BRIEF REPORT

Changes in Fecal Estrogen Levels and Sexual Behavior in Captive Sichuan Snub-Nosed Monkeys (\textit{Rhinopithecus roxellana}) Following a Male Replacement

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\textit{Rhinopithecus roxellana} are generally seasonal breeders, although copulation can occur throughout the year. Previous studies suggest that estradiol modulates female sexual behavior during the mating season. However, the effects of social context on estrogen levels and behavior have not been fully explored. We studied the relationship between sexual behaviors and fecal estrogens in a group of captive \textit{R. roxellana} during a period of social instability. We collected behavioral data for six months and collected fecal samples at 2–3-day intervals for four months spanning the mating and nonmating seasons, and analyzed fecal estrogen levels via RIA. Females showed clear cyclic solicitation and copulation peaks in the mating season, which corresponded with sharp peaks in fecal estrogens. During the nonmating season, solicitation rates, copulation rates, and fecal estrogens were generally low. However, one nonpregnant female displayed a sharp peak in solicitations, copulations,
and estrogens during the nonmating season 10–14 days after a male replacement. Our results provide preliminary evidence that social and behavioral changes affect estrogen levels in *R. roxellana*. *Zoo Biol.* 00:1–9, 2012. © 2012 Wiley Periodicals, Inc.

**Keywords:** seasonal breeding; copulation frequency; fecal estrogens; male replacement

**INTRODUCTION**

Breeding seasonality is characteristic of many mammals in which females face seasonally fluctuating food resources [Brockman and van Schaik, 2005; Campbell, 2007; Knott, 2001]. In vertebrates, gonadal steroid hormones generally regulate sexual behavior, and mating tends to be concentrated in the periovulatory period during which conception is possible [Adkins-Regan, 2005]. Nevertheless, many primate taxa, including seasonally breeding species, have been observed mating outside of the female fertile period [Wallen, 2001; Ziegler, 2007], and there is substantial evidence that primate sexual behavior is influenced by social context [e.g., Matsumoto-Oda et al., 2007; Pereira, 1991; Wallen, 2001]. Several reasons for copulation during periods when conception is unlikely have been proposed, including reinforcement of pair-bonds [Yan and Jiang, 2006; Ziegler, 2007], paternity confusion, and minimization of infanticide risk [Hrdy and Whitten, 1987].

*Rhinopithecus roxellana* are endemic to temperate forests (30–35° N) in mountainous areas of central China [Zhang et al., 2000], an area with pronounced seasonal fluctuations in food availability and climatic conditions [Li et al., 2000; Li, 2001]. Accordingly, *R. roxellana* are seasonal breeders, with mating peaks in August–November and birth peaks in March–May [Bao-Ping et al., 2003; Ren et al., 1995; Qi et al., 2008; Yan and Jiang, 2006; Zhang et al., 2000]. Wild *R. roxellana* generally live in large groups consisting of several one-male units (OMU), and males typically disperse from their natal groups before achieving sexual maturity [Qi et al., 2009; Zhang et al., 2006].

Female behavior appears to be the primary driver of seasonal variation in copulation frequencies in *R. roxellana*. Females frequently solicit copulations from males in their OMU during the mating season, initiating ~95% of copulations [Ren et al., 1995; Zhao and Li, 2009]. Estradiol levels in captive *R. roxellana* are correlated with the frequency of female sexual solicitations during the mating season [Yan and Jiang, 2006]. However, Yan and Jiang [2006] did not detect a relationship between hormones and behavior outside of the mating season, and the effects of social context and season on the relationship between female hormone levels and behavior in this species are not yet well-understood.

We examined the relationship between female sexual behavior and fecal estrogen levels in a group of captive *R. roxellana*. We predicted that if changes in circulating estradiol levels are drivers of female sexual behavior, there should be significant positive correlations between fecal estrogens and female solicitation and copulation rates. The group composition changed several times during the course of the study, and ultimately the adult male was removed from the group and replaced with another male in January 2009. This provided an unexpected opportunity to examine the effects of a male replacement on female behavior and estrogen levels.
TABLE 1. Study individuals

<table>
<thead>
<tr>
<th>Name</th>
<th>Sex</th>
<th>Age (2008)</th>
<th>Body mass (January 2008)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogong</td>
<td>Male</td>
<td>11 years</td>
<td>17 kg</td>
</tr>
<tr>
<td>Changjang</td>
<td>Male</td>
<td>5 years</td>
<td>8 kg</td>
</tr>
<tr>
<td>Shaoring</td>
<td>Female</td>
<td>8 years</td>
<td>7 kg</td>
</tr>
<tr>
<td>Lili</td>
<td>Female</td>
<td>5 years</td>
<td>5.5 kg</td>
</tr>
</tbody>
</table>

METHODS

Study Site and Study Subjects

Our study was conducted on a group of *R. roxellana* in the Everland Zoological Garden in Gyeonggi-do, South Korea. Seasonal changes in day-length and temperatures at Everland are comparable with those in the animals’ natural habitat. The monkeys, which are on breeding loan from China, are housed at night in two enclosures (2.6 × 3.6 × 4.2 m and 2.8 × 3.6 × 4.2 m) and are on display from 09:30 to 16:00 h in a ca. 152 m² outdoor enclosure. At the beginning of the study, the group included an adult male (Ogong), a subadult male (Changjang), an adult female of prime breeding age (Shaoring), and a young adult female (Lili; Table 1). Both females were nulliparous. All individuals were unrelated to each other and could be identified using their physical features.

The group composition changed during the study because Ogong (the alpha male) attacked and injured first Changjang and then Lili, who were subsequently removed from the group (Table 2). In early January, 2009, the keepers removed Ogong from the group and returned Lili and Changjang.

Behavioral Data and Fecal Sample Collection

We observed the study group four days/week from September 2008 to February 2009, a period that encompassed three months of the mating season and three months of the nonmating season. On each observation day, behavioral data were collected continuously from 10:00 to 12:00 h and 13:00 to 16:00 h using all occurrence sampling [Altmann, 1974]. Generally, data were collected at least once every two days, except during adverse weather conditions. We recorded all observations of copulations, copulation attempts, and copulation solicitations involving any group members throughout the sampling period.

TABLE 2. Composition of the study groups

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogong (♂)</td>
<td>Ogong (♂)</td>
<td>Ogong (♂)</td>
<td>Shaoring (♀)</td>
</tr>
<tr>
<td>Shaoring (♀)</td>
<td>Shaoring (♀)</td>
<td>Shaoring (♀)</td>
<td>Lili (♀)</td>
</tr>
<tr>
<td>Lili (♀)</td>
<td>Lili (♀)</td>
<td>Changjiang (♂)</td>
<td></td>
</tr>
</tbody>
</table>

Dates provided in day/month/year format.
We collected fecal samples on each observation day from October 2008 to February 2009. We only included samples produced between 14:00 and 16:00 daily to control for diurnal fluctuation in hormone secretion. Samples were labeled with individual identity, date, and defecation and collection time, and stored in a deep freezer (−54°C).

Hormone Analysis

Hormone analyses were conducted at the reproductive endocrinology laboratory at Hanyang University (Seoul, South Korea). Before an assay, fecal samples were lyophilized and ground. Dried feces (0.5 g) were then mixed with 8 ml diethyl ether in falcon tubes, vortexed, and then centrifuged for 15 min (3000 rpm, 4°C). Extracted samples in the ether layer were then transferred to 50 ml corn tubes by pipette, and evaporated in a shaking water bath at 36°C until only one drop was left. We mixed the evaporated samples with 1 ml of PBS buffer in 1.5 ml vials, and stored them at −70°C prior to analysis.

Previous studies have shown that estradiol levels reflect female ovarian function in Sichuan snub-nosed monkeys [Yan and Jiang, 2006]. As far as we know, fecal estrogens have not been previously studied in this species, but studies in several other Old World monkeys have shown strong and significant correlations between circulating estradiol and fecal estradiol [Risler et al., 1987; Shideler et al., 1993; Whitten and Russell, 1996]. In Old World monkeys, estradiol is excreted in feces primarily as free estradiol and estrone with a stable estradiol/estrone metabolite ratio, suggesting that assays for fecal estradiol effectively characterize the estrogen profiles of these species [Shideler et al., 1993; Wasser et al., 1994].

We measured baseline fecal estradiol levels without dilution using radioimmunoassay (RIA) with Coat-A-Count (SIEMENS, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) iodinated steroid RIA kits following manufacturer-supplied protocols. For fecal samples collected from females during solicitation peaks (which may have corresponded with the periovulatory period), we diluted the samples fivefold for RIA. Cross-reactivity was 0.29% with 17β-estradiol-2-monosulfate, 0.32% with estriol, and 10.0% with estrone. Assay sensitivity for estradiol was 0.43ng/g. Intra- and interassay coefficients of variation were 7.88% ($n = 10$) and 8.43% ($n = 4$), respectively. Fecal estrogen assays using the same methods have been validated in a variety of primates [Hernández-López et al., 2010; Kugelmeier et al., 2011; Torres-Pelayo et al., 2011], and studies in a closely related colobine monkey have validated the use of fecal estradiol RIA to measure concentrations of fecal estrogens to monitor ovarian function [Lu et al., 2010, 2012].

RESULTS

Solicitation and Copulation Frequencies in the Mating and Nonmating Seasons

Female solicitation and copulation frequencies are shown in Table 3. When both males were housed with the females, both females solicited copulations and copulated more frequently with adult male Ogong than with subadult male Changjang, although both females copulated with both males (Table 3). Shaoring copulated with Ogong significantly more frequently during the mating season than the nonmating season.
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**TABLE 3.** Mean copulation and solicitation rates per observation day for females Shaoring and Lili during the mating season (September–November) and the nonmating season (December–February)

<table>
<thead>
<tr>
<th>Season</th>
<th>Female</th>
<th>Interactions with O Gong</th>
<th>Interactions with Changjang</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Solicitations/ day</td>
<td>Copulations/ day</td>
</tr>
<tr>
<td>Mating season</td>
<td>Shaoring</td>
<td>5.4 ± 9.3</td>
<td>4.0 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>Lili</td>
<td>0.15 ± 0.44</td>
<td>0.94 ± 1.14</td>
</tr>
<tr>
<td>Non-mating season</td>
<td>Shaoring</td>
<td>0.47 ± 0.84</td>
<td>0.89 ± 0.88</td>
</tr>
<tr>
<td></td>
<td>Lili</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Lili was not housed with O Gong during the nonmating season; Shaoring was housed with O Gong in December and early January, but with Changjiang and Lili in January and February.

![Fig. 1. Solicitation and copulation frequencies for Shaoring](image)

(F<sub>1.85</sub> = 8.955343, P < 0.01, ANOVA). As Lili was isolated for much of the nonmating season, we did not make comparisons between seasons for Lili.

There were significant correlations between daily solicitation and copulation frequencies for Shaoring (with O Gong: r = 0.741, P < 0.01, n = 67; with Changjiang: r = 0.504, P < 0.01, n = 41; Fig. 1). For Lili, sample sizes were lower, and the relationship between daily solicitation and copulation frequencies directed at O Gong approached significance (r = 0.323, P = 0.062, n = 33), but the relationship was not significant for behaviors directed at Changjiang (r = –0.080, P < 0.621, n = 41).

**The Relationship Between Fecal Estrogens and Copulation Frequency**

Shaoring’s copulation frequency was positively correlated with her fecal estrogens (r = 0.71, P < 0.01, n = 50), and her copulation frequency varied cyclically during the mating season, reaching peaks at ~26-days intervals (Fig. 2). There was not cyclical variation in Shaoring’s fecal estrogens during the nonmating season (from December; Fig. 2). However, after O Gong was removed and replaced with Changjiang, Shaoring’s estrogen levels showed a sharp increase, approaching the levels seen during the mating season (Fig. 2), and Shaoring and Changjiang copulated frequently (Table 3).

Only six fecal samples were collected from Lili prior to her injury and isolation from the group. Six days after Lili was isolated, however, her estrogen levels peaked (Fig. 2). After Lili was returned to the group in January, she was observed to copulate only once, and her fecal estrogen levels remained consistently low. Neither female conceived during the study period, but Shaoring conceived in the subsequent year and gave birth to an infant on April 1, 2010. Lili gave birth in 2011.
DISCUSSION

Seasonality of Mating

Captive *R. roxellana* in Korea showed a clear mating season from September to November like their wild conspecifics [Ren et al., 1995; Qi et al., 2008], although females in Everland were provisioned year-round and did not experience seasonal variation in food availability or quality. Our results and those of other studies of captive *R. roxellana* [Li and Zhao, 2007; Ren et al., 2003b; Zhang et al., 2000], suggest that the seasonality of mating and births in *R. roxellana* is mediated by changes in climatic variables (e.g., light or temperature regimes), rather than diet.

Seasonal and Social Influences on Estrogen Levels and Sexual Behavior

Our results show regular and concordant peaks in fecal estrogens, solicitation frequencies, and copulation frequencies in female *R. roxellana* in the mating season. This suggests that cyclical changes in estrogen levels, which are probably associated with ovulation, affect female sexual behavior in this species, as noted by Yan and Jiang, [2006]. However, behavioral changes may also trigger changes in hormone levels, and there is abundant evidence that female physiology and behavior can be influenced by social circumstances. For example, ovulation is generally suppressed in subordinate female callitrichids [Abbott, 1984; Ziegler et al., 1987], and the timing of estrus in several primate species appears to be affected by other females [French and Stribley,
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1987; Matsumota-Oda et al., 2007; Pereira, 1991; Yan and Jiang, 2006]. Changes in female behavior and physiology following male takeovers, including sexual swellings in pregnant and lactating females [Swedell, 1999; Zinner and Deschner, 2000] and postconception estrus [Struhsaker and Leland, 1987] have also been reported in several primate species. These responses may confuse paternity and thus reduce the risk of infanticide [Struhsaker and Leland, 1987; van Schaik and Janson, 2000; Xiang and Grueter, 2007].

We observed pronounced elevation of fecal estrogens, solicitation rates, and copulation rates of one female, Shaoring, immediately after the group male was replaced by another despite the fact that the replacement occurred during the nonmating season. *R. roxellana* in the Shanghai Wild Animal Park also showed high copulation rates outside of the mating season following male takeovers [Ren et al., 2002], although endocrine correlates of these behavioral changes were not documented. These observations suggest that sexual behavior in the nonmating season serves an important social function in this species. For example, copulation may be involved in the formation or maintenance of social relationships between males and females, as well as confusing paternity [Yan and Jiang, 2006; Zhao et al., 2008]. Our results also indicate that fecal estrogens are affected by social changes in this species, and suggest that the relationship between female estrogen levels and sexual behavior in *R. roxellana* is not restricted to the mating season.

CONCLUSIONS

1. In the mating season, the frequency of female copulation solicitations is correlated with copulation frequency, and copulation frequencies are correlated with fecal estrogen levels.
2. Female fecal estrogen levels are affected by social as well as seasonal cues, and estrogen peaks can occur outside of the mating season.

ACKNOWLEDGMENTS

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REFERENCES


