Infant bystanders modulate the influence of ovarian hormones on female socio-sexual behaviour in free-ranging rhesus macaques

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Summary

It has long been established that one of the driving factors underlying changes in female socio-sexual behaviour across the ovarian cycle is variation in the hormones oestrogen and progesterone. However, the effect that the social environment, and specifically con-specific bystanders, exerts on social relationships is far less clear. Here we explore the modulating effects of infant bystanders on relationships between female ovarian cycling and socio-sexual behaviour in free-ranging rhesus macaques during the 6-month mating season on Cayo Santiago, Puerto Rico. We used non-invasive hormone assessment to time ovulation in females, and analysed measures of social and sexual behaviour with respect to a 2-day ovulation window. Rates of copulation and ejaculation varied relative to ovulation, with female–male sexual interactions peaking around ovulation. Moreover, the presence of an infant bystander affected these rates, with fewer sexual interactions occurring for a given day with respect to ovulation when infant bystanders were more frequently in close proximity to the female. Other bystander categories (adult females, adult males, and adult female & infant groupings) did not have the same effect on female mating behaviour. These results suggest that mother–offspring conflict might manifest not only as direct interactions between mother and infant (e.g., weaning or carrying conflict), but also through indirect interactions.

Keywords: Bystander effects, mother–offspring conflict, socio-sexual behaviour, rhesus macaque.

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1. Introduction

Reproduction among female mammals is driven by variation in underlying physiology and behaviour, but may also be influenced by the social environment. For example, field voles (*Microtus agrestis*) who breed in matrilineal kin clusters experience increased reproductive success compared to voles breeding separately (Pusenius et al., 1998), while African elephants (*Loxodonta africana*) living in social groups with weaker female bonds experience decreased reproductive output compared to elephants living in more connected groups (Gobush et al., 2008). A variety of factors may drive relationships between the social environment and reproduction. In African elephants, individuals with weak social bonds exhibited elevated basal faecal glucocorticoid levels, reflecting chronic stress in females for whom social support was not available (Gobush et al., 2008), and chronic stress has been shown to have deleterious effects on growth, immune response, and reproduction (Romero, 2004). Other important factors could include: changes in coalitionary support for females (Sterck et al., 1997), levels of protection from harassment from conspecifics (Smuts, 1985), tolerance from higher-ranking group members (Henzi & Barrett, 1999), and infanticide risk (Palombit, 1999). Although relationships between various components of social context (e.g., number of kin in breeding group or strength of social bonds) and reproduction have been documented in several species (e.g., chacma baboons (*Papio cynocephalus ursinus*): Silk et al., 2009), how variation in particular social surroundings influences mating behaviours at specific times is relatively unknown for most social animals.

In contrast to the context-specific consequences of social setting, the underlying hormonal regulation of mating behaviour is remarkably consistent across taxa, and is well documented. Among catarrhine primates, both experimental and observational studies on captive populations have demonstrated that female social and sexual behaviours are highly correlated with levels of ovarian hormones (e.g., humans (*Homo sapiens*): Dennerstein et al., 1980; chimpanzees (*Pan troglodytes*): Nadler et al., 1994; gorillas (*Gorilla gorilla*): Atsalis et al., 2004; pigtailed macaques (*Macaca nemestrina*): Short et al., 1989; Japanese macaques (*Macaca fuscata*): Soltis et al., 1999; Fujita et al., 2001). One species in which such changes have been particularly well studied in captive settings is the rhesus macaque (*Macaca mulatta*) (e.g., Zumpe & Michael, 1970; Johnson & Phoenix, 1976; Gordon,

1981; Wilson et al., 1982; Wallen et al., 1984; Wallen & Tannenbaum, 1997; Zehr et al., 1998; Wallen, 2001). In captive rhesus, rates of female proceptive and receptive behaviours increase as oestradiol levels rise, and decrease as oestradiol levels fall and progesterone levels rise, with the frequency of socio-sexual interactions with males peaking around ovulation and the period of maximum fertility (e.g., Gordon, 1981; Wallen et al., 1984). Advances in non-invasive hormone assessment over the last few decades have made it possible to determine hormone metabolite levels from urine and faeces in wild and free-ranging populations, with many studies of catarrhine primate populations now using such methods to estimate likely ovulation windows, and to investigate relationships between female fertility, and female and male reproductive activity. Such studies have found relationships between female ovarian hormones and socio-sexual behaviours, demonstrating that these behaviours either correlate with levels of oestrogens (positively) and progestogens (negatively) (e.g., olive baboons (*Papio hamadryas anubis*): Higham et al., 2009a), vary specifically with respect to the timing of ovulation (so potentially revealing fertility to males) (e.g., Tonkean macaques (*Macaca tonkeana*): Aujard et al., 1998; Japanese macaques: O'Neill et al., 2004), or both (e.g., chimpanzees: Emery Thompson, 2005; long-tailed macaques (*Macaca fascicularis*): Engelhardt et al., 2005).

The types and frequencies of socio-sexual interactions between captive male and female rhesus macaques are not only proximately regulated by hormones, but are affected by group size and composition as well (e.g., Gordon, 1981; Wallen & Winston, 1984). As female rhesus monkeys live naturally in large multi-male, multi-female social groups, and dyadic interactions between males and females in this gregarious species rarely occur in a social vacuum, behaviours and patterns of associations exhibited by female rhesus macaques across the ovarian cycle are necessarily affected by social context. For example, experiments conducted on captive rhesus macaque male-female dyads have demonstrated that the presence of female conspecifics during testing influences the type and rate of social and sexual interactions with males across the ovarian cycle. Specifically, females displayed higher rates of copulation and proceptive behaviours when female–male dyads were isolated than when in a group setting (Wilson et al., 1982; Wallen & Winston, 1984).

Despite such evidence for social effects on primate mating, very few studies of either captive or free-ranging primate populations have investigated

the effects that specific social contexts, and in particular, specific bystanders, have on socio-sexual behaviours. Many studies investigating the effects of bystanders focus on communicative contexts, such as alarm or food calls. For example, red-bellied tamarins (*Saguinus labiatus*: Caine et al., 1995) and chimpanzees (Brosnan & de Waal, 2003) display higher rates of food calls when in the visual presence of conspecifics, and solitary male Thomas langurs (*Presbytis thomasi*) produce alarm calls less frequently than group-living males (Wich & Sterck, 2003). Recently, a few studies have investigated the influence of bystanders on non-human primate behaviour in a reproductive context. For example, free-ranging rhesus macaque mothers on Cayo Santiago were more likely to allow suckling when a higher-ranking, "high-risk" female was present (Semple et al., 2009), and non-alpha bonobo males (*Pan paniscus*) experienced increased mating success with oestrous females when their mothers were in close proximity (Surbeck et al., 2010). Such studies suggest that behaviours aside from calls are susceptible to bystander effects and that both the presence of bystanders and their relationship to the focal animal are important.

One particularly interesting bystander effect might be that of infants on their mother's behaviour as she resumes cycling. Due to parent-offspring conflict (Trivers, 1974), it is in the interest of the current infant to delay their mother's future reproduction, such that we might predict infant bystanders to have a dampening effect on their mother's mating behaviour in order to gain extended maternal investment. Here, we investigate the effect that infant bystanders have on female socio-sexual behaviour around the time of ovulation in the seasonally reproducing, free-ranging rhesus macaque population on Cayo Santiago, Puerto Rico. This population exhibits an inter-birth interval of approximately one year (Hoffman et al., 2010), meaning that most females are still nursing or weaning the previous year's infant as they regain reproductive capability and become sexually active. Thus, even during the mating season, many females spend a large proportion of their time in close proximity to an infant, such that infant presence might act as a constraint on female social and sexual interactions with males. This could occur either if the infant actively interfered in female–male interactions, or if the infant indirectly and passively interfered, with the mere presence of an infant distracting or arousing the female, male, or both. Females in this population do not always become pregnant every year (Hoffman et al., 2010), and experience several cycles before conception (e.g., during the 2007 mating season,

an average of 2.96 cycles to conception (range 1–6, $N = 22$) Higham et al., 2011). As such, infants could obtain extended maternal investment by delaying reproduction of their mothers for one or more cycles, or even potentially for an entire year if mothers fail to conceive during that mating season.

We collected behavioural data on female–male social and sexual interactions while documenting the presence of bystanders at all times. We also measured oestrogen and progesterone metabolites non-invasively from faeces in order to estimate the ovulation window in individual female cycles. We analysed these data with two specific aims: (1) to determine relationships between female–male socio-sexual interactions and the ovulation window, predicting that rates of copulation, ejaculation, grooming, and male interest will increase as a female approaches ovulation; and (2) to test the hypothesis that infant bystanders can modulate the influence of female fertility on female–male social and sexual interactions; specifically, we predicted that infant proximity to a female–male consortship pair would decrease the frequency of sexual behaviour, as well as affiliative and agonistic interactions, with respect to each day of the ovarian cycle. We also assessed the effects of other categories of bystanders to act as controls to any effects related to infants.

2.   Methods

2.1.   Study site and subjects

This study was undertaken on Cayo Santiago, a 15.2 ha island located 1 km off the east coast of Puerto Rico (Rawlins & Kessler, 1986). The Cayo Santiago colony contains approximately 1000 free-ranging rhesus macaques living in six naturally formed social groups containing between 80 and 300 individuals. Macaques on Cayo Santiago are provisioned with monkey chow and rainwater, but also forage naturally on vegetation. Behavioural and physiological data were collected in Group R, which contained approximately 240 individuals at the time of data collection (approximately 35 adult males, 75 adult females, 35 sub-adults, 45 juveniles and 50 infants).

Rhesus macaques are seasonal breeders (Gordon, 1981), with the population on Cayo Santiago currently mating during a 6-month window between March and August (Hoffman et al., 2008). Data were collected for the present study from April to the end of July 2009, encompassing the majority

of one full mating season. Behavioural data and faecal samples permitted us to time ovulation to a 2-day window in 8 adult females.

2.2. *Behavioural data collection*

Females were observed between 7:00 and 14:30 h using a combination of continuous focal-animal and instantaneous scan sampling (Altmann, 1974). Individuals were observed for multiple 30-min periods with at least 5 min between consecutive observation periods. Females became focal subjects as soon as they entered behavioural oestrus, defined as the first day mating was observed after an extended period with no sexual interactions. Focal subjects were observed daily for as long as mating persisted, and for four days following cessation of all mating behaviour to ensure that we obtained sufficiently regular faecal samples around ovulation (given that there is a 2–3 day excretion lag for hormone metabolites in faeces, see below). Focal subjects were then observed on three different days over the following 2 weeks to capture post-oestrous behaviours and associations. Behavioural data were collected during both behavioural oestrus and post-oestrus periods in order to study changes in female–male interactions across the ovarian cycle and relative to the ovulation window (e.g., chimpanzees: Emery Thompson, 2005; long-tailed macaques: Engelhardt et al., 2005; Japanese macaques: O'Neill et al., 2004; Tonkean macaques: Aujard et al., 1998).

Continuous behavioural data were collected using a Psion Workabout (www.psionteklogix.com) loaded with "Behaviour" software (Syscan International, Montreal, QC, Canada). We here focus on several key social and sexual behaviours (Table 1) that are commonly analyzed in studies examining the relationship between ovulation and behaviour in macaques and baboons (e.g., macaques: Manson, 1996; Wallen & Tannenbaum, 1997; O'Neill et al., 2004; Engelhardt et al., 2005; baboons: Higham et al., 2009a). These include female–male interactions, as well as females' social interactions with other group members. Following precedent for behavioural research in macaques, we recorded various expressions of sexual behaviour (i.e., mounting, muzzle-ups, copulation, and copulation with ejaculation) (as in Manson, 1996). Each of these four variables were analyzed separately, as it has been shown that each has the potential to indicate a component of female sexual behaviour (Beach, 1976). Additionally, we recorded all instances of affiliative (grooming) and agonistic behaviour (threats, lunges, chases, and physical aggression) with all conspecifics (as in Widdig et al., 2002; Brent et al.,

Table 1. Ethogram of social and sexual behaviours analyzed in the present study.

Social behaviours	Sexual behaviours
Groom: Grooming exchanged between two individuals	Mount: Mounting without intromission
Non-contact aggression	Muzzle-up: Male approach and quick presentation of his face and neck
Threaten: Open-mouth threats without any body movement	Copulate: Mounting with intromission
Lunge: Aggressive movement forward of less than 5 m with no observed physical contact	Copulate with ejaculation: Mounting with intromission and confirmed ejaculation
Chase: Chasing or being chased with no observed physical contact	
Contact aggression	
Any physical aggression given to another individual	

2010) as both male–female and female–female interactions vary across the ovarian cycle in captive rhesus macaques (Wallen & Tannenbaum, 1997). For all social and sexual behaviours, with the exception of chases, the interaction partner (either age/sex category or identity, if known) as well as the direction of the behaviour (e.g., whether the female was grooming *or* being groomed) were recorded. Due to the speed and somewhat chaotic nature of chases, we were able to record directionality but not interaction partner with sufficient regularity for analysis.

A total of 109 h of observational data were collected by T.M and J.H., with each of the 8 females being observed for, on average, 12.1 h over 25 observation sessions (with, on average, each female being observed for approximately 1.5 h per day across the behavioural oestrus and post-oestrus periods). In conjunction with continuous data collection, instantaneous scan samples were taken every 5 min during a focal observation, in which the identity (if known) or age/sex class of individuals within 2 m of the focal animal was recorded. Female dominance ranks are known on Cayo Santiago as they are maternally inherited, but we additionally collected data on the outcome of dyadic agonistic interactions for both males and females to determine sex-specific dominance hierarchies and to allow us to control for rank effects in analyses. These dyadic interaction data were placed into a winners-losers matrix and MatMan was used to generate statistically significant dominance hierarchies for each sex separately (see Higham & Maestripieri, 2010 for

male data). Males of different ranks employ different mating strategies (e.g., Berard et al., 1994), with high-ranking males often mate-guarding and monopolizing the female in a way that middle and low-ranking males do not. Since the different mating strategies used by high versus non-high ranking males could result in large variation in the amount of time a male and female have to interact, males of different rank categories were treated both separately (for copulating, ejaculating and grooming behaviours — i.e., those behaviours that are affected most by proximity and time in association) and together ("adult males" category, assessed for all behaviours). To conduct the rank analyses, males were placed in rank categories with the top, middle, and bottom third of males categorized as high, middle, and low-ranking, respectively.

2.3. *Faecal collection and analysis*

Faecal samples were collected from females between 7:00 and 14:30, as studies often show low diurnal variation in endocrine metabolite values measured from faeces (e.g., Higham et al., 2009b), and expected changes in progesterone metabolite levels in response to ovulation are much greater than any documented diurnal variation. We collected 146 faecal samples in total, including one sample per individual every 1–2 days across the behavioural oestrus period (faecal sample collection frequency mean and SEM across all females during the oestrus period $= 1.7 \pm 0.1$ days) to enable accurate estimation of an ovulation window, as well as less frequent samples during a 2-week post-behavioural oestrus period. Faecal samples (uncontaminated with urine) were collected only after defecation was observed directly. Samples were then homogenized, non-faecal debris was removed, and a small bolus of approximately 0.5–2 g wet weight was placed in a 20 ml tube, which was in turn placed into a cooler containing ice packs (Hodges & Heistermann, 2003). Upon return to the mainland (at either 11:30 or 14:30), tubes were frozen at $-20°C$ until they were shipped on ice to the German Primate Center. All faecal samples arrived in Germany still frozen.

Faecal samples were prepared for Enzyme-Immuno Assay (EIA) by being lyophilized and pulverized followed by extraction of an aliquot (50–70 mg) of the faecal powder with 3 ml 80% methanol by vortexing for 15 min (Heistermann et al., 1995). All faecal samples were analyzed using an EIA for 5β-pregnane-3alpha, 20alpha-diol (PdG), a major progesterone metabolite in rhesus macaque faeces (Shideler et al., 1990). The assay has been

previously validated for assessing female reproductive status and timing of ovulation in the rhesus (Shideler et al., 1990; Dubuc et al., 2009). In addition, samples from four cycles for which the ovulation window could not be narrowed down to a 2-day window were tested for levels of oestrone conjugates (E1C: Heistermann et al., 1995), in order to help to define the ovulation window more accurately for cycles in which the frequency of faecal samples was insufficient to use PdG alone. Inter-assay variation, measured by repeated measurement of high and low value quality controls on each microtiter plate, was 11.5% (High) and 15.1% (Low) (PdG), and 3.7% (High) and 8.8% (Low) (EIC), while intra-assay variation was 6.8% (High) and 8.7% (Low) (PdG) and 4.1% (High) and 5.5% (Low) (E1C).

Given variability in the excretion lag of hormone levels in blood into faeces (Wasser et al., 1994), a 2-day ovulation window was calculated. This was defined as the days -2 and -3 before a post-ovulatory rise in PdG levels to a threshold of at least two standard deviations above the mean of the previous three values, which then remained elevated for at least two consecutive samples (Jeffcoate, 1983). In addition, E1C data were used to narrow the ovulation window to 2 days for cycles in which there were faecal sample collection gaps. We took the day of the E1C peak as the day of ovulation given that oestrogen levels peak 1 to 2 days before ovulation in blood (Jeffcoate, 1983), and given the excretion lag of this into faeces (Wasser et al., 1994). Within each cycle, each day relative to this ovulation window (DayRO) was coded, such that both days of the ovulation window were considered DayRO 0, the day preceding the ovulation window DayRO -1, the first day after the ovulation window DayRO $+1$, and so on (as in Higham et al., 2008, 2009a, 2010; Dubuc et al., 2009). As we expected changes in behaviour to be associated with the timing of the ovulation, we squared values of DayRO for analyses (Higham et al., 2008, 2009a, 2010; Dubuc et al., 2009). The use of this method implies that the corresponding days before and after ovulation (e.g., the day before and the day after the ovulation window, etc.) are treated equally in the analysis. However, this approach allows us to look for a non-linear relationship between DayRO and behaviour (i.e., low and rising before ovulation, peaking around ovulation, and then declining again) using linear models (e.g., Higham et al., 2008, 2009a, 2010; Dubuc et al., 2009).

2.4. Data analysis

All state behaviours (e.g., grooming) were calculated as durations per hour, while event behaviours (e.g., copulation) were calculated as frequencies

(number per hour). In addition to examining changes in social and sexual behaviours across the ovarian cycle, we also assessed male rank effects by analyzing female interactions with males of high, middle, and low rank separately. Additionally, we assessed the effects of different social partner categories, analyzing all interactions with specific age/sex groups separately (e.g., grooming with adult females vs. adult males). For ease of analysis and to allow clear quantification of broad scale effects, we created a binary indicator of infant presence by calculating the average proportion of instantaneous scans for which an infant was recorded within 2 m of the focal female across all observation days (average = 54.82%), then coding each day for each female for whether an infant was present more or less than the average. Although all females included in this analysis had a surviving infant from the previous year (aged between 6 and 11 months), we were unable to establish unequivocally at all times whether or not an infant in proximity to an adult female was this female's offspring. However, it seemed extremely likely to be the female's infant the majority of the time, as females still spend much of their time with their infants when they are under one year of age (Hinde & Spencer-Booth, 1967), but typically show little or no interest or engagement in other female's infants during mating. Further, males tolerate close proximity of the female's infant during mating periods, but do not affiliate with and are intolerant of other infants (reviewed in Redican, 1977). To determine whether changes in focal female behaviour were due to the presence of non-infant bystanders, we calculated averages and created binary indicators of the presence of other categories of individuals (e.g., adult males average = 19.85%, adult females = 21.30%, adult female and infant group = 13.15%), again coding each day for whether specific categories of individuals were present more or less than their average.

Behavioural data were analysed using Linear Mixed Models (LMMs) to determine relationships between social and sexual behaviours and DayRO, while at the same time investigating effects of bystanders on these relationships by including our bystander category variables in models, with each model containing only one bystander category at a time. As such, the first set of models tested whether each behaviour (dependent variable) varied with respect to DayRO (covariate), with bystander presence (infant, adult male, adult female or adult female & infant) as a fixed factor. Additionally, for all behaviours for which the first LMM provided a bystander result of $p > 0.1$ across all four bystander categories separately, we undertook a step down

simplification, excluding the bystander variable, and re-running the model. Where significant effects of DayRO and bystander presence on behaviour were found, rates of behaviour were averaged within each female and then across females during the fertile period (defined as the two day ovulation window plus the previous three days to account for the lifespan of sperm, Wilcox et al., 1995), as well as during the 5 days preceding the fertile period, and the 5 days following the fertile period. In all periods, daily average rates of behaviour were calculated separately for days in which bystanders were present more than average and days in which they were present less than average. This was undertaken not for statistical analysis, but merely to demonstrate the strength of effects. In all models, Female ID was included as a random factor to control for repeated observations of the same female. Tests were two-tailed with $p < 0.05$ considered significant. All analyses were performed in SPSS 18.0.

3.   Results

All females showed typical ovarian profiles, with hormone levels showing an average 3-fold increase in PdG values from the follicular to the luteal phase of the cycle (Figure 1). Values of E1C showed, on average, a 3-fold increase from baseline, approximately 3–4 days before the post-ovulatory rise in progestogens. The endocrine pattern found here, thus, resembled the typical cyclical changes of the two reproductive hormones during the course of the rhesus macaque ovarian cycle (cf., Shideler et al., 1990; Dubuc et al., 2009).

For social behaviours, we found that the total rates of chasing varied significantly both relative to DayRO and to infant presence, occurring more around ovulation, and in addition, more when an infant was present less than average (DayRO $F_{1,60.9} = 4.33$, $p = 0.042$; Infant presence $F_{1,62.7} = 7.45$, $p = 0.008$), but without an effect of any other bystander category. This result was driven by interactions for which the female was the chaser, not interactions in which the female was being chased (chasing: DayRO $F_{1,65.0} = 4.01$, $p = 0.049$; Infant presence $F_{1,65.0} = 5.28$, $p = 0.025$; being chased: both $p > 0.1$). Grooming bouts in which females groomed high-ranking males were significantly related to both ovulation and to the bystander category adult female and infant group, with grooming of high-ranking males occurring significantly more often around ovulation but less
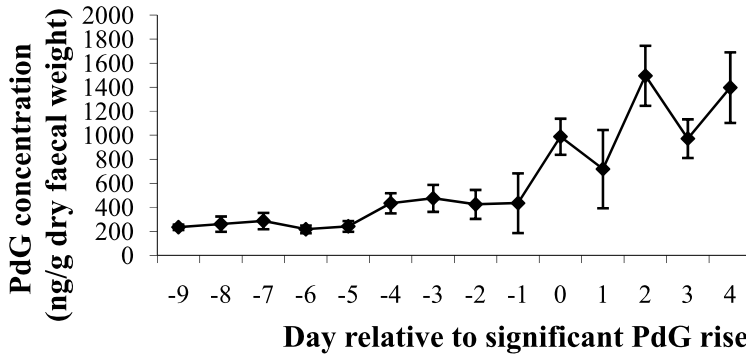
Figure 1. Composite hormonal profile of mean ($\pm$ SEM) 5β-pregnane-3α, 20α-diol (PdG: $N = 8$ cycles). Individual profiles were aligned to the day of significant PdG rise (day 0).

often when an infant and another adult female were nearby (DayRO $F_{1,59.1} = 4.66$, $p = 0.035$, Adult female & Infant presence $F_{1,64.5} = 8.08$, $p = 0.006$). Grooming given to or received from middle and low-ranking males was not affected by the presence of any category of bystander or DayRO (all $p > 0.1$). Finally, the rate of threats received from adult females was significantly elevated around the ovulation window, with no effect of any bystanders (DayRO $F_{1,63.89} = 6.73$, $p = 0.012$). No other social behaviours tested varied significantly relative to DayRO regardless of whether infant or non-infant bystanders were included or excluded from the models (all $p > 0.1$).

For sexual behaviours, we found that copulations ending in confirmed ejaculation occurred significantly more often both around ovulation and when an infant was present less than average (DayRO $F_{1,60.8} = 4.61$, $p = 0.036$; Infant presence $F_{1,61.7} = 8.12$, $p = 0.006$), with rates of copulation without ejaculation approaching significance for DayRO, but still with a significant negative effect of infant presence (DayRO $F_{1,61.4} = 3.26$, $p = 0.076$; Infant presence $F_{1,63.9} = 4.96$, $p = 0.030$). The effect of infant bystanders was particularly pronounced during the fertile phase of the cycle (Table 2). The presence of non-infant bystanders did not exert a significant effect on the rates of copulations or copulations ending in ejaculation (all $p > 0.10$). Additionally, the rate of muzzle-ups was significantly higher with respect to the timing of ovulation (DayRO $F_{1,61.6} = 4.57$, $p = 0.037$), but without an effect of infant or non-infant bystander presence. No other sexual behaviours varied significantly relative to DayRO, regardless of whether infant or other bystanders were included or excluded from

Table 2. Effect of infant bystander presence and ovarian cycle phase on rates of sexual behaviours.

	Rate of confirmed copulation with ejaculation (N/h)			Rate of confirmed copulation without ejaculation (N/h)		
	Pre-fertile	Fertile	Post-fertile	Pre-fertile	Fertile	Post-fertile
Infant present less than average	1.44	1.03	0.31	1.44	19.30	0.92
Infant present more than average	0.80	0.24	0.00	2.99	2.67	0.25

models (all $p > 0.1$), and there was no effect of male rank in any of the analyses.

4. Discussion

Our results indicate that certain female socio-sexual interactions with males are shaped by temporal proximity to ovulation as well as the presence of an infant bystander. Specifically, the frequencies of copulations and copulations with ejaculations were significantly lower for each day with respect to ovulation when a bystander infant was near the female more often than average. Rates of agonistic chasing in which the focal female was involved were also higher near ovulation, again with this behaviour occurring at a lower rate for each day of the cycle when infants were present more than average. Female grooming of high-ranking males significantly increased when females were closer to ovulation, but was significantly lower when in the presence of the grouping of an adult female and infant. Other behaviours such as the frequencies of male muzzle-ups (a potential indicator of male interest) and the frequencies of threats received from other females increased closer to ovulation but were not significantly affected by the presence of infants or other bystanders.

The observed changes in sexual, grooming and agonistic behaviour in relation to the timing of ovulation are consistent with the findings from previous studies of socio-sexual behaviour in group-living rhesus macaques (e.g., Gordon, 1981). Increased male copulation rate near ovulation probably reflects a combination of higher female attractiveness, proceptivity and receptivity. Increased female–male grooming may reflect higher female proceptive

behaviour or simply be a result of a greater amount of time spent in proximity to an adult male. Finally, higher frequencies of chases and threats may reflect, at least in part, greater female–female mating competition around the time of ovulation (see also Wallen & Tannenbaum, 1997). As previous research has shown, the above described changes in female socio-sexual behaviour are likely the result of changes in concentrations of circulating oestradiol and progesterone around the time of ovulation (Gordon, 1981). Interestingly, our study suggests that these hormonal effects on behaviour are modulated by the proximity of other individuals, and especially infants.

Of particular importance is that we show that increased infant presence resulted in decreased rates of certain sexual behaviours (copulation and copulations with ejaculations) for a given day relative to ovulation. Although we were unable to confirm the identity of infants in all cases, all focal females had infants from the previous breeding season and, thus, it is likely that the observed infant was their offspring in the vast majority of cases. This suggests that the observed infant bystander effects on female sexual interactions may be an expression of parent-offspring conflict, with infants interfering with their mothers' mating activities and thereby potentially delaying their reproduction in order to obtain more maternal investment (Trivers, 1974; Maestripieri, 2002). Of the eight mothers studied in the present study, two did not give birth in the following birth season, and of the remaining six, only one is thought to have conceived in their first cycle of the mating season, such that there is potential for infants to have delayed their mother's future reproduction. Notably, although we did find that non-infant bystanders exerted a minor effect on one social behaviour (grooming given to high-ranking males), non-infant bystanders did not significantly influence the rates of sexual behaviours. Although mother–offspring conflict is typically conceived of as behavioural conflict between these two actors, and is often measured by examining direct interactions between mother and infant such as time spent feeding, guarding, or carrying the young (e.g., weaning conflict in rhesus macaques: Hinde & Spencer-Booth, 1971; reviewed in Maestripieri, 2002), the present study indicates that mother–offspring conflict might also be manifest as indirect interactions between mother and infant.

The mechanisms underlying the observed infant bystander effects are difficult to determine without more information on infant behaviour during female–male interactions. In previous studies of bystander effects, the bystander presence was associated with an increased (Semple et al., 2009) or

decreased (Surbeck et al., 2010) risk of aggressive interactions for the focal individual, such that in both cases avoidance of aggression may have been the potential mechanism linking bystander proximity and changes in social behaviour. In contrast, our study illustrates behavioural modification in the presence of a non-threatening bystander such as an infant. It is possible that the infant affected behaviour through direct interaction with either social partner (e.g., glances), or interaction with other conspecifics that necessitated maternal intervention (e.g., getting into trouble with others), thereby affecting the outcome of the female–male interactions. Even without direct interference, the presence of the infant might also have increased arousal or nervousness in the female, leading her to decrease her rates of interaction with potentially aggressive partners (i.e., an adult male) or to decrease her rates of risky behaviours (e.g., chases) when an infant was in close proximity. Detailed focal observations on infants are required to distinguish these direct and indirect behavioural mechanisms.

The social interactions data revealed that other bystanders could exert a modest effect on social behaviour, as we found that females groomed high-ranking males significantly more often around ovulation, with a decrease in the frequency of grooming when in close proximity to both an adult female and infant. This result could be due to changes in behaviour of the focal female (e.g., distracted by the close presence of a bystander female or infant) or changes in male behaviour (e.g., male interest in and interaction with the bystander female). The current dataset of female behaviour does not allow for investigation of the first option, while focal observations of the male are necessary to determine the validity of the latter. However, irrespective of the mechanisms regulating the effects of infant or non-infant bystanders, it is important to note that infant bystanders exert an effect on reproductively meaningful behaviours in a manner that non-infant bystanders do not.

In sum, our findings contribute to the growing body of research showing that the effects of sex hormones on socio-sexual behaviour are modulated by social context, as well as to the evidence that dyadic interactions between individuals can be influenced by the presence of third parties. Here we have presented new evidence that infant bystanders may affect dyadic sexual and social interactions between females and males during the mating season, in specific and reproductively meaningful ways that non-infant bystanders do not, highlighting the myriad ways in which parent-offspring conflict may

manifest. Collectively, these findings add a new layer of complexity to our understanding of the regulation of behaviour in social, group-living animals.

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