

**ON THE ORIGINS OF THE WESTERN SLOPE REGION OF
ENDEMISM: SYSTEMATICS OF FIG-EATING BATS,
GENUS *ARTIBEUS***

by

BRUCE D. PATTERSON

*Division of Mammals, Field Museum of Natural History,
Chicago, IL 60605, U.S.A.*

VICTOR PACHECO

*Museo de Historia Natural,
Universidad Nacional Mayor de San Marcos,
Av. Arenales 1256, Apartado 14-0434, Lima-14, Perú*

and

MARY V. ASHLEY

*Department of Biological Sciences,
University of Illinois at Chicago,
Chicago, IL 60680, U.S.A.*

ABSTRACT

A phylogenetic analysis of fig-eating bats (genus *Artibeus*) was undertaken to illuminate the origins and interrelationships of the highly distinctive biota that inhabits the Western Slope of the Andes in Peru. The study focused on three populations of *Artibeus fraterculus*, a species endemic to the Western Slope, and their relationships to each other, as well as to other *Artibeus* species in the Amazon Basin and in Southeastern Brazil. We examined sequence variation in the mitochondrial genes ATPase 8 and ATPase 6 using the polymerase chain reaction (PCR) and both phenetic and cladistic analyses. Sequence divergence among populations of *A. fraterculus* on the western versant and in the Marañón valley was very low (<1%). Contrary to past taxonomy, which treated *fraterculus* as a subspecies of the widespread *A. jamaicensis*, no sister-group relationship exists between these taxa. Instead, analyses suggest that *A. jamaicensis*, *A. planirostris*, and *A. lituratus* are all more closely related to one another than any is to *A. fraterculus*. This indicates that diversification of these Amazonian taxa took place subsequent to the divergence that gave rise to *A. fraterculus*. This phylogenetic perspective is difficult to reconcile with hypotheses of a late-Pleistocene origin of *A. fraterculus* via dispersal across the Huancabamba Deflection. Two alternative hypotheses based on an earlier origin of *A. fraterculus* are proposed: that this species and other elements of the Western Slope biota originated by vicariant events associated with Andean orogeny during the Pliocene, or that Western Slope taxa may be derived from the Middle American biotic province, rather than Amazonia.

RESUMEN

Se realizó un análisis filogenético de los murciélagos frugívoros del género *Artibeus* para elucidar los orígenes e interrelaciones de la biota altamente diferenciada que habita la vertiente Occidental de los Andes del Perú. El estudio examinó tres poblaciones de *Artibeus fraterculus*, una especie endémica de la vertiente Occidental, y sus relaciones entre ellas, así como con otras especies de *Artibeus* en la Hoya Amazónica y en el Sureste del Brasil. Analizamos la variación secuencial en los genes mitocondriales

ATPasa 8 y ATPasa 6 usando la reacción de polimerasa en cadena (PCR) y análisis fenéticos y cladísticos. La divergencia en secuencia entre poblaciones de *A. fraterculus* en la vertiente Occidental y en el valle del Río Marañón fue muy baja (<1%). Contrariamente a la taxonomía pasada, la que trataba *fraterculus* como una subespecie del ampliamente distribuido *A. jamaicensis*, ninguna relación de grupo hermano existe entre estos taxa. Por el contrario, nuestros análisis sugieren que *A. jamaicensis*, *A. planirostris*, y *A. lituratus* están más cercanamente relacionados entre sí que alguno lo está a *A. fraterculus*. Esto indica que la diversificación de estos taxa amazónicos tomó lugar luego de la divergencia que dió lugar a *A. fraterculus*. Esta perspectiva filogenética es difícil de reconciliar con hipótesis de un origen pleistocénico tardío de *A. fraterculus* via dispersión a través de la Deflección de Huancabamba. Dos hipótesis alternativas basadas en un origen más temprano de *A. fraterculus* son propuestas: que esta especie y otros elementos de la biota de la vertiente Occidental se originaron por eventos vicariantes asociados con la orogenia andina durante el Plioceno, o que los taxa de la vertiente Occidental pueden haberse derivado de la provincia biótica de Meso America en vez que de la Amazonía.

INTRODUCTION

For much of their length, the Andes Mountains constitute an insurmountable barrier to the dispersal of lowland organisms. Many plants and animals are widely distributed with non-coincident ranges in the eastern lowlands of Amazonia but have geographic ranges sharply and coincidentally truncated on the lower slopes of the mountains. On the other hand, the Andes offer a spectrum of dispersal opportunities for many highland species. Some montane species exhibit geographic ranges as much as 800 times longer than they are wide, occurring in ribbon-like bands along the slopes of the mountains (Graves, 1988). The Andes have few equals as a natural theater for evolutionary experiments, given their biological diversity and geographic extent.

Correspondence between biotic distributional limits and the Andes has long been appreciated (Tschudi, 1847). Analyses of mammalian distributions include those of Baker (1967), Hershkovitz (1972), and Koopman (1978, 1982). Most studies identify a region of limited diversity but high endemism along the Pacific coast of Peru. Koopman's (1982) study of South American bats (Mammalia: Chiroptera) is illustrative. In six of the seven faunal subregions he recognized, endemic species amount to 3-13% of the standing diversity. However, fully 37% of the species in the Western Slope biota are endemic to that region. Information on other groups is meager, but generally supports the biotic distinction of the Western Slope. Studying distributions of Neotropical birds, Cracraft (1985) recognized three centers of endemism in western Peru (his Marañón, Tumbes, and Peruvian Arid Coastal Centers). In a detailed account of butterfly distributions in Peru, Lamas (1982) identified no fewer than 48 different biogeographic units in Peru, 12 or 13 of which are situated on the Western versant. Plant species endemism in the coastal "lomas" formations of the Peruvian and Atacama deserts exceeds 40% (Rundel *et al.*, 1991).

The endemism shown by various groups in the Western Slope region is readily explicable, given the region's isolation by high elevations and their distinctive habitats. Only in northern Peru, in an area termed the Huancabamba Deflection (because the cordilleran axis shifts from northwest-southeast to northeast-southwest) are there east-west passes lower than 4000 m at tropical latitudes. Indeed, the lowest pass in the deflection, Abra Porculla, is only 2145 m high. These low-elevation passes and the adjacent arid valley of the Río Marañón constitute significant barriers to the north-south distributions of páramo and puna (or "jalca") birds. Vuilleumier (1984) found that 63% of the avian species found on either side of the deflection have differentiated, and 31% of the species are absent from highlands on the other side. On the other hand, many species of lower-elevation birds range widely throughout the dry vegetation

types of the passes and the adjacent Western and Marañón slopes (Chapman, 1926; Vuilleumier, 1984).

In a distributional synopsis based on more than 380 days of field observation, Parker *et al.* (1985) identified two components of geographic isolation in the Huancabamba region: low passes along the western cordillera and the arid basin of the Río Marañón to the east. Their analysis identified 18 species pairs separated by the Marañón valley but found no instances of species replacement on opposite sides of passes in the western cordillera. On these bases, Parker *et al.* (1985) questioned the efficacy of low-lying passes as barriers to montane species, especially those inhabiting shrub-steppe vegetation.

The purpose of this study is to investigate the biogeographic significance of montane passes and the Marañón valley to the distributions of lower-elevation species. Patterns of evolutionary divergence are assessed relative to these barriers, because such patterns provide cumulative and integrated records of past and present distribution, ecology, and gene flow. We chose to study bats of the genus *Artibeus* (Chiroptera: Phyllostomidae) because they are: 1.) widely distributed in the Neotropics, ranging from southern México and the Greater Antilles to northern Argentina, 2.) highly diversified, with at least 10 species (in the restricted sense of Owen, 1987), including one (*fraterculus*) that is endemic to the Western Slope, and 3.) generally restricted in ecological range to lower elevations, typically in mesic and semi-arid forests. Genetic analyses were conducted because of the utility of molecular characters in elucidating the phylogenetic relationships of closely related species and in offering information directly related to patterns of dispersal and gene flow.

A BIOGEOGRAPHIC HYPOTHESIS

Present distributions suggest that many lowland organisms are unable to surmount the Eastern or Western cordilleras of the Peruvian Andes. Two less formidable geographic barriers separate lowland inhabitants of the Amazon Basin from the western lowlands along the most direct axis of colonization: the arid valley of the lower Río Marañón, isolating Amazonian forests from gallery forests along this inter-Andean valley, and the Huancabamba passes, separating the Marañón valley from the Western Slope. These barriers are arranged in sequence as a distributional gauntlet. That is, having colonized the Marañón valley, an Amazonian species would have to surmount the arid montane passes in the Huancabamba region before becoming established in the Western lowlands. Within each of the three lowland regions bounded by these barriers, dispersal and gene flow presumably occur freely. Given that all species of *Artibeus* save *A. fraterculus* occur in the eastern lowlands (or else in Central America and the Antilles), any tree of presumed relationships should be rooted in the east.

Given these assumptions, geographic relationships among areas can be expressed in the form of an area cladogram (Fig. 1). Correspondence between the area cladogram and genetic divergence patterns of *Artibeus* can be interpreted as corroborating the causal assumptions underlying the area cladogram.

For most of its history, *A. fraterculus* was considered to be a race of *Artibeus jamaicensis* (e.g., Ortiz de la Puente, 1951; Cabrera, 1958; Tuttle, 1970; Jones & Carter, 1976) until Koopman (1978) showed it was a distinct species. Therefore, we expected *A. jamaicensis* populations in the Amazon Basin to form the sister group to *A. fraterculus*. However, we included various other species of *Artibeus* in our analysis to clarify the nature of relationships between *fraterculus* and *jamaicensis*.

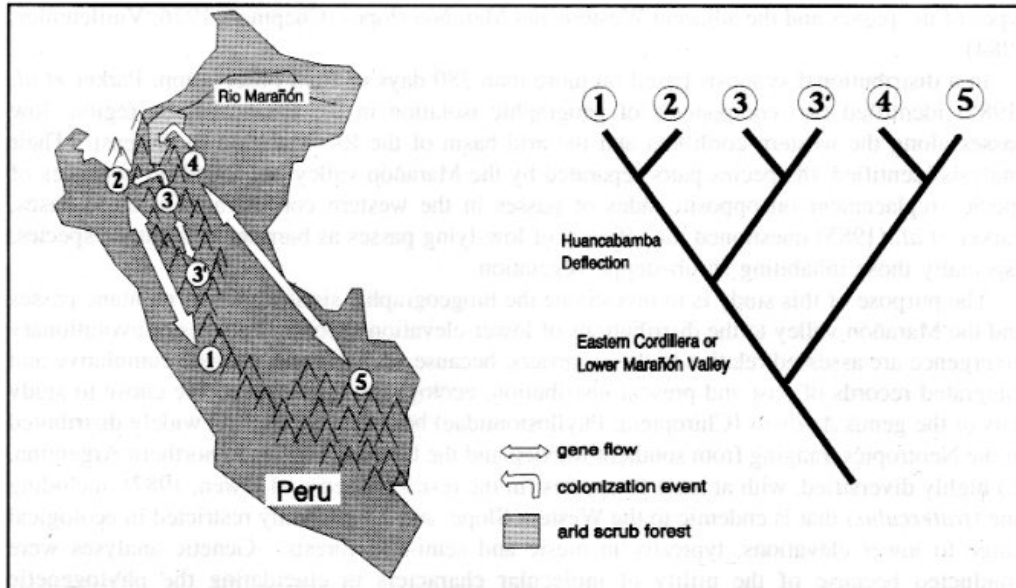


Fig. 1. Area cladogram for lowland species in northern Peru. The main cordilleras currently isolate populations on the Western Slope, in the Marañón valley, and in the Amazon Basin, but dispersal and gene flow take place unimpeded within each of these areas. Historically, Amazon Basin ancestors are presumed to have colonized the Western Slope in two successive stages: colonizing the lower Marañón valley, and surmounting the western passes. The area cladogram summarizes these postulated relationships, predicting patterns of evolutionary divergence within monophyletic clades.

METHODS AND MATERIALS

During fieldwork in 1987, we collected *Artibeus fraterculus* on the Western Slope east of Lima (San Bartolomé) and northeast of Trujillo (near Montesecco), as well as in the lower Marañón valley (Hacienda Limón). *A. fraterculus* was taken 19 km (by road) E of Balsas, on the east side of the Río Marañón, but was not taken on the eastern versant from 1100-2800 m along the Río Utcubamba, an east-slope tributary of the Marañón in Amazonas. We also failed to find this species at high elevations (2950 m) along the Río Mosna, a headwater tributary of the Marañón (locality 3' in Fig. 1). Collections made in Rondônia, Brazil in 1986 provided Amazonian samples of *A. lituratus*, *A. jamaicensis*, *A. planirostris*, and possibly *A. obscurus*. (The systematic relationships of the last three species are enigmatic and under active investigation by C. O. Handley, Jr. and associates.) Collections made in São Paulo, Brazil in 1989 provided Atlantic forest samples of *A. lituratus* and *A. jamaicensis*, as well as an Atlantic forest endemic, *A. fimbriatus*. Finally, a sample of *Artibeus planirostris*, collected in southeastern Peru in 1979, was borrowed from J. L. Patton at the University of California (see Appendix). Museum vouchers and their catalogue numbers are used to ensure that this study can be related to future, refined views of species limits within the genus.

Fresh-frozen tissues were stored at -70° to -100°C until used. Tissues were then minced with a razor blade then Dounce homogenized in STE buffer. Proteinase K and SDS were added and the sample was incubated overnight at 55°C with shaking. Three phenol/chloroform extractions were performed, followed by EtOH precipitation of DNA. DNA pellets were washed with 70% EtOH, dried, and resuspended in TE buffer.

Oligonucleotide primers L8384 (5'-ATGCCGCAACTAGACACGTC-3') and H8764 (5'-GCCTAGTAGGTTTGTGATCC-3') were used to flank a 381 b.p. region of the mitochondrial ATPase 8 and ATPase 6 genes. Double-stranded amplifications (dsPCR) were conducted in 0.5 ml microcentrifuge tubes, containing 10 mM tris, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 150 μM dNTPs, 0.5 μM each amplification primer, and 10 units *Taq* polymerase (Perkin-Elmer/Cetus). Genomic DNA (10-1000 ng) was added after overlaying the reaction mixture with mineral oil. Reaction conditions consisted of 35 cycles of 1 minute at 93°C, 1 minute at 46°, and 2 minutes at 72°, for denaturation, annealing, and extension steps, respectively. The first cycle was preceded by a 3 minute denaturation step and the last cycle was followed by a further 3 minute extension.

Five microliters of the dsPCR product were then applied to a 3% NuSieve (FMC) agarose minigel and separated from free primers and nucleotides by electrophoresis at 60V in 40 mM tris-acetate buffer, pH 8.0. The gel was then stained with ethidium bromide, and the amplified PCR product was excised from the gel and melted in 300 μl water. Two to four microliters of a 1:20 dilution of this solution were then used as template in a 100 μl asymmetric PCR for the purpose of generating single-stranded DNA template for direct sequencing (Gyllenstein & Erlich, 1988). The amplification conditions were similar to those for dsPCR except for a 50-fold dilution of one of the primers and the use of an annealing temperature of 47°. Following 45 cycles of amplification, excess primers, salts, and free nucleotides were removed by three cycles of centrifugal dialysis (Centricon-30, Amicon). An aliquot of the washed and concentrated PCR product was then sequenced by the dideoxy method (Sanger *et al.*, 1977), using a commercially available kit (Sequenase, United States Biochemical) with the oligonucleotide primer that had been limiting in the second-stage PCR. The sequencing reactions were loaded on a 60 cm x 20 cm field gradient 6% polyacrylamide gel, and subjected to electrophoresis for 3 hours at 55 W. After being fixed in 5% methanol and 5% acetic acid, the gel was dried and exposed to X-ray film (Kodak X-OMAT AR) for 1-3 days.

DNA sequences were aligned using ESEE (Eyeball Sequence Editor, written by E. L. Cabot) against published cow, mouse, and human sequences. Percent sequence divergence and variable sites were determined using FIX_DNA, a Turbo Pascal program written by S. M. Lanyon. For any set of sequences, the program identifies and retains all variable positions, as well as only those at the first, second, or third codon positions. Amino acid sequences and distance matrices (calculated as percent pair-wise substitutions) can also be generated. Output from FIX_DNA was analyzed using PAUP 3.0 on a MacIntosh and NT-SYS 1.40 on an IBM-clone.

RESULTS

DNA sequences from 27 specimens of *Artibeus* were obtained from a region of the mitochondrial genome encoding for ATPase 8 and ATPase 6. Sequences were aligned by eye. This operation was facilitated by the presence of a 44 b.p. region where the two genes overlap with a shift in reading frames; no substitutions were observed in this region of overlap. Sequences from individual specimens averaged 303 b.p. in length, and had mean base compositions of adenosine 35.93%, cytidine 24.55%, guanosine 7.88%, and thymidine 31.65%. Of the 417 base-pair sites (counting sites in the region of overlap twice, once with each gene), 49 proved to be variable. Sixteen sites were variable in the first codon position, 8 in the second position, and 25 in the third position. As reported for other vertebrates (e.g., Moritz *et al.*, 1987), substitutions were heavily biased, with transitions (A \leftrightarrow G, C \leftrightarrow T) accounting for 47 of

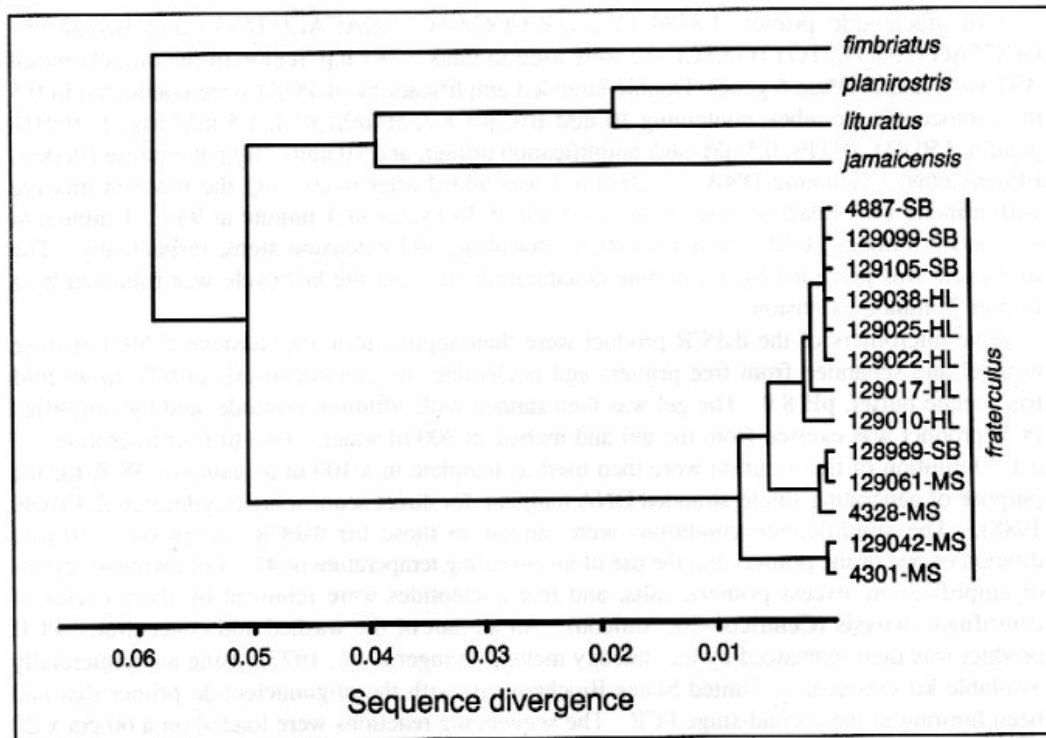


Fig. 2. UPGMA dendrogram of percent divergence in a 417 base-pair region of the mitochondrial genes for ATPase 8 and ATPase 6. The coefficient of cophenetic correlation is 0.97. Numbers refer to Field Museum and Museo de Historia Natural catalogue numbers of *Artibeus fraterculus*. Locality abbreviations are: SB, San Bartolomé; HL, Hacienda Limón; and MS, Montesecco.

the 49 variable sites; only two transversions were observed. Twenty of the substitutions resulted in amino acid replacements.

Percent sequence divergence was used in a genetic distance analysis. Divergence values among samples ranged from 0, in the case of several samples of *A. fraterculus* that had identical base sequences, to a high of 0.11, separating an *A. fimbriatus* from an *A. obscurus*. However, only 110 b.p. of sequence were obtained from the last individual. Average interspecific divergence was about 0.05. The distance matrix was subjected to a UPGMA cluster analysis, which identified *A. fimbriatus* samples as the most divergent of those analyzed. Conspecific samples tended to be nearest neighbors, although samples identified as *A. jamaicensis*, *A. planirostris*, and *A. obscurus* were intermixed. All *A. fraterculus* samples formed a single cluster before joining other *Artibeus*. Contrary to expectation, the cluster of *fraterculus* samples did not join to one of *A. jamaicensis*. Rather, the cluster of *fraterculus* joined with a cluster comprising *fimbriatus* (0.053), and this cluster joined at a slightly higher level (0.058) with the remainder. Numerous ties limit the resolution of this analysis, especially with regard to the resolution of relationships among samples of *A. fraterculus*.

Another cluster analysis was conducted, employing all *fraterculus* samples with single representatives of other taxa to maximize information relating to the divergence of *fraterculus* samples (Fig. 2). Here, *fraterculus* samples cluster together, then with a group containing *planirostris*, *jamaicensis*, and *lituratus*; *fimbriatus* represents the basal element of this analysis.

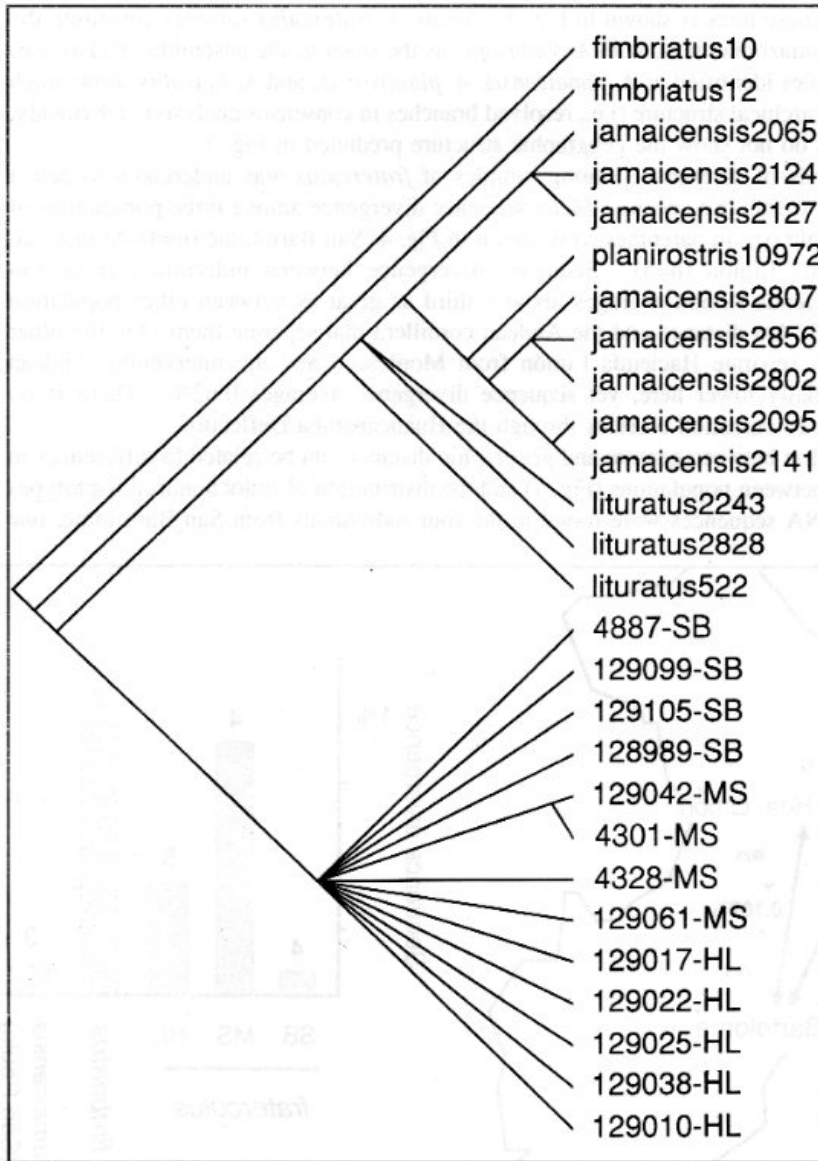


Fig. 3. Strict-consensus tree resulting from phylogenetic analysis of substitutions at 49 variable sites. The tree requires 67 steps. Numbers after species names refer to specimen numbers (see Appendix); other conventions as in Fig. 2.

Within *fraterculus*, most specimens from San Bartolomé (sample 1 in Fig. 1) are joined with members of Hacienda Limón (sample 3), whereas another individual from San Bartolomé clusters with members of Montesecco (sample 2). All of the clusters in this analysis save one containing two individuals from Montesecco proved to be geographically heterogeneous. The topology of this tree fails to support the area relationships predicted in Fig. 1.

A very similar tree topology results from phylogenetic analysis of the same data. *A. fimbriatus* was treated as an outgroup because of its high average divergence from other species. Substitutions at the 49 variable sites were analyzed using the TBR, MULPARS, and heuristics options of PAUP. A total of 2,110,211 rearrangements produced 250 trees with 67 steps; the

strict consensus of these trees is shown in Fig. 3. Again, *A. fraterculus* samples constitute the sister-group to all Amazonian taxa, with *A. fimbriatus* as the sister to the ensemble. Relative to *A. fraterculus*, samples identified as *A. jamaicensis*, *A. planirostris*, and *A. lituratus* show much more consistent hierarchical structure (i.e., resolved branches in consensus analysis). Obviously, *fraterculus* samples do not show the geographic structure predicted in Fig. 1.

A refined analysis of divergence among samples of *fraterculus* was undertaken to detect geographic patterns within this species. Mean sequence divergence among three populations of *A. fraterculus* (sample size in parentheses) is shown in Fig. 4: San Bartolomé (n=4), Montesecco (n=4), and Hacienda Limón (n=5). Sequence divergence between individuals from San Bartolomé and Hacienda Limón averages about a third as great as between other population pairs, despite the 595 km distance and the Andean cordillera that separate them. On the other hand, only 120 km separate Hacienda Limón from Montesecco and the intervening Andean passes are substantially lower here, yet sequence divergence averages 0.62%. There is no evidence here for a colonization corridor through the Huancabamba Deflection.

Lack of correlation between genetic and geographic distance can be related to differences in genetic variability between populations (Fig. 4) and the distribution of mitochondrial haplotypes (Table 1). Two DNA sequences were found in the four individuals from San Bartolomé; one

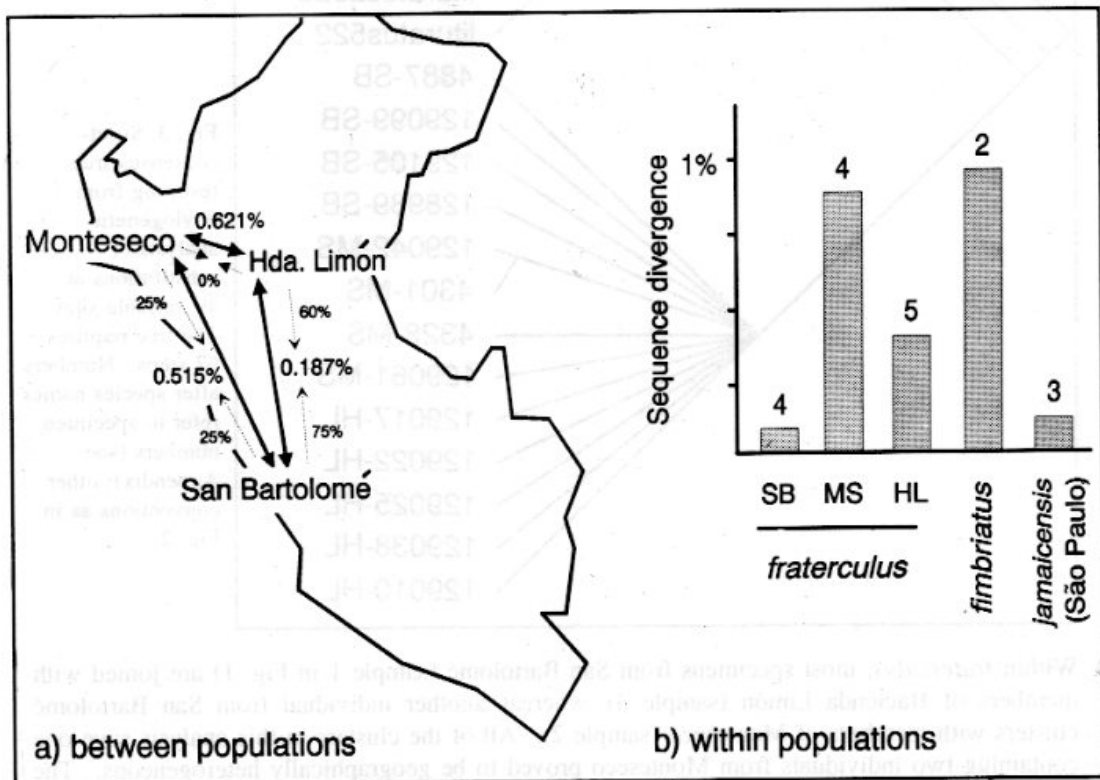


Fig. 4. Sequence variation among and within populations of *Artibeus fraterculus*: a.) analysis of sequence divergence (percent substitutions at surveyed sites) among three populations. Solid lines and larger fonts indicate mean percent sequence divergence between population pairs. Dotted lines and smaller fonts give the proportions of individuals in each population sample that were identical in base sequence to one or more individuals in another population. b.) within population analysis, comparing levels of individual variation in *A. fraterculus* populations to that found in related species of *Artibeus*.

Table 1. Geographic and taxonomic distribution of mitochondrial haplotypes of *Artibeus* in the region of ATPase 8 and ATPase 6. Detailed information on provenience is given in the Appendix. Sample number refers to Fig. 1.

Species	haplotype	Catalogue number	Source	Sample no.
<i>fraterculus</i>	A	MUSM 4887	San Bartolomé	1
	A	FMNH 129099	San Bartolomé	1
	A	FMNH 129105	San Bartolomé	1
	A	FMNH 129022	Limón	3
	A	FMNH 129025	Limón	3
	A	FMNH 129038	Limón	3
	B	FMNH 128989	San Bartolomé	1
	B	FMNH 129061	Montesecco	2
	C	FMNH 129042	Montesecco	2
	C	MUSM 4301	Montesecco	2
	D	MUSM 4328	Montesecco	2
	E	FMNH 129017	Limón	3
	F	FMNH 129010	Limón	3
	<i>fimbriatus</i>	G	MZUSP (SFC10)	Southeastern Brazil
H		MZUSP (SFC12)	Southeastern Brazil	-
<i>jamaicensis</i>	I	MZUSP (BDP2065)	Amazon Basin	-
	J	MZUSP (BDP2124)	Amazon Basin	-
	K	MZUSP (BDP2127)	Amazon Basin	-
	L	MZUSP (BDP2802)	Southeastern Brazil	-
	M	MZUSP (BDP2807)	Southeastern Brazil	-
	N	MZUSP (BDP2856)	Southeastern Brazil	-
<i>lituratus</i>	O	FMNH 129111	Eastern versant, north	4
	P	MZUSP (BDP2243)	Amazon Basin	-
	Q	MZUSP (BDP2828)	Southeastern Brazil	-
<i>jamaicensis/obscurus?</i>	R	MZUSP (BDP2095)	Amazon Basin	-
	S	MZUSP (BDP2141)	Amazon Basin	-
<i>planirostris</i>	T	MVZ 166533	Eastern versant, south	5

of these also appeared in an individual from Montesecco, and the other haplotype, shared by three individuals, was also found in three individuals from Hacienda Limón (Fig. 4). Thus, bats from San Bartolomé exhibited no unique haplotypes. Identical sequences in 75% of this sample and 60% of that from Hacienda Limón accounts for the very low genetic divergence between them. On the other hand, three different sequences were found in four individuals from Montesecco, only one of which was shared, accounting for its high levels of divergence. Mean divergence within populations of *fraterculus* is shown in Figure 4b alongside similar population estimates for other species. Because of the distribution of shared haplotypes (Table 1), sequence divergence among individuals from the Montesecco population is greater than that between any pair of *fraterculus* populations.

DISCUSSION

GENETIC RELATIONSHIPS

Data and analyses presented in this paper offer no support for the hypothetical area cladogram (Fig. 1). Despite our use of several Amazon Basin and Atlantic Forest species of *Artibeus*, we were unable to ally the Western Slope endemic *A. fraterculus* to any one of these. Nor is there evidence for deriving the two Western Slope samples from the Marañón valley population. Rather, phenetic and phylogenetic analyses suggest that the divergence event that gave rise to *A. fraterculus* predated speciation of *Artibeus* in the Amazon.

It is often found that insular and geographically peripheral populations exhibit low genetic variability, whether measured as heterozygosity, mean polymorphism, alleles per locus, or in numbers of mitochondrial haplotypes (Patton, 1984; Ashley & Wills, 1987; Fooden & Lanyon, 1989; Sullivan, in press). Reduced variability in nuclear genes can be readily correlated with the small population sizes that peripheral and isolated populations typically exhibit (Brown, 1984), thence to reduced numbers of mutations and higher rates of drift (Wright, 1978). In addition, factors such as founder effects, differential dispersal by males, reduced effective population sizes, and stochastic lineage extinctions might further erode mt-DNA variability relative to nuclear variability in insular populations (Ashley & Wills, 1987). Thus, mitochondrial haplotypes suggest that both the Hacienda Limón and San Bartolomé populations may be peripheral or isolated. The greatest diversity of haplotypes in *A. fraterculus*, where variation is not limited by small population size, infrequent gene flow, or founder effects, appears to be in northern Peru on the Western Slope. Chapman (1926, p. 72) also noted greater variation in birds from the coast, relative to the Marañón valley and used this pattern to postulate western origins for avian members of this biota.

We were unable to resolve the interrelationships of Amazonian *Artibeus* because of current difficulties in identifying and delimiting species. Tremendous geographic variation in widespread species such as *A. jamaicensis*, polymorphism in taxonomic characters (e.g., presence-absence of third molars), and the discovery of new or previously unrecognized species all contribute to this confusion. Handley (1987, 1989, in press) and coworkers continue to resolve this picture, so we defer conclusions regarding the relative positions of *jamaicensis*, *planirostris*, *obscurus*, and *lituratus* except to say that all appear to be more closely related to one another than any is to *A. fraterculus* or to *A. fimbriatus*. Additionally, several species of *Artibeus* not analyzed here may be closer relatives of *A. fraterculus*. Patten (1970) argued that *fraterculus* was allied with two Middle American species *A. hirsutus* and *A. inopinus* in a clade distinct from *jamaicensis* and *planirostris* on one hand and *lituratus* on another. The position of *A. amplus* Handley, 1987, newly described from the Venezuelan llanos, also needs to be resolved.

BIOGEOGRAPHIC IMPLICATIONS

Low sequence divergence between Western Slope and Marañón populations of *Artibeus fraterculus* suggests that this species may be able to disperse through the western passes. Divergence between Western Slope and Marañón populations is even lower than the within-population divergence of individuals at Montesecco (Fig. 4). Frequent dispersal in this region is also suggested by our collecting this species 12 km SSW of San Miguel in Cajamarca, at 1830 m elevation, at a site intermediate to the Montesecco and Hacienda Limón populations. Evidently,

A. fraterculus is not limited to low elevation forests and may achieve near-continuous distributions in inter-Andean valleys near the deflection.

A review of *A. fraterculus* specimens in museum collections shows that this species occurs in favorable habitats as high as 2800 m near Huaylas, Ancash, on the Western Slope (MUSM 1528). Many other mammals inhabiting both the lower Marañón valley and the Western Slope have similar elevational ranges. Pearson (1982) showed that several species of mice (*Phyllotis amicus*, *P. andium*, *Akodon orophilus*, and *A. mollis*) occur in both areas, as well as the intervening highlands. Pacheco & Patterson (1992) found that a simple isolation-by-distance model explained electrophoretic variation in *Sturnira erythromos* populations from the same localities studied here. Geographic distance between localities explained a significant amount of genetic variation, suggesting that specific geographic features (e.g., Andean passes) play a subordinate role in the differentiation of these higher-elevation bats. In fact, no species of lowland mammals are known to replace others on the east and west sides of the Huancabamba Deflection.

Palynological evidence also suggests that western passes in the Huancabamba Deflection played a minor role in the colonization of the Western Slope by lowland taxa during the Pleistocene. Late-glacial temperatures in Panama were roughly 5°C lower than at present, judging from a 650 m forest that resembled in composition those now found at 1500-1800 m (Piperno *et al.*, 1990). Data from two sites in Amazonian Ecuador suggest that, 30,000 years ago, temperatures in that area were as much as 7.5°C lower than today (Bush *et al.*, 1990). Bush *et al.* also documented a sequence of cold-dry climates >30,000 years BP, followed by a cool-moist period lasting at least 4,000 years, giving way to cold-dry climates. This same sequence of events during the latest Pleistocene glaciation has also been documented in tropical Africa (e.g., Taylor, 1990), and a 3.5 million year pollen sequence from the basin of Bogotá (Hooghiemstra, 1989) indicates no significant amelioration of highland temperatures in the recent past. In all these scenarios, temperature depressions during glacial episodes would have led to the contraction, not expansion, of low-elevation forests, meaning the Huancabamba Deflection (and other Andean passes) would have been effectively 700-1100 m higher than they are today (Liu & Colinvaux, 1985). Such changes would have rendered the passes all but insurmountable to most lowland species. However, cool, moist climates during the Pleistocene would have ameliorated the habitat barrier posed by the Marañón valley to the western dispersal of mesophilic species. This seems to be the most plausible mechanism for the colonization of the Western Slope by eastern taxa (see Haffer, 1967; Czaplewski, 1990).

In an analysis of plants of the Colombian Chocó, Gentry (1982) found little evidence for trans-Andean dispersal by plants. Nearly all of the species in the Río Palenque flora that are found in Amazonia also occur in Panama; only 4% of the Río Palenque flora are Amazonia-Chocó disjuncts. Colonization through lowland habitats at the northern end of the Andes is clearly implicated in these patterns, probably via the Río Atrato and Río San Juan, which mark the axis of the Bolívar geosyncline (see Hershkovitz, 1972; Alberico, 1990a). The southern limits of this northern Pacific biota coincide with major ecological changes. The Andes in Colombia mostly lie within the Equatorial trough; as a consequence, annual precipitation follows a bimodal distribution and the lower slopes (500-1500 m) do not suffer regular moisture deficits. In Southern Ecuador, however, this bimodal precipitation pattern changes to a unimodal one, characterized by a pronounced dry season extending from April to August and associated moisture deficits (Simpson, 1979).

Interestingly, the 35 trans-Andean disjuncts in the Río Palenque flora include a number of dry forest species that may have crossed the Andes through the Marañón valley (Gentry, 1982).

The arid Marañón valley appears to have acted as an east-west filter barrier, preventing the dispersal of forms dependent on mesic forest, but permitting more xeric-adapted taxa to cross (see also Table 3 in Koopman, 1978). These same taxa are well suited for crossing the arid passes of the Huancabamba Deflection. Thus, the arid Marañón valley marks the eastern limits of the Western Slope biota (see also Chapman, 1926; Haffer, 1967).

ALTERNATIVE BIOGEOGRAPHIC HYPOTHESES

Data from *Artibeus* provide strong evidence to reject the notion that this species arose from an Amazonian ancestor during Pleistocene climatic fluxes. Certain other members of this biota are so distinctive that their relationships, if any, to Amazonian taxa are unclear (e.g., *Tomopeas*, *Platalina*). For these species at least, alternate biogeographic scenarios must be considered.

An alternative hypothesis compatible with our data is that this species, and many other elements of the Western Slope biota, originated via Andean orogeny in the Pliocene or early

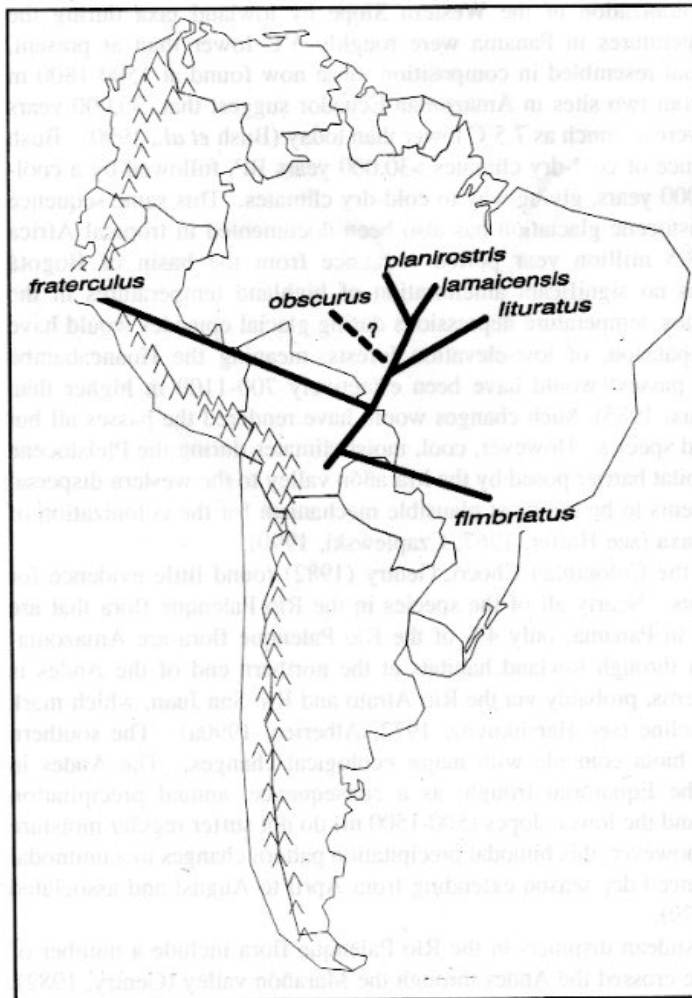


Fig. 5. Phylogenetic relationships among fig-eating bats, genus *Artibeus*, in relation to regions of endemism in South America. Divergence of Amazonian bats appears to be a recent phenomenon, predated by the divergence of *fraterculus* on the Western Slope. According to this analysis, the Atlantic Forest region of Southeastern Brazil is home to the sister-species to all other *Artibeus* analyzed, *A. fimbriatus*. The phylogenetic positions of other regional endemics, especially the llanos endemic *A. amplus* and Middle American *A. hirsutus*, *A. intermedius* and *A. inopinatus*, remain unresolved.

Pleistocene, prior to the divergence of Amazon Basin taxa. According to Simpson (1979), Andean uplift to elevations in excess of 2000 m was not achieved until the last 2-5 million years. Moreover, there is accumulating evidence that much of the Amazon Basin was flooded by a vast freshwater lake during the late Pleistocene (Campbell, 1990). This would have prevented allopatric divergence of terrestrial taxa for much of the middle Pleistocene. Pliocene vicariance of an ancestral form that gave rise to *fraterculus*, and late Pleistocene divergence of *jamaicensis*, *planirostris*, and *lituratus*, agrees well with patterns of divergence documented in this paper. Alberico (1990b) offered a similar vicariant explanation for diversification of bats of the genus *Vampyrops* (= *Platyrrhinus*) in the Chocó of Colombia. Both cases appear to document Haffer's (1967) hypothesis of "pre-Andean" components of the Arid Pacific biota.

Direct corroboration of this hypothesis is difficult, given the paucity of South American bat fossils (Koopman, 1982). However, should fossil *Artibeus* be recovered, *A. fraterculus* is sufficiently distinctive morphologically that its remains could be reliably distinguished. More hopeful is the use of "molecular clocks," which presents certain other analytical problems. Also needed are broader taxonomic surveys (see below).

A second hypothesis is that the Western Slope biota represents a southern derivative of the Middle American biotic province. Koopman (1982) placed the southern limits of this biota in northernmost Peru in Piura, but individual components of this biota range farther south. For example, opossums of the genus *Didelphis* are found as far south as Lima (MUSM specimen from Bosque de Zaraté), and may range into Ica and Arequipa. More highly vagile species range considerably farther south in favorable microhabitats within the Peruvian and Atacama deserts. Hellmayr (1932) recorded several birds in northern Chile (Tacna and Tarapacá) that are typical of Ecuador and northern Peru: *Volatinia jacarina peruviansis*, *Pyrocephalus rubinus obscurus*, *Crotophaga sulcirostris*, and *Zenaida asiatica meloda*. Campbell (1982) postulated late-Pleistocene climates with annual monsoon-type rains in the northern Peruvian desert. Such a climate would have permitted Middle American and western Colombian species to colonize western Peru, whereas modern climates would have fragmented these distributions into remnant pockets situated in the most favorable microhabitats.

To test this distributional hypothesis using *Artibeus*, phylogenetic analyses of the Middle American species (*hirsutus* and *inopinatus*) are needed. The hypothesis would be corroborated if *fraterculus* and one or both of the Middle American taxa formed a distinct clade, sister to Amazonian taxa. It would be refuted if *hirsutus* and *inopinatus* were members of the clade defined here by Amazonian taxa. Phylogenetic studies of other groups of bats, especially the Glossophaginae to determine the systematic position of the Western Slope endemic *Platalina*, would also be useful in evaluating this hypothesis.

ACKNOWLEDGMENTS

Field work for this study was supported by the Eppley Foundation, a grant-in-aid of research from the American Society of Mammalogists (to V.P.), and the Barbara E. Brown Fund for Mammal Research, the Ellen Thorne Smith Bird and Mammal Study Center, and the Continental Coffee Fund of Field Museum of Natural History. M. A. Rogers, R. Arana and S. Fazzolari Corrêa provided indispensable assistance in the field. The generous cooperation and support of Drs. P. E. Vanzolini, H. de Macedo, and A. Sagástegui made fieldwork possible for us. We thank J. L. Patton and B. Stein of the Museum of Vertebrate Zoology for the loan of frozen tissues from Madre de Dios bats, and S. M. Lanyon for making his software available to us. Museum and laboratory portions of the work were supported in part by the National Science

Foundation (BSR-8501595 and BSR-8821834 to BDP; BNS-9008896 to MVA) and the E. T. Smith Fund of Field Museum. We thank C. J. Phillips and A. Williams for their generous help and cooperation in selecting the biochemical characters used in this paper. P. K. Austin deserves much credit for making the most of limited time in the biochemical lab, aided by the expertise of J. Hall. J. Hall and T. S. Schulenberg commented on the manuscript.

LITERATURE CITED

- ALBERICO, M. S. 1990a. A new species of pocket gopher (Rodentia: Geomyidae) from South America and its biogeographic significance, pp. 103-111. In: Peters, G. & R. Hutterer, (Eds.), *Vertebrates in the Tropics: Proceedings of the International Symposium on Vertebrate Biogeography and Systematics in the tropics, Bonn, June 5-8, 1989*. Alexander Koenig Zoological Research Institute and Zoological Museum, Bonn.
- ALBERICO, M. S. 1990b. Systematics and distribution of the genus *Vampyrops* (Chiroptera: Phyllostomidae) in northwestern South America, pp. 345-354. In: Peters, G. & R. Hutterer (Eds.), *Vertebrates in the Tropics: Proceedings of the International Symposium on Vertebrate Biogeography and Systematics in the tropics, Bonn, June 5-8, 1989*. Alexander Koenig Zoological Research Institute and Zoological Museum, Bonn.
- ASHLEY, M. V. & C. WILLS. 1987. Analysis of mitochondrial DNA polymorphisms among Channel Island deer mice. *Evolution* 41: 854-863.
- BAKER, R. H. 1967. Distribution of Recent mammals along the Pacific coastal lowlands of the Western Hemisphere. *Systematic Zoology* 16: 28-37.
- BROWN, J. H. 1984. On the relationship between abundance and distribution of species. *American Naturalist* 124: 255-279.
- BUSH, M. B., P. A. COLINVAUX, M. C. WIEMANN, D. R. PIPERNO, & K.-B. LIU. 1990. Late Pleistocene temperature depression and vegetation change in Ecuadorian Amazonia. *Quaternary Research* 34: 330-345.
- CABRERA, A. 1958. Catálogo de los mamíferos de América del Sur. *Revista del Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," Ciencias Zoológicas* 4(1): 1-307.
- CAMPBELL, K. E., JR. 1982. Late Pleistocene events along the coastal plain of northwestern South America, pp. 423-440. In: Prance, G. T. (Ed.), *Biological Diversification in the Tropics*. Columbia University Press, New York.
- CAMPBELL, K. E., JR. 1990. The geological basis of biogeographic patterns in Amazonia, pp. 33-43. In: Peters, G. & R. Hutterer (Eds.), *Vertebrates in the Tropics: Proceedings of the International Symposium on Vertebrate Biogeography and Systematics in the tropics, Bonn, June 5-8, 1989*. Alexander Koenig Zoological Research Institute and Zoological Museum, Bonn.
- CHAPMAN, F. M. 1926. The distribution of bird-life in Ecuador: a contribution to a study of the origin of Andean bird-life. *Bulletin of the American Museum of Natural History* 55: 1-784.
- CRACRAFT, J. 1985. Historical biogeography and patterns of differentiation within the South American avifauna: areas of endemism, pp. 49-84. In: Buckley, P. A., M. S. Foster, E. S. Morton, R. S. Ridgely, & F. G. Buckley (Eds.), *Neotropical Ornithology. Ornithological Monographs* 36.
- CZAPLEWSKI, N. J. 1990. Late Pleistocene (Lujanian) occurrence of *Tonatia silvicola* in the Talara Tar Seeps, Peru. *Anais Academia Brasileira de Ciencias* 62: 235-238.
- FOODEN, J. & S. M. LANYON. 1989. Blood-protein allele frequencies and phylogenetic relationships in *Macaca*: a review. *American Journal of Primatology* 17: 209-241.

- GENTRY, A. H. 1982. *Phytogeographic patterns as evidence for a Chocó refuge*, pp. 112-136. In: Prance, G. T. (Ed.), *Biological Diversification in the Tropics*. Columbia University Press, New York.
- GRAVES, G. R. 1988. Linearity of geographic range and its possible effect on the population structure of Andean birds. *Auk* 105: 47-52.
- GYLLENSTEN, U. B. & H. A. ERLICH. 1988. Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the HLA-DQA locus. *Proceeding of the National Academy of Science (USA)* 85: 7652-7656.
- HAFFER, J. 1967. Zoogeographic notes on the "nonforest" lowland bird faunas of northwestern South America. *El Hornero* 10(4): 315-333.
- HANDLEY, C. O., JR. 1987. New species of mammals from northern South America: fruit-eating bats, genus *Artibeus* Leach, pp. 163-172. In: Patterson, B. D & R. M. Timm (Eds.), *Studies in Neotropical Mammalogy: Essays in Honor of Philip Hershkovitz*. *Fieldiana (Zoology)* (N.S.) 39.
- HANDLEY, C. O., JR. 1989. The *Artibeus* of Gray 1838. *Advances in Neotropical Mammalogy* 1989: 443-468.
- HANDLEY, C. O., JR. 1991. The identity of *Phyllostoma planirostre* Spix, 1823 (Chiroptera: Stenodermatinae), pp. 12-17. In: Griffiths, T. A. & D. Klingener (Eds.), *Contributions to Mammalogy in Honor of Karl F. Koopman*. *Bulletin of the American Museum of Natural History* 206.
- HELLMAYR, C. E. 1932. The birds of Chile. *Field Museum of Natural History Zoological Series* 19: 1-472.
- HERSHKOVITZ, P. 1972. *The recent mammals of the Neotropical realm: a zoogeographic and ecological review*, pp. 311-431. In: Keast, A., F. C. Erk, & B. Glass (Eds.), *Evolution, Mammals, and Southern Continents*. State University of New York Press, Albany, New York.
- HOOGHTEMSTRA, H. 1989. Quaternary and upper-Pliocene glaciations and forest development in the tropical Andes: evidence from a long high-resolution pollen record from the sedimentary basin of Bogotá, Colombia. *Palaeogeography, Palaeoclimatology, Palaeoecology* 72: 11-26.
- JONES, J. K., JR. & D. C. CARTER. 1976. Annotated checklist, with keys to subfamilies and genera, pp. 7-38. In: Baker, R. J., J. K. Jones, Jr., & D. C. Carter (Eds.), *Biology of Bats of the New World family Phyllostomatidae. Part I. Special Publications, The Museum, Texas Tech University* 10.
- KOOPMAN, K. F. 1978. Zoogeography of Peruvian bats with special emphasis on the role of the Andes. *American Museum Novitates* 2651: 1-33.
- KOOPMAN, K. F. 1982. Biogeography of the bats of South America, pp. 273-302. In: Mares, M. A. & H. H. Genoways (Eds.), *Mammalian Biology in South America. Special Publication, Pymatuning Laboratory of Ecology* 6.
- LAMAS, G. 1982. A preliminary zoogeographical division of Peru, based on butterfly distributions (Lepidoptera, Papilionoidea), pp. 336-357. In: Prance, G. T. (Ed.), *Biological Diversification in the Tropics*. Columbia University Press, New York.
- LIU, K.-B. & P. A. COLINVAUX. 1985. Forest changes in the Amazon Basin during the last glacial maximum. *Nature* 318: 556-557.
- MORITZ, C., T. E. DOWLING, & W. M. BROWN. 1987. Evolution of animal mitochondrial DNA: relevance for population biology and systematics. *Annual Review of Ecology and Systematics* 18: 269-292.

- ORTIZ DE LA PUENTE, J. 1951. Estudio monográfico de los quirópteros de Lima y alrededores. *Publicaciones del Museo de Historia Natural "Javier Prado," U.N.M.S.M. (A)7*: 1-48.
- OWEN, R. D. 1987. Phylogenetic analysis of the bat subfamily Stenodermatinae (Mammalia: Chiroptera). *Special Publications, The Museum, Texas Tech University* 26: 1-65.
- PACHECO, V. & B. D. PATTERSON. 1992. Systematics and biogeographic analyses of four species of *Sturnira* (Chiroptera: Phyllostomidae), with emphasis on Peruvian forms. In: Young, K. R. & N. Valencia (Eds.), *Biogeografía, Ecología y Conservación del Bosque Montano en el Perú. Memorias del Museo de Historia Natural, U.N.M.S.M. (Lima)* 21:57-81.
- PARKER, T. A. III, T. S. SCHULENBERG, G. R. GRAVES, & M. J. BRAUN. 1985. The avifauna of the Huancabamba Region, northern Peru, pp. 169-197. In: Buckley, P. A., M. S. Foster, E. S. Morton, R. S. Ridgely, & F. G. Buckley (Eds.), *Neotropical Ornithology. Ornithological Monographs* 36.
- PATTEN, D. R. 1970. *A review of the large species of Artibeus (Chiroptera: Phyllostomatidae) from western South America*. Ph.D. dissertation, College Station, Texas.
- PATTON, J. L. 1984. Genetical processes in the Galapagos, pp. 77-95. In: Berry, R. J. (Ed.), *Evolution in the Galapagos Islands. Biological Journal of the Linnean Society* 21 (1&2).
- PEARSON, O. 1982. *Distribución de pequeños mamíferos en el altiplano y los desiertos del Perú*. In: Salinas, P. (Ed.), *Zoología Neotropical. Actas del VIII Congreso Latinoamericano de Zoología*, Mérida.
- PIPERNO, D. R., M. B. BUSH, & P. A. COLINVAUX. 1990. Paleoenvironments and human occupation in late-glacial Panama. *Quaternary Research* 33:108-116.
- RUNDEL, P. W., M. O. DILLON, B. PALMA, H. A. MOONEY, S. L. GULMON, & J. R. EHLERINGER. 1991. The phytogeography and ecology of the coastal Atacama and Peruvian deserts. *Aliso* 13: 1-50.
- SANGER, F., S. NICKLON, & A. R. COULSON. 1977. DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* 74: 5463-5467.
- SIMPSON, B. B. 1979. Quaternary biogeography of the high montane regions of South America, pp. 157-188. In: Duellman, W. E. (Ed.), *The South American Herpetofauna: its Origin, Evolution, and Dispersal. Monograph of the Museum of Natural History, University of Kansas* 7.
- SULLIVAN, R. M. In press. Evolutionary genetics and gene flow among populations of coniferous forest mammals in relation to macrohabitat distribution: a test of the dispersal hypothesis. *Evolution*.
- TAYLOR, D. M. 1990. Late Quaternary pollen records from two Ugandan mires: evidence for environmental change in the Rukiga Highlands of southwest Uganda. *Palaeogeography, Palaeoclimatology, Palaeoecology* 80: 283-300.
- TSCHUDI, J. J. VON. 1847. *Travels in Peru During the Years 1838-1842. On the coast, in the Sierra, across the Cordilleras and the Andes into the Primeval Forests*. English translation from the original German by Thomasina Ross, Wiley and Putnam, New York.
- TUTTLE, M. D. 1970. Distribution and zoogeography of Peruvian bats, with comments on natural history. *University of Kansas Science Bulletin* 49(2): 45-86.
- VUILLEUMIER, F. 1984. Zoogeography of Andean birds: two major barriers; and speciation and taxonomy of the *Diglossa carbonaria* superspecies. *National Geographic Society Research Reports* 16: 713-731.
- WRIGHT, S. 1978. *Evolution and the genetics of populations. Vol. 4. Variability within and among natural populations*. University of Chicago Press, Chicago.

APPENDIX

Voucher specimens (either skins, skulls, and skeletons or alcoholic specimens) for specimens used in the genetic analyses are listed below. The following museum acronyms are used: FMNH = Field Museum of Natural History, Chicago; MUSM = Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima; MZUSP = Museu de Zoologia, Universidade de São Paulo; MVZ = Museum of Vertebrate Zoology, University of California, Berkeley.

Artibeus fimbriatus—BRAZIL: São Paulo; Estacao Biológica de Boracéia (MZUSP uncatalogued—SFC 10, SFC 12).

Artibeus fraterculus—PERU: Depto. Cajamarca; Prov. Celendín, Hacienda Limón, 6720' (FMNH 129010, 129017, 129022, 129025, 129038). Prov. Santa Cruz, Rio Zaña, 2 km N Monteseo, 4080' (FMNH 129042, 129061; MUSM 4301, 4328). Depto. Lima, Prov. Huarochiri, San Bartolomé, 1560 m (FMNH 129099, 129105; MUSM 4887) 1600 m (FMNH 128989).

Artibeus jamaicensis—Brazil: Rondônia; Cachoeira Nazaré, W bank Rio Ji-Paraná, 100 m (MZUSP uncatalogued—BDP 2065, BDP 2124, BDP 2127). São Paulo; Ilha do Cardoso, 10 m (MZUSP uncatalogued—BDP 2802, BDP 2807); Ilha do Cardoso, Rio Pereque, 300 m upstream (MZUSP uncatalogued—BDP 2856).

Artibeus lituratus—BRAZIL: Rondônia; Cachoeira Nazaré, W bank Rio Ji-Paraná, 100 m (MZUSP uncatalogued—BDP 2243). São Paulo; Ilha do Cardoso, 10 m (MZUSP uncatalogued—BDP 2828). PERU: Depto. Amazonas, Prov. Luya, Río Utcubamba, 15 km (by road) W of Pedro Ruiz, 3600' (FMNH 129111).

Artibeus jamaicensisobscurus? BRAZIL: Rondônia; Cachoeira Nazaré, W bank Rio Ji-Paraná, 100 m (MZUSP uncatalogued—BDP 2095, BDP 2141).

Artibeus planirostris—PERU: Depto. Madre de Dios, Prov. Manú, Río Alto Madre de Dios opposite Salvación, Hacienda Erika, 350 m (MVZ 166533).