined (Fig. 2 and Fig. S1). Addition of individuals collected from the southern Lesser Antilles, including those morphologically and mitochondrially identified as *A. schwartzi*, to our phylogenetic analyses resulted in reduced statistical support between *A. jamaicensis* and *A. planirostris* and the formation of a single clade among these species (Fig. 2). This topological pattern would be expected if gene flow via hybridization were occurring among these species (28, 29). Furthermore, the presence of only three AFLP bands unique to *A. schwartzi* with respect to *A. jamaicensis* and *A. planirostris* is evidence of hybridization among these species. However, the mitochondrial genome typical of *A. schwartzi* complicates the lack of structure in our AFLP results because it is distinct from those of either *A. jamaicensis* or *A. planirostris* and exhibits a genetic distance to all other species of *Artibeus* typical of levels that distinguish other species within the genus (~6% in cyt-*b* sequence data) (Fig. 2) (20, 22, 27, 30). This distinct mitochondrial genome is most common within populations of *Artibeus* distributed across an ~300-km zone in the southern Lesser Antilles, the region of primary contact and hybridization between *A. jamaicensis* and *A. planirostris* (Fig. 1). Considering recent documentation of potential mitochondrial recombination events in mammals (31), we tested for mitochondrial recombination among these species, and our results failed to identify potential mitochondrial recombination among *A. jamaicensis*, *A. planirostris*, and/or *A. schwartzi* (*SI Materials and Methods*). Additionally, previous analyses have rejected incomplete lineage sorting with regard to *A. schwartzi* mtDNA haplotypes (32).

The most parsimonious explanation for the lack of structure in our AFLP data and the existence of a distinct mitochondrial genome in southern Lesser Antillean populations is that the mtDNA genome was present in a now-extinct or uncharacterized lineage that hybridized in the Caribbean with *A. jamaicensis* and/or *A. planirostris*. This hypothesis would require the nuclear genome of the now-extinct species to be swamped through extensive hybridization and its mitochondrial genome subsequently to introgress into populations of *A. jamaicensis* and *A. planirostris* in the Lesser Antilles and Venezuela. Although the evolutionary origin is unclear, the distinct mtDNA genome is found at high frequencies in populations of *A. schwartzi* on St. Vincent and surrounding islands (Fig. 1). Previous (22) and current analyses identify a complex pattern of mitochondrial introgression in Lesser Antillean populations of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* (Fig. S1). Thus, nuclear admixture, mitochondrial capture, and unique mtDNA variation indicate a reticulate origin for *A. schwartzi*.
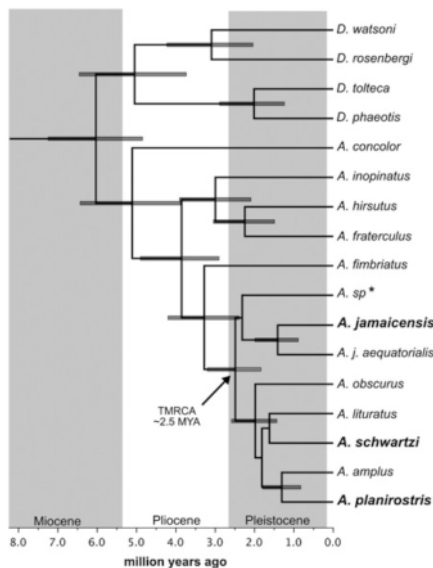
Fig. 4. Timescale of diversification of *Artibeus*. Chronogram depicts diversification of four species of *Dermanura* and 12 species of *Artibeus* based on complete cyt-*b* sequences. Nonreproductively isolated taxa (*A. jamaicensis*, *A. planirostris*, *A. schwartzi*) are in bold. TMRCA for these species was estimated at ~2.5 Mya. Gray bars indicate 95% highest posterior density for divergence estimates. *, Unrecognized species-level variation in Brazil (26). The timescale of diversification presented here is compatible with major paleogeographic events in the Neotropics: the split between Central American endemics (*A. inopinatus*, *A. hirsutus*) and the remainder of the genus coincides with the closure of the Panamanian Isthmus (~3.5 Mya) (39); the split between *A. hirsutus* and *A. fraterculus* (a southwestern Andean endemic) occurs subsequent to the closure of the Panamanian Isthmus; the split between the *A. jamaicensis* complex (distributed west of the Andes Mountains) and the remainder of the genus is compatible with isolation resulting from uplifts of the Andean chain (from ~5 Mya to present) (40).

Morphological data from southern Lesser Antillean populations of *Artibeus* reinforce this hypothesis. Specifically, the presence/absence of the upper third molar (M3) is a species-level character distinguishing northern South American and northern Lesser Antillean populations of the putative parent taxa *A. jamaicensis* and *A. planirostris*, respectively (23). This character is absent within northern Lesser Antillean populations of *A. jamaicensis* but is present within populations of *A. planirostris* in Venezuela and Trinidad (23, 33). Although the St. Vincent population of *A. schwartzi* exhibits size variation outside that of either putative parent species (Fig. 3), ~12.9% of population has the M3, indicating historical genetic influence from *A. planirostris* (23).

The morphological phenotype of *A. schwartzi* also reflects a common signature of hybrid speciation. Specifically, the morphometric variation in this species is characteristic of that predicted by transgressive segregation (29, 34–37). The predicted pattern of hybridization-induced transgressive segregation is that the hybrid lineage possesses a range of variability outside of that of the parental taxa (34). Fig. 3 shows that the phenotypes of the extant parental taxa [*A. jamaicensis* ($n = 10$) and *A. planirostris* ($n = 12$)] overlap in their morphologies, but the distribution of variation in *A. schwartzi* from the island of St. Vincent ($n = 36$) is outside that of both its putative (extant) parents. The distinctiveness of the St. Vincent population of *A. schwartzi* also was recovered in the structure analysis of AFLP data (Fig. 1 and Fig. S3). We identified sympatric phenotypes of *A. jamaicensis* and *A. schwartzi* on the islands north (St. Lucia) and south (the Grenadines) of St. Vincent (Fig. 3). Notably, only one parental phenotype (*A. jamaicensis*) was identified in our sample of the St. Vincent population, and mtDNA haplotypes on St. Vincent

($n = 129$) were <7% for *A. jamaicensis* and <1% for *A. planirostris*. There are two alternative hypotheses regarding whether the phenotype of *A. schwartzi* is representative of transgressive segregation: (*i*) the morphology reflects that of the progenitor within which the distinct mitochondrial genome typical of *A. schwartzi* evolved, or (*ii*) the morphology reflects heterosis arising from recent hybridization between *A. jamaicensis* and *A. planirostris*. The first hypothesis seems plausible when considering the morphological variation observed within closely related species (i.e., *A. lituratus*); however, it is likely that this hypothesis can be tested only with fossil data from St. Vincent, because the phenotype of the extinct progenitor is unknown. It should be noted that a recent study has shown that the production of transgressive hybrid offspring was more likely between distantly related but phenotypically similar species (38). This observation is important with respect to the hybridizing Caribbean populations of *Artibeus*, because *A. jamaicensis* and *A. planirostris* have a similar phenotype (Fig. 3) despite sharing a common ancestor ~2.5 Mya (as discussed later). Regarding the hypothesis of heterosis, the geographic structure of the morphological characteristics of *A. schwartzi* from St. Vincent (Fig. 3), in combination with a nearly complete absence of parental types on the island, provides evidence of a directional evolutionary process that goes beyond the initial production of hybrids. The AFLP analyses herein indicate potential F1s and F2s on the islands north (St. Lucia) and south (the Grenadines) of St. Vincent (Fig. 1 and Fig. S3); however, these populations do not show the morphological distinctiveness of *A. schwartzi* on St. Vincent (Fig. 3). Collectively, the data from St. Vincent do not suggest that hybridization with pure parental types is ongoing. These data indicate that the *A. schwartzi* hybrid lineage is a stable, self-sustaining population at an advanced stage rather than one generated by recurrent hybridization between parental species.

Our hypothesis of hybridization among multiple species of *Artibeus* requires an absence of reproductive isolating mechanisms among distantly related (nonsister) species. A lack of reproductive isolating mechanisms among species distributed in the Caribbean is likely the result of an absence of the evolution of such isolating barriers while the parental species were in allopatry. *A. jamaicensis* (*sensu stricto*) and *A. planirostris* are at the terminal branches of two respective lineages and have evolved allopatrically, a hypothesis supported by the phylogeography of each species (20), the presence of sister lineages (*A. jamaicensis aequatorialis* and *A. amplus*, respectively; Fig. 4), and the timescale of diversification for the genus (Fig. 4). The maintenance of distinctive phenotypes and genotypes among sympatric mainland species (e.g., *A. amplus*, *A. obscurus*, *A. lituratus*, and *A. planirostris* east of the Andes Mountains and *A. lituratus* and *A. jamaicensis* west of the Andes and in Central America) provides evidence that reproductive isolation (prezygotic or postzygotic) is present among mainland populations. Furthermore, *A. lituratus* is a widely distributed congener and is sympatric with nearly all species of *Artibeus* in the Neotropics as well as *A. jamaicensis*, *A. planirostris*, and *A. schwartzi*. Our analyses fail to provide any evidence of hybridization between *A. lituratus* and any other species of *Artibeus* (including on small Caribbean islands). This result is compatible with our hypothesis of differential rates of reproductive isolation within the radiation of *Artibeus*. The lack of sympatric populations of *A. jamaicensis* (*sensu stricto*) and *A. planirostris* suggests that these species have not interacted with each other ecologically on the mainland. The mitochondrial genome typical of the hybrid lineage *A. schwartzi* most likely evolved within a species that invaded the Caribbean from northern South America during the early Pleistocene, before the origin of either *A. jamaicensis* or *A. planirostris* (Fig. 4). Furthermore, our maximum estimate of the TMRCA among these species is ~3.5 Mya, a value younger than the hypothesized rate of hybrid inviability for mammals (18, 41). The patterns of differential rates of reproductive isolation in allopatry described above

are compatible with theoretical models of speciation (18, 42, 43). Theoretical treatments of hybrid speciation have shown that speciation is likely to occur faster when the chromosomal differences between the parental taxa were low and the hybrids were common in a given population (44). We posit that empirical data from the genus *Artibeus* meet these criteria, because all species of *Artibeus* possess a highly conserved karyotype (45), and the available data indicate that *A. schwartzi* is of hybrid origin and that the St. Vincent population is self-sustaining. A critical observation is that bats of the genus *Artibeus* are one of the youngest and most specious lineages of the family Phyllostomidae (46, 47). Thus, the patterns of differential rates of reproductive isolation described herein are comparable to empirical studies of young radiations that have identified similar evolutionary patterns regarding the presence/absence of pre- and postmating isolating mechanisms and introgressive hybridization (48–50).

A bifurcating model of evolutionary relationships accounts for the genetic variation observed between sympatric mainland species of *Artibeus* but does not account for the variation observed within Caribbean *A. jamaicensis*, *A. planirostris*, and *A. schwartzi*. We document that natural hybridization among species in the genus has resulted in a novel lineage, because our genetic and morphological data for *Artibeus* provide support for a unique three-way hybrid origin of *A. schwartzi*. Our data indicate restricted gene flow from parental species into the insular populations of this hybrid lineage, thus contributing to local adaptation of the newly produced taxon. Restricted gene flow by parental species into the St. Vincent population is likely the result of rising sea levels since the last glacial maximum. Geographic isolation of the St. Vincent population of *A. schwartzi* is critical to our hypothesis of hybrid speciation, because theoretical models have shown that the likelihood of hybrid speciation increases with isolation of derived lineages (51). We posit that the unique geography of the southern Lesser Antilles (23), in combination with oscillating glacial periods during the late Quaternary, has contributed to the formation and stabilization of a hybrid species.

Although studies of relatively young radiations of animal taxa continually identify patterns of historical introgression and/or reticulated evolution (1–4, 6–8, 29, 48, 49, 52, 53), the significance of such patterns with respect to speciation and other evolutionary processes remains poorly studied. When considering mammals, few researchers have hypothesized that natural hybridization has contributed to extant species-level diversity (14–17). The putative hybrid origin of the red wolf (*Canis rufus*) (parental species *C. latrans* and *C. lupus*) (14) is perhaps the most contentious of these hypotheses, and more recent analyses indicate that *C. rufus* evolved independently in North America during the late Pleistocene (54). Genetic and morphological signatures of reticulate evolution have been identified in studies focused on primate evolution (53, 55), and at least two have suggested hybrid speciation events [*Macaca arctoides* (15) and *Rungwecebus kipunji* (16)]. Both these studies hypothesize that expansion and contraction of forest refugia during the Pleistocene facilitated hybrid speciation events. The majority of well-documented cases of animal hybrid speciation events are from insects (1–4) and fish (5–7), and most of these studies hypothesize the existence of a hybrid species that exhibits some combination of (*i*) an intermediate or transgressive phenotype with respect to parental species, (*ii*) a restricted distribution or distribution within a ecology distinct (in allopatry or parapatry) from that of parental species, and/or (*iii*) isolation from parental species by either allopatry or assortative mating (e.g., allochrony or mate choice). We provide comparable data for the genus *Artibeus* and hypothesize that interspecific hybridization has contributed to extant species-level diversity. An important observation of the data from primates and *Artibeus* is that traditional aspects of island biogeography (discussed above) likely have contributed to the formation of hybrid taxa. Moreover, although

not presented within the framework of species-level variation, a similar situation has been documented for Japanese subspecies of *Mus* whereby separate colonization events of Japan by parental taxa likely contributed to the formation of the hybrid *M. musculus molossinus* (13). It is probable that additional studies targeting contact zones among recently evolved mammalian species that meet certain criteria [i.e., primary/secondary contact of closely related species (TMRCA <4 Mya) within mainland/oceanic insular settings] will identify similar patterns of reticulate evolution.

There is no consensus among evolutionary biologists regarding the definition of "species." Traditionally the concept of a species is envisioned as a "closed system" with a discrete beginning and end (56). However, the evolutionary processes occurring within the genus *Artibeus* perhaps are best conceptualized within the framework of an "open system," whereby allopatric diversification, reinforcement during periods of sympatry, and hybrid speciation have contributed to contemporary diversity. In the evolution of animal taxa, such patterns are not unique to *Artibeus*. Therefore, criteria for species recognition formulated within the framework of a closed system (i.e., reproductive isolation) are inappropriate for defining species of *Artibeus* and perhaps for other species of mammals. Indeed, with the available data, we cannot prove that the St. Vincent population of *A. schwartzi* is reproductively isolated from either *A. jamaicensis* or *A. planirostris*. However, our data show that *A. schwartzi* is evolving on an evolutionary trajectory separate from other species of *Artibeus*, and therefore *A. schwartzi* is a species under criteria that most biologists accept. These observations document that reticulated evolution is a viable mechanism for speciation in mammals.

Materials and Methods

AFLP Data. AFLPs were generated from 73 specimens of *Artibeus*. Methodologies followed those previously published (57, 58). Genotyping was performed using an ABI 3100-Avant genetic analyzer. AFLP fragments were scored for presence or absence using GeneMapper v4.0 software (Applied Biosystems). Fifteen primer combinations were examined, of which six (*Eco*RI plus three bases; *Ase*I plus three bases) (58) produced scorable fragments within a 400-bp region. AFLPs were scored blindly at least three times, and scoring was performed on two separate datasets. The first dataset examined variation within the genus (73 individuals of eight species of *Artibeus*); the second examined variation among *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* (51 individuals). Both binary AFLP datasets were analyzed using previously published methods (58). *A. concolor* was chosen as an outgroup following previous studies (20, 27). We conducted a tree-based homoplasy excess test (29) on the first AFLP dataset to investigate homoplasy introduced by hybridization. One species at a time was removed, and nodal support was calculated based on 1,000 bootstrap replicates for each remaining species-level node. We examined the genetic structure of 218 AFLPs from mainland and Caribbean populations of *A. jamaicensis*, *A. planirostris*, and *A. schwartzi* using the software Structure version 2.3 (59). We performed 10^4 burn-in generations and 10^6 Markov chain generations, altering the number of clusters from one to six with and without admixture. Structure analyses were performed with and without collecting locality data (LOCPRIOR; for *A. jamaicensis* and *A. planirostris* collected outside the putative hybrid zone). The admixture model with two and three clusters best fit the data (Fig. S2). Additionally, SplitsTree v4.10 (60) was used to examine genetic structure and reticulation in the AFLP data.

Mitochondrial DNA Sequencing and Phylogenetic Analyses. Cyt-*b* gene sequences were generated from 230 specimens (GenBank numbers GQ861587–GQ861814; GU356393; Tables S1 and S2). Methods for amplifying and sequencing followed those previously published (20). Sequencing was performed using an ABI 3100-Avant genetic analyzer. Sequences were verified and assembled using Sequencher v4.9 (Gene Codes Corporation) and were further checked in Mac-Clade v4.05 (61). Multiple sequence alignments were performed using ClustalW v2.0 (62). Phylogenetic analyses were performed using PAUP v4.0b10 (63). Species identifications were confirmed by morphological analyses and by comparison with previously published genetic data (20, 26, 27).

Morphological Analyses. Seventeen cranial and mandibular measurements were taken from 67 specimens [nearest 0.01 mm with digital calipers (Fowler Ultra-Cal Mark IV)] to assess variation among *A. jamaicensis*, *A. planirostris*, and

A. schwartzi. Only adult specimens were used. Variables included greatest length of skull, condyloincisive length, height of skull, mastoidal breadth, greatest width of braincase, zygomatic arch breadth, postorbital constriction width, breadth across upper canines, breadth across the outer edges of the second upper molars, palatal length, maxillary toothrow length, mandible length, mandibular toothrow length, breadth across lower canines, height of coronoid, mandibular depth, and condylar mandibular breadth. A MANOVA was performed to test for differences among species; probabilities were estimated based on 1,000 bootstrap iterations and an α level predefined at 0.05. A PCA was performed, using 1,000 bootstrap iterations to evaluate the morphological variation among the species. Statistical analyses were performed using Matlab v6.5 (using functions developed by R. E. Strauss, Department of Biological Sciences, Texas Tech University) and SPSS version 13.0.

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