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## Social and hormonal mechanisms underlying male reproductive strategies in black howler monkeys (*Alouatta pigra*)

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### ABSTRACT

We investigated the social and hormonal mechanisms underlying male reproductive strategies in two multimale–multifemale groups of black howler monkeys (*Alouatta pigra*) during a 14-month study in Palenque National Park, Mexico. Fecal glucocorticoid (fGC) and androgen (fA) levels were analyzed for 343 fecal samples collected from 14 males during their presence in the study groups. Neither immigrating males nor resident males that remained in the group had elevated fGC and fA levels during 11 observed male migration events, suggesting that competition over group membership was not associated with variation in the fecal hormonal levels of males. Instead, fGC and fA levels were significantly higher in males who maintained a central position in the group and had almost exclusive access to fertile females than in other resident males. These “central” males were responsible for maintaining close spatial associations and cultivating strong affiliative relationships with cycling, sexually active females but not with noncycling, sexually inactive females. “Noncentral” males did not form strong social relationships with either cycling or noncycling females and had no or very few mating opportunities. Our findings suggest that male black howler monkeys compete nonaggressively by fostering relationships with cycling females and that the elevated fGC levels of central males may be indicative of the social challenges involved in their indirect competition.

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### Introduction

Socioendocrine studies that investigate the interactions between hormones, behavior, and the social environment are critical for our understanding of proximate, physiological mechanisms underlying male reproductive strategies and individual variation in reproductive success (Bercovitch, 1999; Bercovitch and Ziegler, 2002). Such studies have generally investigated how direct male–male competition over access to mates is associated with male androgen levels (sensu challenge hypothesis; Wingfield et al., 1990, 2000) or glucocorticoid levels (sensu allostatic load; Goymann and Wingfield, 2004; see also Creel, 2001; Abbott et al., 2003). For example, in many social mammals in which males compete over high rank through frequent agonistic interactions, dominant males have higher androgen and glucocorticoid levels than subordinate males (e.g., African wild dogs, *Lycaon pictus*, Creel et al., 1997; wolves, *Canis lupus*, Sands and Creel, 2004; chimpanzees, *Pan troglodytes*, Muehlenbein et al., 2004; Muller and Wrangham, 2004a,b; mandrills, *Mandrillus sphinx*, Setchell et al., 2008; African elephants, *Loxodonta africana*, Rasmussen et al.,

2008; gray-cheeked mangabeys, *Lophocebus albigena*, Arlet et al., 2009). In other species, however, no rank-related differences in androgen or glucocorticoid levels have been observed (e.g., dwarf mongooses, *Helogale parvula*, Creel et al., 1996; alpine marmots, *Marmota marmota*, Arnold and Dittami, 1997; mountain gorillas, *Gorilla beringei*, Robbins and Czekala, 1997; Japanese macaques, *Macaca fuscata*, Barrett et al., 2002; meerkats, *Suricata suricatta*, Carlson et al., 2004; chacma baboons, *Papio hamadryas ursinus*, Beehner et al., 2006; red-fronted lemurs, *Eulemur fulvus rufus*, Ostner et al., 2002, 2008a).

These variable responses may be explained by the high costs and potentially detrimental effects associated with chronically elevated androgen levels (Grossman et al., 1991; Folstad and Karter, 1992; Marler et al., 1995; Braude et al., 1999; Muehlenbein and Bribiescas, 2005; Hau, 2007) and glucocorticoid levels (Sapolsky, 2002, 2005). For example, rank-related differences in hormonal levels may only be apparent during periods of heightened male–male aggression, such as during social instability due to rank reversals (e.g., chacma baboons, Bergman et al., 2005), immigration events (e.g., Verreaux's sifakas, *Propithecus verreauxi*, Brockman et al., 2001; chacma baboons, Bergman et al., 2005; ursine colobus monkey, *Colobus vellerosus*, Teichroeb and Sicotte, 2008; gray-cheeked mangabeys, Arlet et al., 2009), or reproductive competition (e.g., dwarf mongooses, Creel

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et al., 1992; ring-tailed lemurs, *Lemur catta*, Cavigella and Pereira, 2000; Gould and Ziegler, 2007; Verreaux's sifakas, Brockman et al., 1998, Fichtel et al., 2007, Assamese macaques, *Macaca assamensis*, Ostner et al., 2008b).

Consistent with the frameworks of the challenge hypothesis and allostasis, androgen and glucocorticoid levels do not differ among males in species with little aggressive competition over access to receptive females (northern muriquis, *Brachyteles hypoxanthus*, Strier et al., 1999; tufted capuchin monkeys, *Cebus apella nigrinus*, Lynch et al., 2002; spotted hyenas, *Crocuta crocuta*, Goymann et al., 2003a,b; Dloniak et al., 2006; moustached tamarins, *Saguinus mystax*, Huck et al., 2005). In these species, male fitness may be mediated by factors other than direct male–male aggression, and the hormone–behavior interaction underlying male reproductive strategies may be affected by additional variables such as nonaggressive forms of male competition and male–female relationships (Bercovitch, 1999; Dloniak et al., 2006). For example, androgen levels of male Japanese macaques were positively correlated with rates of noncontact aggression directed toward females, a behavioral tactic presumed to represent a form of sexual solicitation (Barrett et al., 2002). Similarly, despite low rates of male–male aggression in tufted capuchin monkeys, androgen and glucocorticoid levels of both dominant and subordinate males increased during the breeding season and were positively correlated with the frequency of female-maintained consortships and sexual behavior (Lynch et al., 2002). In spotted hyenas, where male dominance rank is maintained through a queuing convention and males rarely interact agonistically, males compete indirectly over mates by fostering long-term relationships with particular females and mate guarding them against other resident males during both receptive and nonreceptive periods (East and Hofer, 2001). Irrespective of their social status in the clan, male spotted hyenas that courted, mate guarded, or simply associated with a receptive female 30–60 min before blood sample collection (Goymann et al., 2003a) or up to 3 days before fecal sample collection (Dloniak et al., 2006) had significantly higher androgen levels than males that had not engaged in these interactions. In addition, fostering relationships with females and mate guarding might be sufficiently socially challenging to cause elevations in male glucocorticoid concentrations (e.g., Barrett et al., 2002; Lynch et al., 2002; Strier et al., 2003; Fichtel et al., 2007, Arlet et al., 2009).

In this study, we examine the influence of male–male competition and male–female relationships on male fecal androgen (fA) and glucocorticoid levels (fGC) in black howler monkeys (*Alouatta pigra*). Black howler monkeys live in relatively small groups of 1–3 adult males and 1–3 adult females (Van Belle and Estrada, 2006). Overt male–male competition is mostly confined to immigration events and intergroup encounters, which may result in injuries or even death (Horwich et al., 2000; Van Belle et al., 2008). Both single males and pairs of males have been observed to successfully immigrate into established groups, but coalitions of males are more successful than single males at taking over groups and evicting resident adult males (Horwich et al., 2000; Van Belle et al., 2008). Takeover attempts may be accompanied by infanticidal attacks (Brockett et al., 1999; Crockett, 2003) and both takeover attempts and intergroup encounters may involve extragroup copulations (Horwich, 1983; Horwich et al., 2000; Van Belle et al., 2009), indicating that encounters with extragroup males may pose substantial threats to the reproductive success of resident central males. The associated stressors during encounters with adjacent groups or male immigration attempts might be expected to lead to elevated fGC and fA levels in both remaining and immigrating males (Alberts et al., 1992; Sapolsky 1993; Brockman et al., 2001; Bergman et al., 2005; Arlet et al., 2009).

Resident black howler males seldom engage in agonistic and affiliative interactions with one another, and no agonistic dominance hierarchy can be discerned. Nevertheless, one resident male, herein referred to as the “central” male, has almost exclusive access to fertile

females, whereas “noncentral” males have few or no mating opportunities (Van Belle et al., 2008). Most sexual interactions occur during the periovulatory phases of ovarian cycles, and the sexual monopolies of central males are the result of the close spatial associations they maintain with ovulating females. Concurrently, females exhibit preferences for central males by actively soliciting them for sexual interactions during their periovulatory phases (Van Belle et al., 2009).

Little is known about the social relationships between male and female black howler monkeys or how these relationships and male hormone levels vary. If central males cultivate strong relationships with females as part of their reproductive strategies, then we predict that central males should engage in affiliative interactions with females more than noncentral males do, and should direct social interactions more frequently toward females than females do toward them. Such a reproductive strategy could lead to higher fGC and fA levels in central males compared to noncentral males, especially during periovulatory phases of female ovarian cycles when interacting with females might become increasingly more important for gaining sexual access to them. Alternatively, central males might not rely on cultivating strong relationships with females as part of their reproductive strategies. Under this hypothesis, we predict that central males should not affiliate with females more than noncentral males do or more than females affiliate with them and there should be no differences in the fGC and fA levels of central and noncentral males at any time.

## Methods

### Study site and subjects

This study was conducted from June 2006 through July 2007 in Palenque National Park (PNP), Chiapas, Mexico (17° 28'N, 99° 03'W). PNP encompasses 1771 ha of which 900 ha are covered by primary tall evergreen tropical rainforest and forest vegetation in various stages of regeneration. The remaining land consists of human-induced pasture lands (Díaz Gallegos, 1996). Mean annual rainfall is 2200 mm with a dry season between January and April (mean monthly rainfall = 62 ± 18 mm) and a wet season between May and December (mean monthly rainfall = 240 ± 106 mm; Estrada et al., 2002).

We studied two multimale–multifemale black howler groups (Balam and Motiepa groups), both of which underwent several changes in group memberships involving 13 males (Balam: seven changes involving 11 males; Motiepa: two changes involving two males; Van Belle et al., 2008). A 14th male was present in the Motiepa group throughout the study period. All individuals could be identified by permanent scars, broken fingers, or botfly marks. Based on the changes in male group membership, we recognized eight distinct periods in the history of the Balam group and three distinct periods in the history of the Motiepa group with 1–3 males present in each period (Table 1). For five of the nine periods with multiple males, one central male could be distinguished from one or two noncentral males by their behaviors, but no particular male maintained significantly closer associations with resident females in the other four multimale periods (Van Belle et al., 2008).

### Data collection

Behavioral data were collected on a total of 306 days (0630–1730 h) from June 11, 2006, through July 24, 2007, alternating observations between the two study groups every 2 days. We used 60-min focal animal samples (Altmann, 1974) to record continuously all activities of adults and subadults of both sexes in each group, along with the identity of all participants and directionality of social interactions. Activities were classified into the following general categories: resting, feeding, moving within and traveling between feeding or resting patches, vocalization, scent marking, social behavior, and out of sight. Social behaviors included agonistic interactions (pushing, chasing,

**Table 1**

The 11 distinct periods in the two study groups based on changing male group membership across the 14-month study.

Balam Group							
Period 1	Period 2	Period 3	Period 4	Period 5	Period 6	Period 7	Period 8
6 Jun 06– 25 Jul 06	26 Jul 06– 6 Aug 06	7 Aug 06– 27 Aug 06	28 Aug 06– 24 Sep 06	25 Sep 06– 27 Oct 06	28 Oct 06– 22 Nov 06	23 Nov 06– 17 Dec 06 <sup>c</sup>	11 Jan 07– 20 Jul 07
Central M						Central M	Central M
AM							
BA <sup>a</sup>	BT	PE	BT	VI	VI	DV <sup>a</sup>	GZ <sup>a</sup>
RO	RO	LO	PE	PE		LF	KR
PA	PA	PA	LO	LO			
AF							
LU <sup>b</sup>	LU	LU	LU	LU	LU	LU <sup>b</sup>	LU <sup>b</sup>
TE	TE	TE	TE	TE	TE	TE	TE <sup>b</sup>
MI <sup>b</sup>	MI	MI	MI	MI			
Motiepa Group							
Period 9	Period 10	Period 11					
19 Jul 06– 14 Mar 07 <sup>c</sup>	15 Mar 07– 2 Apr 07	3 Apr 07– 24 Jul 07					
Central M	Central M	Central M					
AM							
JP <sup>a</sup>	JP <sup>a</sup>	JP <sup>a</sup>					
BO		HG					
AF							
IS	IS	IS <sup>b</sup>					
MO	MO	MO					

The time spans and identities of males (AM) and females (AF) are given. In five multimale periods and one single male period, a central male (see text) could be distinguished from other males.

<sup>a</sup> Central males.

<sup>b</sup> Cycling females.

<sup>c</sup> No data were collected between 18 Dec 06 and 10 Jan 07.

grabbing, and fighting), affiliative interactions (playing, touching, and allogrooming), and sexual interactions (soliciting and copulating). We analyzed a total of 1832 focal hours (Balam: 1027 h; Motiepa: 805 h). We carried out instantaneous scan samples (Altmann, 1974) at 10-min intervals throughout the focal animal samples to record the identity of all independent neighbors at 0–1 m and at >1–5 m of the focal individual. We analyzed a total of 11,117 scan samples (Balam: 6328 scan samples; Motiepa: 4789 scan samples). Data on spatial dynamics were collected by recording the individuals responsible for all approaches and retreats within 1-m radius of the focal subject during the focal samples. We also recorded occurrences of encounters with adjacent groups and extragroup males ad libitum (see Van Belle et al., 2008 for more details on the data collection protocol).

#### Fecal sample collection and steroid analyses

Fresh fecal samples were collected from each adult male whenever the individual could be unambiguously identified and samples were not contaminated with urine (Hodges and Heistermann, 2003). This resulted in 343 fecal samples collected from 14 males at  $4.0 \pm 2.9$ -day intervals (no fecal samples were collected in July 2007). Fecal material was collected in 50-ml polypropylene vials, labeled with information on individual identity, hour and date of defecation, and immediately stored on ice. Steroids were extracted as wet feces at the field research house (after Strier and Ziegler, 1997; Strier et al., 1999; Ziegler and Wittwer, 2005). Samples were homogenized and weighed. A small amount (0.1 g) of wet fecal material was suspended in 5 ml distilled water:ethanol (50:50), followed by manually shaking for 5 min and centrifuging for 10 min at  $1000 \times g$ . The fecal pellet was discarded, while 1 ml of the aliquot was slowly pushed through a Preveil C18 Solid Phase Extraction (SPE) cartridge (Alltech Associates, Inc., Deerfield, IL). The SPE cartridges were stored at room temperature until analyses.

Steroid analyses were conducted at the Wisconsin National Primate Research Center—Assay Services Unit. The samples were washed with 1 ml distilled water, eluted from the SPE columns with 2 ml methanol, subsequently dried and resuspended in 1 ml ethanol. We used cortisol (Ziegler et al., 1995) and testosterone (Ginther et al., 2001) assays that have shown to provide reliable information on endocrine adrenal function and testicular function, respectively, in many platyrrhine, catarrhine, and prosimian primate species (e.g., Sousa and Ziegler, 1998; Strier et al., 1999; Ziegler et al., 2000; Lynch et al., 2002; Ziegler et al., 2004; Gould et al., 2005; Gould and Ziegler, 2007; Teichroeb and Sicotte, 2008). No captive animals were available for adrenocorticotrophic hormone (ACTH) or gonadotropin releasing hormone (GnRH) challenge tests for validation of the responsiveness of glucocorticoids and androgens. Previously, Martínez-Mota et al. (2008) have shown that black howler monkeys respond with elevated serum and fecal glucocorticoids after anesthesia was applied as a stressor. In addition, we performed High Pressure Liquid Chromatography (HPLC) analysis on fecal extracts to obtain information on the characteristics of glucocorticoid and androgen metabolites measured in the assays. For this, a 2 ml pool of male samples in ethanol solution was split in two halves. One half of the pool was fractionated by HPLC at 1 ml/min for a 60-min run, as reported in Strier et al. (1999). The other half of the pool was first subjected to solvolysis to free steroid metabolites from conjugates, as described by Ziegler et al. (1996) and Ziegler and Wittwer (2005), prior to fractionation by HPLC. Each fraction of both halves of the male pool was divided in two parts. One part was assayed by enzyme immunoassay (EIA) for cross-reactivity with the cortisol antibody, while the other part was assayed by EIA for cross-reactivity with the testosterone antibody. Comparing antibody reactivity between fractions subjected to solvolysis with those without solvolysis revealed that solvolysis was necessary prior to cortisol EIA but not prior to testosterone EIA. Cortisol activity occurred only in fractions containing cortisone and other glucocorticoids, while

testosterone activity occurred in fractions containing testosterone, dihydrotestosterone (DHT), and androstenedione. fGC and fA levels were positively correlated in only nine of the 14 males (range Pearson  $r = 0.51–0.82$ ,  $P \leq 0.05$ ). The absence of a correlation in the other five males ( $P > 0.05$ ), together with the absence of cross-reaction between the two assays indicate that they reliably detected adrenal glucocorticoid and testicular androgen output in black howler monkeys. For testosterone EIAs, sample volumes of 50  $\mu$ l of ethanol solution were dried and resuspended in assay buffer/enzyme solution, and aliquoted as 33% into each well. The standards were prepared in the same way. The same procedure was used for cortisol EIAs, except that sample volumes were 200  $\mu$ l.

For testosterone assays, mean  $\pm$  SE percent accuracy of howler monkey pooled fecal extracts added to the standard curve points was  $119.87\% \pm 3.89\%$  and serially diluted fecal extracts were parallel to the standard curve with no difference in slope ( $t_{27} = -1.49$ ,  $P > 0.05$ ). Intraassay and interassay testosterone coefficients of variation (CV) for a low pool were 1.7% and 15.2% and for a high pool 2.8% and 11.2%, respectively ( $n = 11$ ). Mean recoveries of added tritiated testosterone to wet fecal samples that were extracted as described above was 96.9% ( $n = 3$ ). For cortisol assays, mean  $\pm$  SE percent accuracy of howler monkey pooled fecal extracts added to the standard curve points was  $115.35\% \pm 2.51\%$  and serially diluted fecal extracts were parallel to the standard curve with no difference in slope ( $t_{24} = 1.77$ ,  $P > 0.05$ ). Intraassay and interassay cortisol CV for a low pool were 3.3% and 15.4% and for a high pool 2.7% and 15.8%, respectively ( $n = 10$ ). Mean recoveries of added tritiated cortisol to wet fecal samples that were extracted as described above was 50.6% ( $n = 3$ ).

#### Data analyses

Values of hormone concentrations were  $\log_{10}$  transformed to normalize the distribution and equalize the variances (Kolmogorov-Smirnov tests and Levene's tests:  $P > 0.05$ ), allowing the use of parametric tests. We report transformed hormone values throughout the text and figures. Samples were collected opportunistically because it was not feasible to collect all samples at roughly the same time of the day ( $n = 144 < 12:00$  h,  $n = 199 > 12:00$  h). Because the time of collection can influence fecal hormone concentrations (e.g., Sousa and Ziegler, 1998; Lynch et al., 2002; Muller and Wrangham, 2004a,b), we compared mean fGC and fA levels in samples collected before and after 12:00 h for each individual male via unpaired samples  $t$ -tests. No diurnal variation was found for either hormone in 12 of the 14 males. Significantly lower levels were found in morning than in afternoon samples for fGC values in one male (JP;  $t_{60} = -2.71$ ,  $P = 0.009$ ) and fA values in another male (PA;  $t_{20} = -3.11$ ,  $P = 0.006$ ). Separate analyses of morning and afternoon samples of these two males compared to samples from other males provided similar results, and we therefore report the data of their morning and afternoon samples combined. In order to examine possible seasonal influences on hormone concentrations, we calculated mean fGC and fA levels per male per month and compared the overall monthly means of individual males across the 13 months during which fecal samples were collected via one-way ANOVA tests. Monthly mean fGC and fA levels did not differ across the study months, indicating the absence of a seasonal pattern in hormonal levels (fGC:  $F_{12,41} = 1.71$ ,  $P = 0.10$ ; fA:  $F_{12,41} = 1.35$ ,  $P = 0.23$ ).

We used general linear mixed models (GLMM) to analyze whether hormone levels of immigrant males ( $n = 9$ ) differed during 1–15 days versus 16–30 days following their immigration and whether hormone levels of males remaining in their group throughout the immigration of other males ( $n = 5$  males, including three males who experienced two successive immigration events) differed between the 2 weeks prior to and the 2 weeks after immigration events. The rapid sequence of immigration events did not allow us to examine periods longer than 2 weeks. Random factors in the GLMM included male identity to

account for the repeated sampling of the same individual and immigration events nested within groups to account for the possibility of males migrating together in the same study group had correlated hormonal levels. In addition, we calculated rates of encounters with adjacent groups or extragroup males by dividing the number of encounters by the sum of contact hours for each study group per 2-week periods. If the study groups met with  $> 1$  social group or extragroup male, we counted these incidents separately. We used Pearson correlations to examine a possible correlation between biweekly rates of encounters and corresponding biweekly mean fGC and fA levels per male.

To examine whether central and noncentral males had different fGC and fA concentrations, we used a GLMM with male identity and periods nested within groups as random factors and male centrality as the predictor variable. We conducted these analyses during the periovulatory phases and the nonperiovulatory phases of female ovarian cycles separately and combined. At least one resident female was cycling during four of the five periods in which a central male was distinguishable (Table 1). Female fecal hormonal profiles indicated a mean ( $\pm$  SE) ovarian cycle length of  $18.3 \pm 1.4$  days, including a periovulatory phase defined as the estimated ovulation day  $\pm 3$  days (Van Belle et al., 2009). To examine whether male hormonal levels changed across different female reproductive phases, we conducted a GLMM with male identity and periods nested within groups as random factors, and periovulatory versus nonperiovulatory phases as the predictor variable for central males and noncentral males separately.

For the five multimale periods with a central male, we calculated the Hinde's index, the percentage of spatial associations, the rate of approaches, and the rate of social interactions for each central male–cycling female dyad ( $n = 6$ ), central male–noncycling female dyad ( $n = 6$ ), noncentral male–cycling female dyad ( $n = 8$ ), and noncentral

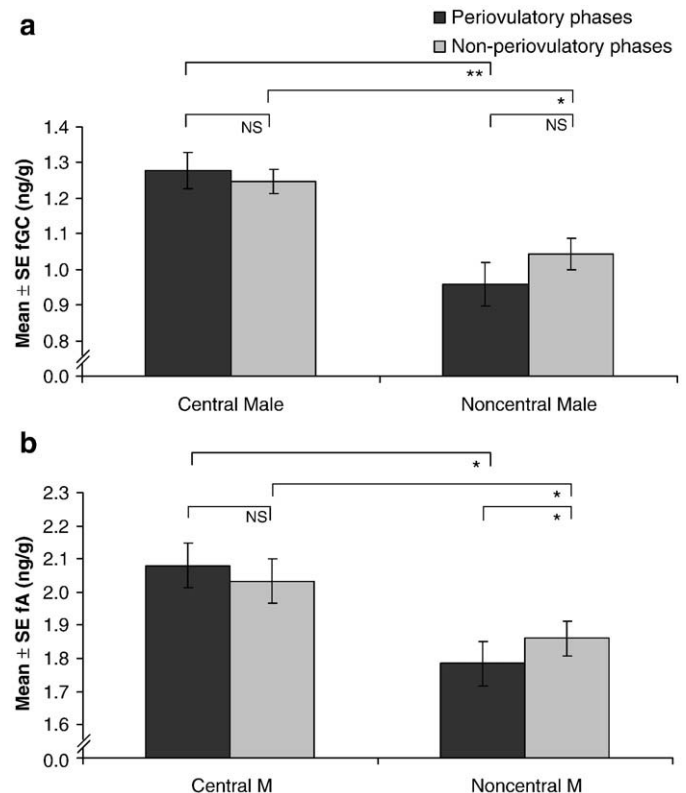
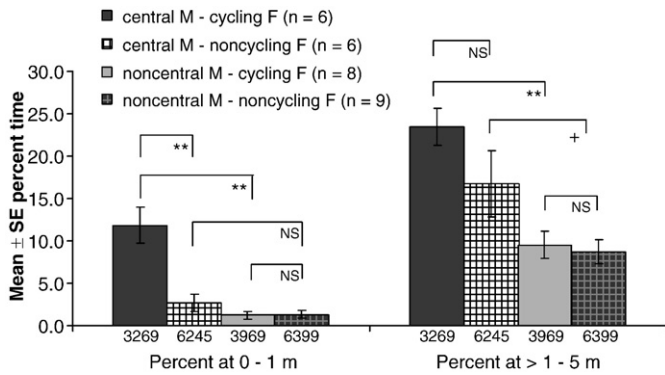


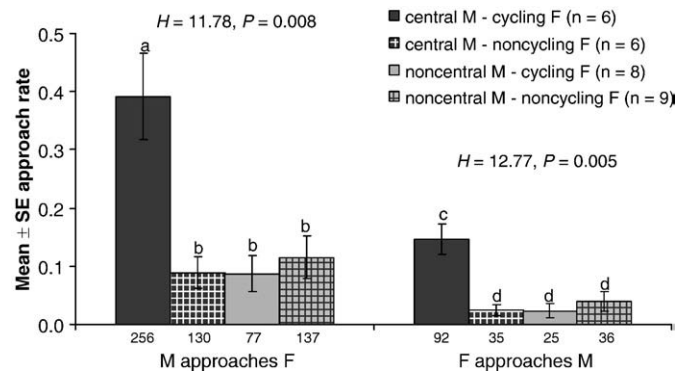
Fig. 1. Male mean  $\pm$  SE (a) fGC levels and (b) fA levels during periovulatory and nonperiovulatory phases of female ovarian cycles. Significance levels of general linear mixed models (GLMM) are shown. (\* $0.05 < P \leq 0.01$ , \*\* $0.01 < P \leq 0.001$ , NS  $P > 0.05$ ).



**Fig. 2.** Mean  $\pm$  SE percentage of time male-female dyads spent at 0–1 m and >1–5 m. Significance levels of pairwise Mann-Whitney *U*-tests are indicated (\*\* $P \leq 0.01$ ; + $P = 0.077$ ; NS  $P > 0.10$ ). Sum of scan samples for each male-female category is provided below bars. M = male, F = female.

male–noncycling female dyad ( $n = 9$ ). The Hinde's index (Hinde and Atkinson, 1970) measures which member of each dyad was responsible for maintaining close proximity and is the proportion of all the dyad's approaches displayed by the male minus the proportion of all the dyad's retreats displayed by the male. The index ranges between  $-1$  and  $+1$ , and a positive index indicated that the male was responsible for maintaining close proximity, while a negative index indicated that the female was responsible. Following Hill (1990), we calculated the Hinde's index only for dyads with a total of  $\geq 10$  observations of approaches and retreats by both individuals. This excluded two central male–noncycling female dyads, three noncentral male–cycling female dyads, and three noncentral male–noncycling female dyads for the Hinde's index analyses.

Dyadic spatial associations were calculated as the percentage of time a dyad was observed at 0–1 m and at >1–5 m during scan samples of both individuals. Dyadic rates of approaches or social interactions were calculated as the number of approaches or social bouts observed between dyads divided by the total sum of focal hours on both individuals (male as actor and recipient separately). We defined a social bout as one or more events of social interaction of the same category (i.e., agonistic or affiliative) between the same individuals separated by  $\leq 5$  min (Neville, 1972). Social bouts involving reciprocal interactions were partitioned between the two individuals relative to the proportion of time each engaged in the interaction. We compared mean percentages of spatial associations and mean rates of approaches and social interactions among the four dyad types with Kruskal-Wallis tests. If these tests were statistically significant, Mann-Whitney *U*-tests were used for post hoc pairwise comparisons. All statisti-



**Fig. 4.** Mean  $\pm$  SE dyadic approach rates of males toward females and females toward males among central male-female dyads and noncentral male-female dyads when females are cycling versus noncycling. Kruskal-Wallis statistics ( $H$ ,  $df = 3$ ) and corresponding  $P$ -values are shown. Post hoc pairwise comparisons (Mann-Whitney *U*-tests,  $df = 1$ ) are indicated with letters (a–b, c–d). Sum of approaches across dyads within each male-female category is provided below bars. M = male, F = female.

cal analyses were two-tailed with  $P \leq 0.05$ . Results are presented in mean  $\pm$  SE.

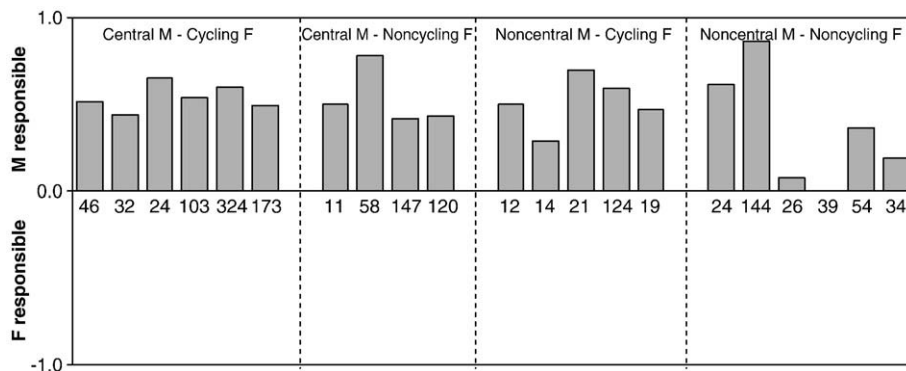
The research complied with protocols of the Animal Care Committee of the University of Wisconsin-Madison and the legal requirements of Mexico.

**Results**

*Hormonal correlates of male-male competition*

Immigrant males ( $n = 9$ ) had no significant differences in their fGC (1–15 days =  $1.19 \pm 0.07$  ng/g, 16–30 days =  $1.21 \pm 0.06$  ng/g, GLMM  $F_{1, 70.41} = 0.001$ ,  $P = 0.980$ ) and fA levels (1–15 days =  $1.93 \pm 0.05$  ng/g, 16–30 days =  $1.93 \pm 0.07$  ng/g,  $F_{1, 69.76} = 0.015$ ,  $P = 0.904$ ) between the first 2 weeks compared to the following 2 weeks after their immigration. Similarly, remaining males ( $n = 5$ ) did not differ in their fGC (before =  $1.08 \pm 0.09$  ng/g, after =  $1.16 \pm 0.07$  ng/g,  $F_{1, 67.58} = 2.80$ ,  $P = 0.099$ ) and fA levels (before =  $1.97 \pm 0.08$  ng/g, after =  $1.98 \pm 0.08$  ng/g,  $F_{1, 67.36} = 0.016$ ,  $P = 0.900$ ) between the 2 weeks before and after male immigrations into their groups.

Encounters with adjacent groups occurred at a rate of 0.029 ( $n = 45$ ) and 0.018 events/contact hour ( $n = 21$ ) for the Balam and Motiepa groups, respectively. The encounters with extragroup males occurred at a rate of 0.019 ( $n = 30$ ) and 0.004 events/contact hour ( $n = 5$ ) for the two study groups, respectively. Biweekly rates of encounters with either adjacent groups or extragroup males were not associated with changes in male hormonal levels (all  $P$ -values  $> 0.05$ ).



**Fig. 3.** The Hinde's index for each central male-female dyad and each noncentral male-female dyad when females were cycling versus noncycling. Positive indices indicate that the male was responsible for maintaining close proximity, whereas negative indices indicate that the female was responsible. Sum of approaches and retreats between each dyad is provided below bars. M = male, F = female.

### Hormonal differences between central and noncentral males

Central males had significantly higher fGC levels (mean =  $1.27 \pm 0.03$  ng/g, range = 1.16–1.35 ng/g) than noncentral males (mean =  $1.02 \pm 0.05$  ng/g, range = 0.85–1.14 ng/g; GLMM:  $F_{1,8.59} = 13.48$ ,  $P = 0.006$ ). Central males also had significantly higher fA levels (mean =  $2.08 \pm 0.05$  ng/g, range = 1.96–2.14 ng/g) than noncentral males (mean =  $1.84 \pm 0.06$  ng/g, range = 1.61–1.98 ng/g;  $F_{1,8.39} = 9.18$ ,  $P = 0.015$ ). These hormonal differences were evident independent of female reproductive states. Central males had higher fGC and fA levels than noncentral males during both periovulatory phases (fGC:  $F_{1,7.61} = 12.43$ ,  $P = 0.008$ ; fA:  $F_{1,6.80} = 8.47$ ,  $P = 0.023$ ; Figs. 1a–b) and nonperiovulatory phases of ovarian cycles (fGC:  $F_{1,6.23} = 10.95$ ,  $P = 0.015$ ; fA:  $F_{1,6.80} = 7.04$ ,  $P = 0.034$ ; Fig. 1a–b).

Changes in hormonal levels during times when at least one resident female was cycling versus times when no resident females were cycling could only be evaluated for one central male (JP) who was present in the Motiepa group throughout the study. His hormone levels did not change when resident females were cycling versus noncycling (fGC: lactating =  $1.38 \pm 0.04$  ng/g, cycling =  $1.27 \pm 0.05$  ng/g, pregnant =  $1.21 \pm 0.04$ , GLMM:  $F_{2,52} = 2.47$ ,  $P = 0.094$ ; fA: lactating =  $2.13 \pm 0.03$  ng/g, cycling =  $2.18 \pm 0.06$  ng/g, pregnant =  $2.24 \pm 0.05$  ng/g,  $F_{2,52} = 0.97$ ,  $P = 0.384$ ). The fGC and fA levels of central males did not differ between female periovulatory versus nonperiovulatory phases (fGC:  $F_{1,19.68} = 2.65$ ,  $P = 0.106$ ; fA:  $F_{1,25.27} = 0.25$ ,  $P = 0.618$ ; Fig. 1a–b). There were also no significant differences in the fGC levels of noncentral males across female ovarian cycles ( $F_{1,32.63} = 2.42$ ,  $P = 0.122$ ; Fig. 1a), but their fA levels were significantly lower during the periovulatory than during nonperiovulatory phases ( $F_{1,31.34} = 5.42$ ,  $P = 0.021$ ; Fig. 1b).

### Behavioral differences between central and noncentral males

Central males spent significantly more time in close proximity (0–1 m) to cycling females than noncentral males did. Central males spent significantly less time in close proximity to noncycling females than to cycling females, and they did not differ from noncentral males in their associations with noncycling females (Fig. 2). Central males spent significantly more time in the vicinity (>1–5 m) of cycling females and tended to spend more time in the vicinity of

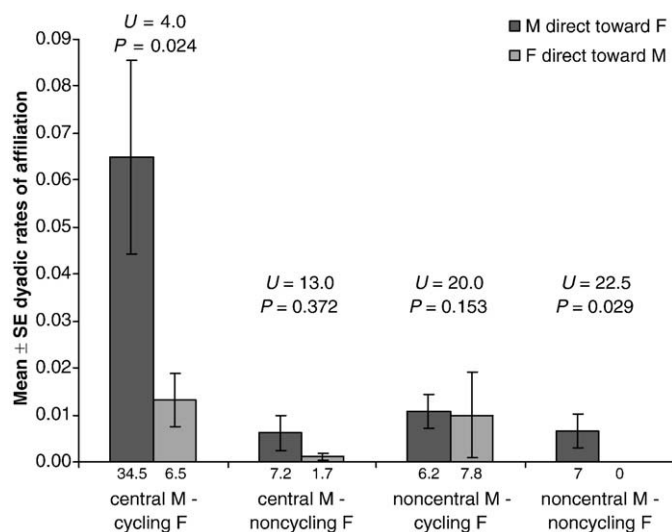


Fig. 5. Mean  $\pm$  SE dyadic affiliation rates of males directing toward females versus females directing toward males for central male–cycling female dyads ( $n = 6$ ), central male–noncycling female dyads ( $n = 6$ ), noncentral male–cycling female dyads ( $n = 8$ ), and noncentral male–noncycling female dyads ( $n = 9$ ). Mann-Whitney  $U$  statistics ( $df = 1$ ) and corresponding  $P$ -values are shown. Sum of affiliative bouts across dyads within each male–female category is provided below bars.

noncycling females than noncentral males spent with either cycling or noncycling females (Fig. 2). Yet, despite the differences in time males spent in close proximity to females, both central males and all but one noncentral male were responsible for maintaining close proximity to both cycling and noncycling females (Fig. 3).

Consistent with the greater time spent in close proximity, central males approached cycling females at significantly higher rates than noncentral males did. Central males approached noncycling females at significantly lower rates, similar to the approach rates of noncentral males to cycling and noncycling females alike (Fig. 4). Interestingly, females approached central males at significantly higher rates when they were cycling than when they were not, but they did not change their approaches rates toward noncentral males across different reproductive states (Fig. 4).

Central males also engaged in affiliative interactions with cycling females at higher rates than noncentral males did ( $n_1 = 6$ ,  $n_2 = 8$ ,  $U = 4.0$ ,  $P = 0.010$ , Fig. 5). Central males engaged in affiliative interactions with noncycling females at significantly lower rates than with cycling females ( $n_1 = n_2 = 6$ ,  $U = 1.0$ ,  $P = 0.006$ , Fig. 5), similar to the rates at which noncentral males affiliated with both cycling ( $n_1 = 6$ ,  $n_2 = 9$ ,  $U = 26.5$ ,  $P = 0.949$ , Fig. 5) and noncycling females ( $n_1 = 6$ ,  $n_2 = 9$ ,  $U = 26.5$ ,  $P = 0.949$ , Fig. 5).

Overall, males tended to direct affiliation toward females at higher rates than females did toward them. This was statistically significant for central male–cycling female dyads and noncentral male–noncycling female dyads, but not for central male–noncycling female dyads and noncentral male–cycling female dyads due to the low rates of affiliative interactions (Fig. 5). Males and females engaged rarely in agonistic interactions, and there were no significant differences among the four types of male–female dyads (central M–cycling  $F = 0.13 \pm 0.05$  bouts/h,  $n = 9$  bouts, noncentral M–cycling  $F = 0.03 \pm 0.02$  bouts/h,  $n = 2$  bouts, central M–noncycling  $F = 0.04 \pm 0.02$  bouts/h,  $n = 3$  bouts, and noncentral M–noncycling  $F = 0.08 \pm 0.05$  bouts/h,  $n = 4$  bouts, Kruskal-Wallis  $H = 3.74$ ,  $df = 3$ ,  $P = 0.291$ ).

### Discussion

Our results suggest that the reproductive strategies of male black howler monkeys involve indirect competition based on the relationships males foster with sexually active females. Although complete biological validations of our hormonal assays for this species were precluded by the absence of captive black howler monkeys, the behavioral data are consistent with the lack of evidence of direct competition from our analyses of male hormones. In addition, these results were thoroughly validated for measurement in black howler monkey feces and indicate there is no interference with measuring the hormones for this species.

### Hormonal correlates of male–male competition

In our study, hormone levels of immigrant males did not change during their first month in the group, nor did those of remaining males change between the weeks before and after the immigrations of other males. These preliminary findings suggest that male–male competition over group membership is not reflected in the fecal hormonal levels of black howler males. However, the unusual rapid turnover of male group membership in one of the study groups might have affected our ability to evaluate the influence of immigration on male hormonal levels. Furthermore, we cannot exclude the possibility that changes of male hormonal levels during these events are so transient that they are not reflected in hormonal measures from fecal material (Whitten et al., 1998).

Nonetheless, differences in hormonal responses to male migration events among primate species seem to reflect differences in male reproductive strategies. For example, in chacma baboons with pronounced male hierarchies, male immigrations and takeovers of the

alpha position were associated with increased male–male aggression and elevated fGC levels in both high and low ranking males. Furthermore, dispersing males had higher fGC levels in the month following immigration but not in the month preceding emigration or at other times (Bergman et al., 2005). However, hormonal levels of male moustached tamarins, who do not establish male hierarchies, did not significantly increase after the death of the breeding female and the subsequent immigration of a new female and emigration of two males (Huck et al., 2005), similar to black howler monkeys.

We also found no association between rates of intergroup or solitary male encounters and mean fGC and fA levels in either central or noncentral males. Similarly, neither male moustached tamarins nor male redfronted lemurs responded to intergroup encounters with elevated fA levels. In these studies, intergroup encounters occurred relatively frequently, and might not have posed unpredictable, threatening events (Ostner et al., 2002, 2008a; Huck et al., 2005). In ursine colobus monkeys, however, encounters with extragroup males may form a threat to resident males because extragroup copulations, attacks on immatures, and takeover attempts may occur (Sicotte and MacIntosh, 2004), as is the case in black howler monkeys. Androgen levels of ursine colobus males were not correlated with encounter rates of bisexual groups, but were positively correlated with encounter rates of neighboring males temporarily traveling without females (Teichroeb and Sicotte, 2008). In mantled howler monkeys (*Alouatta palliata*), fA levels, but not fGC levels, averaged across all group males were positively correlated with the number of extragroup males living in the same forest fragment, suggesting that mantled howler males respond to potential threats posed by the extragroup males (Cristóbal-Azkarate et al., 2006, 2007). The differences between mantled howler and black howler males may be due to differences in the frequency of encounters with extragroup males or may reflect variation among howler monkey species.

#### *Behavioral and hormonal correlates of male–female relationships*

Our results indicate that the central male position in a black howler group involves maintaining close spatial associations and fostering strong affiliative relationships with cycling females. This is consistent with our prediction that central males' relationships with cycling females are part of their reproductive strategies to gain sexual access to females. The central male position is further reinforced by cycling females who approached central males at significantly higher rates than they approached noncentral males, indicating their preference for central males as close neighbors. This preference is even more pronounced during periovulatory phases when females approached and sexually solicited central males at higher rates than they did during nonperiovulatory phases of their ovarian cycles (Van Belle et al., 2009). Although there were no differences between central and noncentral males in their spatial associations at 0–1 m or social relationships with noncycling females, central males did tend to spend more time in the vicinity (1–5 m) of noncycling females than noncentral males did. Both central and noncentral males were largely responsible for maintaining their spatial associations with noncycling females, suggesting that males generally take the initiative to establish relationships with females.

Our findings that central males had significantly higher fGC and fA levels than noncentral males during both the periovulatory and nonperiovulatory phases, as well as when no resident females were cycling suggest that fostering relationships with females may represent a nonaggressive, but nonetheless socially challenging, form of male–male competition in black howler monkeys. These findings are consistent with the proposal by Booth et al. (2006) that androgen levels should be correlated with behaviors and displays intended to indicate dominance rank and not just aggression per se. For example, male gelada baboons (*Theropithecus gelada*) display a red patch of skin on their chests to indicate their social status. Leader males of female

harems had redder patches, higher fA levels, and higher reproductive success than bachelor (prereproductive) and follower (postreproductive) males, and leader males with the reddest patches had more females in their harem than paler leader males (Beehner and Bergman, 2009). Similarly, male rock hyraxes (*Procapra capensis*) compete indirectly by singing to each other. Males that incorporated singing behavior into their reproductive strategies had higher rank, attained higher copulation rates, and had higher GC levels, as well as higher rates of aggression, than silent males (Koren et al., 2008). In our study groups, central black howler males howled alone and with other group members at similar rates as noncentral males did, but central males initiated the majority of howling bouts in all but one of the five periods with central and noncentral males (Van Belle et al., 2008). Initiative and risky behaviors that are important in achieving and maintaining social status might be associated with elevated androgen levels (Booth et al., 2006). Hence, the initiatives taken by central males to howl and to establish relationships with cycling females might be reflected in their elevated androgen levels.

Males may also respond with elevated hormonal levels to visual or olfactory cues from females. For example, captive male marmosets exposed to scents of novel ovulating females showed considerably sexual interest by licking and smelling the scent at higher rates, had erections more frequently, and responded with elevated serum testosterone levels, but not cortisol levels, compared to when they were exposed to control odors (Ziegler et al., 2005). Central black howler males monitored females' reproductive status by sniffing their genitals at higher rates than noncentral males did, and they did so at higher rates during the periovulatory phases than outside these phases (Van Belle et al., 2009). Yet, central male hormonal levels did not increase during times when at least one resident female was cycling or during the periovulatory phases of cycling females. In contrast, noncentral males had significantly lower average fA levels during the periovulatory phases, which could be indicative of some suppression of testicular endocrine function at times when resident females are most likely to conceive. One noncentral male was observed to copulate with both resident females during their periovulatory phases (Van Belle et al., 2009), suggesting that sexual function is not fully suppressed. It is unclear whether cues from the coresident central male or cycling females might have caused the reduced androgen levels in noncentral males, but it might have led to noncentral males becoming less attractive to females during their periovulatory periods (Van Belle et al., 2009). Investigating how male hormonal levels fluctuate with changes in male relationships with females would elucidate the causal effect among male hormonal levels and reproductive strategies.

We conclude that the reproductive strategies of male black howler monkeys include behavioral tactics that allow a male to establish himself in a bisexual group and subsequently to attain a central position that enhances his probability of gaining access to receptive females. Our findings suggest that male fecal hormonal levels are not associated with heightened male–male competition during changes in group membership. Instead, male fecal hormonal levels are associated with the affiliative relationships they foster with cycling females and that result in almost exclusive sexual access. These nonaggressive forms of male–male competition might be socially challenging as indicated by the higher fGC levels in central males.

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