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GENE GENEALOGY AND DIFFERENTIATION AMONG ARBOREAL SPINY RATS (RODENTIA: ECHIMYIDAE) OF THE AMAZON BASIN: A TEST OF THE RIVERINE BARRIER HYPOTHESIS

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Abstract.—Sequence variation in the mitochondrial cytochrome *b* gene was examined in the arboreal spiny rat, *Mesomys hispidus*, collected at 15 sites along the Rio Juruá in western Amazonia, Brazil, to determine the importance of riverine barriers in the diversification of this taxon. Twenty individual haplotypes were uncovered, most of which were unique to single localities but some of which were shared among adjacent sites either along or across the river. Genealogical analyses suggest that gene flow is limited and, in combination with the unique distribution of most haplotypes, suggest that populations of this species are strongly substructured along the river. Thus, most sharing of haplotypes between adjacent localities is probably caused by historical association rather than to ongoing gene flow. Two haplotype clades were uncovered, but these correspond to headwaters versus mouth areas, not to opposite sides of the river, as would be expected by the Riverine Barrier Hypothesis. Moreover, haplotype sharing across the river was greater at its mouth than in the headwaters, a pattern opposite that expected if the river were a substantive barrier. Broader scale phylogeographic patterns of this species show that both clades have relationships to areas well outside the Rio Juruá basin. This suggests that the basin represents a relatively recent point of invasion between two more broadly distributed and differentiated geographic units of the species.

Key words.—Amazon Basin, arboreal spiny rat, cytochrome *b*, *Mesomys*, mitochondrial DNA haplotype, phylogeography, river barriers.

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Several hypotheses have been advanced to explain the high levels of species diversity observed in the lowland tropical forests of the Amazon Basin. Although most recent attention has centered on the Refuge Hypothesis (Haffer 1969; Vanzolini and Williams 1970; see Prance 1982 and Whitmore and Prance 1987 for general reviews) and the alternative Ecological Gradient Hypothesis (Endler 1977, 1982), others have strong empirical support as well as deep historical roots. For example, the Riverine Barrier Hypothesis was first advanced by Alfred Russell Wallace in 1849, when he argued that primate distributions were affected by river barriers and showed that the Basin was divisible into four major geographic areas bounded by the Amazon, Negro, and Madeira rivers. This hypothesis, although not mutually exclusive from others, has received recent attention and support. Ayres (1986) and Ayres and Clutton-Brock (1992) have confirmed Wallace's original observation by documenting the correlation between the degree of primate community similarity on opposite banks of Amazonian rivers and river width, or flow rate. Additionally, Capparella (1987, 1988, 1992)

has shown that the degree of genetic divergence among samples of understory bird species is related to river width.

One explicit expectation of the Riverine Barrier Hypothesis is that increasing divergence should relate positively to river size (width, flow rate, etc.). Hence, differentiation should increase along both sides of a given river, from its headwaters to the mouth, as the barrier widens and the potential for cross-river gene flow diminishes (Hershkovitz 1977). However, the expectation for any given taxon is likely to be complicated by the dynamic nature of floodplain rivers, because populations have the potential for passive transfer from one side to the other by river-bend cutoffs, or oxbow lake formation, through time (see, for example, Hershkovitz 1983). Consequently expectations of the potential force of riverine barriers are likely to vary among taxa that occur in the river floodplain (the seasonally flooded forest, or "várzea" of the Amazon Basin) as opposed to those that are limited to upland, nonflooded forest, or terra firme. The pattern and degree of divergence may also depend on other ecological characteristics, such as the degree of

arboreality, and taxa may respond to the river barrier, including its associated seasonally flooded margins, in different ways.

We have begun a general research program that examines the effectiveness of rivers as barriers for terrestrial and arboreal small mammals in western Amazonia, and in 1991–1992 censused populations along the Rio Juruá in the Brazilian states of Amazonas and Acre (fig. 1). The Rio Juruá is a broad floodplain river, coursing nearly 1000 km from the Peruvian-Brazilian border to the south bank of the Amazon upriver from Manaus. That this river might be an effective barrier for Amazonian taxa is suggested by its identification as a major avian contact zone (Haffer 1974) and as a presumptive zone of rapid environmental transition (Brown 1982; Endler 1982). Two elements of the history of the Rio Juruá are minimally pertinent to the questions we ask: namely, the timing and origin of the current basin and the structural and ecological dynamism of the river channel itself. Although little specific geological or ecological data are available for any part of this river system, its basin is similar to others in western Amazonia in that the present floodplain probably owes its origin to the postglacial Quaternary. Moreover, the forests of the floodplain are continuously subjected to lateral erosion and channel migration, both processes that create a mosaic of successional forests within the present meander plain and offer the opportunity for the passive transfer of faunal elements from one side to the other by channel meanders (see Sioli 1984 for general review, and Salo et al. 1986 for the importance of river dynamics on Amazonian diversity).

We are making comparisons among assemblages of species (in relation to both terra firme and várzea habitats) and at the genetic level for specific taxa of rodents and marsupials at paired sites along the river. For the latter, our approach is to combine sequence variation in the mitochondrial genome with recently developed genealogical, or coalescent, theory specifically designed to predict expected patterns in phylogenies of alleles, or haplotypes (reviewed by Hudson 1990 and Kreitman 1991). These types of analyses permit the explicit counting of historical events, such as episodes of migration (Slatkin 1989; Slatkin and Maddison 1989, 1990). Thus, they allow one to address indirectly the importance of rivers as functional barriers to gene flow and, consequently, as active participants in evolutionary divergence among populations of ro-

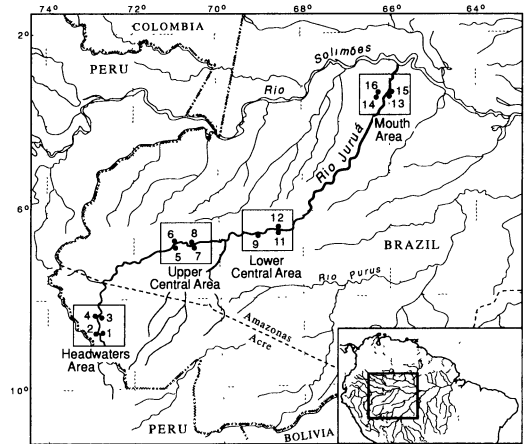


FIG. 1. Fifteen sample sites for the arboreal spiny rat, *Mesomys hispidus*, along the Rio Juruá, Acre, and Amazonas states, in western Brazil. Individual sites are numbered and grouped into regional units, as in the list of localities in the Specimens Examined section.

dent. Our approach is thus different from that used by other authors (e.g., Capparella 1987, 1988, 1992) who have relied on statistical relationships between genetic and geographic distance to assess the strength of riverine barriers within the Amazon Basin.

Here we report results of sequence divergence in the mitochondrial cytochrome *b* gene for the arboreal spiny rat *Mesomys hispidus*, a member of the caviomorph rodent family Echimyidae. We sampled four sets of localities along the entire length of the Rio Juruá from its mouth to the headwaters (see fig. 1). According to the Riverine Barrier Hypothesis, rivers have served actively as barriers to promote differentiation. However, because they may also be merely convenient meeting places of populations that have differentiated elsewhere, we examine the degrees of differentiation along the Rio Juruá for this species in relation to more broadly based patterns that encompass most of its distributional area in the Amazon Basin (see da Silva and Patton 1993).

MATERIALS AND METHODS

Liver tissue was used as a source of DNA. It was either frozen in the field on liquid nitrogen and maintained in the laboratory at - 70°C or preserved in the field in 95% ethyl alcohol and maintained at ambient temperature. Extractions were made using either the sodium dodecyl sulfate-proteinase K/RNase/chloroform-phenol

method (Maniatis et al. 1982) or with sodium chloride (Miller et al. 1988). DNA sequence was obtained for segments of the cytochrome *b* gene (cyt *b*) via the polymerase chain reaction (PCR) using various combinations of primers. The initial 399 base pairs (bp) of light strand sequence (133 codons) were obtained for all individuals collected ($N = 95$) to identify haplotype variation within and among those populations sampled along the Rio Juruá. For each haplotype identified per locality from this initial analysis, the data set was then expanded to 798 bp (266 codons). Sequences of the 798 bp fragment were available for samples of *Mesomys* from localities in Bolivia, Peru, Venezuela, and other sites in Brazil away from the Rio Juruá. The shorter sequence was used to examine patterns of differentiation along the Rio Juruá, and the longer segment was used to infer phylogenetic relationships among all populations throughout the range of the species. Primer sequences, amplification conditions, and sequencing strategy are given in da Silva and Patton (1993).

Sequences were aligned manually and translated using the EyeBall Sequence Editor (ESEE, version 1.09d; Cabot and Beckenbach 1989) on an IBM-compatible 386 computer. Pairwise estimates of percent sequence divergence, the absolute number of transition and transversion differences by codon position, and the proportions of each base at each codon position were performed on the same computer using version 0.01a of the MoSy program written by Christopher A. Meacham. Maximum parsimony analyses using PAUP, version 3.0s (Swofford 1991) and distance-based analyses using PHYLIP, version 3.4 (Felsenstein 1989) were conducted on a Macintosh fx computer. The DNADIST subroutine was used to calculate maximum-likelihood distances, and various subroutines in the PHYLIP package were used to generate Fitch-Margoliash (FITCH), Kitsch (KITSCH), and Neighbor-Joining (NEIGHBOR) trees based on those distances (for a discussion of tree-building methods, see Nei 1991). The maximum-likelihood distances were calculated by assuming a 10:1 transition to transversion ratio (close to that empirically observed) and a 2:1:7 rate variance (at 1st, 2nd, and 3rd positions, respectively) among sites within codons. The DNAML program was also used to evaluate the log-likelihood values for user-supplied tree topologies that specified (1) no gene flow across the river or (2) total panmixia among local sample sites. Log-likelihood evolutions of

the three distance trees and those generated by alternative hypotheses followed the approach of Kishino and Hasegawa (1989), in which a tree topology differs significantly from the best tree if the 95% confidence interval (log-likelihood value ± 1.96 times the standard error) does not include the log-likelihood value of the best tree. Sequence data from *Mesomys cf. stimulax* from the Rio Xingu in Pará state in eastern Amazonia (da Silva and Patton 1993) were used as outgroups in all tree building procedures for the Rio Juruá samples.

Specimens Examined

Numbers prefixed by MNFS, JLP, and JUR are field-catalog numbers of specimens collected during the authors' 1991–1992 expedition to the Rio Juruá. Vouchers for all of these specimens are available and will be catalogued eventually either in the collection at the Instituto Nacional de Pesquisas da Amazônia, Manaus, Brazil; the Museu Paraense Emílio Goeldi, Belém, Brazil; or the Museum of Vertebrate Zoology, University of California, Berkeley. The initial 399 bases of the light strand are available from the authors for all specimens examined; an asterisk identifies those specimens for which a total of 798 bases are also available (GenBank accession numbers L23365 to L23398). Localities are identified by number, as in the map, figure 1. Provenience data for other samples included in the parsimony analysis are given in da Silva and Patton (1993).

Mesomys hispidus.—Rio Juruá ($N = 95$)—BRAZIL: Acre; HEADWATERS AREA (1) Porongaba, right bank Rio Juruá, ($N = 5$ —MNFS 1230*, 1231*, 1294, 1363*, 1416). (2) Porongaba, left bank Rio Juruá, ($N = 3$ —MNFS 1257*, 1258, 1353*). (3) Nova Vida, right bank Rio Juruá, ($N = 9$ —MNFS 1519*, 1531, 1539, 1596, 1615, 1636, 1658, JUR 221, 249). (4) Sobral, left bank Rio Juruá, ($N = 1$ —MNFS 1573*). Amazonas; UPPER CENTRAL AREA (5) Sacado, right bank Rio Juruá ($N = 8$ —MNFS 568*, 569, 583, 592, 593, 612, 637, 654). (6) Condor, left bank Rio Juruá ($N = 7$ —MNFS 564*, JLP 15624, 15651*, 15678, 15701, 15708, 15725*). (7) Penedo, right bank Rio Juruá ($N = 6$ —MNFS 436*, 470, JLP 15367, 15424, 15465, 15501). (8) Nova Empresa, left bank Rio Juruá ($N = 8$ —MNFS 432*, 464, 485, 517, JLP 15385, 15431, 15444, 15445). LOWER CENTRAL AREA (9) Altamira, right bank Rio Juruá ($N = 8$ —MNFS 909*, 910, 911, JLP 16047, 16048, 16049, 16066, JUR 194). (11) Jainu, right bank Rio Juruá ($N = 1$ —

TABLE 1. Sequence variation within and among populations of *Mesomys hispidus* from the Rio Juruá in western Brazil, based on 399 base pairs of the mitochondrial cytochrome *b* gene. Haplotype diversity (*h*) is from Nei (1987). Population samples are identified as in the Specimens-Examined section and as plotted on the map, figure 1; right (R) or left (L) bank localities along the river are indicated, as is their respective habitat types (V, várzea or seasonally flooded forest; TF, terra firme, or nonflooded, upland forest).

Sample	Bank	Habitat	<i>N</i> ₁ *	<i>N</i> _H †	Mean divergence (%)	Maximum divergence (%)	<i>h</i> ‡
Headwaters							
Porongaba (1)	R	TF	5	3	1.22	1.90	0.800
Porongaba (2)	L	V	3	2	0.01	0.01	0.663
Nova Vida (3)	R	V	9	2	1.40	1.40	0.220
Sobral (4)	L	TF	1	1	—	—	—
Upper central							
Sacado (5)	R	V	8	1	—	—	—
Condor (6)	L	TF	7	3	0.72	1.10	0.714
Penedo (7)	R	TF	6	1	—	—	—
Nova Empresa (8)	L	V	8	1	—	—	—
Lower central							
Altamira (9)	R	TF	8	1	—	—	—
Jainu (11)	R	V	1	1	—	—	—
Barro Vermelho (12)	L	TF	4	3	3.55	6.50	0.833
Mouth							
Ipaxiuba (13)	R	V	4	2	1.40	1.40	0.500
Vira-Volta (14)	L	TF	14	5	1.08	1.90	0.780
Vai-Quem-Quer (15)	R	TF	12	3	1.40	1.40	0.318
Ilhazinha (16)	L	TF	5	3	0.56	1.40	0.700

* Number of individuals sampled.

† Number of haplotypes per sample.

‡ $h = (1 - \sum x_i^2) / n - 1$, where x_i is the frequency of the *i*th mitochondrial DNA type.

MNFS 754). (12) Barro Vermelho, left bank Rio Juruá (*N* = 4—MNFS 729, 745, JLP 15852, 15853). MOUTH AREA (13) Ilha Paxiuba, right bank Rio Juruá (*N* = 4—JUR 291, 325, 337, 369). (14) Vira-Volta, left bank Rio Juruá (*N* = 14—MNFS 1739, JUR 416, 453*, 457, 461, 462, 471, 488, 498, 499, 503, 533, 540, 541). (15) Vai-Quem-Quer, right bank Rio Juruá (*N* = 12—JUR 268, 269, 284, 285, 321, 322, 332, 333, 334, 335, 370, 398). (16) Ilhazinha, left bank Rio Juruá (*N* = 5—MNFS 1784, JUR 414, 415, 459*, 500).

RESULTS AND DISCUSSION

Haplotype Diversity

Twenty cytochrome *b* haplotypes were detected within the total sample of 95 individual *Mesomys hispidus* collected at the 15 sites along the Rio Juruá, and for which 399 base pairs (bp) of sequence were available. The number of haplotypes and sample sizes per locality, the level of sequence divergence among haplotypes cooccurring at each site, and a measure of haplotype diversity are given in table 1. Average amounts

of sequence divergence among sites within each of the four geographic sample areas, and among those areas, are given in table 2. In general, localities at the headwaters in Acre state (localities 1–4) and at the river’s mouth (localities 13–16) exhibit both substantial haplotype diversity and sequence divergence within local populations. In both areas, more than one haplotype was present at each locality for which sample size was greater than one. For example, five haplotypes are present at locality 14 at the mouth (*h* = 0.780); these differ by a maximum of 1.9%. In the headwaters, three haplotypes were found among five individuals at locality 1 (*h* = 0.800), which also differ by a maximum of 1.9%. In contrast, only at two of the seven localities in the mid stretches of the river (localities 5–12) did we recover more than one haplotype despite generally equivalent sample sizes. The greatest degree of haplotype diversity and differentiation within a given locality was found at one of these two mid-river areas, Barro Vermelho (locality 12, *h* = 0.833), where three haplotypes that differed by a maximum of 6.5% were uncovered among the four individuals sampled (table 1).

TABLE 2. Average percent sequence difference among haplotypes of the arboreal spiny rat, *Mesomys hispidus*. Data are given as the average in comparisons between sites at each regional sampling area and among those areas, corrected for within-area variation.

Comparison	Mean divergence (%)
Within area	
Headwaters	1.57
Upper central	0.83
Lower central	2.68
Mouth	0.97
Among areas	
Headwaters-upper central	1.63
Headwaters-lower central	5.05
Headwaters-mouth	6.42
Upper-lower central	5.17
Upper central-mouth	6.79
Lower central-mouth	2.06

Average sequence differentiation among sites within each of the four regional sampling areas was generally low to moderate, with a maximum of 2.68% within the lower central area that includes the Barro Vermelho site (table 2). Other than this exception, the degree of differentiation between any pair of sites within regional areas averaged less than that among the areas themselves [a range of 0.83%–1.57% within areas (excluding Barro Vermelho) as opposed to a range of 1.63%–6.79% among them].

Haplotype Sharing along and across the River

The Riverine Barrier Hypothesis requires that differentiation among localities be distributed predominantly across as opposed to along the banks of a given river for any series of sampling points. Of the 20 *cyt b* haplotypes identified here for *M. hispidus*, 12 are unique to given localities, and thus exhibit no pattern of sharing either across or along the river (table 3). However, four haplotypes are found at two or more adjacent localities on the same side of the river, one is shared across the river at sites on opposite banks, and three are found at sites that are adjacent both along and across the river (table 3). Figure 2 illustrates the haplotype distributions at the four sites at the mouth of the Rio Juruá, where sample sizes and haplotype diversity are moderately high, and where expectations for sharing across the river are the least under the Riverine Hypothesis. As is readily apparent, haplotypes are shared between adjacent localities, not surprisingly so for

those on the same side of the river, as these are only a kilometer or less apart. But, haplotypes are also shared between localities on opposite sides as well. For example, each haplotype present on the right bank is also present on the left, even though these localities are more than 25 km apart by airline distance and are separated not only by the river's width but also by an extensive area of várzea on both banks.

The pattern depicted among the mouth sites is the most extreme degree of haplotype sharing for adjacent localities. In the central portion of the river (localities 5–12), three of the seven haplotypes uncovered are shared along the river and one is shared across, the remainder are unique to single sites (table 3). Surprisingly, all eight haplotypes found in the four headwaters sites (localities 1–4) are unique to each single site. Consequently, by simply matching haplotype patterns, there is substantial sharing of the same haplotype among localities on both sides of the river at the mouth of the Rio Juruá but none in the headwaters. This is opposite to the expectation of the Riverine Barrier Hypothesis, where divergence should be less in the headwaters and increase progressively towards the mouth.

Cladistic Relationships among Haplotypes and Localities

The distributional pattern of single haplotypes, however, is only one means to detect the potential effects of riverine barriers on divergence. A second is to examine the genealogical pattern of relationship among the haplotypes themselves. For example, whereas all haplotypes in the river's headwaters are unique to each site, their genealogy may reflect a history indicative of either across-river gene flow or across-river barrier.

Figure 3 illustrates tree topologies reflecting haplotype genealogies based on three different distance methods: Fitch-Margoliash (A), Kitch (B), and Neighbor-Joining (C). The locality (or localities) and side of the river (right and/or left banks) are indicated for the terminal twigs (that is, individual haplotypes) of each tree. Two, and the same two, clades of haplotypes and localities are identified in each tree (and in parsimony analyses as well, see below and da Silva and Patton 1993). Each of these has geographic continuity, with one defining a group of localities in the headwaters and upper central portion of the river (localities 1–8) and the other grouping those from the lower central portion and mouth of the

TABLE 3. Pattern of geographic distribution of individual cytochrome *b* haplotypes of *Mesomys hispidus* along the Rio Juruá.

Haplotype	Area*	Unique	Shared localities	
			Same side	Opposite side
1	Mouth		X	X
2	Mouth		X	X
3	Mouth		X	X
4	Mouth		X	
5	Mouth	X		
6	Lower central	X		
7	Lower central	X		
8	Lower central		X	
9	Lower and upper central		X	
10	Upper central	X		
11	Upper central		X	
12	Upper central			X
13	Headwaters	X		
14	Headwaters	X		
15	Headwaters	X		
16	Headwaters	X		
17	Headwaters	X		
18	Headwaters	X		
19	Headwaters	X		
20	Headwaters	X		

* The Mouth sites are localities 12–15, lower central are localities 9–11, upper central are localities 5–8, and headwaters sites are localities 1–4 (see map, fig. 1, and Specimens Examined).

river (localities 9–16). The two clades overlap at one site in the central Rio Juruá at Barro Vermelho (locality 12). Of the four individuals examined from this locality, three belong to the headwaters clade and one to that from the mouth, a mixing of clades that results in the high degree of sequence divergence found at this locality (see table 1), and the relatively high level of divergence between it and those immediately adjacent in the same geographic area (table 2).

Multiple haplotypes from single localities are genealogically adjacent, suggesting a monophyletic origin, in only two instances: the pairs of haplotypes at both locality 2 (haplotypes 16 and 17) and locality 3 (haplotypes 19 and 20) are each phylogenetically linked in all three trees. In all other cases, haplotypes from single localities do not form monophyletic units. For example, the three haplotypes (13–15) from locality 1 are distributed among three separate subclades within the headwaters unit, and in no case are those haplotypes monophyletic at any single locality at the mouth of the Rio Juruá.

The phylogenetic sharing of haplotype lineages among localities, as with the interpopulation sharing of individual haplotypes (see above), suggests that there is, or has been, some genetic contact among localities along the Rio Juruá, at least within the context of each major clade and among

those sites that make up each of the four sample areas (fig. 1). However, the generally low haplotype diversity, especially among those localities in the middle part of the river where nearly every site has a single, unique haplotype, suggests that the species is compartmentalized into sub-

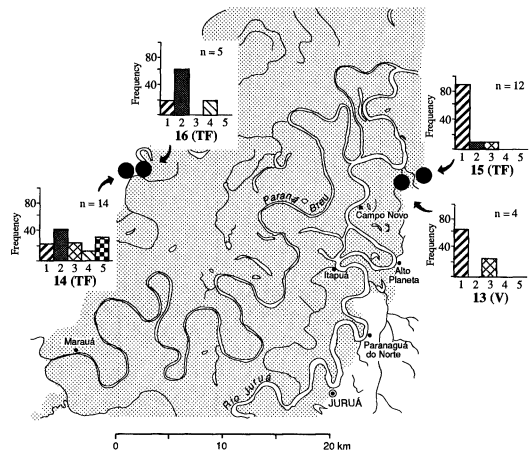


FIG. 2. Details of the four sample sites at the mouth of the Rio Juruá, western Brazil, with frequency histograms of mitochondrial DNA (mtDNA) cytochrome *b* haplotypes at each site indicated. The stippled area on both sides of the river illustrates the extent of the várzea or seasonally flooded forest. The map is based on photographic images from the 1978 Projeto RADAMBRASIL, 1:250,000 series.

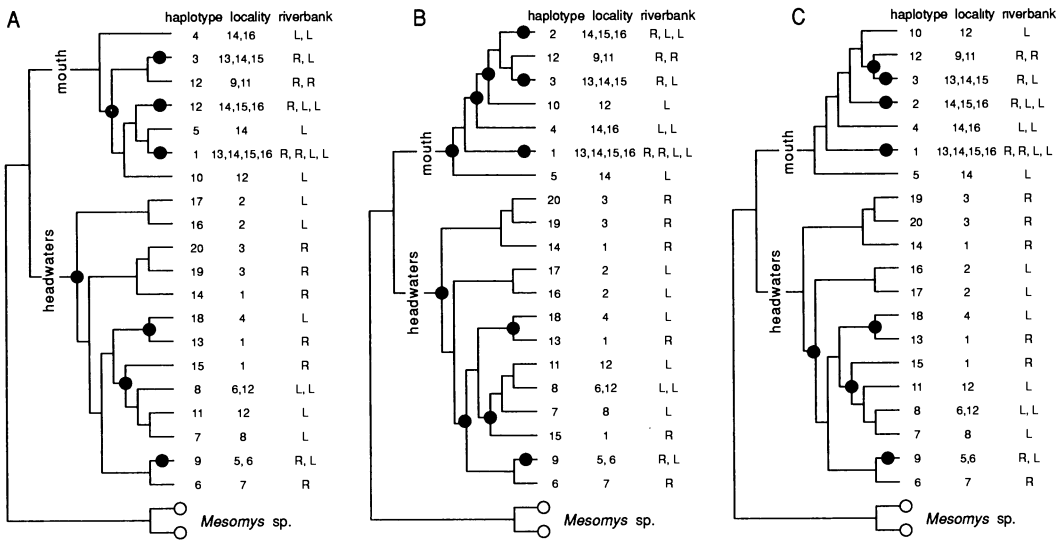


FIG. 3. Three phylogenetic trees based on maximum-likelihood divergence estimates between the 20 *cyt b* haplotypes recovered among 95 individual *Mesomys hispidus* from 15 localities along the Rio Juruá, western Brazil. In each case, the locality (or localities) at which a given haplotype was found, and the river bank [right (R) or left (L)] of each locality, are indicated. Trees were rooted using sequences from *Mesomys cf. stimulax* from the Rio Xingu in Pará state, eastern Brazil (see da Silva and Patton 1993). The number of inferred between-population coalescent events, s , is indicated for each tree by large solid circles. The trees are not drawn proportional to the amount of sequence divergence.

divided local units along the river (see Tajima 1990). Complete local compartmentalization would be expected if all within-locality haplotypes were monophyletic (Neigel and Avise 1986; Slatkin 1989).

Gene Flow or the Retention of Mitochondrial Lineages?

The patterns of paraphyly of haplotypes at the population level seen in each tree in figure 3 could be caused either by ongoing gene flow (Slatkin and Maddison 1989, 1990) or by historical association, the persistence (incomplete "sorting") of ancestral alleles in contemporary populations (Takahata 1989). As a means to distinguish between these two possibilities, the number of between-population points of common ancestry (coalescent events, s) were determined for each tree in figure 3 using a parsimony reconstruction of geographic states as described in Slatkin and Maddison (1989). Because the assignment of coalescent events to specific branches of a tree can be ambiguous, s values were determined by both working down the tree from the terminal tips and up from the basal nodes. The two haplotype clades were analyzed separately, because of their apparent evolutionary in-

dependence, with total s values being sums for both clades. Values of s calculated in this fashion correspond to the number of potential historical migration events.

The number of s events was eight for both the Fitch and NJ trees and 11 for the Kitsch tree (mean = 9, fig. 3). Four of these events occurred in all three trees and are attributable to the sharing of the same haplotype at sites on opposite banks; these can be taken as irrefutable evidence of movement (or persistence) of genes across the river, unless one allows for convergent evolution of the same mitochondrial DNA (mtDNA) sequence. The remainder were genealogical coalescent events.

The few individuals sampled and the relatively low haplotype diversity per sample site hampers the utility of the genealogical coalescence approach. However, interpolation from table 1 of Slatkin and Maddison (1989, p. 609), rescaled by the number of populations examined, provides estimates of Nm , the product of the effective population size, and migration rate per generation, of 0.15 for the entire data set and a range of from 0.10 to 0.25 if the two major clades are analyzed separately. A similar analysis applied only to the four sites at the river's mouth yielded

an Nm of only 0.4, despite the clear evidence of haplotype sharing across the river. Consequently estimates of current gene flow rates in *M. hispidus* along the Rio Juruá are quite low, supporting other evidence that each clade is well substructured geographically and suggesting that most genealogical sharing of haplotype lineages is probably caused by historical retention. Although no data exist on the population demography of these animals, the rather small estimates of Nm are expected. Arboreal spiny rats are relatively small bodied rodents (about 200 g, on average) whose movements must be somewhat localized, and our samples are limited to but four sets of points distributed along more than 900 km of river.

Is There a Riverine Barrier?

Although our data suggest that gene flow rates in *M. hispidus* within the Rio Juruá drainage are relatively low, this applies equally to gene flow along each bank of the river as well as across it. Consequently, these data do not address the Riverine Barrier Hypothesis directly; they suggest only that gene flow rates are sufficiently low such that the river could be an effective barrier. The best single piece of evidence that it does not represent a strong barrier lies in the haplotype distribution across sites at the mouth of the river (fig. 2).

To further examine this issue, we performed log-likelihood tests (Kishino and Hasegawa 1989) for alternative tree topologies, in which user-defined trees were compared with those generated by the phylogenetic distance analyses (fig. 3). Two user-defined topologies were produced under different historical models. In the first case, a tree was designed that allowed for gene flow along the river, between adjacently placed localities, but where the haplotypes present on a single side were forced to be monophyletic. This case would represent that of the most severe expectation of a riverine barrier, in which no gene flow across the river has occurred subsequent to founding. The second tree was one that permitted regional panmixia, with haplotypes both along and across the river at each of the four sample stations constrained to be monophyletic relative to those at the other three stations.

The Fitch (log likelihood = -1272.7 ± 8.04 95% confidence limits), Kitch (ln L = -1279.3 ± 16.18), and NJ (ln L = -1279.3 ± 16.18) topologies were not significantly different from each other. However, all of these trees were sig-

nificantly better than the two hypothesis trees (along river ln L = -1538.3 ± 76.56 , and across river ln L = -1442.5 ± 55.15). The same results are obtained if the headwaters and mouth clades are examined separately in log-likelihood analyses. Clearly, the divergence pattern observed among all haplotypes is neither one of complete panmixia at each sampling point across the river nor complete separation of samples on opposite banks. Rather, it is somewhat intermediate between these two extremes.

Thus, even when analyses are restricted to each haplotype clade, there is little evidence that the Rio Juruá has provided a significant barrier in the differentiation of these arboreal spiny rats. However, neither is there substantial support for the significance of present-day gene flow across the river. The low gene flow estimates, haplotype distribution, and diversity levels suggest that populations of this species are strongly structured geographically, as strongly so along the river as across it.

Differentiation of M. hispidus in the Amazon Basin

The Rio Juruá basin represents one of the four zones of hybridization identified by Haffer (1974) for neotropical birds, as well as a region of rapid environmental transition (Brown 1982; Endler 1982), suggesting that it may represent a generalized barrier to nonvolant mammals as well. However, this clearly does not appear to be the case, at least for the arboreal echimyid rodent *M. hispidus*. Populations of this species are aligned into headwater and mouth clades of mtDNA haplotypes, rather than ones whose distribution coincides with opposite banks of the river, as would be expected if the river were a substantive barrier along its length. Gene flow rates, moreover, between populations both along and across the river appear sufficiently low such that populations are well structured genetically. Certainly, no evidence exists to suggest that gene flow is less intense across the river than along it, such that divergence patterns are unlikely to reflect only a right bank–left bank division, even along short segments of the river.

The division of haplotypes along the Rio Juruá into two major clades, and thus the pattern of haplotype distribution along the entire river, must be viewed in the context of more global geographic patterns of haplotype phylogeny for the species as a whole. Figure 4 illustrates this pattern, based on analyses of 798 bp of sequence

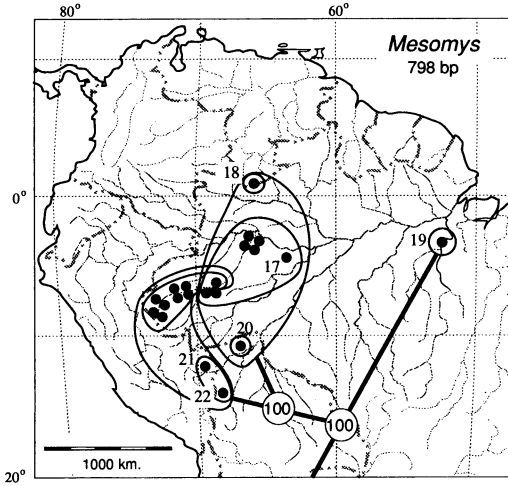


FIG. 4. Gene-geography of *Mesomys hispidus* samples from throughout the western Amazon Basin. The mitochondrial DNA *cyt b* clades identified are linked based on the strict consensus tree from a maximum-parsimony analysis of 798 base pair of sequence, using a heuristic search in PAUP, with *Mesomys cf. stimulax* from the Rio Xingu as an outgroup. Circled numbers at the two nodes are bootstrap values in an analysis of 100 bootstrap runs, with replacement. Tree length = 100 steps; consistency index = 0.810. Localities along the Rio Juruá are not numbered (but see fig. 1); those extralimital to the Rio Juruá are numbered as follows: 17, Rio Urucu, Amazonas, Brazil; 18, Serra Neblina, Amazonas, Venezuela; 19, Rio Xingu, Pará, Brazil; 20, San Juan de Nuevo Mundo, Pando, Bolivia; 21, Cusco Amazonico, Madre de Dios, Perú; and 22, Rio Madidi, La Paz, Bolivia. See da Silva and Patton (1993) for further details.

given in da Silva and Patton (1993, and unpubl. data). The headwaters and mouth clades are sister units in the broader picture of population relationship throughout Amazonia. However, haplotypes in the headwaters region of the Rio Juruá associate closely with samples in southeastern Peru and northwestern Bolivia, and the mouth clade associates with haplotypes to the east (Rio Urucu in central Brazil), north (Sierra Neblina in southern Venezuela), and south (Pando department, Bolivia). Clearly, both haplotype clades present along the Rio Juruá have more broadly based geographic relationships to units beyond the river basin, and these broader geographic patterns must be known before a complete understanding of the evolutionary diversification of this species within the Rio Juruá can be achieved.

It is tempting to suggest that the two clades within the Rio Juruá each owe their origin to

areas outside of the river basin and have entered the present basin from opposite directions in the relatively recent past. Under this scenario, the headwaters clade invaded down the river, perhaps from areas in southeastern Peru and northern Bolivia; the mouth clade entered the river at its mouth, spreading upwards until it encountered the other unit at Barro Vermelho (locality 12). Minimal support for this scenario is the general loss of haplotype diversity within both clades at sample sites along the central portion of the river, as well as their geographic relationships to areas outside of the Juruá basin. A pattern of decreased haplotype diversity would be expected in a wave-front migration pattern extending from a more ancient "home" region. In any event, the distribution pattern of the two haplotype clades within the basin, and their relationships to others outside of it offer no support that the Rio Juruá has been a substantial barrier in the divergence patterns present within the species. It remains to be seen if the Rio Juruá has been an effective barrier in other small mammals.

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