Roaring Behavior of Two Syntopic Howler Species (*Alouatta caraya* and *A. guariba clamitans*): Evidence Supports the Mate Defense Hypothesis

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Abstract Long-distance calls are loud vocalizations involved in within and between group communication in animals. These calls may maintain cohesion with group members or communicate the ownership of valuable resources such as territory, food, or mates to individuals from other groups. In howlers (*Alouatta* spp.), three non-mutually exclusive hypotheses suggest that the ultimate function of roaring (howling) behavior is to protect resources from neighboring groups. The space/food defense hypothesis maintains that roars are used to gain exclusive access to food; the mate defense hypothesis holds that roars ensure exclusive access to mates; and the infanticide avoidance hypothesis states that the roaring behavior serves to avoid male takeovers and infanticide. To test these three hypotheses, we conducted a study on black-and-gold howlers (*Alouatta caraya*) and brown howlers (*A. guariba clamitans*) living in syntopy at El Piñalito provincial park in northeast Argentina. We recorded 12 mo of data on the roaring behavior of two groups of each species, along with data on food availability and diet. Although all four groups overlapped extensively in their use of food resources during the study period, roars occurred much more frequently during interactions between groups of the same species than between groups of different species. Roaring frequency was not higher during the lean season, when high-quality food was less abundant. Howlers did not roar more frequently at home range boundaries, nor were roaring bouts spatially associated with valuable feeding resources. Males participated in all of the roaring bouts, whereas females participated in only 29% of them. Adults did not roar more frequently when there were unweaned infants in the group, as expected if roaring behavior decreases infanticide risk. These

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results provide no support for the food defense or infanticide avoidance hypotheses, but support predictions derived from the mate defense hypothesis.

Introduction

Many animal species rely on long-distance calls to communicate, e.g., insects (Alexander 1967), deer (Clutton-Brock and Albon 1979), frogs and toads (Gerhardt 1994), primates (Cowlishaw 1992; Zuberbühler *et al.* 1997), and carnivores (Harrington and Mech 1978). In group-living animals, loud calls can be used to communicate with individuals within the group to maintain cohesion, as has been shown in wolf packs (*Canis lupus*: Mech 1966), lion prides (*Panthera leo*: Grinnell and McComb 1996), or elephant bond groups (*Loxodonta Africana*: Leighty *et al.* 2008). Long-distance calls are also involved in intergroup communication in terms of territorial defense, e.g., lions (Grinnell *et al.* 1995; McComb *et al.* 1994) and wolves (Harrington and Mech 1978); signaling the use of a food resource, e.g., black crested gibbons (*Nomascus concolor jingdongensis*: Peng-Fei *et al.* 2009); or defending sexual partners, e.g. savannah baboons (*Papio cyanocephalus ursinus*: Kitchen *et al.* 2004a).

Howlers (*Alouatta* spp.) are folivorous–frugivorous primates (Milton 1998), that live in uni- or multimale groups (Di Fiore and Campbell 2007). Howlers are not usually seasonal breeders (Di Bitetti and Janson 2000; Kowalewski and Zunino 2004; Strier *et al.* 2001), although researchers have reported a degree of reproductive seasonality for some study sites (Crockett and Rudran 1987; Zunino 1996). Both sexes emigrate from the natal group to another established group, or to form a new one (Rudran 1979; Rumiz 1990; Sekulic 1982a). All 10 species in this genus (Groves 2001) are capable of performing powerful long-distance roars (Whitehead 1995). Males have an enlarged hyoid bone, which conforms a hyolaryngeal apparatus that works as a resonance chamber (Kelemen and Sade 1960), amplifying their roars. These loud calls are most often initiated and led by adult males, which may be joined by the rest of the group to form a chorus (Carpenter 1934; Kelemen and Sade 1960). Although howlers can use these vocalizations to locate members of their own group (Oliveira 2002; Steinmetz 2005), most evidence seems to support a role in intergroup communication (Chiarello 1995; Kitchen 2004; Sekulic 1982a; Whitehead 1987).

Howler roars are aggressive displays involved in the regulation of the use of space between neighboring groups (Altmann 1959; Baldwin and Baldwin 1976; Carpenter 1934; da Cunha and Byrne 2006; da Cunha and Jalles-Filho 2007; Whitehead 1987, 1989). How this behavior is used to mediate the use of space varies among the different howler species and even among populations of the same species (Baldwin and Baldwin 1976; da Cunha and Byrne 2006; da Cunha and Jalles-Filho 2007; Kitchen 2004; Sekulic 1982a,b; Whitehead 1987, 1989). For example, roars may discourage solitary individuals or groups from approaching, resulting in the segregation of groups and avoidance of intergroup encounters (Kitchen 2004; Kitchen *et al.* 2004b; Sekulic 1982b; Whitehead 1987, 1989), or, conversely, may attract solitary

individuals or groups to the roars, resulting in an approach and promotion of intergroup encounters (Kitchen 2004; Kitchen *et al*. 2004b; Whitehead 1987, 1989). In *Alouatta pigra*, *A. guariba clamitans*, and *A. seniculus arctoidea*, individuals assess the relative fighting ability of groups or the physical strength of opponents through their roars (Chiarello 1995; Kitchen 2004; Kitchen *et al*. 2004b; Sekulic 1982a, b).

Although roars regulate spacing among howler groups, it is not clear how this benefits the individuals that produce the calls. Three non-mutually exclusive hypotheses have been considered in this context. First, the space/food defense hypothesis maintains that howlers roar to gain exclusive access to resources, e.g., food. Defense of space/food could be achieved by constantly announcing the occupancy of an area through roars, as in *Alouatta caraya* (da Cunha and Byrne 2006); through the active patrol of borders with vocalization sessions, as in *A. guariba clamitans* (da Cunha and Jalles-Filho 2007); or by roaring more frequently during the food-limited dry season, as in *A. guariba clamitans* (Chiarello 1995) and *A. seniculus arctoidea* (Sekulic 1982b). Second, the mate defense hypothesis holds that howlers roar to ensure exclusive access to sexual partners. Long-distance calls during intergroup encounters in *Alouatta seniculus arctoidea* have been argued to deter outsider males from entering the group, in the context of male–male competition for females (Sekulic 1982a). Third, the infanticide avoidance hypothesis considers that roaring behavior may reduce the likelhihood of male takeovers and infanticide. Males of *Alouatta pigra* answered roars heard during playback experiments only if they outnumbered the simulated group, unless unweaned infants were present in the group, indicating that infant presence was an important factor in deciding when to roar (Kitchen 2004).

All previous studies examining the ultimate function of roars were conducted in areas inhabited by only one howler species. In this situation, it is difficult to disentangle whether groups roar to repel other groups from space/food resources or mates because both targets are of interest to all individuals within the same population. Syntopy, i.e., the co-occurrence of different species in the same habitat within an area of sympatry is an uncommon situation among howler species and has been reported in only a few contact zones (Agostini *et al*. 2008; Aguiar *et al*. 2007; Bicca-Marques *et al*. 2008; Cortés-Ortiz *et al*. 2007; Chames and Olmos 1997; Defler 2004; Di Bitetti *et al*. 1994; Gregorin 2006; Iwanaga and Ferrari 2002; Pinto and Setz 2000; Wallace *et al*. 1998). Natural hybridization has been reported for some howler species (Agostini *et al*. 2008; Aguiar *et al*. 2007, 2008; Bicca-Marques *et al*. 2008; Cortés-Ortiz *et al*. 2007) and in most sites where syntopy between howler species was recorded, a relatively high proportion of mixed groups (composed by individuals of both species) and putative hybrids are observed (Aguiar *et al*. 2007; Bicca-Marques *et al*. 2008; Cortés-Ortiz *et al*. 2007). In areas of syntopy with a low frequency of mixed species groups and reduced natural hybridization, members of different species potentially face high competition for food resources (Agostini *et al*. 2010a) but low competition for sexual partners. Thus, these areas provide a unique opportunity to conduct a comparative study to test the ultimate explanations of the roaring behavior of howlers.

We here test the predictions of the space/food, mate defense, and infanticide avoidance hypotheses using a field study of the comparative feeding ecology and

vocal behavior of two howler species, the black-and-gold howler (*Alouatta caraya*) and the brown howler (*A. guariba clamitans*) in El Piñalito Provincial Park, a protected area in the Atlantic Forest of Argentina where these two species live in syntopy but with low frequencies of mixed species groups and interspecific hybridization. We derived and tested the following predictions of the space/food, mate defense, and infanticide avoidance hypotheses.

Food/Space Defense Hypothesis

The diets and the spatial and temporal patterns of habitat use of the two howler species at our study site overlap amply, indicating a high potential for food competition between the two species (Agostini *et al.* 2010a, b; see Discussion for more details). For this reason, if howlers roar to defend food resources within their home ranges, we predict that 1) encounters between groups of different howler species (heterospecific encounters), as well as encounters between groups of the same howler species (conspecific encounters) should involve roars; 2) roars produced by groups out of sight should be answered by both species; and 3) roaring frequency should exhibit seasonal variation, with howlers of both species roaring more frequently during times of food scarcity. Food defense could also be achieved by two alternative strategies: either by 4) exhibiting some degree of territoriality and roaring more from territory boundaries than from interior positions or 5) indicating ownership of valuable resources by performing more roaring bouts in their immediate vicinity than elsewhere.

Mate Defense Hypothesis

If howlers roar to defend their access to mates, we predict that: 1) only encounters with conspecific groups should be aggressive and include roars; 2) howlers should respond only to conspecific roars produced by another group or individual out of sight; 3) males should roar more often than females; and 4) males should roar more often when females in the group are in estrous than at other times.

Infanticide Avoidance Hypothesis

Infanticide constitutes a well established reproductive strategy in howlers (Crockett 2003; Crockett and Janson 2000; Rudran 1979) and incoming adult males tend to kill unweaned infants (infants ≤9 mo old; Mack 1979). Killing an unweaned infant reduces the interbirth interval by returning the female to estrous in howlers (Crockett 2003). If roars provide honest information about the minimal number of roaring individuals in a chorus (Kitchen 2006; Kitchen *et al.* 2004a) and, because females with infants in a vulnerable stage are threatened by the entry of another male into the group (Sekulic 1982a), the degree of participation of adult females in roaring choruses may vary in relation to their reproductive status because female roars increase the group's perceived fighting ability, making the group less attractive to potentially invading males. If howlers roar to avoid infanticide, then: 1) males should roar more when unweaned infants are present within the group; 2) females with unweaned infants should roar with the alpha male more than females without infants

do; and 3) if groups with a higher number of reproductive females are more attractive to potentially invading males (Crockett and Janson 2000), mothers of unweaned infants should roar less than females without helpless infants, to hide their presence and protect their infants against infanticide.

Our test of these hypotheses relies on two assumptions. First, howlers can finely adjust their roaring behavior in response to local conditions, e.g., the degree of food or mate competition exerted by potential competitors, including individuals from another howler species. Second, howlers will respond to, i.e. will be aroused by, howls emitted by another howler species with the same intensity as to those emitted by same species. This assumption is justified by the apparent lack of specificity in the eliciting stimuli, e.g., the frequently reported observations of roaring behavior in response to deep and loud sounds produced by thunder, chain-saws, and motors (Baldwin and Baldwin 1976; Chiarello 1995; Horwich and Gebhard 1983).

Materials and Methods

Study Site

We conducted this study in El Piñalito Provincial Park (26°30′S, 53°50′W), a strictly protected area of Atlantic Forest of 3796 ha, located in the province of Misiones, northeastern Argentina, from December 2006 to November 2007. Here, black-and-gold howler and brown howler groups occur in syntopy (Agostini *et al.* 2008). All censused groups in the park ($N=9$) were monospecific, except for one brown howler group that contained a single black-and-gold howler female from 2005 until the end of the study. Of a total of 62 individuals observed in El Piñalito groups, only two were putative hybrids (both born to the same black-and-gold howler female mentioned earlier), neither of which survived beyond the age of 2 mo.

We studied two groups of black-and-gold and two groups of brown howlers. The size and group composition of the study groups are detailed in Table I. I. Holzmann and I. Agostini followed each of the groups 3 (SD=0.6, range 1–5) d/mo to gather data on feeding and roaring behavior. All group members were identifiable, with the

Table I Age and sex composition of the four focal groups during the study period

Group	Composition						
	Adult males	Adult females	Subadult males	Subadult females	Juvenile males	Juvenile females	Infants
BR1	1	3	1	0	2–3	0	0–2
BR2	1	3[a]	0	0	0	0	2–3
BL1	1–2	5	1	0	0–2	4–5	1–3
BL2	1–2	1	1	0–1	1	1–2	0–1

BR = brown howler groups 1 and 2; BL = black-and-gold howler groups 1 and 2.

[a]Includes one black-and-gold howler female that lived in the BR2 group. (Modified from Agostini *et al.* 2010a.)

exception of the adult females of one group of black-and-gold howlers (group BL1), which we classified as belonging to same age/sex class owing to their similarity.

Vegetation Survey

To estimate the seasonal pattern of plant productivity, we established two phenological trails that partially crossed the home ranges of the focal groups, along which we selected trees from species consumed by howlers. I. Holzmann and I. Agostini monitored the selected trees on a monthly basis and estimated the abundance of each phenophase (young leaves, mature-senile leaves, flower buds, flowers, unripe fruits, and ripe fruits) (Agostini *et al.* 2010a). Based on the observed pattern of food availability, we divided the study into two periods: an "abundant season" (September–April) and a "lean season" (May–August; Agostini *et al.* 2010a).

Roaring Behavior

For each focal group, we recorded the occurrences of all roars, with the individual identity and the activity/context in which the vocalization was produced (feeding, resting, traveling, and intergroup encounter). When we heard a roar produced by another group that was out of sight, we identified the howler species that produced the roar based on acoustic differences (Whitehead 1995) and noted whether the focal group replied or not. We considered a roar to be in answer to a similar vocalization from another group if it was given from the start of the potentially eliciting signal until 10 min after the latter ended, based on our experience during an extensive preliminary study period (January 2005–December 2006).

We recorded encounters that took place among the four focal groups plus encounters between these groups and other neighboring groups (or solitary individuals). We included data collected in January 2005–November 2007 to increase the sample size since the number of intergroup encounters during the study period was low (31 intergroup encounters on 145 days for the four groups). We defined an intergroup encounter as an occasion when two or more groups or individuals came closer than 50 m to one another, with visual contact between them. We recorded if roars occurred and the identity of the individuals that participated, if so.

Spatial Data

For each scan sample and roaring bout, we also recorded the spatial position of the center of the group using a portable Garmin® GPS unit. We recorded readings only if the estimated position error was <20 m, obtaining readings for >90% of total scans for each study group. We analyzed ranging data using the Animal Movement Analysis Extension in ArcView 3.2. We estimated home range size for each focal group using the fixed kernel method (Agostini *et al.* 2010b), which represents the home range as the smallest area that incorporates a set percentage of the utilization distribution (Kernohan *et al.* 2001; Worton 1989). The 95% contour represents the home range, and the 50% contour represents the most intensively used areas or core areas (Hooge *et al.* 1999).

Statistical Analyses

We performed χ^2 tests to assess the independence of roaring bouts during conspecific and heterospecific group encounters and responses to roars from groups out of sight (conspecific and heterospecific). In some cases, and depending on the hypothesis tested, we pooled groups and species because the data set was too small to conduct one analysis for each group/species. We considered the roaring bouts in these analyses as independent events because 1) >10 min had passed between successive howling bouts of the same group, 2) successive howling bouts of the same group occurred at different locations, and 3) we recorded roars from two different groups that occurred one in response to the other as a single event.

We also used a χ^2 test to compare observed vs. expected roaring frequencies between the lean and the abundant season (expected frequencies were based on the amount of time spent following the groups in each season).

To test whether howlers roar more frequently in the vicinity of clumped food sources than a same number of randomly chosen positions, we compared the distance from the roaring positions and from the same number of randomly chosen locations (from the scan sample data set) to the closest clumped food resource (ripe fruits or new leaves) for each group. We measured the distances from roaring bouts and random positions to the closest clumped food resource for each monthly sample during which howlers both produced a roaring bout and used clumped food sources. We defined clumped food resources as spatially confined food sources, e.g. a tree crown, where the majority of the group spent at least two consecutive scans, averaging >20 min of food consumption. We measured distances from roaring locations and random positions to the closest clumped resource using ArcView, where we mapped all three elements — roaring, random, and clumped resources positions— within the home range of each group for the entire study period. To test whether roaring positions were closer to spatially clumped feeding resources than to the same number of random positions, we performed a 2-way ANOVA with season as another independent factor. We transformed distances to natural logarithms to conform to a normal distribution.

We also used χ^2 tests to evaluate whether the frequency of roars of adult males and participation in roaring bouts by adult females is dependent on the presence of unweaned infants in the group or not.

We report 2-tailed tests with α set at 0.05, performed using Statistica 5.5 (Statsoft, Inc.).

All research reported in this manuscript complied with the protocols approved by the appropriate institutional Animal Care and Use Committee, and adhered to the legal requirements of Argentina. All research protocols were reviewed and approved by the Ministry of Ecology and Natural Resources of the province of Misiones.

Results

We recorded a total of 79 vocalization bouts over 145 d in the four groups over the study period: $N=45$ by brown howler groups and $N=34$ by black-and-gold howler

groups. Roars occurred in the following contexts: spontaneous roars ($N=53$), inter-group encounters ($N=17$), and responses to roars from other groups that were out of sight ($N=9$).

We recorded 47 intergroup encounters during the study and the preliminary periods combined (January 2005–November 2007): $N=7$ between brown howler groups, $N=11$ between black-and-gold howler groups, and $N=29$ between groups of both species. Encounters were mostly between established groups ($N=43$) and occasionally between an established group and a solitary male ($N=4$). Howler monkeys roared more during encounters with a group or individual of the same species (83.3% of 18 conspecific encounters) than during encounters with groups or individuals from the other howler species (20.6% of 29 heterospecific group encoun-ters, $\chi^2=17.63$, df=1, $P<0.0001$; Fig. 1a).

We recorded 49 interactions in which our focal group heard a roar from another group or individual (as ascertained by our hearing ability) with no encounter or visual contact ($N=4$ between brown howler groups/individuals, $N=10$ between black-and-gold howler groups/individuals, and $N=35$ between groups/individuals from both species). Groups of both species showed a clear pattern of response: they answered 57.1% of conspecific roars, but ignored roars from groups or individuals of different species except for one occasion ($\chi^2=19.65$, df=1, $P<0.0001$; Fig. 1b).

Fig. 1 Number of group interactions. (**a**) Conspecific and heterospecific group encounters during the preliminary study period and the study period combined. (**b**) Number of conspecific and heterospecific vocalizations answered.
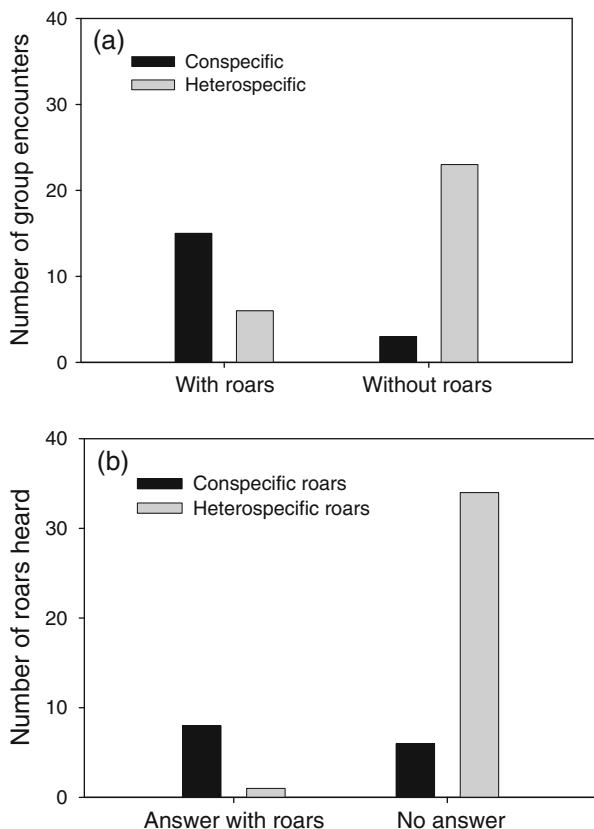
We found no seasonal pattern in vocalization bouts. The frequency of roars recorded in the abundant season and the lean season were not different from those expected by chance for either howler species ($\chi^2=1.58$, df=1, $P=0.20$ for brown howlers; $\chi^2=0.50$, df=1, $P=0.82$ for black-and-gold howlers; Fig. 2).

Roaring bouts did not show any relationship with the boundaries of the home ranges (Fig. 3). The distribution of roaring bouts was random for one group of each species (BL1 and BR2; Fig. 3a,d) and clumped in the other group of each species (BL2 and BR1; Fig. 3b,c). Roaring bouts were not spatially associated with clumped food resources within the home range. The distance of roar locations to clumped food sources was no different from the distance to random points ($F_{1, 112}=2.47$, $P=0.119$; Fig. 4); there was no seasonal effect on this pattern ($F_{1, 112}=2.40$, $P=0.124$) and no interaction with season ($F_{1, 112}=0.84$, $P=0.363$).

At least one adult female roared with the alpha male in 29.1% ($N=23$) of cases. Most bouts with female participation were spontaneous ($N=14$, including 9 from brown howler females and 5 from black-and-gold howler females) or intergroup encounters ($N=8$, including 6 during conspecific group encounter and 2 during heterospecific group encounters). On only one occasion in which the alpha male responded to another group out of sight (a conspecific vocal interaction) did a female roared with him.

Fig. 2 Roaring frequency in the lean and the abundant season for (a) the two brown howler groups and (b) the two black-and-gold howler groups.
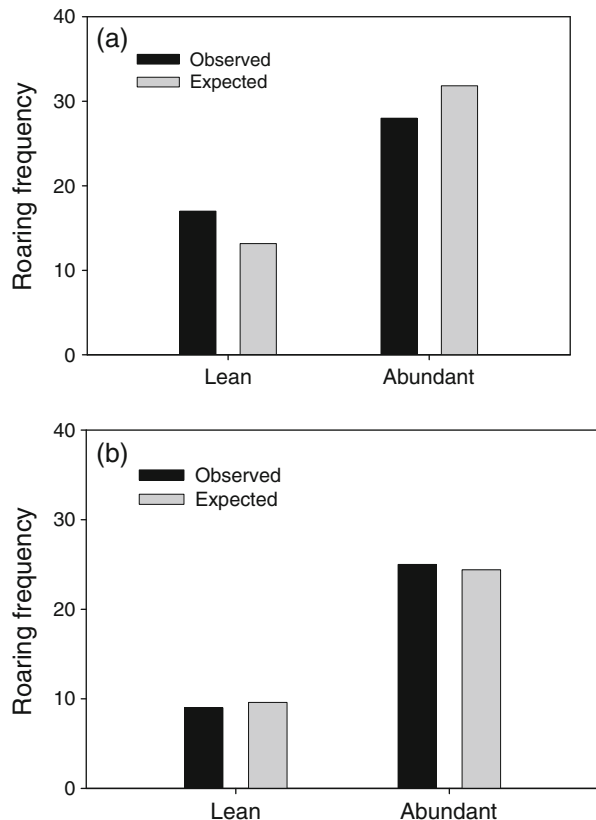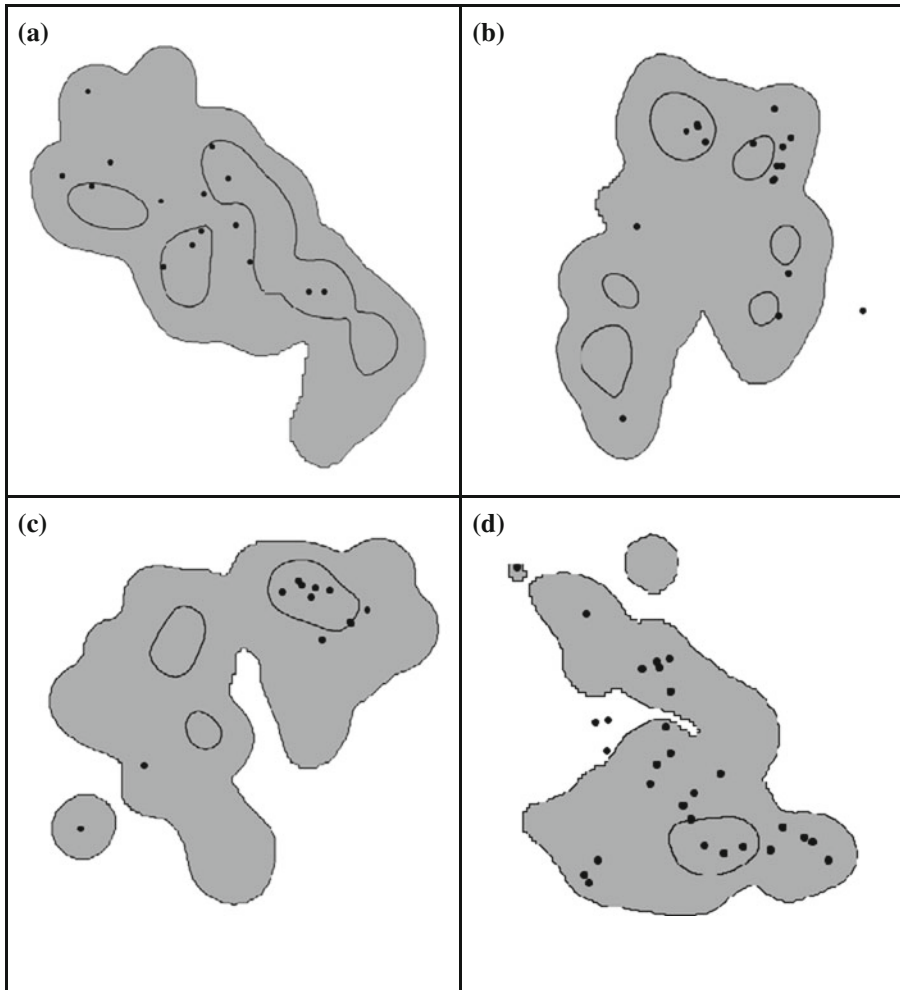
Fig. 3 Spatial distribution of roaring bouts (black dots) for each study group within its home ranges. (**a**) Black-and-gold howler group BL1. (**b**) Black-and-gold howler group BL2. (**c**) Brown howler group BR1. (**d**) Brown howler group BR2. Gray areas depict 95% kernel home ranges of each group; inner black lines depict the core areas (50% of the fixes; Agostini *et al.* 2010b).

Males did not roar more frequently when unweaned infants were present in the group in either brown howlers ($\chi^2 = 2.12$, df=1, $P=0.14$; Fig. 5a) or black-and-gold howlers ($\chi^2 = 019$, df=1, $P=0.88$; Fig. 5b). We found no differences between the roaring frequency of females with and without infants in either species ($\chi^2 = 0.01$, df=1, $P=0.92$ for brown howlers and $\chi^2 = 0.15$, df=1, $P=0.69$ for black-and-gold howlers; Fig. 6).

Discussion

Our data are consistent with the mate defense hypothesis: howlers roared consistently more during conspecific group encounters than during heterospecific ones, they
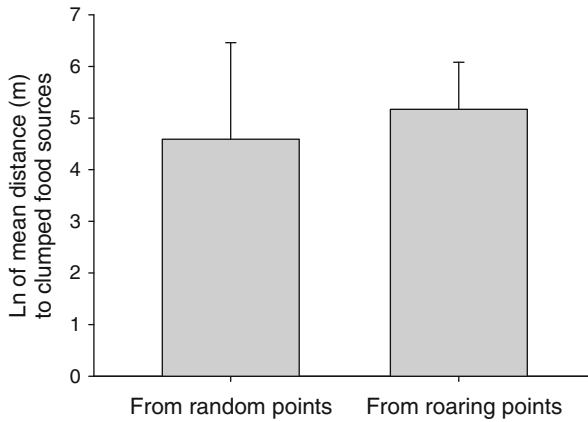
Fig. 4 Natural logarithm of the mean distance (m) to clumped food sources (ripe fruits or new leaves) from roaring locations and from the same number of random points ($N=58$). Whiskers above the bars represent the standard deviation.

roared almost exclusively in response to roars from conspecific individuals out of sight while ignoring most heterospecific roars, and whereas males called in all the roaring bouts, females participated in only a small portion of them. Our findings did
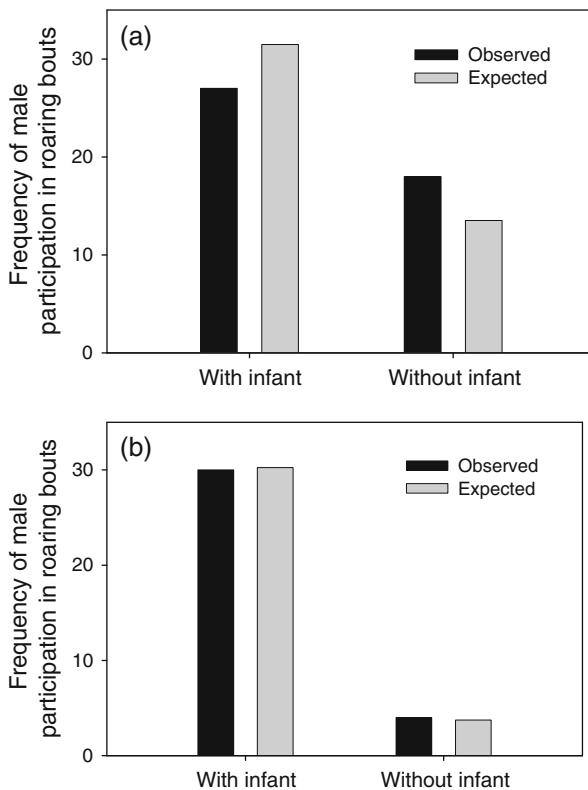

Fig. 5 Male roaring behavior with and without infants in the group. (**a**) Brown howler males. (**b**) Black-and-gold howler males.
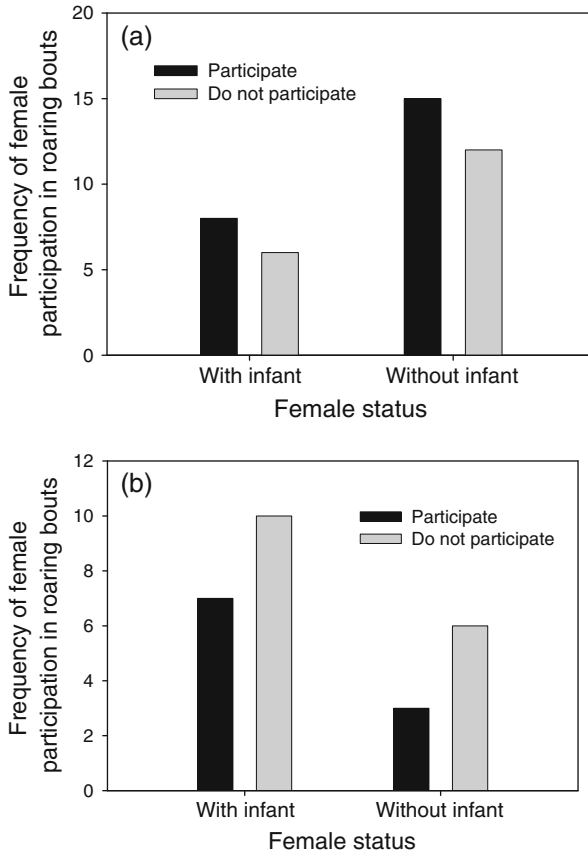
Fig. 6 Roaring behavior of mothers with unweaned infants and females without offspring. (a) Brown howler females. (b) Black-and-gold howler females.

not support the space/food defense hypothesis because neither species roared more during the time of the year when food was less available, howlers did not roar more from the boundaries of their home ranges, and there was no spatial relationship between clumped food resources and roaring bouts. Our results did not support the infanticide avoidance hypothesis because the presence of unweaned infants in the group did not influence the roaring behavior of males or females.

There is an ample trophic niche overlap between the two howler species at our study site, with high potential for interspecific food competition (Agostini *et al*. 2010a). Dietary overlap of specific food items was high: 45.64% (SE=2.97) according to the percentage index and 0.60 (± 0.05 SE) according to the Morisita-Horn index (C_H) (Agostini *et al*. 2010a). This overlap did not vary significantly between the abundant and the lean season and was similar between groups of the same species and groups of different howler species (Agostini *et al*. 2010a). Indeed, the dietary overlap between the two species in El Piñalito was among the highest in comparisons with other primate species pairs studied in sympatry (Agostini *et al*. 2010a). If roaring behavior in howlers evolved to ensure exclusive access to food resources, we would expect howler groups to consider any other neighboring groups as potential

competitors, irrespective of species identity. In contrast, our results show that, during group encounters, roars were produced almost exclusively between groups of the same species.

Howler groups at El Piñalito did not encounter one another more often during times of the year when food was less available (Agostini *et al*. 2010b). Further, despite the fact that the howlers' food availability changed notably throughout the year, marking distinct seasons of food abundance and food scarcity (Agostini *et al*. 2010a,b), roaring frequency did not vary between seasons, indicating that howlers at our site did not roar more to protect valuable food in times of food shortage. These results differ from studies of *Alouatta guariba clamitans* (Chiarello 1995) and *A. seniculus arctoidea* (Sekulic 1982b), which roar more during the dry season as a result of more frequent intergroup encounters over scarce food resources, e.g., trees bearing new leaves or fruiting figs. These differences in the differential use of roars during times of food scarcity may arise as a result of different patterns of food distribution or competitive regimes (direct vs. indirect) among sites. Trees with fruits and new leaves, the main food sources used by howlers in Chiarello's (1995) and Sekulic's (1982b) study sites during the lean season, tend to be spatially clumped resources that could be defended and monopolized by a group. Roars may be used to signal ownership of these concentrated sources and may help accrue uncontested access to them. However, at our study site, during the lean season, both howler species rely heavily on mature leaves, a less spatially clumped resource, and much less on fruits and new leaves (Agostini *et al*. 2010a).

Howlers at El Piñalito seem not to use a territorial strategy to defend their food resources within the home range, unlike *Alouatta guariba clamitans* in Brazil and *A. pigra* in Belize (da Cunha and Jalles-Filho 2007; Horwich and Gebhard 1983) because groups did not roar more frequently from the home range boundaries, as would be expected as the least costly strategy to defend an area of exclusive use of food resources (Brown 1964). Despite the nonrandomness of roaring bouts clearly evident in two of the groups, the pattern of roars of both groups was more clumped in some places within the home range. These clumped roars can be explained by the overlap between conspecific home ranges of neighboring groups. Group BL2 apparently concentrates its roaring sessions in the small portion of its home range overlapping with the only other black-and-gold howler group (BL1) in the area (no other groups of this species overlap with groups BL1 and BL2; Fig. 7). Group BR1 (Fig. 3c) roared frequently in a part of the home range where several conspecific group encounters with a group not under study took place. This portion of the home range of the BR1 group may also have overlapped with a third conspecific group. This further suggests that roars are used to communicate with neighboring groups of the same species.

If the function of roars is to defend access to adult females within each group, then the targets of the roars should be conspecific males (Sekulic 1982a), which are potential competitors for the resident alpha male. Nonbreeding adult males (either solitary or resident in other howler groups) can be considered the main challengers to the alpha male's control of females in a group (Sekulic 1982a). These potential threats were present in our study: Three of our focal groups contained nonbreeding adult males, and four of the encounters reported here were between an established group and a solitary male. Extragroup matings have been observed in howler monkey
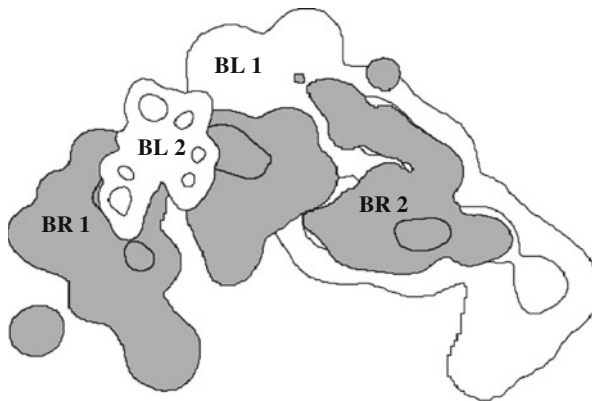
Fig. 7 Home ranges of the four focal groups.

species (Kowalewski and Garber 2010; Sekulic 1983), which further indicates that the alpha male should avoid allowing other adult males to approach his group of females. During our study, we observed two extragroup mating events, both during conspecific group encounters (Holzmann, *pers. obs.*). The nature of conspecific group encounters with roars compared with the almost absence of roars during heterospecific ones supports the mate defense hypothesis. The fact that howlers almost never responded to roars produced by another group that was out of sight when they belonged to a different species provides further evidence for the mate defense hypothesis.

Most of the group encounters (29 of 47) and the vocalizations putatively heard by the focal groups emitted by groups out of sight (35 of 49) reported here were between groups of different species. This may result from the fact that groups of different howler species showed an extensive home range overlap at El Piñalito, while groups of the same species exhibit little or no spatial overlap (Fig. 7; Agostini *et al.* 2010b), reducing the opportunities to encounter or hear groups of the same species. This pattern of home range overlap also supports the idea that howlers tolerate individuals that do not represent competitors for mates despite the fact that they may represent food competitors (Agostini *et al.* 2010a,b), providing further support for the mate defense hypothesis.

As we expected under the mate defense hypothesis, males participated in roaring bouts more frequently than females. Strong male agonistic behavior toward foreign groups, with low female participation, observed in other howler species (*Alouatta pigra, A. seniculus arctoidea*: Kitchen 2006; Sekulic 1983) also supports the hypothesis that roars act as an aggressive display more related to mate competition than to food competition.

The strongest test of whether roaring serves as a defense of females would be to look at roaring behavior during periods when at least one resident female is reproductively active. However, we observed only six copulations over the entire study period. Further, although Van Belle *et al.* (2009) showed that black howler females concentrate copulations during the periovulatory period, when conception is most likely, there is also evidence that in some howler populations females copulate during the entire cycle (Herrick *et al.* 2000; Kowalewski and Garber 2010). Reproductive

activity thus may not be a reliable indicator of probability of conception/paternity. Finally, the absence of a definite breeding season in howlers (Di Bitetti and Janson 2000; Strier *et al.* 2001) precluded an analysis of a general correlation between vocal behavior and breeding season.

Males at El Piñalito did not roar more frequently when unweaned infants were present in the group, unlike in *Alouatta pigra* (Kitchen 2004). In the case of females, roaring with the males, may be important to strengthen the alpha male–female bond, increasing the possibility that the male acts protectively toward their infant, as proposed for *Alouatta seniculus arctoidea* (Sekulic 1983). If female participation in choruses helps signal higher fighting ability of the group, this may also decrease the likelihood of group takeover attempts by outside males. If this is the case, females with unweaned infants should be expected to roar more frequently than other adult females. As an alternative, the probability of male takeover of a group and consequent infanticide may be positively related to the number of females in a howler's troop (Crockett and Janson 2000). Thus, females with unweaned infants may do better by hiding their presence in the group and not roaring together with their group mates. Our results do not support any of these predictions: females brown and black-and-gold howlers with unweaned infants do not differ from other adult females in their probability of roaring. A study of *Alouatta pigra* shows similar results and suggests that the best predictor for female participation in aggressive intergroup interactions is not the presence of unweaned infants, but the number of males in their group in relation to the number of males in an intruding group as simulated by playbacks: Females do not participate when the numeric odds are against their group, demonstrating that females were able to assess the group fighting ability through the roars and decide when to participate or not (Kitchen 2006). Thus, the presence of unweaned infants seems not to be a predictor of the female participation in roaring bouts. Almost all infanticide in howlers is related to alpha male replacements (Crockett 2003; Crockett and Sekulic 1984). During the almost 3 yr we spent following the groups we saw no alpha male replacements and no attempts by an outsider male to enter any of the focal groups. It is possible that this stable social milieu explains the lack of a relationship between roaring behavior and the presence of unweaned infants in the group: Males and females had no need to protect their infants through their roars and that results would differ under different social conditions.

Finally, howlers of both species at our study site had a low howling rate when compared to howler groups elsewhere (Chiarello 1995; da Cunha and Byrne 2006; da Cunha and Jalles-Filho 2007). This difference may result from the low population density at El Piñalito: 0.10 ind/ha for brown howlers and 0.15 ind/ha for black-and-gold howlers (Agostini *et al.* 2010b). During a line transect survey we conducted at this site, we observed a very low encounter rate with howler groups in comparison with other sites (Arditi and Placci 1990; Chiarello and de Melo 2001): 0.3 groups/10 km for brown howlers and 0.4 groups/10 km for black-and-gold howlers (Agostini 2009). The low population density at our study site may explain why roaring behavior is apparently used to defend mates but not food, as proposed for other study sites. It is possible that in sites where population densities are higher, the food competition function of the roaring behavior becomes more prominent.

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