Relationship Between Ovarian Cycle Phase and Sexual Behavior in Female Japanese Macaques (*Macaca fuscata*)

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ABSTRACT   We conducted behavioral observations simultaneously with fecal sample collection on eight non-lactating females 2–3 times per week, October 1997–March 1998, to examine the relationship between ovarian hormones and the sexual behavior of female Japanese macaques (*Macaca fuscata*) during the mating season. We analyzed samples by enzyme immunoassay for fecal hormone levels. Hormone profiles of estrone-glucuronide (E1) and pregnanediol-glucuronide (PdG) were used to separate ovarian cycles into three phases (follicular, periovulatory, and luteal). Hormonal profiles indicate average cycle lengths of 27.6 ± 4.2 days ($\pm$ SD; n = 26). Average lengths of the luteal and follicular phases were 12.3 ± 3.8 days ($\pm$ SD) and 8.3 ± 3.4 days ($\pm$ SD), respectively. We observed female Japanese macaques engaging in sexual activity throughout the ovarian cycle, with the highest rates occurring during the follicular and periovulatory phases as compared to the luteal phase. The attractivity of female Japanese macaques increased significantly during the follicular and periovulatory phases of the ovarian cycle, when E1 levels are peaking and PdG levels drop to baseline. In addition, females displayed a significant increase in proceptive behavior during the follicular and periovulatory phases. Grooming bouts, as well as proximity between female and male macaques, also increased significantly during the follicular and periovulatory phases. We conclude that fluctuating levels of ovarian hormones in different phases of the cycle are significantly associated with variable rates of copulatory and pericopulatory behaviors in these Japanese macaque females. Am J Phys Anthropol 125:352–362, 2004. © 2004 Wiley-Liss, Inc.

The sexual behavior of nonprimate mammals is thought to be under relatively strict hormonal control (Baum et al., 1977; Beach, 1942, 1976). However, the extent to which primate sexual behavior is influenced by hormones is not yet fully known. Many highly controlled studies of captive social groups and of individuals paired for short periods of time have contributed to our knowledge of the behavioral and physiological aspects of primate reproduction. Field studies also provide an essential foundation for investigating the reproductive repertoires and social systems of primates in the environments for which they are adapted. However, few opportunities exist in the field for the techniques required to analyze underlying physiological mechanisms. One exception is a set of noninvasive techniques in fecal steroid extraction and analysis that allows researchers to monitor hormone levels in free-ranging animals (e.g., Brockman et al., 1995; Fujita et al., 2001; Shideler et al., 1994; Strier et al., 1999; Wasser, 1994; Yoshida et al., 2001; Ziegler et al., 1989, 1996, 1997, 2000; Zucker et al., 1995). Through enzyme immunoassay (EIA) analysis of fecal steroid metabolites, researchers are able to determine the reproductive status of their subjects with minimal disruption of routine behavioral patterns. The present study employs such techniques in order to analyze the relationship between ovarian hormones and the sexual behavior of female Japanese macaques (*Macaca fuscata*) in a semifree-ranging environment during the mating season.

Following Dixson (1998), we conceptualize sexual behaviors as those actions that are directly part of copulation (e.g., mounting, holding, or hip-touch-

ing), as well as those patterns of pericopulatory behavior that serve to bring the sexes into proximity and maintain them in close physical contact (e.g., estrous vocalizations, approaches, or grooming). Sexual behavior in Japanese macaques is concentrated in a distinct mating season that lasts 4–6 months, beginning in early autumn (Fedigan and Griffin, 1996). During the mating season, females experience monthly menstrual cycles, during which time they are periodically predisposed to mate (Enomoto, 1981). Throughout this season, they form close associations with sexually mature males (hereafter referred to simply as males) in pairs that are inclined to isolate themselves from the rest of the group, and engage in repetitive bouts of courtship and copulation. We refer to these temporary but intense attachments between a male and female based on sexual attraction as "consortships" (Fedigan, 1992; Manson, 1997). The consort pair spends most of its time mating, traveling, eating, and grooming together, and copulations almost never occur outside these consorts. Consortships may last for as little as a few hours to as long as 2 weeks or more. During the mating season, the behavior of the female is modified, and she will actively seek out males, make sexual invitations, and groom males more than at other times of the year (Enomoto, 1981). The male's behavior toward females also changes; he seeks females out, follows them closely, grooms them, and makes more sexual invitations.

Solicitations and copulation are reported to vary with ovarian cycle phase in a number of primates (e.g., *Brachyteles arachnoides*, Strier and Ziegler, 1997; *Cebus apella*, Linn et al., 1995; *Macaca mulatta*, Cochran, 1979; Herbert, 1970; Johnson and Phoenix, 1976; Michael et al., 1968; *Macaca fuscata*, Enomoto et al., 1979; Oshima et al., 1977; Wolfe, 1979; *Macaca arctoides*, Murray et al., 1985; *Erythrocebus patas*, Loy, 1981; mangabeys, Gordon et al., 1991; *Papio ursinus*, Saayman, 1970; and *Microcebus talapoin*, Herbert, 1970). Changes in intensity of sexual activity during the ovarian cycle are commonly observed in nonhuman primates, with higher mating frequencies occurring at midcycle, or in the periovulatory period during the presumed time of ovulation, as well as during the follicular phase of the cycle immediately prior to ovulation (e.g., Beach, 1976; Hodges, 1987; Bercovitch and Goy, 1990; Soltis et al., 1999; Strier and Ziegler, 1997; Dixson, 1998; Zehr et al., 1998). However, only a minority of these studies provide hormonal evidence (rather than behavioral inference) for ovarian cycle phase. The present study is the first to examine the relationship between ovarian cycle phase determined from fecal steroid samples and rates of sexual activity in female Japanese macaques.

Beach (1976) proposed three facets of sexual behavior in female mammals: attractivity, proceptivity, and receptivity. Attractivity refers to the female's ability to evoke sexual responses from the male. Proceptivity concerns female-initiated behaviors directed toward the male that serve to invite, establish, and maintain sexual interaction. Receptivity is defined as a female's willingness to accept the male's attempts to copulate.

These three patterns have been linked to levels of circulating hormones in the following ways: increased attractivity in females was demonstrated during the periovulatory phase of the cycle in rhesus monkeys (Michael and Welegalla, 1968), at a time when estrogen levels reach their peak. In addition, the attractivity of ovariectomized monkeys increased significantly when injected with estrogen. It was also demonstrated in several mammal species that estrogen-induced attractivity is reduced or eliminated by progesterone (Beach, 1976). Proceptivity is manifested through various behavioral interactions initiated by the female, including contact, affiliative, and solicitational behaviors *directed* toward the male. Females tend to exhibit proceptive behaviors when they are stimulated by estrogen during the periovulatory phase of the cycle. Conversely, increased levels of progesterone during the luteal phase function to decrease proceptive behavior in the female. Finally, it was observed that sexual receptivity depends on estrogenic stimulation, but the degree of dependence varies considerably in different species (Beach, 1976). It was also suggested that testosterone, primarily of adrenal origin in the female, is the main libidinal hormone responsible for receptivity in primates (Dixson et al., 1973; Trimble and Herbert, 1968; Johnson and Phoenix, 1976). In some nonhuman primates, such as the chacma baboon, receptivity in the female is apparently limited to the periovulatory period (Saayman, 1970), whereas rhesus macaque females may permit copulation at any stage of the cycle (Johnson and Phoenix, 1978). Thus, the expression and functional significance of receptive behavior vary from species to species. Nevertheless, continual receptivity in anthropoid primates has been widely reported and is now generally accepted, whereas proceptivity and attractivity have been found to fluctuate (Dixson, 1998). Given this premise of continual receptivity in most Old World monkeys, especially macaques, and given the dearth of unambiguous behavioral indicators of receptivity and its converse (see Materials and Methods) in these monkeys, we elected to focus this study on attractivity and proceptivity.

This study examines the relationship between aspects of female sexual behavior and ovarian cycle phase during the mating season in the semifree-ranging Arashiyama West group of Japanese macaques. The following questions are addressed. 1) Does the attractivity of the female macaque vary depending on ovarian cycle phase? 2) Does the proceptivity of the female macaque vary across the ovarian cycle? 3) Do patterns of pericopulatory behavior (approaching, grooming, and sitting in proximity) vary according to cycle phase?

MATERIALS AND METHODS
Study site and animals

This study was conducted on a group of Japanese macaques (*Macaca fuscata*) kept under semifree-ranging conditions in a 65-acre enclosure at the Arashiyama West Texas Snow Monkey Sanctuary (TSMS). The study group, composed of over 350 monkeys, is well-suited for a behavioral study due to their semifree-ranging status, which makes them accessible to the researcher, while at the same time maintaining fairly normal behavior patterns in the animals. The Arashiyama West troop of Japanese macaques has been well-studied (see references in Fedigan and Asquith, 1991; Pavelka, 1993), and the availability of complete genealogies and life histories on the monkeys (plus the tattoo system of identification) provides valuable assistance to researchers.

The subjects for this study consisted of eight non-lactating Japanese macaque females. Each female was between 10–11 years of age during the study period, and all females were of similar rank. Seven females were multiparous, and one was primiparous. None of the eight females had offspring in the prior birth season (April–July, 1997). In this population, females give birth on average every other year, and usually nurse infants for at least 12 months (Fedigan and Pavelka, 2001; Fedigan, unpublished data). Because lactation can affect hormone levels and copulatory activity during the mating season, we deliberately chose a sample of females that had not given birth in the previous year and were all of similar rank and age. The most homogeneous sample available by those criteria was from the lowest third of the female dominance hierarchy. Previous studies by Fedigan et al. (1986) showed that low-ranking females do not experience different reproductive success from high-ranking females in this group of monkeys.

Data and fecal sample collection

Fecal samples were collected 2–3 times per week from the eight target females between 8:00 AM–8:00 PM, during the 1997–1998 mating season (October–March). Fecal collection was carried out during focal animal follows, as well as opportunistically. Samples were collected within 10 min of defecation and placed in 30-ml sterile plastic vials and frozen for preservation. Frozen samples were transported to the Wisconsin Regional Primate Research Center (WRPRC) in a plastic cooler containing dry ice, and stored in a freezer until analysis.

Behavioral data were collected using focal animal sampling, and in total, 352 hr of focal animal data were collected (ranging from 41–48 hr per subject) between October 1997–April 1998. Only data collected prior to conception (239.5 hr) were used in these analyses. Normally, focal subjects were rotated throughout the week, although this depended somewhat on the schedule of fecal collection.

Sample extraction and analysis

Extraction of ovarian steroids was performed according to the procedure described by Strier and Ziegler (1997). Aliquots of 0.1 g wet feces were extracted with 5 ml of distilled water and ethanol (50:50). We examined the level of conjugation of the steroids, and found that estrone-glucuronide (E1) and pregnanediol-glucuronide (PdG) steroids were excreted in freeform. Therefore, we used ether extraction to prepare samples for EIA.

High-pressure liquid chromatography (HPLC) was used to help identify which estrogens and progestins were excreted in higher amounts in Japanese macaque feces. The technique was described in Strier et al. (1999). The sample was separated on HPLC and collected in fractions to be run in the EIA. Ninety percent of the cross-reactivity for estrogen was found at the retention time of 22.6 min, where E1 elutes. Fractions from HPLC showed that PdG contributed to the majority of activity in the assay.

The addition of tritiated steroids of known concentrations to a few representative fecal samples before extractions was used to determine procedural losses for extraction techniques. Tritiated 20 alpha hydroxyprogesterone (for pregnanediol) and tritiated estrone (18,000 cpm) were added to 0.1-g samples of feces.

Pooled fecal samples were used to validate the PdG and E1 assays. PdG and E1 concentrations were determined by EIA methods, running assays with volumes from 5–100 μl from ether-extracted pools. The accuracy with which the assay measures steroids in each sample was assessed by adding small volumes of the fecal pool to the standard curve points. Parallelism was determined through serial dilutions of the fecal pool along the standard curve. In addition to running a standard curve, fecal pool samples were run in duplicate on each assay in order to establish a mean intra- and interassay coefficient of variation (CV) for E1 and PdG.

Mean steroid recoveries were found to be high (PdG = 90.00%; E1 = 91.80%). Accuracy assessment was also found to be within acceptable parameters (above 90%). A mean percent accuracy was determined for PdG ($102.69 \pm 3.38\%$) and E1 ($91.76 \pm 1.44\%$) assays. Pooled samples were found to be parallel to the standard curve for both PdG and E1 (PdG: T = -0.50, df = 28, $P < 0.05$; E1: T = -1.70, df = 28, $P < 0.05$). In addition, mean intra- and interassay coefficients of variation (CV) values were found to be within acceptable limits (no difference in slope; PdG: intra = 3.92%, inter = 11.41%; E1: intra = 7.62%, inter = 21.40%).

Estrone was measured in feces, employing an E1 EIA previously reported (Ziegler et al., 1997). Pregnanediol was measured in feces by a PdG EIA also described previously (Carlson et al., 1996).
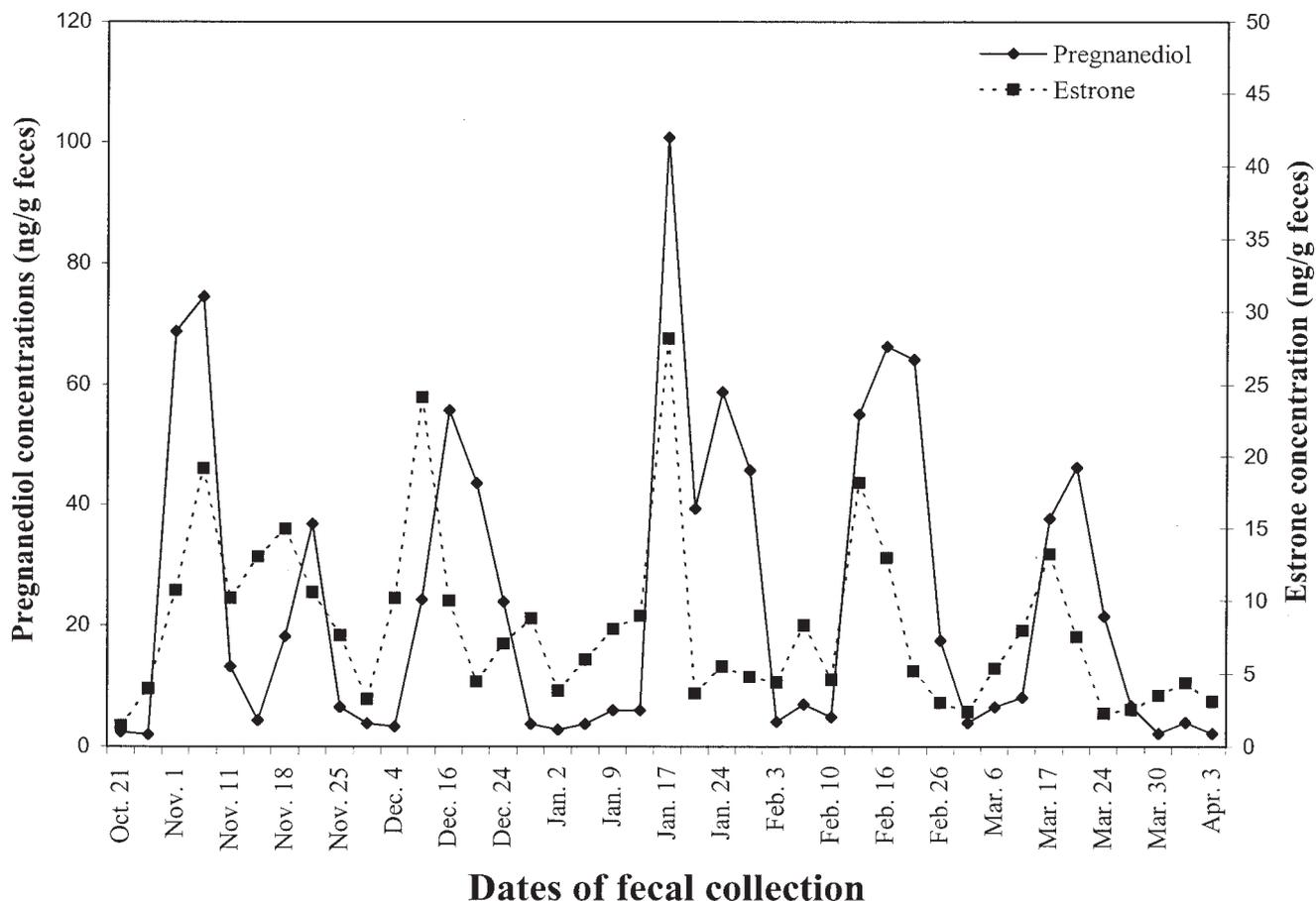
Fig. 1. Representative hormone profile for one female Japanese macaque during 1997–1998 mating season. Base levels of pregnanediol indicate follicular phase of cycle, day of estrone surge (or first day that pregnanediol levels begin to rise) ± 3 days represents periovulatory phase, and sustained increase in pregnanediol represents luteal phase. Figure represents six complete cycles for one female.

Interpretation of hormonal data

Due to the delayed excretion of E1 (approximately 24 hr in macaques; Dixson, 1998; Fujita et al., 2001) and the infrequency of fecal collection, the onset of PdG increase was considered to be the best estimate of day of ovulation (Strier and Ziegler, 1997). Progesterone synthesis from the ovary actually begins to increase prior to ovulation, within hours of the gonadotropin surge (Espey and Lipner, 1994). Consequently, the sample preceding the first day of the PdG increase was considered to represent the most likely day of ovulation. The periovulatory phase was therefore conservatively defined as estimated day of ovulation ± 3 days (as per Strier and Ziegler, 1997).

The earliest possible day of conception was considered the estimated day of ovulation if steroid levels failed to return to their lowest baseline levels. Length of gestation was calculated as the interval between estimated ovulation during the cycle in which conception occurred and the date of parturition. Parturition date was defined as the first day on which a female was observed with a new infant (parturition dates for the troop were collected by local management personnel and were accurate to

within 2 days). Cycle lengths were calculated as intervals between successive PdG surges. There were 1–6 cycle lengths calculated for each female (Fig. 1). Figure 2 represents one complete ovarian cycle.

Behavioral analysis

As noted earlier, receptivity is difficult to measure in this species; in fact, receptivity appears continual during consorts. Copulation and male attempts to mount occur only in the context of consorts in Japanese macaques, and females almost never refuse mount attempts from their male consort partners. What females may refuse are the prior male courtship overtures that were necessary to establish consortships in the first place, but these often take place long before the pair comes under observation. Thus, in the absence of mount refusals, it was not possible to document fluctuations in receptivity.

Analyses were carried out on behavioral indicators of attractivity and proceptivity. These behaviors included mounts, holding behavior associated with copulation, "other" sexual behaviors (presenting for mount, lip-smacking, and hip-touching), estrous
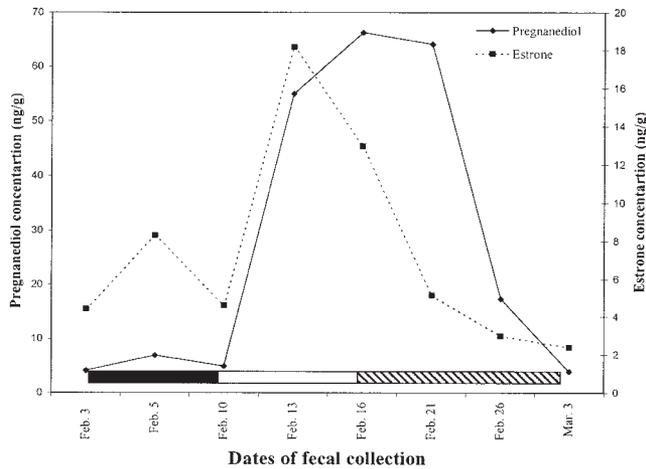
Fig. 2. One representative ovarian cycle for a Japanese macaque female during 1997–1998 mating season.

General hormone levels during three phases of the ovarian cycle are represented as follow:

Hormone	Follicular ■	Periovulatory □	Luteal ▨
Pregnanediol (PdG)	Low	Rising	Peak
Estrone (E1)	Rising	Peak	Low

calling, approaching, and grooming (Beach, 1976; Fedigan, 1992; Fedigan and Gouzoules, 1978; Huffman, 1991; Pavelka, 1993). Behaviors indicating the female's attractivity were defined as those copulatory and pericopulatory actions *received* by the target female from males, such as mounts, holds, lip-smacks, hip-touches, approaches, and grooms. Proceptive activities were defined as behaviors *directed* by the target female toward males, such as mounts, holds, lip-smacks, hip-touches, estrous calls, approaches, and grooms. Estrous calls are distinctive vocalizations given by females during mating and consort activity (Pavelka, 1993). In addition, spatial proximity between the female subject and male troop members was analyzed using three categories (contact, less than 1 m, and between 1–4 m).

The results of the hormonal analysis were used to create hormone profiles for each female (e.g., Fig. 1). Hormone profiles were used to calculate ovulatory cycle and gestation lengths. Cycles were divided into three phases: follicular, when PdG has dropped to base line levels; periovulatory, when PdG levels begin to slightly rise and E1 levels reach their peak; and luteal, when PdG levels stay well above baseline for an extended duration and E1 levels return to baseline (Fig. 2).

A mean frequency per hour score for each behavioral variable was calculated for each of eight subjects for each of the three phases of the cycle. These mean scores were compared for cycle phase variation, using Friedman's analysis of variance by ranks. Behaviors showing significant variation across the three cycle phases were further analyzed with Wilcoxon signed rank tests to determine where

the variation existed. In addition to copulatory behaviors, the rate of approaches *directed* toward and *received* by the target female from male troop members was analyzed. Data were also analyzed via Friedman and Wilcoxon tests to determine whether females were more likely to be found in proximity to males during each phase of the ovarian cycle. This was accomplished by tallying the total time the target female was in proximity to a male during focal samples, and calculating a mean percent of time for each female for each phase of the cycle. Finally, the grooming behavior of target females in relation to male troop members was analyzed via Friedman and Wilcoxon tests for cyclic patterns. This was achieved by calculating mean percent of time each female spent directing or receiving grooming from males, and separating the data into the three phases of the cycle. Level of significance was set at $P < 0.05$, and all tests were two-tailed. All statistical analyses were performed using an SPSS 8.0 statistical software package.

RESULTS

Hormone profiles

The profiles of immunoreactive E1 and PdG showed clear cyclic patterns in which the follicular and luteal components of cycles could be clearly distinguished. One to six ovarian cycles were calculated for each of the eight target females, for a total of 27 complete cycles (Table 1). The ovarian cycle lengths averaged 27.6 ± 4.2 days ($\pm$ SD; range, 19–35 days). Cycle lengths did not differ significantly among the eight females ($\chi^2 = 5.31$, df = 7, $P = 0.623$). The lengths of the follicular and luteal phases averaged 8.4 ± 3.4 and 12.3 ± 3.8 days ($\pm$ SD), respectively, and did not differ significantly among females (follicular: $\chi^2 = 5.694$, df = 7, $P = 0.576$; luteal: $\chi^2 = 6.092$, df = 7, $P = 0.529$). Four of the eight subjects conceived after 1–3 ovarian cycles, and they were removed from hormonal and behavioral analyses following their estimated conception dates. The mean gestation length for females in this study was calculated as 175.25 ± 12.8 days ($\pm$ SD; range, 163–192 days; median, 173 days).

Attractivity

Target females were observed to engage in sexual behavior during all three phases of the ovarian cycle (with the exception of one female who was not observed participating in sexual activity, but who did conceive during this mating season). The reception of mounts by females occurred frequently throughout the mating season, and varied significantly across the ovarian cycle (Table 2 and Fig. 3). Females were mounted significantly more during the follicular and periovulatory phases compared to the luteal phase of the cycle, and although there was no significant difference in the rate of mounts *received* in the follicular compared to the periovulatory phase ($P = 0.53$), the mean rate of mounts per hour showed

TABLE 1.
Ovarian cycle and gestation lengths in days for Japanese macaque females

Female	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Gestation
Trisha	30						192
Saskia	35	22	25				170
Zoe	29	27					176
Tyler	33						163
Shy Mug	22	19	28	34	28	27	
Tantalis	32	26	35	28			
Lisa[1]	27		29	29	21		
Bo Nose	23	24	27	31	28	28	

[1] Sample collection for Lisa was incomplete for cycle 2, and was consequently removed from analysis.

TABLE 2. *Summary statistics for Friedman and Wilcoxon tests*

	Differences across three cycle phases (Friedman analyses)	Follicular vs. periovulatory (Wilcoxon analyses)	Follicular and periovulatory vs. luteal (Wilcoxon analyses)
Behaviors indicating attractivity			
Mount receive	$\chi^2 = 8.00$, df $= 2$, $P = 0.02$*	$Z = -0.63$, $P = 0.53$	$Z = -2.20$, $P = 0.03$*
Hold receive	$\chi^2 = 8.27$, df $= 2$, $P = 0.02$*	$Z = -1.78$, $P = 0.08$	$Z = -2.80$, $P = 0.005$*
Other receive	$\chi^2 = 2.00$, df $= 2$, $P = 0.37$	N/A	N/A
Behaviors indicating proceptivity			
Mount direct	$\chi^2 = 6.71$, df $= 2$, $P = 0.03$*	$Z = -0.11$, $P = 0.91$	$Z = -2.02$, $P = 0.04$*
Hold direct	$\chi^2 = 7.18$, df $= 2$, $P = 0.02$*	$Z = -1.36$, $P = 0.17$	$Z = -2.80$, $P = 0.005$*
Other direct	$\chi^2 = 6.33$, df $= 2$, $P = 0.04$*	$Z = -0.83$, $P = 0.41$	$Z = -1.89$, $P = 0.06$
Estrus call	$\chi^2 = 9.33$, df $= 2$, $P = 0.01$*	$Z = -0.82$, $P = 0.41$	$Z = -2.27$, $P = 0.02$*
Behaviors facilitating mating			
Approach receive	$\chi^2 = 1.23$, df $= 2$, $P = 0.54$	N/A	N/A
Approach direct	$\chi^2 = 6.07$, df $= 2$, $P = 0.05$*	$Z = -0.07$, $P = 0.94$	$Z = -2.08$, $P = 0.04$*
Groom receive	$\chi^2 = 11.08$, df $= 2$, $P = 0.001$*	$Z = -1.69$, $P = 0.09$	$Z = -2.41$, $P = 0.02$*
Groom direct	$\chi^2 = 8.86$, df $= 2$, $P = 0.01$*	$Z = -0.85$, $P = 0.40$	$Z = -2.43$, $P = 0.02$*
Proximity (contact)	$\chi^2 = 10.90$, df $= 2$, $P = 0.001$*	$Z = -0.30$, $P = 0.76$	$Z = -2.43$, $P = 0.02$*
Proximity (< 1 m)	$\chi^2 = 10.40$, df $= 2$, $P = 0.01$*	$Z = -0.10$, $P = 0.27$	$Z = -2.25$, $P = 0.02$*
Proximity (1–4 m)	$\chi^2 = 6.65$, df $= 2$, $P = 0.04$*	$Z = -0.65$, $P = 0.52$	$Z = -2.20$, $P = 0.03$*

*Significant difference at $P < 0.05$.

a 56% increase in the periovulatory compared to the follicular phase.

Holding behavior in relation to copulation varied significantly across the cycle, with considerably more holds received from males during the periovulatory and follicular compared to the luteal phase (Table 2 and Fig. 3).

"Other" sexual behaviors (presenting for mount, lip-smacking, and hip-touching) received by females from males occurred infrequently. As a consequence, these behaviors were not found to show significant differences among the three phases of the cycle (Table 2). Nevertheless, the few times these behaviors were displayed, they occurred during the follicular and periovulatory phases.

Proceptivity

All behaviors indicating proceptive behavior in the female were found to vary significantly across the three phases of the cycle (Table 2). Females displayed increased sexual activity (mounting, holding, and estrous calling) toward males during the follicular and periovulatory compared to the luteal phase (Table 2 and Fig. 4). Once we broke the "other" sexual behaviors down into paired Wilcoxon tests (follicular vs. periovulatory; follicular and periovulatory vs. luteal), there were no significant differences.

Behaviors facilitating mating: pericopulatory patterns

The rate of approaches *received* by target females from male troop members did not differ significantly among the three phases of the cycles (Table 2). Conversely, the rate of approaches *directed* by target females toward male troop members did vary significantly across the three phases of the cycle (Table 2), with the highest frequencies occurring in the follicular and periovulatory compared to the luteal phase of the cycle (Fig. 5a).

The proportion of time which females spent grooming and being groomed by male troop members varied significantly across the three cycle phases (Table 2). Grooming between females and males increased substantially during the follicular and periovulatory compared to the luteal phase (Table 2 and Fig. 5b). In addition, the amount of time females spent in proximity to males increased significantly during the follicular and periovulatory compared to the luteal phase (Table 2 and Fig. 5c).

DISCUSSION

The results of our study show that these Arashiyama West Japanese macaque females experience cycle and gestation lengths similar to those previously reported for this species (Nigi, 1976; Fujita et
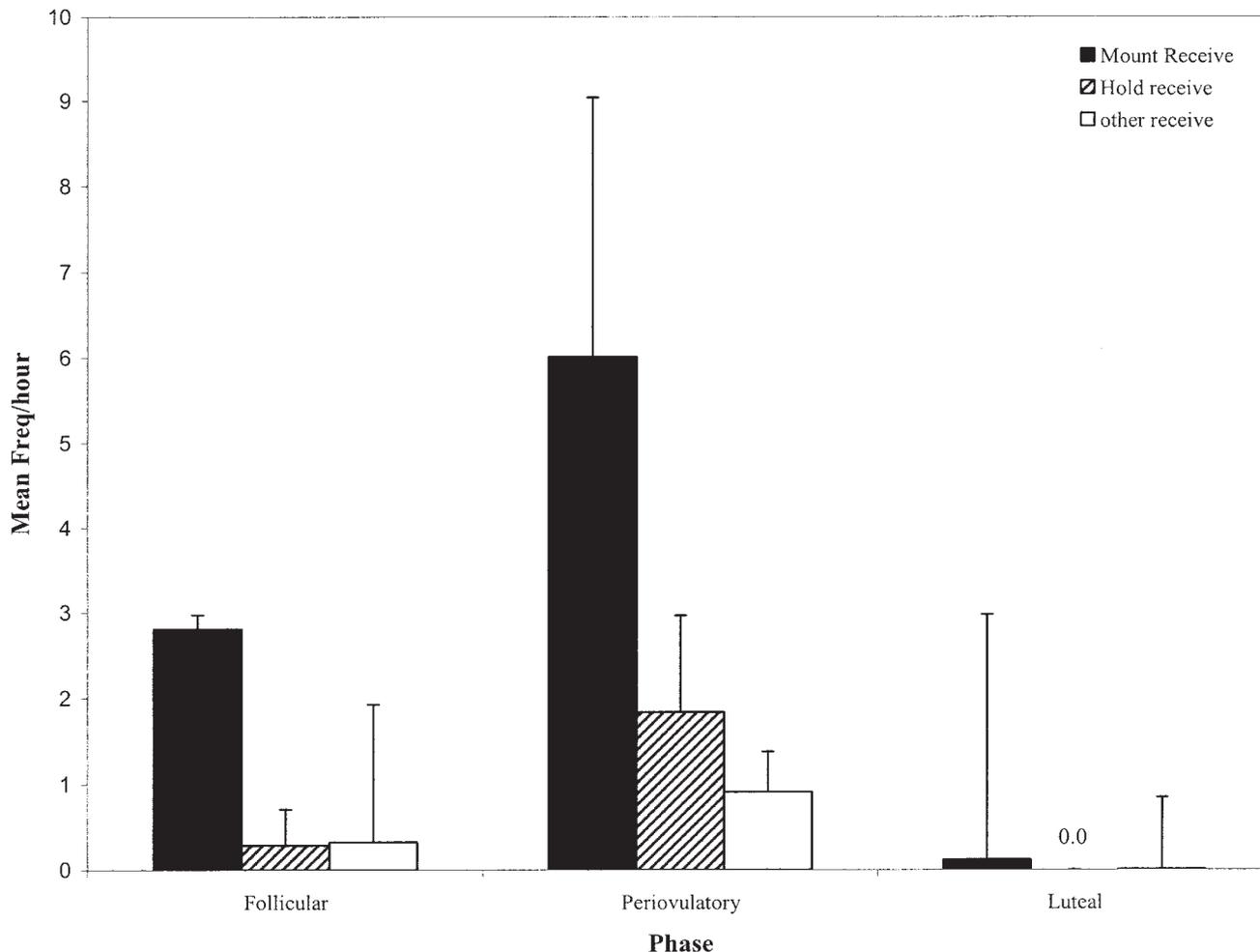
Fig. 3. Attractivity of female Japanese macaques during three phases of ovarian cycle (mean ± SEM). ■: Mounts received by focal females from male partners. ▨ : Holds received by focal females from male partners. □: Other sexual behaviors received by focal females from male partners. Friedman statistical analysis: a, $\chi^2 = 8.00$, df = 2, $P < 0.02$; b, $\chi^2 = 8.27$, df = 2, $P < 0.02$; c, $\chi^2 = 2.00$, df = 2, $P < 0.37$.

al., 2001). Our findings also demonstrate that almost all the sexual behaviors we examined are exhibited/received by these female monkeys at significantly different rates across the ovarian cycle. Behaviors indicating attractivity and proceptivity are much more likely to occur during the follicular and periovulatory than the luteal phase of the cycle, a consistent finding that is further discussed below.

Enzyme immunoassays and hormone profiles

The employment of a simple extraction technique, coupled with established enzyme immunoassay methods, proved very successful for measuring fecal steroids in these study animals. This method yielded high total steroid recoveries (>90%). The sustained increase in estrogens which typically occurs in other macaques in a 1–2-day period at time of ovulation (Dixson, 1998; Fujita et al., 2001) was not always present in the sample collection. Therefore, given the irregular rate of fecal sample collection (i.e., not daily), PdG levels proved the most reliable means of determining cyclic patterns in the ovarian cycle. Av-

erage cycle lengths were calculated at 27.6 ± 4.2 days, which is comparable to earlier studies by Nigi (1975) (26.3 ± 5.4 days) that were based on vaginal swabs and sexual swellings. Although not statistically significant, ovarian cycle lengths were found to vary throughout the mating season for each individual female, as well as among female subjects. Gestation lengths were found to vary considerably among the four females who conceived. Mean gestation length for females in this study, as determined by hormonal profiles, was 175.25 ± 12.8 days (± SD; median, 173 days), which was also comparable to earlier nonhormonal estimates reported in Japanese macaques (173 ± 6.9 days; Nigi, 1976), as well as hormonal estimates previously reported (170–189 days; Fujita et al., 2001).

Attractivity

Previous studies documented attractivity in primates as being regulated by circulating estrogen levels (Enomoto et al., 1979; Johnson and Phoenix, 1976; Michael and Zumpe, 1993). Estrogens begin to
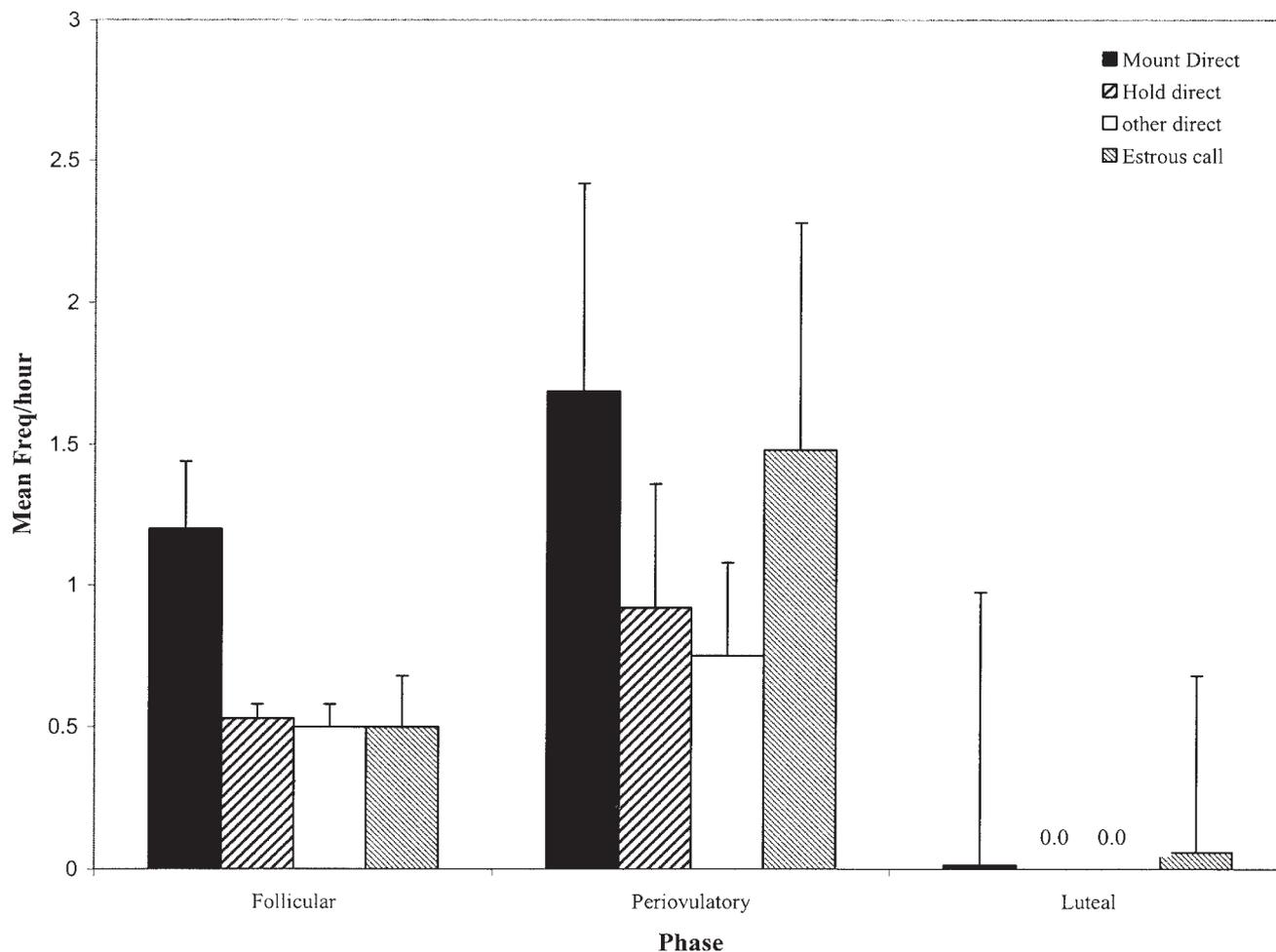
Fig. 4. Proceptivity of female Japanese macaques during three phases of ovarian cycle (mean ± SEM). ■: Mounts directed by focal females toward male partners. ▨ : Holds directed by focal females toward male partners. □: Other sexual behavior directed by focal females toward male partners. ▨ : Estrous calls directed by females. Friedman statistical analysis: a. $\chi^2 = 6.71$, df = 2, $P < 0.03$; b, $\chi^2 = 7.18$, df = 2, $P < 0.02$; c, $\chi^2 = 6.33$, df = 2, $P < 0.04$; d, $\chi^2 = 9.33$, df = 2, $P < 0.01$.

increase in Japanese macaques late in the follicular phase, and peak toward midcycle (Enomoto et al., 1979). In this study, estrone levels were found to increase toward the periovulatory phase of the cycle. The attractivity of females appears to increase in the follicular and periovulatory phases, as evidenced by a significant increase in the rate of mounts and holds received from males in these two phases, followed by an absence of this behavior in the luteal phase of the cycle.

The role of progesterone in the expression of sexual behavior is not entirely clear. This study found that pregnanediol (PdG) increases during the luteal phase, while maintaining low levels in the follicular and periovulatory phases of the cycle. Earlier findings on rhesus macaques showed that progesterone had an inhibitory effect on attractivity (Baum et al., 1977; Michael and Zumpe, 1993). In this study, mounts were primarily restricted to the follicular and periovulatory phases, when PdG concentrations are low, followed by a significant decrease in the behavior during the luteal phase, when PdG levels

reach their peak. Holding behavior generally occurred during a mount series, and as a consequence, the rate of this behavior appeared to follow a similar pattern to that observed in "mounts received" by the female. "Other" sexual behaviors, such as hip touches, lip-smacking, and presents, were rarely observed in target females. Consequently, no significant patterns were detected in the rate of these sexual behaviors *received* by target females.

Proceptivity

Proceptivity in primates has not been linked to any single hormone (Baum, 1983). However, it has been suggested that females are not only more attractive during the periovulatory phase, when estrogen levels are increased, but that they also show increases in proceptive behavior during this phase (Beach, 1976; Chambers and Phoenix, 1987; Wallen and Tannenbaum, 1997; Wallen et al., 1984). We found significant increases in proceptive behaviors during the follicular and periovulatory phases of the cycle. Females mounted males more often in the
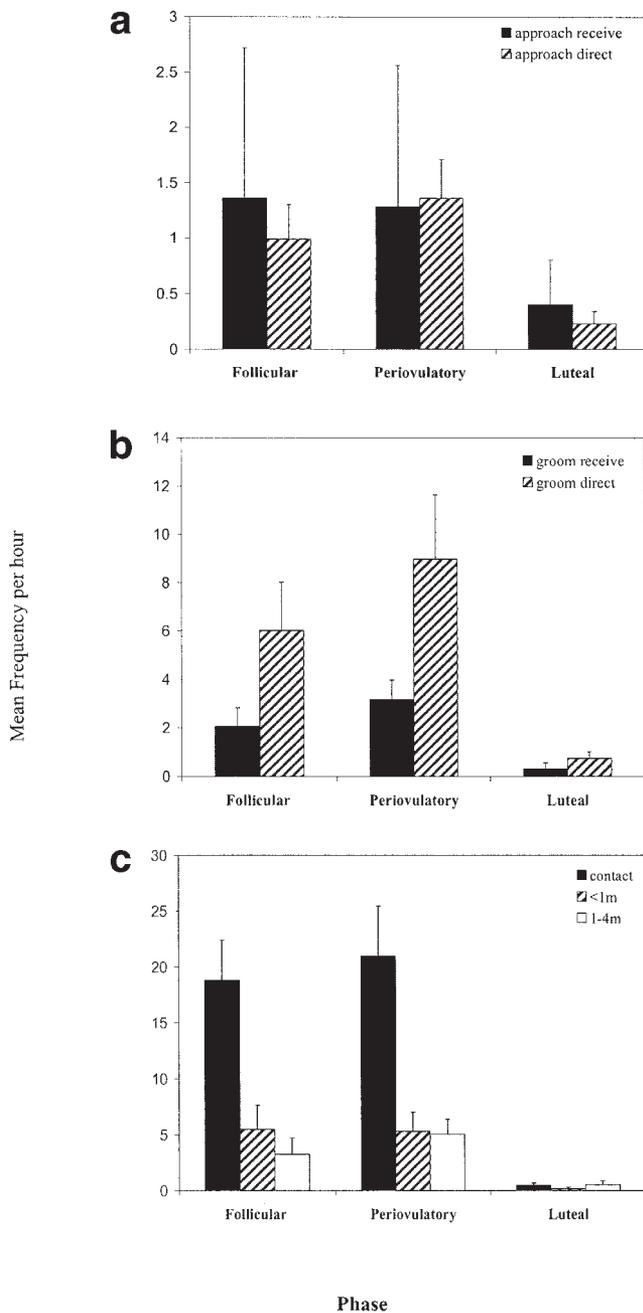
Fig. 5. a: Mean frequency per hour of approaches received from males and directed by target females to male sexual partners during three phases of ovarian cycle (mean ± SEM). Friedman statistical analysis: received $\chi^2 = 1.23$, df = 7, $P < 0.54$; directed $\chi^2 = 6.07$, df = 7, $P < 0.05$. b: Mean proportion of time focal females spent grooming and being groomed by male sexual partners during three phases of ovarian cycle. Friedman statistical analysis: received $\chi^2 = 11.08$, df = 7, $P < 0.00$; directed $\chi^2 = 8.86$, df = 7, $P < 0.01$. c: Mean proportion of time focal females spent in proximity to male troop members during three phases of ovarian cycle. Friedman statistical analysis: contact, $\chi^2 = 10.90$, df = 7, $P < 0.00$; <1 m, $\chi^2 = 10.40$, df = 7, $P < 0.01$; 1–4 m, $\chi^2 = 6.65$, df = 7, $P < 0.04$.

follicular and periovulatory phases, during the decrease in PdG and towards the E1 peak. Females also held and *directed* "other" sexual behaviors to-

ward males during the follicular and periovulatory phases, and showed a complete absence of these behaviors during the luteal phase. Females also exhibited a higher rate of estrous calls during the follicular and periovulatory compared to the luteal phase, during which PdG levels were elevated.

The expression of estrous calls appeared to be specific to certain females, and showed a significant increase in rate during the follicular and periovulatory phases and a decline during the luteal phase. Estrous calls generally occurred in conjunction with consortships, often when the male had moved temporarily out of proximity during the consortship, or after the consortship ended abruptly with the male moving on to another female. This pattern of estrous calls supports previous observations that females use estrus calls to stimulate the continuation of male proximity and sexual behaviors (Fedigan, 1992).

Behavior facilitating mating: pericopulatory patterns

Although copulatory activity is the best indicator of the attractive and proceptive state of the female, pericopulatory behaviors may also serve to indicate attractivity and proceptivity in the female. The attractivity of the female can often be ascertained by the rate with which she is approached by male troop members. In addition, grooming among consorts is frequently observed throughout the mating season, and the amount of time a male spends grooming a female may be an indicator of the female's attractivity.

Approaches received by females from male troop members did not vary significantly throughout the ovarian cycle. Grooming behavior between male and female Japanese macaques during the mating season is closely linked to consortships. When a male and a female are engaged in a consortship, they spend most of their time together and groom each other frequently between mounts in a mount series. Grooming between male and female macaques is not always proportionate, and often the female directs significantly more grooming than she receives. This appears to be the case with the females in this study. Grooming patterns for some females were not consistent, and not all females received grooming from male partners during consortships. Nevertheless, females were groomed by males significantly more during the follicular and periovulatory compared to the luteal phase. These results are consistent with our other findings indicating increased attractivity of the female during these phases of the cycle.

Approaching and grooming behaviors may also function to indicate the proceptivity of the female Japanese macaque. Female Japanese macaques were observed to approach and groom males more often, as well as spend more time in close proximity to males, during consortships (Enomoto, 1981). In this study, females approached males more often in the follicular and periovulatory than in the luteal

phase, suggesting increased proceptivity when PdG levels are low and E1 levels are increasing. In addition, females groomed males significantly more in the follicular and periovulatory phases, with a notable, but not significant, increase in the periovulatory phase.

A further analysis was conducted to examine spatial proximity between males and females during the three phases of the cycle. Although spatial proximity cannot be attributed to either individual, it does require the cooperation of both individuals to maintain proximity. The results of this analysis clearly demonstrate that females spend significantly more time in proximity (contact, <1 m, and 1–4 m) with adult males during the follicular and periovulatory phases, when E1 levels peak.

CONCLUSIONS

As determined in prior studies of primate sexual behavior (Dixson, 1998), we found that rates of copulatory and pericopulatory behavior were consistently higher during the follicular and periovulatory phases and lower during the luteal phase. Table 2 shows that it is clearly the luteal phase that is behaviorally distinct from the other two phases of the cycle. Why might this be so? We suggest three (nonmutually exclusive) explanations. First are the proximate effects of fluctuating hormone levels. Estrone levels are on the rise and reaching a peak in the late follicular/early periovulatory phase, and this hormonal pattern has been associated with increases in both attractivity and proceptivity. Conversely, in the luteal phase, pregnanediol levels are at their peak and estrone levels are falling, both of which are associated with decreases in attractivity and proceptivity. Second is the possibility that there *are* differences between the follicular and periovulatory phases in the expression of these sexual behaviors, but we were unable to document them. We did find some increases in sexual behaviors during the periovulatory phase as compared to the follicular phase, but these differences did not reach significance. Much of the sexual activity that was exhibited during the follicular phase of the cycle occurred near the end of that phase and close to the onset of the periovulatory phase, suggesting that the boundary of the two phases may not be behaviorally discrete. Third and finally, there may be a functional reason to attract males just prior to, as well as during, ovulation. As Dixson (1998) suggested, attractivity and proceptivity may begin to increase during the follicular phase in order to locate and attract males who will then be in place and mating when ovulation does occur.

In sum, we found ovarian cycle phases, with their different hormonal patterns, to be significantly associated with variable rates of sexual behavior in these Japanese macaque females. Consistent cyclic changes in behavioral indicators of attractivity and proceptivity show that these female monkeys are more likely to invite and attract male sexual partners immediately prior to, as well as during, ovulatory periods.

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