

**SUBORDINATE MALE BLACK HOWLER MONKEY (ALOUATTA
PIGRA) RESPONSES TO LOUD CALLS: EXPERIMENTAL
EVIDENCE FOR THE EFFECTS OF INTRA-GROUP MALE
RELATIONSHIPS AND AGE**

by

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Summary

When assessing a group's overall fighting ability, functional group size (*i.e.* the number of cooperative members willing to confront opponents) may be more important than actual group size. Despite obvious benefits, group members do not always act in a collective manner. For example, participation by subordinate male black howler monkeys (*Alouatta pigra*) in natural group-level contests is highly variable. Using playback experiments, we examined whether alpha-subordinate relationships, number of intruders, or age affected subordinate contest participation. Long-term census data were used to estimate the nature and duration of intra-group male associations. Some subordinate males had long-term relationships with

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the alpha that existed prior to residence in the current group or they were the alpha male's grown sons. Other subordinate males were in more recent associations with the alpha that formed under seemingly antagonistic circumstances (e.g. the deposed alpha or his grown sons). We found that subordinate males in long-term alpha-subordinate associations had stronger howling and approach responses than males in short-term associations. Younger long-term associates had the strongest reactions to simulated intruders, similarly aged short-term associates rarely responded, and older males in both association categories responded at intermediate levels. We discuss the variable strategies of subordinate male black howlers and suggest that males who were more likely to participate in group-defence might be gaining direct or indirect fitness benefits by group living.

Keywords: black howler monkey, loud calls, multi-male, group-level contests.

Introduction

Experimental evidence has demonstrated that asymmetry in relative group size can predict the pattern of escalation in group-level contests in some animals (lions: McComb *et al.*, 1994; chimpanzees: Wilson *et al.*, 2001; black howler monkeys: Kitchen, 2004) and observational evidence suggests that this may be a determining factor in the outcome of inter-group disputes in many species (e.g. carnivores: Harrington & Mech, 1979; Kruuk & Macdonald, 1985; Packer *et al.*, 1990; primates: Cheney, 1987; Isbell, 1991; birds: Black & Owen, 1989). However, a group of cooperating individuals should be more successful in contests than an equal sized group of non-cooperators. Hence, functional group size may be more important than actual group size when assessing competitors. If both cooperating and non-cooperating strategies were more successful when rare, defectors might be maintained in a population by frequency-dependent selection (e.g. Barnard & Sibly, 1981; Boyd & Richerson, 1988). Consequently, various strategies might exist among individuals within the same population.

In multi-male societies with reproductive skew, alpha males are predicted to contribute more to group-defence than subordinate males (e.g. Nunn, 2000; Nunn & Lewis, 2001), but subordinate male participation might be influenced by their relationship with the alpha male. Based on benefits derived through inclusive fitness (Hamilton, 1964), subordinates in coalitions with male relatives should participate in inter-group contests more than those unrelated to their male companions. Among unrelated males, subordinates who have disproportionately low access to reproductive females should be less

likely to assist the alpha during inter-group encounters than subordinates in non-kin groups with only minor reproductive skew (e.g. Vehrenkamp, 1983). For example, although both kin and non-kin coalitions of male African lions demonstrate fairly unconditional cooperation during group defence (Grinnell *et al.*, 1995), coalitions of relatives have higher reproductive skew than groups of unrelated males (Gilbert *et al.*, 1991; Packer *et al.*, 1991). Finally, alpha males might be more likely to foster such cooperative relationships (e.g. tolerate mating by subordinates) with familiar, long-term companions than with recent or antagonistic partners (e.g. Reeve *et al.*, 1998; Johnstone *et al.*, 1999; Eshel & Shaked, 2001).

In multi-male groups of black howler monkeys (*Alouatta pigra*), intra-group relationships among the two to three co-resident males range from antagonistic to affiliative (Horwich & Gebhard, 1983). When confronted by another group or by coalitions of one to three 'take-over' males, any or all adult members of a howler group may respond by participating in loud call (howling) displays (Horwich & Lyon, 1990; Kitchen, 2000). In such encounters, individuals risk being supplanted by other groups from feeding resources or home ranges, or risk being evicted by take-over males and losing the benefits gained by group living. For individuals with a reproductive investment in the group, the threat of infanticide in this species (Brockett *et al.*, 1999) further increases the cost of failure to resist a take-over.

Group-defence by howling is an inherently risky behaviour in that it reduces opponents' uncertainty about the defender's location and fighting ability. In addition, loud (88 dB at 5 m) and prolonged howling bouts (sometimes in excess of 1 h), accompanied by vigorous physical displays are probably energetically costly to produce. This is particularly likely given that a large portion of a howler's diet consists of leaves laden with secondary compounds (Glander, 1978; Silver *et al.*, 1998), yet their digestive tracts are not highly specialized and are more similar to those of frugivorous primates (Chivers & Hladik, 1980; Milton, 1980). Based on the lack of ready energy available from their diets, howlers are reported to minimize even basic activities such as travel (Milton, 1980; reviewed in Sekulic, 1982a). Howler monkeys can further escalate howling displays by approaching their rivals, thereby increasing their risk of chases and physical fights with more proximate opponents. Although likely energetically expensive, howling displays are less costly than physical fights, where individuals risk injury and even

death (Kitchen, 2000; see also red howlers, *A. seniculus*: Crockett & Pope, 1988).

Howling choruses are not only potentially costly, but they are also honest indicators of the minimum number of males; a single male cannot mimic multiple calling males because individual contributions are staggered. According to game theoretical models (*e.g.* Maynard Smith & Parker, 1976), howler groups could use a reliable signal such as a howling display to assess relative group-level fighting ability and avoid contests they would likely lose. In natural group-level black howler interactions, one male (defined here as the 'alpha' male, see Methods) participates in group-defence more frequently than other group members. Despite the fact that additional assistance by 'subordinate' males might be crucial in size-sensitive confrontations with intruders, their participation in howling displays is facultative. Of 112 howling bouts produced by alpha members of multi-male groups, only 58.9% included a howling subordinate male (Kitchen, 2000). Additionally, some subordinate males cooperated more consistently than others. In one group a subordinate joined the alpha in more than 80% of 22 natural inter-group encounters, in another group a subordinate male participated during 59% of 22 encounters, and in a third group a subordinate never joined the displaying alpha during 13 encounters.

Predictions

Using experimental playback techniques, we explored whether the high variability in subordinate male contributions toward group-defence could be explained based on their relationship with the alpha, the number of intruders they faced, or their age.

Intra-group male relationships

Data from the related red howler monkey (Pope, 1998) suggest that, as in the African lion model, male howler monkey coalitions may be made up of related or unrelated partners. Whereas among male lions participation in group-defence is invariant but reproductive skew is low among non-kin, reproductive skew is high in all coalitions of red howlers (Pope, 1990). Assuming the same reproductive skew in black howler monkeys (reviewed in Kitchen, 2004), we examined whether the relationship among males in the same group explained their variable participation in group-defence.

When presented with recordings of howling strangers, we predicted that subordinate males in 'long-term associations' with alpha males, presumably their relatives or their coalitional partners, would have stronger responses (in terms of howling or approaching) than subordinates in relatively new alpha-subordinate associations, formed under seemingly antagonistic circumstances (association categories defined in Methods).

Number of intruders

If animals assess relative group-level fighting ability, then their response should change based on the number of rivals they face. Playback studies on the responses of African lions (females: McComb *et al.*, 1994; males: Grinnell *et al.*, 1995) and chimpanzees (Wilson *et al.*, 2001) provide evidence that members of social units use loud calls to gauge the size of opposing groups. In black howlers, experimental evidence suggests that alpha males avoid escalating contests when they are outnumbered (Kitchen, 2004; see also red howlers: Sekulic, 1982b). Similarly, we predicted that subordinate male black howlers would participate in group-level contests (by howling and/or approaching speakers) more when their group faced a single rather than multiple simulated intruders, thus when their group outnumbered intruders and their personal risks of fighting were low. Alternatively, subordinate males might be more likely to join in group-defence when faced with multiple simulated intruders, thereby participating when their help was most valuable in preserving the integrity of the group.

Subordinate male age

Finally, age could affect stamina and condition, and we predicted that younger subordinate male black howlers would be more likely to invest in potentially costly howling displays and approaches than older males. Any effect of age is unlikely independent of the male-male relationship factor described above. In fact, the most powerful test of the relationship factor would be a comparison of young males in each relationship category. Because the births of these males are indicated in the census data, we know their ostensible kin relationships. We predicted that young males born during an alpha male's tenure would have stronger responses to playback stimuli than similarly aged males born just prior to the alpha male's residence in the group.

Methods

Study site and population

More than 1000 black howler monkeys live in the Community Baboon Sanctuary, along an approximately 8 km stretch of the Belize River (Horwich & Lyon, 1990). Individual monkeys in the section of the sanctuary surrounding the village of Bermudian Landing, approximately 53 km northwest of Belize City, have been followed since 1990 (Horwich *et al.*, 2001). We can identify many adults in this subset of the population by the coloured beads on their ankle bracelets (James *et al.*, 1997; Horwich *et al.*, 2001).

Study subject

Black howler monkeys live in groups with one to three adult males, one to four adult females, and juveniles of various ages. It is difficult for human observers to establish a hierarchy among male black howler monkeys because of the paucity of intra-group interactions (reviewed in Crockett & Eisenberg, 1987). However, patterns of male 'take-over' and infanticide (*e.g.* red howlers: Crockett & Sekulic, 1984; Crockett & Janson, 2000; mantled howlers, *A. palliata*: Clarke *et al.*, 1994; black howlers: Crockett *et al.*, 1999; Horwich *et al.*, 2000) and reproductive skew (red howlers: Pope, 1990) suggest that there are salient differences among males in multi-male groups of howler monkeys. For that reason, we systematically classified males prior to this study as either 'alpha' or 'subordinate' based on two independent lines of evidence: the frequency of involvement in natural group-level interactions and male-female proximity. First, during systematic collection of recordings in 1994 we found that in multi-male groups, one male howled more frequently than the others. Whereas these 'alpha males' were frequently the only ones to howl either at dawn or during natural encounters, 'subordinate males' were never observed howling without accompaniment. Second, as in mantled howler monkeys (Jones, 1982; Zucker & Clarke, 1986), our prior observations suggested that males designated as alphas spent more time close to females than those classed as subordinate males. To confirm this observation, we randomly chose males and followed them for one-hour intervals throughout this study. Every 20 min during these 1 h time periods, we recorded the identity of the animal(s) positioned closest (within 10 m) to the focal male. We collected 801 nearest neighbour samples from 22 males in 13 groups, including nine pairs where both sampled males were living in the same group. For all nine pairs we found that the male previously categorized as 'alpha' had an adult female as a nearest neighbour more frequently than the males regarded as 'subordinate.' Additionally, although male-male approach-retreat interactions were observed on only five occasions, all corroborated these alpha-subordinate classifications. Finally, the fact that 'alpha' and 'subordinate' males differed in age (see age categories below) further substantiates the distinction between them. In this paper, we focus on the responses of males designated as subordinate. Only one male per group was designated as alpha and the responses of these males are presented elsewhere (Kitchen, 2004).

As in the well-studied red howler monkeys (*e.g.* Pope, 1998; reviewed in Crockett & Eisenberg, 1987; Crockett & Janson, 2000), long-term observational data on individually marked black howler monkeys indicates that alpha-subordinate relationships vary within and between groups (R. Horwich, R. James, K. Glander & R. Crockett, unpubl. data; Horwich *et al.*, 2000). First, if an alpha male's tenure is long enough his reign may coincide with the sexual maturity of his sons. Second, black howler males may take-over a group alone or as members of two- or three-male cooperative coalitions (Horwich *et al.*, 2000). Although at

TABLE 1. Design of experimental playbacks on ten subjects

Long-term alpha-subordinate associations			Short-term alpha-subordinate associations		
Identity	Age	Simulated intruders	Identity	Age	Simulated intruders
UB3	Young	X	X	Young	X
UB2	Young	X	X	Young	X
UWE	Young	X	O*	Young	X
LWA	Old	X	X	Old	X
LWE	Old	X	X	Old	X

* One male was not tested in the three-intruder condition because he emigrated before the completion of experimental trials.

least some multi-male coalitions consist of animals from the same natal group, natal coalitions are not necessarily made up of relatives because of bisexual dispersal in this species (Brockett *et al.*, 2000). Third, although intruding male black howlers that successfully take control of females within a group often oust the alpha and other male residents, sometimes the deposed alpha and/or his grown sons continue to reside in the group.

Using ten years of census data collected by two of us (R.H.H. & R.A.J.) we were able to estimate the origin of the relationship between 10 subordinate males and the alpha male in their group (Table 1). Five subordinates were classified in a 'short-term' association category because they had been with the alpha male for less than 4 years at the beginning of the study and had been resident in the group prior to his take-over (see also Pope, 2000, for mean duration of related versus unrelated partnerships in red howler monkeys). All subordinate males that we tested in this category were former alpha males (two males) or the sons of the former alpha males (three males) that were deposed by the current alpha male. The remaining five subordinate males were classified in a 'long-term' association category. Three of these males had been born during the alpha male's tenure and were therefore his purported sons. The two other subordinate males had been associated with the alpha for more than 5 years at study onset. One of these subordinate males had initially been captured and marked several years prior to this study while residing in a different group with the alpha male, possibly their natal group. The second subordinate male moved into the current group at the same time as another male (who subsequently became the alpha), resulting in the eviction of the two male residents. This pattern suggested that these two intruders had formed a coalition prior to residence in this group. Of the five males in each alpha-subordinate association category, four lived in three-male groups. Two other subordinate males in the population were not used in this study because there was not enough information on their relationship with the alpha male to use for classification.

Age was determined using long-term census data and tooth wear estimates made during previous capture (R. Horwich, R. James & K. Glander, unpubl. data). When trials began, alpha males in this population ranged from 9 to 18 years (average = 13.6 years), whereas all subordinate males in this population were either <8 y old (*i.e.* 'young' mean = 5.1 years) or >14 y old (*i.e.* 'old' mean = 16.7 years). In each alpha-subordinate association

category, three subordinate males were classified as young and two as old (Table 1). We saw no indication that males otherwise differed in terms of their health (e.g. based on bot fly infestation).

Small juvenile black howler monkeys (less than nine months of age) are vulnerable to infanticide (reviewed in Kitchen, 2004). We had planned to test the effect of vulnerable juvenile presence on subordinate males hearing the calls of potentially infanticidal non-neighbours. However, only three of ten subjects resided in groups in which juvenile presence changed during the study period. Five other subjects always had juveniles present in their group and the two remaining subjects resided in groups without juveniles (subjects in both conditions were equally distributed between the two alpha-subordinate association categories). Although preliminary analysis pooling males showed no significant effects of juvenile presence (Kitchen, 2000, unpubl. data), the lack of individuals tested under both circumstances precludes a proper test of hypotheses.

Modification of loud calls

Despite their small size (males average 7 kg and 1136 mm, $N = 42$; R. James, R. Horwich and K. Glander, unpubl. data), howling bouts are extremely loud, prolonged and low in frequency (mean emphasised frequency = 689 Hz; Kitchen, 2000; see also Whitehead, 1995). The hyoid apparatus and larynx are extremely enlarged in males of this genus and the air sac that extends into the hollow hyoid likely acts as a resonating chamber (Schön Ybarra, 1988; Fitch & Hauser, 2003). Howling bouts contain loud call periods separated by pauses up to 60 s in duration (Kitchen, 2000). Loud call periods consist of almost continuous, intense vocalisations for up to 114 s (mean = 8.7 second, $N = 290$ loud calls from 15 males). During loud call periods, 'roars' are intermittently produced. Roars are the lowest frequency and most sustained vocalisations in howler repertoires and are accompanied by vigorous physical displays (Whitehead, 1989, 1995; Kitchen, 2000). Because female loud calls are distinct, playback stimuli used here did not include female vocalizations; however, the responses of females to playback stimuli are presented elsewhere (Kitchen, 2000, unpubl. data).

One of us (D.M.K.) recorded male howling bouts from August–September of 1994 using a Sennheiser MKH-70 directional microphone (frequency response: 50–20,000 Hz) and a Sony TCD-D7 digital audio tape-recorder. All recordings were made directly below and facing the caller. From these recordings, we chose bouts for stimulus construction from five single males and two pairs of males that were all elicited within a few seconds of the onset of another group howling. These recordings were all made when groups were 100–200 m apart because so few good quality recordings were collected when interacting groups were closer. Take-overs are only rarely observed, and as a consequence it was not feasible to restrict stimuli to this context. However, because new groups can move into an area (Horwich *et al.*, 2001) and infanticidal take-overs can often be by neighbours (Horwich *et al.*, 2000), both lone males and males residing in bisexual groups present a similar threat. Still, recordings of males responding to distant bisexual groups might contain acoustic features that signal a 'low intensity contest' and using these recordings might have weakened responses to all playback conditions.

Using Sound Edit 16 bio-acoustic software (Walker *et al.*, 1987), we cut approximately 3 min of good quality loud calling (*i.e.* containing roar vocalizations and free from background noise) from original howling bouts and then repeated this sequence once to create modified howling bouts (mean duration = 6.12 min). Using Canary (Charif *et al.*, 1980), we

found no significant differences between original and modified bouts based on measurements including bout duration, duration of loud-call periods, percentage of bout comprised of loud calling, roaring rate, and mean roar duration (Kitchen, 2000).

It is still probable that acoustical differences exist among calling bouts produced by individual males, and these differences could indicate fighting ability or motivation (e.g. howlers: Kitchen, 2000; D. Kitchen, S. Kenney, R. Wilson, unpubl. data; baboons: Kitchen *et al.*, 2003; Fischer *et al.*, 2004). To control for this potentially confounding effect, we mixed single-male calls with two-male calls to produce pairs of recordings in which the same male called alone and in a group of three (*i.e.* A_1/A_3 , B_1/B_3 , C_1/C_3 , D_1/D_3 , E_1/E_3). We used a natural multi-male howling bout as a template, with onset of individual voices offset by a few seconds. Each of the ten howling bouts was a sequence of loud calls (mean loud call duration \pm SE = 20.14 \pm 8.17 s) interspersed with pauses (mean pause duration \pm SE = 4.34 \pm 0.73 s).

When measured under field conditions, amplitudes of recordings did not differ statistically from natural vocalization levels, and there was no effect of number of callers or caller identity on amplitude (measured using a Realistic™ 33-2055 sound level meter, C-weighting, referenced to 20 μ P). There was no statistical difference between single- and three-male calls in several measures including bout duration, duration of loud call periods, roaring rate, and mean duration of roar (Kitchen, 2000). However, the percentage of the total bout comprised of loud calling naturally increased with additional staggered callers.

Experimental design

With the aid of field assistants, one of us (D.M.K.) completed playback experiments during the dry seasons (January–June) of 1995–1997. Subordinate males in each association category were played the howling bouts of one and three unfamiliar males (Table 1). We attempted to test each male with the maximum number of paired recordings possible, depending on their location within the study site (*i.e.* males in the southwest could hear two pairs of recordings from the northeast). This resulted in 45 trials (sample size by pair: $N_A = 8$; $N_B = 11$, $N_C = 15$, $N_D = 6$, $N_E = 5$). However, to avoid pseudoreplication (Hurlbert, 1984), we used the mean male response in each condition (one or three intruders) and no male was played the same pair of recordings twice.

Playback stimuli were broadcast with two Bose 151 Environmental speakers raised on 8 m collapsible fibreglass poles and concealed in vegetation fairly close to subjects (mean = 56.1 m, range = 31–92 m; following Whitehead, 1987, 1989). Speakers were placed 5 m apart when broadcasting three-male recordings, which realistically simulated many natural displays (see Kitchen, 2004).

Although intruding males can challenge residents from anywhere in the home range, there is some indication that encounters between neighbouring groups tend to occur along home range boundaries or in overlap areas, and that they are most intense near important feeding resources (Horwich & Gebhard, 1983; Horwich & Lyon, 1990; unpubl. data; see also red howlers: Sekulic, 1982a; mantled howlers: Whitehead, 1989). We attempted to conduct all trials in these types of areas. In a further attempt to maximize responses, we played only calls of non-neighbours to each focal group. Accordingly, groups in the northeast end of the study site were presented only with recordings collected in the southwest end and vice versa. Because a large pasture (approximately 0.5 km wide, the diameter of one to two home ranges) separates the northeastern and southwestern ends of this otherwise continuous site, it is unlikely that groups from the extreme ends see or hear each other regularly. However, calls

can be heard for over a kilometre (Horwich & Gebhard, 1983) depending on the direction a male faces when calling, habitat, and ambient conditions. To further ensure that males treated these playback recordings as non-neighbours, we attempted to set up broadcasting equipment in directions from focal groups that would be a unique azimuth from which to hear the male(s) on the recording (*i.e.* anywhere in an area approximately 270° surrounding the focal group). Although subjects may have heard the recorded males in the past, they were not currently neighbours and thus callers represented a threat to residents (*see above*).

We randomised the order in which recordings were played to any given group. All experiments were conducted in the morning, when animals were quietly resting or feeding, and followed a search of the surrounding area to avoid conducting trials when other groups were nearby. We also avoided testing any groups that had howled in the 1-hour interval preceding the experiment. We aborted any trial during which the focal group had a natural interaction with another group ($N = 1$) or began moving off into impenetrable bamboo forest just prior to a trial ($N = 2$). To reduce habituation, we maintained a minimum 7-day interval between consecutive experiments on the same group and a 2-day minimum period between experiments with adjacent groups or with use of the same recording. This simulated encounter rate was far less frequent than the natural encounter rate between groups (Horwich & Gebhard, 1983; Silver *et al.*, 1998; Kitchen, 2004). Monkeys never responded to equipment alone, which for various reasons (*e.g.* dead batteries) was frequently set-up without a trial being conducted ($N > 10$).

Data collection

During playback trials, an observer recorded all occurrences of vocalisations and movements (of at least 5 m out of original tree toward or away from the speaker) by a focal subordinate male as an estimate of his willingness to escalate in a contest. Start-times of responses were subtracted from onset of playback recordings to determine latency. If no reaction occurred within one-hour after recording completion, latency to respond was recorded as 3960 s (*i.e.* the mean duration of recordings plus 1-hour response time). We measured duration of responses from the onset of behavior. Approach rate was estimated as meters per second, facilitated by the sanctuary's marked trail system (Horwich *et al.*, 2001). The proportion of speaker-subject distance covered during approaches was determined after experiments using a cloth measuring tape.

Statistics

We used non-parametric analyses (SYSTAT, 1997) to test for an effect of number of intruders, alpha-subordinate association category, subordinate male age, and two potentially confounding variables (speaker-subject distance and number of females per group) on subordinate male responses. To examine the relative influence of predictor variables, we used an analysis of covariance (Sokal & Rohlf, 1981). A backward stepwise selection process (using the general linear model command in SYSTAT, 1997) selected the best model. Although alpha was set at 0.05, we also discuss any marginally significant results (*i.e.* $0.050 < \alpha < 0.100$).

Results

General results

Of the 45 trials, ten resulted in an approach, six of which included a howling response, and one trial resulted in only a howling response. Males who approached covered an average of 57% of the speaker-subject distance (range = 17–100%). Those that advanced to within view of the speaker paid no apparent attention to the equipment and often continued to move past it with vigilant posture, seeming to search for the source of the sound. Six of the seven howling responses began before the end of the approximately 6-min recordings. Thus, in most trials subordinates who escalated contests by howling were 'answered' by the recorded male(s).

Seven of the ten males tested responded at least once, including all five males in long-term alpha-subordinate relationships. Subordinate males did not simply mirror the responses of alpha males; subordinate males only responded to 61.1% of the 18 trials in which the alpha male also responded, similar to the rate at which subordinates joined alphas during natural intergroup encounters (58.9% of 112 encounters; Kitchen, 2000).

Intra-group male relationships

Subordinate males in long-term associations with alphas had shorter latencies to start howling (Mann-Whitney test: $U_1 = 22.0$, $N = 10$, $p = 0.034$; Fig. 1). Although males in long-term associations howled longer than those in short-term associations, this result failed to reach statistical significance ($U_1 = 6.0$, $N = 10$, $p > 0.100$; Fig. 1). Subordinates in long-term associations had shorter latencies to start approaching ($U_1 = 25.0$, $N = 10$, $p = 0.007$; Fig. 2), approached at faster rates ($U_1 = 1.0$, $N = 10$, $p = 0.013$; Fig. 2), and ended approaches closer to speakers ($U_1 = 1.0$, $N = 10$, $p = 0.013$; Fig. 2) than subordinates in short-term associations.

Number of simulated intruders

Although males generally had a stronger response to one than to three simulated intruders (Table 2) these results failed to reach statistical significance (Mann-Whitney U test, $N = 19$: howl latency $U_1 = 33.5$, $p > 0.100$; howl duration $U_1 = 53.0$, $p > 0.100$; approach latency $U_1 = 30.0$, $p > 0.100$; approach rate $U_1 = 58.5$, $p > 0.100$; distance travelled toward speakers

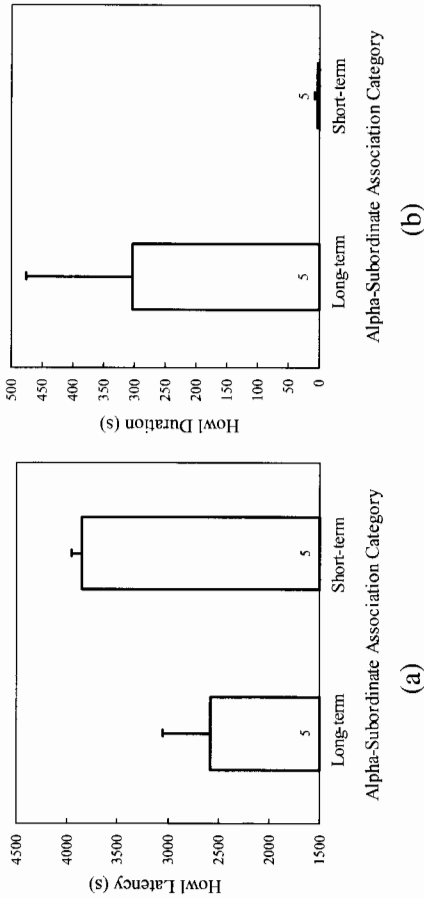


Fig. 1. Mean \pm SE (a) latency and (b) duration of howling bouts by subordinate male black howler monkeys in different associations with the alpha male (defined in text).

TABLE 2. Mean \pm SE subordinate male response based on number of simulated intruders broadcast in playback recordings

Response	One intruder ($N = 10$)	Three intruders ($N = 9$)
Howl latency (s)	2880.93 \pm 459.47	3564.61 \pm 261.77
Howl duration (s)	183.55 \pm 112.49	146.50 \pm 97.55
Approach latency (s)	2727.33 \pm 469.48	3449.72 \pm 367.26
Approach rate (m/s)	1.68 \pm 0.52	0.68 \pm 0.52
Approach distance (%)	16.23 \pm 4.74	7.41 \pm 6.43

$U_1 = 62.0$, $p > 0.100$). It was striking that the only subordinate males to respond to multiple male intruders were those in long-term associations with the alpha.

Subordinate male age

We examined whether the responses of subordinate males in different alpha-subordinate association categories were affected by their age. We found that young males in long-term associations were more likely to howl ($\chi^2 = 6.00$, $N = 6$, $p = 0.014$) and tended to approach the speakers more often ($\chi^2 = 3.00$, $N = 6$, $p = 0.083$) than similarly aged males in the short-term association category (Fig. 3). Older subordinates in both association categories occasionally responded (Fig. 3). Although old long-term associates

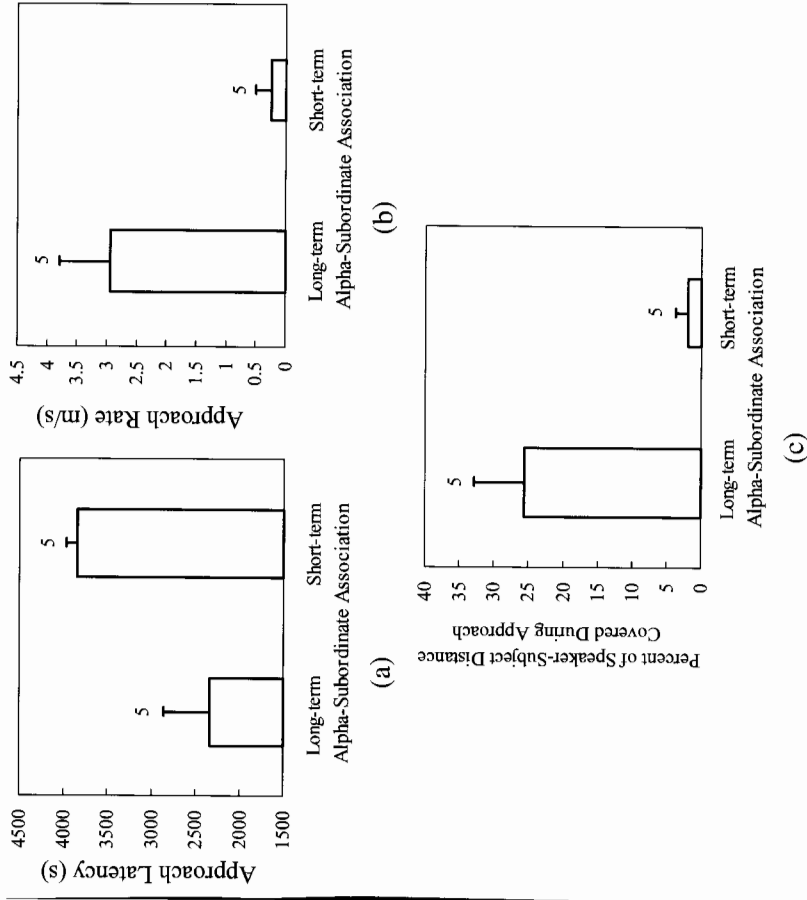


Fig. 2. Mean \pm SE (a) approach latency, (b) approach rate, and (c) percent of speaker-subject distance covered during approaches by subordinate male black howler monkeys in different associations with the alpha male (defined in text).

approached speakers more often than old short-term associates, they vocalized less often and these differences did not reach statistical significance ($\chi^2 = 1.33$, $N = 4$, $p > 0.100$).

Potentially confounding effects

Although male responses had a weak negative relationship with both the number of females in the group (Spearman correlation, $N = 10$: howl latency $r_s = 0.470$; howl duration $r_s = -0.295$; approach latency $r_s = 0.440$; approach rate $r_s = -0.412$; distance travelled toward speakers $r_s = -0.412$) and the mean speaker-subject distance ($N = 10$: howl latency $r_s = 0.357$; howl duration $r_s = -0.075$; approach latency $r_s = 0.213$; approach rate

TABLE 3. Results of an analysis of covariance testing the relative influence of several factors on subordinate male responses

Response variable*	Predictor variable†	R ²	DF	F	p
Howl latency (s)	Alpha-subordinate association category	0.45	1,16	5.99	0.026
	Association × age		1,16	3.64	0.014
Howl duration (s)	Alpha-subordinate association category	0.60	1,15	5.75	0.030
	Subordinate age		1,15	5.75	0.030
	Association × age		1,15	6.21	0.025
Approach latency (s)	Alpha-subordinate association category	0.38	1,17	10.43	0.005
Approach rate (m/s)	Alpha-subordinate association category	0.30	1,17	7.19	0.016
Approach distance (%)	Alpha-subordinate association category	0.38	1,17	10.41	0.005

* For each response variable, a backward stepwise selection identified the best model.

† The number of intruders, speaker-subject distance, and number of adult female group members had no significant effects.

male were more likely to participate in group-level contests (by howling and/or approaching simulated intruders), had shorter latencies to start howling and approaching speakers, howled for longer, approached speakers at faster rates, and ended approaches closer to speakers than subordinates in short-term associations.

Although it is clear that there is something qualitatively different about long-term alpha-subordinate associations in black howler monkeys, the exact nature of these relationships is not fully understood. The most plausible explanation is that at least some subordinate male howlers in the long-term association category were in fact related to the alpha male, just as the long-term census data suggested. Even with a despotic alpha male, the inclusive fitness benefits gained by helping the group deter intruders, and thereby enhancing the future reproductive success of an alpha male relative, might make a 'helper' strategy worthwhile (Hamilton, 1964; Pope, 2000). Accordingly, young males in long-term associations with the alpha were more likely to participate overall (by howling and/or approaching) than similarly aged short-term associates. Because of bisexual dispersal, relatedness within a group of black howlers can presumably be low. Consequently a young subordinate male born in a group prior to the current alpha's tenure may only be related to his mother and her offspring, whereas a young subordinate born during the current alpha's tenure may be his son and thus related to all current and future offspring in the group. Helping to protect the offspring of a

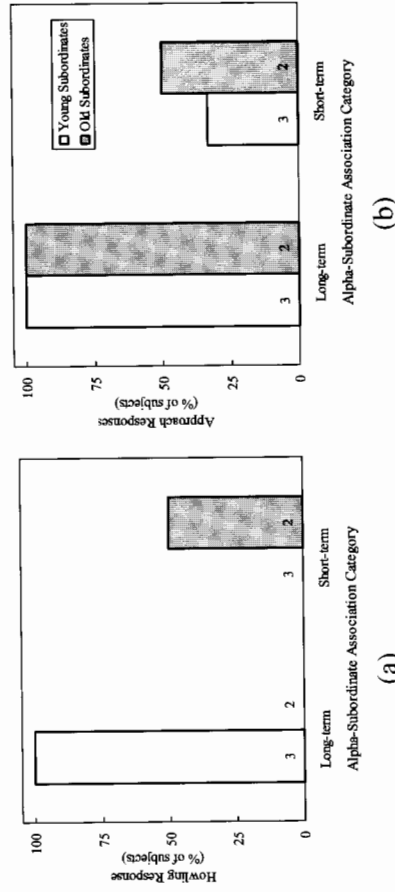


Fig. 3. The percent of males tested that ever (a) howled during playback trials or (b) approached the speakers during playback trials based on their age and their association with the alpha male (association categories defined in text). 'Young' subordinates were <8 y old and 'old' subordinates were >14 y old when trials began.

$r_s = -0.401$; distance travelled toward speakers $r_s = -0.351$), these responses failed to reach statistical significance (*i.e.* all $p > 0.100$).

Relative influence of multiple predictor variables

We used an analysis of covariance to examine the relative influence of the above three predictor and two potentially confounding variables. The alpha-subordinate association category was found to influence all howling and approach responses (Table 3). Subordinate age and the age-association interaction term were found to influence howling responses (Table 3).

Discussion

During observations of natural encounters between groups of black howler monkeys, the participation by subordinate males in howling displays was varied (Kitchen, 2000). Certain subordinate males in this population were never observed joining in group-defence and the participation of others ranged from occasional howling to frequent participation in displays and fights. Experimental evidence indicates that the variation in participation among subordinates may be related to the nature and duration of the alpha-subordinate association (for similar observational evidence in red howlers see Sekulic, 1982b). Subordinates in long-term associations with the alpha

relative is an especially beneficial strategy when dispersal options are limited (reviewed in Krebs & Davies, 1993; see also Pope, 2000). Black howlers at this site occur at high density (170 individuals/km² by study completion; Horwich *et al.*, 2001; see also Treves, 2001) and competition among males for group possession is probably intense.

It is curious that, despite potentially low fitness benefits, older subordinate black howler monkeys in both alpha-subordinate categories occasionally assisted alpha males in group-defence. Whereas older short-term associates began their relationship with the alpha male antagonistically, census data indicated that older long-term associates might be coalitional partners with the alpha male. However, because of potentially low intra-group relatedness in black howler monkeys, even males who form coalitions within their natal group may be unrelated or only distantly related. Further, unlike the lion model where reproductive skew is low in unrelated coalitions (Gilbert *et al.*, 1991; Packer *et al.*, 1991), paternity tests on red howler monkeys revealed that alpha males in multi-male groups father all offspring (Pope, 1990) and observational evidence suggests a similar trend in black howler monkeys (reviewed in Kitchen, 2004).

Perhaps older males in either category employed a 'best of a bad job' strategy (reviewed in Krebs & Davies, 1993); in other words, they occasionally helped in group-defence, particularly when costs were low (*e.g.* when facing one intruder), thereby maintaining the status quo rather than risk eviction by new intruding males. In these circumstances, even if the alpha male was unrelated and despotic there would be potential benefits to a subordinate male living in a group that would be lost if that male was forced out of the group altogether. Perhaps these older subordinate males contribute to group-defence while biding their time waiting to ascend to alpha position (*e.g.* in this population an alpha male killed by a dog was replaced by a subordinate male from within the group; unpubl. data; see also Pope, 2000). However, if howling by subordinate males were solely part of a 'sit-and-wait' strategy then we would predict all males to be equally likely to participate. Instead, young short-term associates rarely participated. While these young males may be well-equipped to disperse and take control of their own group, this might not be an option for older males past their prime, such as deposed alphas (but see Horwich *et al.*, 2000).

Despite the fact that the only subordinate males to approach multiple intruders were those in long-term alpha-subordinate associations, there was

no overall effect of number of intruders on responses. This result might be due to a flaw in the experimental design. While intruders outnumbered some of the subjects tested in the multiple intruder condition, other subjects lived in three male groups and thus were evenly matched with intruders in terms of number of males. The number of subjects available in both types of groups precluded a test of the effect of 'numeric odds' (*i.e.* relative numbers of defending to intruding males).

Because individuals in a group do not typically act in a collective manner, a group's overall fighting ability may be equivalent to the number of cooperative adults within the group who are willing to confront opponents. Therefore, it would be advantageous for group members to be able to predict their companion's behaviour during conflicts and avoid escalating when they were effectively outnumbered. Recent theoretical models have begun to take into account the alpha-subordinate and subordinate-subordinate relationships within a group when predicting collective action by companions during group-level contests (*e.g.* Johnstone *et al.*, 1999; Nunn, 2000; Eshel & Shaked, 2001; Nunn & Lewis, 2001). In black howler monkeys, we found that subordinate males did not simply mirror the alpha male response during simulated inter-group conflicts; rather their responses were affected by their relationship with him. Results presented elsewhere (Kitchen, 2000, unpubl. data) suggest that adult female black howler monkey participation in group-level contests is also influenced by the alpha-subordinate relationship. However, alpha male black howler responses were less influenced by the alpha-subordinate relationship than by the simple numeric odds (Kitchen, 2004). Hence, alpha males are apparently sensitive to the mere presence of subordinate males, perhaps because even uncooperative subordinates provide incidental support when conflicts escalate to physical fighting.

The multiple strategies that exist among adult male black howler monkeys facing extra-group competitors are analogous to the variable strategies observed in African lions. Whereas some lionesses reliably participated in pride defence by approaching intruders ('leaders'), other females consistently lagged behind, and still others joined part of the time (Heinsohn & Packer, 1995). This study suggested that lion 'leaders' could detect cheaters and perhaps they simply couldn't evict these individuals from the group. There is a similar possibility among howlers; based on close intra-group proximity during inter-group encounters, males could monitor the behaviour of co-resident males. Alternatively, 'freeloaders' might be tolerated in

howlers and lions because they assist in group-defence occasionally or because they contribute other benefits to group members (Nunn, 2000; Nunn & Lewis, 2001). For example, based on comparative data within the *Alouatta* genus, Treves (2001) found that the reproductive success of female howlers improved as the number of males per group (relative to the population mean) increased.

Overall, we found that the responses to simulated intruders by various members of black howler social groups were complicated yet predictable (see also Kitchen, 2004, unpubl. data) and cooperation appears to be driven by mutual gains. Responses were not based solely on companion behavior, but also depended on the individual's own costs and benefits of participating against another group of rivals.

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