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Hybridization in Large-Bodied New World Primates

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ABSTRACT

Well-documented cases of natural hybridization among primates are not common. In New World primates, natural hybridization has been reported only for small-bodied species, but no genotypic data have ever been gathered that confirm these reports. Here we present genetic evidence of hybridization of two large-bodied species of neotropical primates that diverged ~3 MYA. We used species-diagnostic mitochondrial and microsatellite loci and the Y chromosome *Sry* gene to determine the hybrid status of 36 individuals collected from an area of sympatry in Tabasco, Mexico. Thirteen individuals were hybrids. We show that hybridization and subsequent backcrosses are directionally biased and that the only likely cross between parental species produces fertile hybrid females, but fails to produce viable or fertile males. This system can be used as a model to study gene interchange between primate species that have not achieved complete reproductive isolation.

HYBRIDIZATION can be viewed as either a breakdown of species boundaries that could eventually result in the loss of pure parental species or a creative force that can lead to the formation of new recombinant lineages (Arnold 1997; Dowling and Secor 1997; Barton 2001; Mallet 2005; Arnold and Meyer 2006). Regardless of which view is taken, studies of hybridization are crucial for understanding the basis of reproductive isolation and the origins of biodiversity (Coyne and Orr 2004). Hybridization among metazoans has traditionally been viewed as an unusual event, but a variety of genetic studies in the past few decades have shown that this phenomenon is rather common, especially between closely related taxa (Mallet 2005). Among primates, natural hybridization occurs in at least 26 of ~233 Old World species (*e.g.*, baboons, guenons, macaques, lemurs) in which hybridization occurs at intraspecific (Groves 1978; Lernould 1988), interspecific (Phillipsconroy and Jolly 1986; Samuels and Altmann 1986; Struhsaker *et al.* 1988; Watanabe and Matsumura 1991; Bynum *et al.* 1997; Evans *et al.* 2001; Wyner *et al.* 2002), and even intergeneric levels (Dunbar and Dunbar 1974; Jolly *et al.* 1997). Among neotropical primates, only 8 of ~132 New World species have been suggested to form hybrids in the wild (Coimbra-Filho *et al.* 1993; Peres *et al.* 1996; Mendes 1997) and these include only small-bodied and very recently separated taxa. Furthermore, of the few reported cases of interspecific hybridization in the wild (Silva *et al.* 1993; Mendes 1997), the taxonomic status of the species is questionable.

Here we present evidence of hybridization of two large-bodied neotropical primates, *Alouatta palliata* and *A. pigra*. These are morphologically (Lawrence 1933; Smith 1970), socially (Crockett and Eisenberg 1987; Treves 2001; Van Belle and Estrada 2006), behaviorally (Crockett and Eisenberg 1987; Neville *et al.* 1988; Whitehead 1995), and genetically (Cortés-Ortiz *et al.* 2003) distinct howler monkey species that diverged ~3 MYA (Cortés-Ortiz *et al.* 2003). *A. palliata* is currently found from southern Veracruz in Mexico through the central part of Guatemala and the southern part of Belize, continuing to the south through Honduras, Nicaragua, Costa Rica, Panama, and the Pacific coast of Colombia and Ecuador. On the other hand, *A. pigra* is confined to the Yucatan peninsula in Mexico, Belize, and the central and eastern part of Guatemala (Figure 1). Although *A. palliata* and *A. pigra* are allopatric in most of their range, Smith (1970) reported an area of sympatry in the state of Tabasco, Mexico. Currently this area is highly deforested and only small patches of vegetation with various degrees of disturbance can be found. During a series of expeditions, we surveyed the area where sympatry had been reported and found troops with individuals of both *A. palliata* and *A. pigra* (based initially on morphological

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characters), as well as individuals that possessed morphological features of both species. Using a multilocus approach, we present genetic data that show that these howler monkeys are hybridizing in Mexico.

MATERIALS AND METHODS

Blood and/or hair samples were collected from *A. palliata* and *A. pigra* individuals from sites in Tabasco, Mexico and other areas throughout Mexico (Figure 1). Genomic DNA was extracted using the DNeasy tissue kit (QIAGEN, Valencia, CA). Primers for eight microsatellite loci [Ap68 (Ellsworth and Hoelzer 1998), Ap74 (Ellsworth and Hoelzer 1998), PEPC8 (Escobar-Páramo 2000), and MapPairs (Invitrogen, Carlsbad, CA) loci D5S111, D6S260, D8S165, D14S51, and D17S804] were used to identify diagnostic alleles in each species and to identify hybrid individuals on the basis of the presence of these alleles. We used primers CB1-5′ and CB2-3′ (Palumbi 1996) to amplify a region of the mitochondrial *cytochrome b* (*cytb*) gene and/or primers LCO-CO2-L and LCO-CO3-H (Cortés-Ortiz *et al.* 2003) to amplify a fragment of the *ATP-synthase 6* and *8* genes (*ATPase*). A fragment of the Y chromosome *Sry* gene was amplified using primers SW2 (Whitfield *et al.* 1993) and SRY (Moreira 2002). To determine whether hybridization and subsequent crosses are directionally biased, we used a chi-square goodness-of-fit test to compare the observed frequencies of genotypes of hybrid individuals to those expected if all possible crosses among hybrids and backcrosses with parental species occur. We also estimated the probabilities of observing the detected genotypes on the basis of equal proportions of alleles/haplotypes in the parental species.

RESULTS AND DISCUSSION

We genotyped 104 individuals of *A. palliata* and *A. pigra* and putative hybrid individuals for the eight microsatellite loci. These individuals include 40 *A. palliata* and 28 *A. pigra* individuals from outside of the putative hybrid zone and 36 individuals from within this zone (Figure 1). On the basis of the genotypes of *A. palliata* and *A. pigra* outside of the zone, three loci contained alleles that were distinct for each species (Ap68, D5S111, and D8S185) (Table 1). Sequences of these alleles confirmed that size differences are due to differences in the number of repeat units. The two species shared alleles at the other loci examined or potentially diagnostic alleles occurred at low frequencies in one or the other species. Several alleles showed clines in allele frequencies through the hybrid zone. We also sequenced a 307-bp region of the mitochondrial *cytb* gene and/or an 817-bp fragment of the *ATPase* locus from the same 104 individuals listed above (GenBank accession nos. DQ875685–DQ875741 and DQ875611–DQ875672, respectively). Sequences from the two parental species have fixed differences at 14 sites for the *cytb* fragment and 46 sites for the *ATPase* fragment, and each locus showed ~5% sequence divergence among species. On the basis of these levels of sequence divergence, *A. palliata* and *A. pigra* likely separated ~3 MYA (Cortés-Ortiz *et al.* 2003).

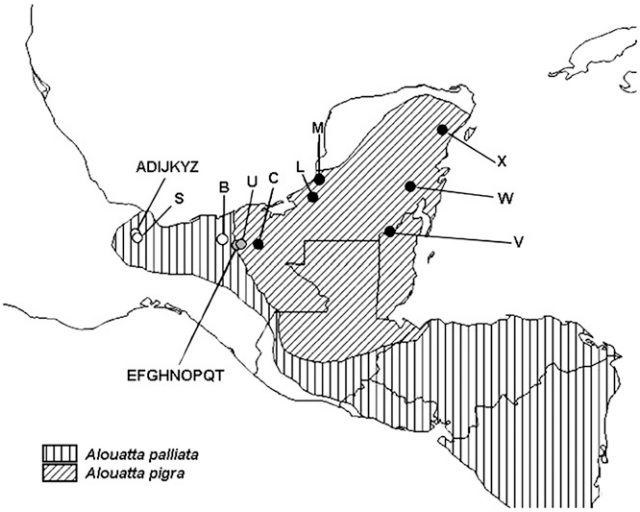

Figure 1.—Geographic distribution of *A. palliata* and *A. pigra*, and approximate location of troops sampled. Letters represent different troops. Localities: open circles contain *A. palliata* individuals, solid circles contain *A. pigra* individuals, and shaded circles contain individuals that have been genetically characterized as hybrids/backcrosses.

In total, 23 individuals from the putative hybrid zone wholly possessed alleles of either *A. palliata* ($n = 11$) or *A. pigra* ($n = 12$) and contained the respective species' mitochondrial haplotype; this suggests that individuals of both parental species are nearly equally abundant within the hybrid zone. Thirteen other individuals were identified as hybrids on the basis of mitochondrial and microsatellite data (Table 2). The hybrid individuals included seven adult females, one infant female, and five adult males. Twelve individuals possessed microsatellite alleles, diagnostic of both parental species, although no individuals were F_1 hybrids (Table 2). The lack of F_1's may be because these individuals are ephemeral, or the hybrid zone is old, or it could reflect a low incidence of hybridization of pure parental forms (see Goodman *et al.* 1999). All adult hybrids contained the mitochondrial haplotype of *A. pigra*. The infant was the only hybrid that possessed *A. palliata*'s haplotype. The presumed mother of this infant (based on genotypic data and the fact that the female was carrying the infant) was pure *A. palliata* based on the genetic markers used here and her appearance and occurred with a hybrid male that was likely the father of this infant based on the genotypic evidence. Hybrid individuals occurred in fragmented habitats where the two species' distributions overlap and were members of "mixed troops" that contained individuals of both parental species and in some cases individuals with unique or intermediate morphologies (Figure 2).

We attempted amplifications of a region of the *Sry* gene with genomic DNA of 4 *A. palliata* males, 2 *A. palliata* females, 3 *A. pigra* males, and 2 *A. pigra* females from outside of the putative hybrid zone and all 13

TABLE 1

Frequencies of alleles of microsatellite loci of populations of *A. palliata* and *A. pigra* from outside and within the putative hybrid zone (including hybrids)

Locus	Allele size[a]	*Apa*O[b]	*Apa*HZ[c]	*Api*HZ[d]	*Api*O[e]
Ap68	187	—	—	0.11	0.17
	191	—	—	0.64	0.41
	193	*0.99*	*1.00*	*0.20*	—
	195	0.01	—	—	—
	197	—	—	0.05	0.43
Ap74	150	—	—	0.50	0.68
	152	0.99	1.00	0.36	0.25
	154	—	—	0.14	0.07
	156	0.01	—	—	—
D5S111	*163*	*1.00*	*1.00*	*0.11*	—
	167	—	—	0.02	0.13
	169	—	—	0.48	0.27
	174	—	—	—	0.02
	178	—	—	—	0.04
	180	—	—	0.39	0.55
D6S260	171	—	—	—	0.06
	173	—	0.25	0.02	—
	177	0.53	0.75	0.07	0.02
	179	0.44	—	—	0.02
	181	0.04	—	0.18	0.27
	183	—	—	0.07	0.08
	185	—	—	0.16	0.06
	187	—	—	0.50	0.50
D8S165	*119*	—	*0.07*	*0.91*	*1.00*
	143	*1.00*	*0.93*	*0.09*	—
D14S51	143	0.03	0.04	0.50	0.48
	145	0.03	—	0.05	—
	147	0.95	0.96	0.45	0.52
D17S804	157	—	—	—	0.15
	161	1.00	1.00	0.89	0.61
	163	—	—	—	0.04
	165	—	—	0.05	0.07
	167	—	—	0.07	0.06
	169	—	—	—	0.07
PEPC8	239	—	—	0.26	0.43
	244	—	—	0.11	—
	246	—	—	0.05	0.04
	248	1.00	1.00	0.58	0.54

[a] Allele sizes are the sizes of the complete sequence of the microsatellite alleles and include both repeat and flanking regions. Diagnostic alleles are shown in italics.
[b] *ApaO*, *A. palliata* from outside the putative hybrid zone.
[c] *ApaHZ*, *A. palliata* from within the putative hybrid zone.
[d] *ApiO*, *A. pigra* from outside the putative hybrid zone.
[e] *ApiHZ*, *A. pigra* from within the putative hybrid zone.

individuals from within the hybrid zone that were characterized as hybrids. Amplifications were successful only with genomic extractions of males; this and the fact that direct sequencing of amplification products yielded chromatograms without double peaks or other ambiguities strongly imply that the gene amplified occurs on the Y chromosome in these individuals. The sequences obtained from individuals outside of the hybrid zone were ~821 bp in length and showed fixed

TABLE 2

Hybrid individuals in the area of species overlap that showed mixed *A. palliata* and *A. pigra* character states

				Microsatellite locus[e]			
ID[a]	Sex[b]	Phenotype[c]	mtDNA[d]	Ap68	D5S111	D8S165	*Sry*[f]
S096	F	*Apa*	i	a/a	a/a	a/a	NA
S098	M	*Api*	i	a/i	a/i	a/i	I
S154	M	*Api*	i	a/i	i/i	i/i	I
S155	F	*Api*	i	a/i	a/i	i/i	NA
S157	F	*Api*	a	a/a	a/i	a/i	NA
S161	F	*Apa*	i	a/a	a/a	a/i	NA
S162	F	*Apa*	i	a/a	a/a	a/i	NA
S164	F	*Api*	i	a/i	i/i	i/i	NA
S165	M	*Api*	i	a/i	i/i	i/i	I
S166	M	*Api*	i	a/i	a/i	i/i	I
S167	F	*Api*	i	a/i	i/i	i/i	NA
S182	M	*Api*	i	i/i	i/i	a/i	I
S183	F	*Api*	i	i/i	a/i	a/i	NA

[a] Identification code.
[b] All individuals except S157 were adults; S157 was an infant still being carried by its presumed mother.
[c] Phenotype based on size and pelage coloration and texture. *Apa*, *A. palliata*-like; *Api*, *A. pigra*-like individuals.
[d] Mitochondrial haplotype. a, *A. palliata*; i, *A. pigra*.
[e] Identity of alleles for parental species at each diagnostic microsatellite locus. a, *A. palliata*; i, *A. pigra*.
[f] Identity of the Y chromosome *Sry* gene. I, *A. pigra*; NA, primers did not amplify a product and were not expected to on the basis of the sex of this individual.

differences at three sites among species (GenBank accession nos. DQ875673–DQ875684). All male hybrid individuals ($n = 5$) possessed the *Sry* gene of *A. pigra* (Table 2).

If matings of hybrids are random and occur among all possible combinations of hybrids and parental species, we expect to find equal frequencies of the four possible genotypes of males and the two possible genotypes of females at the maternally inherited mitochondrial locus and the paternally inherited nuclear locus located on the Y chromosome of males. Although sample sizes are small, the chi-square goodness-of-fit tests suggest that the observed frequencies of males' and females' genotypes differed significantly from these expectations (Table 3). Moreover, probabilities of detecting 12 adult hybrids with the mitochondrial haplotype of *A. pigra* ($P = 2.4 \times 10^{-4}$), 12 hybrid individuals with the mitochondrial haplotype of *A. pigra* plus 1 hybrid individual with the mitochondrial haplotype of *A. palliata* ($P = 1.6 \times 10^{-3}$), and all 5 hybrid males with the *Sry* gene of *A. pigra* ($P = 3.1 \times 10^{-2}$) are low. These patterns imply that the direction of hybridization and subsequent backcrosses is strongly biased. Only crosses between *A. pigra* females or hybrid females carrying the mitochondrial haplotype of *A. pigra* and *A. palliata* males or hybrid males with the *Sry* gene of *A. palliata* occur and give rise to female offspring (Figure 3). However, no male

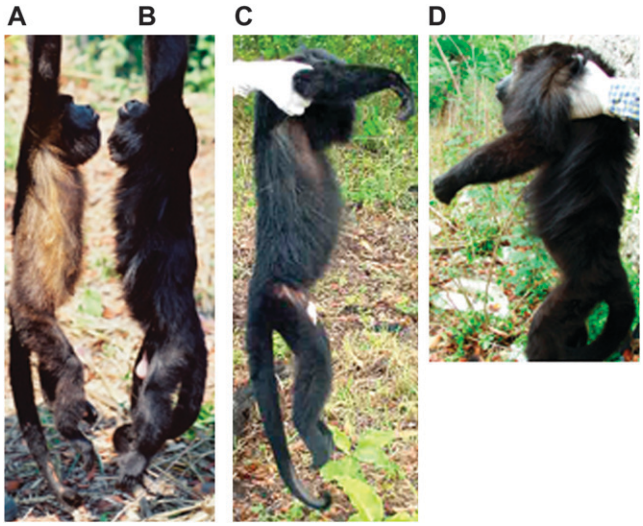

FIGURE 2.—General appearance of *Alouatta palliata*, *A. pigra*, and hybrid individuals. (A) *A. palliata* male from within the hybrid zone; (B) hybrid male that occurred with an *A. palliata* female and a hybrid female (note that males A and B belonged to different neighboring groups); (C) hybrid male with mixed characteristics (size of body and hyoid bone similar to *A. pigra* but note the light hairs in the flanks); (D) *A. pigra* male from outside the hybrid zone.

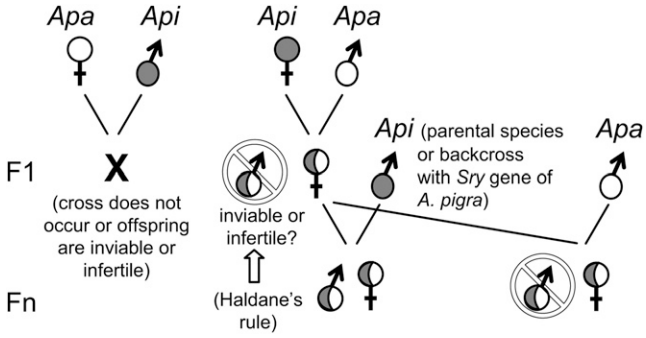

FIGURE 3.—Possible outcomes of crosses between *A. palliata* (*Apa*) and *A. pigra* (*Api*) males and females, according to the specific identification of mitochondrial and Y chromosome markers. See text for explanation.

hybrids with the *Sry* gene of *A. palliata* were observed and so, in accordance with Haldane's rule (HALDANE 1922), the data strongly suggest that the aforementioned crosses fail to produce viable males. Furthermore, on the basis of the low probability of detecting only the mtDNA haplotype of *A. palliata* in 12 adult hybrid individuals, *A. palliata* females and *A. pigra* males or hybrid males either mate infrequently or typically fail to produce viable offspring. Nonetheless, the genotypes of the hybrid infant and its suspected parents imply that this infant (S157, Table 2) was produced from a backcross between a male hybrid (S154, Table 2) and a female *A. palliata*. This demonstrates that such matings occur and that female offspring are produced. However, because no adult females were observed with the mitochondrial haplotype of *A. palliata*, we suspect that such crosses are uncommon or this infant is either infertile or will not survive to reproductive age.

We are currently investigating the potential role of morphological, behavioral, genetic, and cytogenetic differences as causes of the bias in direction of hybridization of these species. This work should advance our understanding of the speciation process and origins of reproductive isolation among primates, as well as the role of hybridization in primate evolution (ARNOLD and MEYER 2006). Moreover, study of the presence of hybrids in fragmented and intact forest tracts will reveal whether human-induced forest fragmentation has instigated hybridization by confining members of both species to small areas and limiting access to conspecific mates.

TABLE 3

Observed and expected frequencies of genotypes if all possible crosses among hybrids and backcrosses with parental species are equally likely

Genotype[a]	Frequencies		Chi-square (d.f.)	*P*-value
	Expected	Observed		
	Males ($n = 5$)			
aI♂	1.25	0	15.0 (3)	1.8×10^{-3}
iI♂	1.25	5		
aA♂	1.25	0		
iA♂	1.25	0		
	Females ($n = 8$)			
a♀	4	1	4.5 (1)	3.4×10^{-2}
i♀	4	7		

[a] Lowercase letters refer to the mitochondrial haplotype of *A. palliata* (a) and *A. pigra* (i); uppercase letters denote the genotype of males at the Y chromosome *Sry* gene for alleles of *A. palliata* (A) and *A. pigra* (I).

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LITERATURE CITED

ARNOLD, M. L., 1997 *Natural Hybridization and Evolution*. Oxford University Press, Oxford.

ARNOLD, M. L., and A. MEYER, 2006 Natural hybridization in primates: One evolutionary mechanism. Zoology **109:** 261–276.

BARTON, N. H., 2001 The role of hybridization in evolution. Mol. Ecol. **10:** 551–568.

BYNUM, E. L., D. Z. BYNUM and J. SUPRIATNA, 1997 Confirmation and location of the hybrid zone between wild populations of *Macaca tonkeana* and *Macaca hecki* in Central Sulawesi, Indonesia. Am. J. Primatol. **43:** 181–209.

Coimbra-Filho, A. F., A. Pissinatti and A. B. Rylands, 1993 Experimental multiple hybridism among *Callithrix* species from eastern Brazil, pp. 95–120 in *Marmosets and Tamarins: Systematic, Ecology and Behaviour*, edited by A. B. Rylands. Oxford University Press, Oxford.

Cortés-Ortiz, L., E. Bermingham, C. Rico, E. Rodríguez-Luna, I. Sampaio *et al.*, 2003 Molecular systematics and biogeography of the Neotropical monkey genus, *Alouatta*. Mol. Phylogenet. Evol. **26:** 64–81.

Coyne, J. A., and H. A. Orr, 2004 *Speciation*. Sinauer, Sunderland, MA.

Crockett, C. M., and J. F. Eisenberg, 1987 Howlers: variations in group size and demography, pp. 54–68 in *Primate Societies*, edited by B. B. Smuts, D. L. Cheney, R. M. Seyfarth, R. W. Wrangham and T. Struhsaker. University of Chicago Press, Chicago.

Dowling, T. E., and C. L. Secor, 1997 The role of hybridization and introgression in the diversification of animals. Annu. Rev. Ecol. Syst. **28:** 593–619.

Dunbar, R. I. M., and P. Dunbar, 1974 On hybridization between *Theropithecus gelada* and *Papio anubis* in the wild. J. Hum. Evol. **3:** 187–192.

Ellsworth, J. A., and G. A. Hoelzer, 1998 Characterization of microsatellite loci in a New World primate, the mantled howler monkey (*Alouatta palliata*). Mol. Ecol. **7:** 657–666.

Escobar-Páramo, P., 2000 Microsatellite primers for the wild brown capuchin monkey *Cebus apella*. Mol. Ecol. **9:** 107–108.

Evans, B. J., J. Supriatna and D. J. Melnick, 2001 Hybridization and population genetics of two macaque species in Sulawesi, Indonesia. Evolution **55:** 1686–1702.

Goodman, S. J., N. H. Barton, G. Swanson, K. Abernethy and J. M. Pemberton, 1999 Introgression through rare hybridization: a genetic study of a hybrid zone between red and sika deer (genus *Cervus*) in Argyll, Scotland. Genetics **152:** 355–371.

Groves, C. P., 1978 Phylogenetic and population systematics of the mangabeys (Primates: Cercopithecoidea). Primates **19:** 1–34.

Haldane, J. B. S., 1922 Sex-ratio and unisexual sterility in hybrid animals. J. Genet. **12:** 101–109.

Jolly, C. J., T. Woolley-Barker, S. Beyene, T. R. Disotell and J. E. Phillips-Conroy, 1997 Intergeneric hybrid baboons. Int. J. Primatol. **18:** 597–627.

Lawrence, B., 1933 Howler monkeys of the *palliata* group. Bull. Mus. Comp. Zool. **75:** 313–354.

Lernould, J. M., 1988 Classification and geographical distribution of guenons: a review, pp. 54–78 in *A Primate Radiation: Evolutionary Biology of the African Guenons*, edited by A. Gautier-Hion, F. Bourliere, J. P. Gautier and J. Kingdon. Cambridge University Press, Cambridge.

Mallet, J., 2005 Hybridization as an invasion of the genome. Trends Ecol. Evol. **2:** 229–237.

Mendes, S. L., 1997 Hybridization in free-ranging *Callithrix flaviceps* and the taxonomy of the Atlantic forest marmosets. Neotrop. Primates **5:** 6–8.

Moreira, M. A. M., 2002 SRY evolution in Cebidae (Platyrrhini: Primates). J. Mol. Evol. **55:** 92–103.

Neville, M. K., K. E. Glander, F. Braza and A. B. Rylands, 1988 The howling monkeys, genus *Alouatta*, pp. 349–453 in *Ecology and Behavior of Neotropical Primates*, edited by R. Mittermeier, A. B. Rylands, A. Coimbra-Filho and G. B. A. Fonseca. World Wildlife Fund, Washington, DC.

Palumbi, S. R., 1996 Nucleic acids II: The polymerase chain reaction, pp. 205–247 in *Molecular Systematics*, edited by D. M. Hillis, C. Moritz and B. K. Mable. Sinauer, Sunderland, MA.

Peres, C., J. L. Patton and M. N. F. da Silva, 1996 Riverine barriers and gene flow in Amazonian saddle-back tamarins. Folia Primatol. **67:** 113–124.

Phillipsconroy, J. E., and C. J. Jolly, 1986 Changes in the structure of the baboon hybrid zone in the Awash National Park, Ethiopia. Am. J. Phys. Anthropol. **71:** 337–350.

Samuels, A., and J. Altmann, 1986 Immigration of a *Papio anubis* male into a group of cynocephalus baboons and evidence for a anubis-cynocephalus hybrid zone in Amboseli, Kenya. Int. J. Primatol. **7:** 131–138.

Silva, B. T. F., M. I. C. Sampaio, H. Schneider, M. P. C. Schneider, E. Montoya *et al.*, 1993 Protein electrophoretic variability in *Saimiri* and the question of its species status. Am. J. Primatol. **29:** 183–193.

Smith, J. D., 1970 The systematic status of the black howler monkey, *Alouatta pigra* Lawrence. J. Mammal. **51:** 358–369.

Struhsaker, T. T., T. M. Butynski and J. S. Lwanga, 1988 Hybridization between redtail (*Cercopithecus ascanius schmidti*) and blue (*C. mitis stuhlmanni*) monkeys in the Kibale Forest, Uganda, pp. 477–497 in *A Primate Radiation: Evolutionary Biology of the African Guenons*, edited by A. Gautier-Hion, F. Bourliere, J. P. Gautier and J. Kingdon. Cambridge University Press, Cambridge.

Treves, A., 2001 Reproductive consequences of variation in the composition of howler monkey (*Alouatta* spp.) groups. Behav. Ecol. Sociobiol. **50:** 61–71.

Van Belle, S., and A. Estrada, 2006 Demographic features of *Alouatta pigra* populations in extensive and fragmented forest, pp. 121–142 in *New Perspectives in the Study of Mesoamerican Primates: Distribution, Ecology, Behavior, and Conservation*, edited by A. Estrada, P. Garber, M. Pavelka and L. Luecke. Kluwer Academic Press, New York.

Watanabe, K., and S. Matsumura, 1991 The borderlands and possible hybrids between 3 species of macaques, *M. nigra*, *M. nigrescens*, and *M. hecki* in the northern peninsula of Sulawesi. Primates **32:** 365–369.

Whitehead, J. M., 1995 Vox Alouattinae—a preliminary survey of acoustic characteristics of long-distance calls of howling monkeys. Int. J. Primatol. **16:** 121–144.

Whitfield, L. S., R. Lovell-Badge and P. N. Goodfellow, 1993 Rapid sequence evolution of the mammalian sex-determining gene SRY. Nature **364:** 713–715.

Wyner, Y. M., S. E. Johnson, R. M. Stumpf and R. Desalle, 2002 Genetic assessment of a white-collared x red-fronted lemur hybrid zone at Andringitra, Madagascar. Am. J. Primatol. **67:** 51–66.

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