

Wild mixed groups of howler species (*Alouatta caraya* and *Alouatta clamitans*) and new evidence for their hybridization

Lucas M. Aguiar · Marcio R. Pie · Fernando C. Passos

Received: 14 February 2007 / Accepted: 21 August 2007 / Published online: 17 October 2007
© Japan Monkey Centre and Springer 2007

Abstract Mixed species groups and hybridization are common among primates, yet these phenomena are rare and poorly understood for the genus *Alouatta*. In this study, we describe the composition of howler groups in a sympatric area of *Alouatta caraya* and *Alouatta clamitans* and provide new evidence for the occurrence of interspecific hybridization. Between October 2006 and April 2007, 11 howler groups were located in a 150-ha forest fragment: two monospecific groups of *A. caraya*, two monospecific groups of *A. clamitans*, two groups composed of *A. clamitans* and hybrid morphotypes (*A. caraya* × *A. clamitans*), and five groups composed of both species together with hybrid morphotypes (mixed species groups). The average size of the studied groups was 5.2 ± 1.2 individuals. Monospecific and mixed groups (mixed species groups + groups with hybrids) did not differ significantly in their sizes. In total, the sex/age ratios were 1 AM:1.5 AF:0.2 SAM:0.5 JUV:0.2 INF and the species ratios were 1 *A. caraya*:1.6 *A. clamitans*:0.4 *A. caraya* × *A. clamitans*. The ratio of immatures to 1AF was larger in the monospecific

groups (0.75 immatures:1AF) than in mixed groups (0.29 immatures:1AF), possibly reflecting a lower viability in the latter. Two features of the hybrid morphotypes of the upper Paraná River support their status as true hybrids: the polymorphism of their coloration patterns and the extremely female-biased sex ratio. The effects of Haldane's rule and population fragmentation on the interactions between both species are discussed.

Keywords Fragmentation · Haldane's rule · Hybrid zone · Sympatry · Sex ratio

Introduction

Mixed species groups are polyspecific associations that are known to occur among both Old World and New World primates. The advantages of living in a mixed species group might include an increase in the efficiency of anti-predator and foraging strategies and enhanced reproductive success, which might include hybridization events (Terborgh 1990; Stensland et al. 2003).

Natural hybridization among primates can represent an important evolutionary mechanism (Detwiler et al. 2005; Ackermann et al. 2006; Arnold and Meyer 2006). The numerous records of hybrid individuals and the persistence of hybrid zones suggest that hybridization is common among primates (Detwiler et al. 2005). Evidence for natural hybridization has also been recorded for both primate groups: in the Old World, hybridization is known among species and subspecies in the genera *Eulemur*, *Propithecus*, *Trachypithecus*, *Papio*, *Macaca*, *Cercopithecus*, *Hylobates*, and between the genera *Theropithecus* and *Papio* (Detwiler et al. 2005; Arckermann et al. 2006; Arnold and Meyer 2006). Cases in the New World include species and

Contribution number 1689 of the Departamento de Zoologia, Universidade Federal do Paraná (UFPR), Caixa Postal 19020, 81531-990, Curitiba, Paraná, Brazil. Contract grant sponsor: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

L. M. Aguiar (✉) · F. C. Passos
Programa de Pós-graduação em Zoologia,
UFPR, Laboratório de Biodiversidade,
Conservação e Ecologia de Animais Silvestres,
Departamento de Zoologia, Universidade Federal do Paraná,
Caixa Postal 19020, 81531-990 Curitiba, Paraná, Brazil
e-mail: lucasmorares@ufpr.br

M. R. Pie
Laboratório de Ecologia Molecular e Parasitologia Evolutiva,
Departamento de Zoologia, UFPR, Curitiba, Paraná, Brazil

subspecies in the genera *Saimiri*, *Callitrix*, *Saguinus* and *Alouatta* (Silva et al. 1992; Peres et al. 1996; Mendes 1997; Cortés-Ortiz et al. 2006).

In the case of the genus *Alouatta* (howlers), the only record of an interspecific association involves the formation of mixed species groups and the natural hybridization between *Alouatta palliata* and *Alouatta pigra* in a fragmented forest in Mexico (Cortés-Ortiz et al. 2000, 2006). In southern Brazil, free-ranging potential hybrids have recently been reported in the sympatric area between *A. caraya* and *A. clamitans*. These hybrids show a coloration pattern that is a mosaic between both species (Aguiar et al. 2007; Gregorin 2006 for museum records). Therefore, the goal of this study is to report the compositions of groups of howlers in a sympatric area of *Alouatta caraya* and *Alouatta clamitans* and to show strong evidence of interspecific hybridization.

Materials and methods

Study area

The study area (23°22'52.3"S and 53°45'39.6"W) is a 150-ha fragment of seasonal semideciduous forest (SSF) of the submontane type that is continuous with the riparian forest of the left margin of the upper Paraná River (see Fig. 1b in Aguiar et al. 2007). This fragment experienced selective cutting in the 1960s and is currently classified as a secondary forest. Locally known as the Mata do Bugio, this fragment belongs to the municipality of Icaraíma and is adjacent to the Ilha Grande National Park, state of Paraná, Southern Brazil. The average annual temperature is 22 °C, with an annual rainfall of between 1,200 and 1,300 mm, and a subtropical climate (Campos 2001). The region of the Mata do Bugio is a sympatric area for the howler species characteristic of the Cerrado (*A. caraya*) and the species characteristic of the Atlantic rainforest (*A. clamitans*) (Aguiar et al. 2007).

Methods

Search efforts for the primates focused only on the Mata do Bugio, given that on a previous field expedition, hybrid morphotypes between *A. caraya* and *A. clamitans* had already been located in that region (Aguiar et al. 2007). During seven months (from October 2006 to April 2007), howler groups were surveyed in field efforts that lasted between five and ten days per month. The search for the groups was carried out using two basic strategies: on foot using the trails in the forest interior, and by locating the animals using their roars. Located animals were always

identified by the same observer (LM Aguiar) using the description of Gregorin (2006). Individuals with coloration patterns that were a mosaic or intermediate between both species were recorded as *A. caraya* × *A. clamitans* (hybrid morphotypes) (Gregorin 2006; Aguiar et al. 2007). Their coloration patterns are mosaics or intermediate between the golden coloration of *A. caraya* females and the dark brown coloration of *A. clamitans* females. These patterns were observed using binoculars and were drawn in a field notebook.

During analyses, groups were divided into two sets: monospecific groups and “mixed groups.” Mixed groups comprised two types of association: groups with hybrid morphotypes and individuals of one of the species, and hybrid morphotypes together with individuals of both species (mixed species groups).

Results

Out of a total of 380 h of field work, 229 h included direct observations of the eleven surveyed groups: two monospecific groups of *A. caraya*, two monospecific groups of *A. clamitans*, two groups comprising individuals of *A. clamitans* and *A. clamitans* × *A. caraya*, and five groups comprising individuals of both species together with *A. clamitans* × *A. caraya* (Table 1). The size of the groups averaged 5.2 ± 1.2 individuals. The compositions of the groups resulted in sex/age ratios of 1 AM:1.5 AF:0.2 SAM:0.5 JUV:0.2 INF. The species ratios were 1 *A. caraya*:1.6 *A. clamitans*:0.4 *A. caraya* × *A. clamitans*. The ratio of immatures [JUVs + INFs (young)] to adult females in this fragment was 0.44 immature:1AF.

The sizes of the monospecific groups [4.5 ± 1.3 individuals ($n = 4$)] and the mixed groups [5.6 ± 1.1 individuals ($n = 7$)] did not differ significantly ($t = 1.383$; $df = 5.67634$; $P > 0.05$). Monospecific groups were unimale and had sex/age ratios of 1 AM:2 AF:1.3 JUV:0.3 INF. The ratio of immatures to adult females was 0.75 immatures:1AF. Mixed groups, on the other hand, were multimale and had sex/age ratios of 1 AM:1.3 AF:0.3 SAM:0.2 JUV:0.2 INF. More detailed ratios were 1 AM (*A. caraya*):1.3 AF (*A. caraya*):1 SAM (*A. caraya*):0.3 JUV (*A. caraya*):3.3 AM (*A. clamitans*):1.7 AF (*A. clamitans*):0.3 SAM (*A. clamitans*):0.7 JUV (*A. clamitans*):0.7 INF (*A. clamitans*):2.7 AF (*A. caraya* × *A. clamitans*). The species ratios were 1 *A. caraya*:1.8 *A. clamitans*:0.7 *A. caraya* × *A. clamitans*. The ratio of immature to adult females was 0.29 immatures:1AF. There was one record of a female of a hybrid morphotype carrying an infant on its back.

The *A. caraya* × *A. clamitans* morphotypes recorded in the present study showed polymorphism with respect to their hair coloration patterns, such that six different

Table 1 Sizes, compositions, and sex–age ratios of 11 *Alouatta* groups in the Mata do Bugio

Groups	Composition										Hours	
	<i>Alouatta caraya</i>				<i>Alouatta clamitans</i>				<i>A. caraya</i> × <i>A. clamitans</i>		Total	
	AM	AF	SAM	JUV	AM	AF	SAM	JUV	INF	AF		
A	1	2	0	2	0	0	0	0	0	0	5	2
B	1	1	0	1	0	0	0	0	0	0	3	18
C	0	0	0	0	1	2	0	1	0	0	4	6
D	0	0	0	0	1	3	0	1	1	0	6	4
E	0	0	0	0	1	1	1	0	1	1	5	2
F	0	0	0	0	2	0	0	0	1	1	4	7
G	1	1	0	1	3	0	0	0	0	1	7	37
H	0	0	1	0	1	1	0	1	0	1	5	11
I	2	1	0	0	0	1	0	1	0	1	6	10
J	0	0	1	0	2	1	0	0	0	1	5	120
K	0	2	1	0	1	1	0	0	0	2	7	12
Total	5	7	3	4	12	10	1	4	3	8	Mean: 5.2	229
Sex–age ratio	1:	1.4:	0.6:	0.8:	2.4:	2.0:	0.2:	0.8:	0.6:	1.6	SD: ±1.2	

The sex–age classes are: adult males (AM), sub-adult males (SAM), adult females (AF), juveniles (JUV), and infants (INF). The sex–age ratio is provided as n:n. Total observation time is provided in hours SD, standard deviation

configurations were identified (Fig. 1B–G); all of these individuals were identified as females.

Discussion

The observation of a larger number of mixed groups or groups with potential hybrids than monospecific groups might indicate a situation of disequilibrium caused by forest fragmentation—an anomalous condition due to the confinement of two closely-related species. Conversely, the continuous riparian forest that is adjacent to the Mata do Bugio has been shown to harbor only monospecific groups (Aguiar et al. 2007).

The average size of the studied groups was small, close to the mean size of the most abundant species in the fragment, *A. clamitans* (Miranda and Passos 2005), which often has smaller groups than *A. caraya* (Rumiz 1990). The proportions among species and hybrid morphotypes (1 *A. caraya*:1.6 *A. clamitans*:0.4 *A. caraya* × *A. clamitans*) in the fragment show that most individuals belong to *A. clamitans* (52.6%), followed by *A. caraya* (33.3%), which might be due to the lower abundance of this species in this type of environment (submontane SSF, Atlantic Rainforest) (Di Bitetti et al. 1994). The proportion of potential hybrids is substantial in the studied forest fragment (14.0%), but below the values observed in hybrid zones between species of several genera, including *Macaca* (Bynum 2002) and particularly *Papio* (Bergman and Bechner 2004; Detwiler et al. 2005), given that, according

to these studies, nearly all of the baboons in those hybrid zones showed some degree of hybridization. Long-term studies are necessary to elucidate the dynamics and the stability of the hybrid zone between *A. caraya* and *A. clamitans* in the upper Paraná River.

The proportion of adult males to adult females is consistent with the proportions observed for both species (Rumiz 1990; Miranda and Passos 2005). However, the proportion of females is smaller in the mixed groups, with most of them represented by hybrid morphotypes. This result suggests that these are the groups where hybrid morphotypes are generated. In addition, there is a lower ratio of immatures to 1AF in the mixed groups than in monospecific groups. These two departures could indicate the existence of a partial prezygotic isolation mechanism, leading to a lower fitness of these groups. Although the record of the potential female hybrid with an immature suggests some level of fertility in hybrids, our data indicate that reproduction in mixed groups is limited, and when it occurs the resulting individuals of interspecific crosses tend to be females, as discussed below.

Specimens of *Alouatta* with a mosaic coloration pattern observed in this and in other studies (Fig. 1) are only known in sympatric areas of both species, which is in itself strong evidence for a hybrid origin. Two other characteristics corroborate their status as actual hybrids. First, specimens are polymorphic with respect to their color patterns, for a total of ten different color configuration patterns (six in the present study, one in Aguiar et al. 2007; and three in Gregorin 2006). Such a high level of morphological variability is a well-known phenomenon in hybrid populations (Ackermann et al. 2006). According to Gregorin (2006), this would indicate that fixation has not occurred in the chromatogenetic fields and so it cannot be considered to be a distinct trait of the two species analyzed. Second, the sex ratio of the known hybrid morphotypes of the upper Paraná River is strongly biased toward females (1 AM:13 AF—eight females in the present study, two females and a male in Aguiar et al. 2007; three museum specimens of females in Gregorin 2006). Such an extreme sex bias is unlikely to have resulted from chance alone (Yates $\chi^2 = 4.375$, $df = 1$, $P = 0.036$), and would be consistent with a lower viability of the heterogametic sex (males), as predicted by Haldane’s rule (Wu et al. 1996). However, the unusual sex determination system of *A. caraya* ($X_1X_2Y_1Y_2$) (Mudry et al. 1998) could affect the dominance patterns of the genes in the sexual chromosomes, limiting the effects of reduced hybrid male viability. Further studies on the genetics of hybrid morphotypes and surveys in other areas are necessary to substantiate these ideas.

The existence of hybrid morphotypes and mixed groups between *A. caraya* and *A. clamitans* indicates incomplete reproductive isolation between the species. If confirmed,

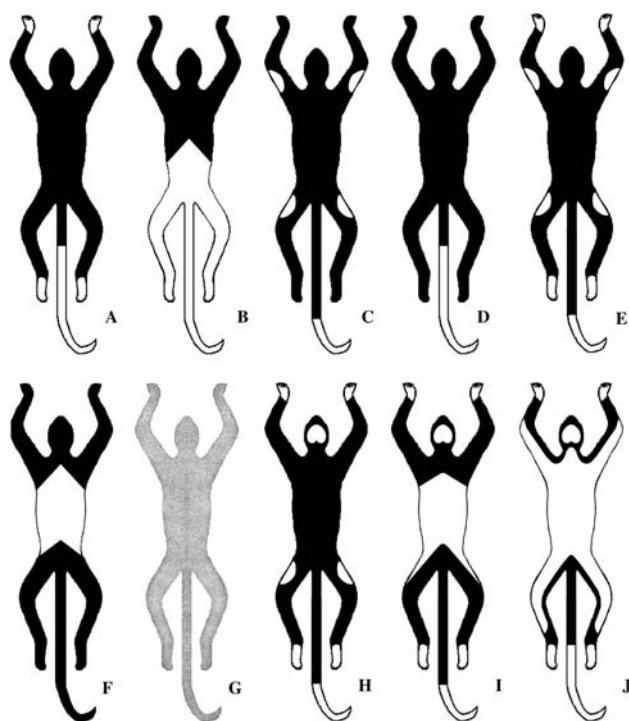


Fig. 1A–J Color polymorphism in the known hybrid morphotypes. **A** Male recorded in Aguiar et al. (2007); **B–G** females recorded in the present study; and **H–J** females from museum collections first reported by Gregorin (2006). *Black filled squares* represent the dark-brown coloration of *A. clamitans* females; *open squares* represent the golden coloration of *A. caraya* females; *gray filled squares* represent intermediate coloration between females of both species

such a phenomenon could increase the probability of genetic introgression between the studied species. As a consequence, the species' isolation mechanisms could be being diluted through hybridization and introgression, possibly leading to the extinction of one or both species at the regional level in the long term (Rhymer and Simberloff 1996; Detwiler et al. 2005). *Alouatta caraya* is regionally abundant in flooded forests (alluvial SSF), whereas *A. clamitans* is rare and appears to show a preference for submontane SSF (LM Aguiar, personal observation). Given that this forest type is almost extinct in the region (Campos 2001) and that the Mata do Bugio is one of the last remnants of submontane SSF in the upper Paraná River, the study of the interaction between *A. clamitans* and its congener should be given a high priority in order to aid its regional conservation.

Acknowledgments We thank CNPq for a scholarship to LM Aguiar and for a research grant to FC Passos, APA Municipal de Alto Paraíso, Ilha Grande National Park, nine research assistants for help during field work, IBAMA for permit 261/2006, and two anonymous reviewers for helpful suggestions about the manuscript. JMD Miranda provided valuable statistical advice.

References

- Ackermann RR, Rogers J, Cheverud JM (2006) Identifying the morphological signatures of hybridization in primate and human evolution. *J Hum Evol* 51:632–645
- Aguiar LM, Mellek DM, Abreu KC, Boscarato TG, Bernardi IP, Miranda JMD, Passos FC (2007) Sympatry of *Alouatta caraya* and *Alouatta clamitans* and the rediscovery of free-ranging potential hybrids in Southern Brazil. *Primates* 48:245–248
- Arnold ML, Meyer A (2006) Natural hybridization in primates: one evolutionary mechanism. *Zoology* 109:261–276
- Bergman TJ, Beehner JC (2004) Social system of a hybrid baboon group (*Papio anubis* × *P. hamadryas*). *Int J Primatol* 25:1313–1330
- Bynum N (2002) Morphological variation within a Macaque hybrid zone. *Am J Phys Anthropol* 118:45–49
- Campos JB (2001) Parque Nacional de Ilha Grande, re-conquista e desafios. IAP/Coripa, Maringá, Brazil
- Cortés-Ortiz L, Bermingham E, Rico C, Rodríguez-Luna E, Sampaio I, Ruiz-García M (2000) Molecular systematics and biogeography of the neotropical monkey genus, *Alouatta*. *Mol Phyl Evol* 26:64–81
- Cortés-Ortiz EB, Canales-Espinosa D, García-Orduña F, Rodríguez-Luna E (2006) Natural Hybridization between howler monkeys in México. *Am J Primatol* 68:136
- Detwiler KM, Burrell AS, Jolly CJ (2005) Conservation implications of hybridization in African cercopithecine monkeys. *Int J Primatol* 26:661–684
- Di Bitetti MS, Placci G, Brown AD, Rode DI (1994) Conservation and population status of the brown howling monkey (*Alouatta fusca clamitans*) in Argentina. *Neotrop Primates* 2:1–4
- Gregorin R (2006) Taxonomia e variação geográfica das espécies do gênero *Alouatta* Lacépède (Primates, Atelidae) no Brasil. *Rev Bras Zool* 23:64–144
- Mendes SL (1997) Hybridization in free-ranging *Callithrix flaviceps* and the taxonomy of the Atlantic Forest marmosets. *Neotrop Primates* 5:6–8
- Miranda JMD, Passos FC (2005) Composição e dinâmica de grupos de *Alouatta guariba clamitans* Cabrera (Primates, Atelidae) em Floresta Ombrófila Mista no Estado do Paraná, Brasil. *Rev Bras Zool* 22:99–106
- Mudri MD, Rahn M, Gorostiaga M, Hick A, Merani MS, Solari AJ (1998) Revised karyotype of *Alouatta caraya* (Primates: Platyrrhini) based on synaptonemal complex and banding analyses. *Hereditas* 128:9–16
- Peres CA, Patton JL, Da Silva MNF (1996) Riverine barriers and gene flow in Amazonian saddle-back tamarin monkeys. *Folia Primatol* 67:113–124
- Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Annu Rev Ecol Syst* 27:83–109
- Rumiz DI (1990) *Alouatta caraya*: population density and demography in Northern Argentina. *Am J Primatol* 21:279–294
- Silva BTF, Sampaio MIC, Schneider H, Schneider MPC, Montoya E, Encarnación F, Salzano FM (1992) Natural hybridization between *Saimiri* Taxa in the Peruvian Amazônia. *Primates* 33:107–113
- Stensland E, Angerbjörn A, Berggren P (2003) Mixed species groups in mammals. *Mammal Rev* 33:205–223
- Terborgh J (1990) Mixed flocks and polyspecific associations: costs and benefits of mixed groups to birds and monkeys. *Am J Primatol* 21:87–100
- Wu C, Johnson NA, Palopoli MF (1996) Haldane's rule and its legacy: why are there so many sterile males? *Tree* 11:281–284