Published in final edited form as:

Horm Behav. 2012 May ; 61(5): 696–705. doi:10.1016/j.yhbeh.2012.03.003.

Do males time their mate-guarding effort with the fertile phase in order to secure fertilisation in Cayo Santiago rhesus macaques?

Constance Dubuc^{1),2),3),4),*}, Laura Muniz²⁾, Michael Heistermann⁵⁾, Anja Widdig^{2),6),†}, and Antje Engelhardt^{1),3),†}

¹⁾Junior Research Group of Primate Sexual Selection, Reproductive Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany

²⁾Junior Research Group of Primate Kin Selection, Department of Primatology, Max-Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany

³⁾Courant Research Center “Evolution of Social Behavior”, Georg-August-Universität Göttingen, 37077 Göttingen, Germany

⁴⁾Département d'anthropologie, Université de Montréal, C.P. 6128, succursale Centre-ville, Montréal, H3C 3J7, Québec, Canada

⁵⁾Reproductive Biology Unit, German Primate Center, Kellnerweg 4, 37077 Göttingen, Germany

⁶⁾Institute of Biology, Faculty of Bioscience, Pharmacy and Psychology, University of Leipzig, Talstrasse 33, D-04103 Leipzig, Germany

Abstract

In contrast to most mammalian species, female sexual activity is not limited to the fertile phase of the ovarian cycle in anthropoid primates, which has long been proposed to conceal the timing of ovulation to males. It is now generally believed that females are still most attractive during the fertile phase, leading to high-ranking males successfully mate-guarding them specifically during this period. While studies conducted in species exhibiting exaggerated sexual swellings (probabilistic signal of the fertile phase) have generally supported this hypothesis, mixed support comes from others. Here, we investigated whether high-ranking males timed mate-guarding effort towards female fertile phases in rhesus macaques (*Macaca mulatta*). In this species, adult females do not exhibit sexual swellings, but undergo facial skin colour variation, an alternative oestrogens-dependent graded-signal of female reproductive status. We collected behavioural, hormonal and genetic paternity data during two mating seasons for one group of the free-ranging population of Cayo Santiago. Our results show that mate-guarding by top-ranking males did not completely cover the entire female fertile phase and that this tactic accounted for only 30-40% of all fertilisations observed. Males tended to prolong mate-guarding into the luteal phase (null probability of fertilisation), which mirrors the pattern of male attraction to female facial colour reported in an earlier study. These findings suggest that males may have limited knowledge regarding the exact timing of females' fertile phase in rhesus macaques, which presumably allows females to gain more control over reproduction relative to other anthropoid primate species.

© 2012 Elsevier Inc. All rights reserved.

*Corresponding author: Constance Dubuc, Tel/Fax: +1-787-852-0690, constance.dubuc@nyu.edu. Current affiliation: Department of Anthropology, New York University, Rufus D. Smith Hall, 25 Waverly Place, New York, NY 10003, USA. .

†Equally contributed

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

Prolonged sexual receptivity; concealed ovulation; mate-guarding; fecal steroids; reproductive strategies; fertile phase; genetic paternity analysis; primates; rhesus macaques

INTRODUCTION

In polygandrous species, males compete with each other over access to females during the period of fertility. A common and efficient behaviour to prevent other males from mating is to mate guard females during that period (e.g. Clutton-Brock, 1989; Isvaran and Clutton-Brock, 2007). In most animal species, female attractiveness and sexual receptivity to males is limited to the short period of the ovarian cycle during which mating can lead to conception, i.e. the oestrus period (Thornton and Finn, 1998). In anthropoid primates, however, females are also sexually active outside the fertile phase of the ovarian cycle, sometimes even during pregnancy, a phenomenon referred to as the loss of oestrus (Dixon, 1998; Hrdy and Whitten, 1987; Martin, 1992; Wallen, 2001). In those species, male mating success can only translate into reproductive success if it is properly timed with the fertile phase and males depend on female cues and/or signals other than sexual receptivity to detect this period.

Over the past several decades, much debate has been centred around the proximate consequences and ultimate function of the loss of oestrus in anthropoid primates. While it has long been assumed that it concealed the timing of ovulation to males (Sillén-Tullberg and Møller, 1993; reviewed in van Schaik et al., 2000), it is now generally believed that males are still more attracted to females when the probability of fertilisation is maximal (i.e. fertile phase) (e.g. van Schaik et al. 1999, 2000). The “female-dilemma hypothesis” (van Schaik et al., 2000; van Schaik et al., 2004; van Schaik et al., 1999) posits that sexual activity extending beyond the cycle’s fertile phase is part of a strategy by which females may decrease the risks of infanticide through paternity confusion, while still maximizing the probability of fertilisation by the highest ranking male, who is presumably the best protector against infanticidal attacks and/or the highest quality male. According to this hypothesis, this dual function is reached through a combination of oestrogens-dependant signals and cues that makes females (i) sexually attractive to males for a period much longer than the actual period of fertility – facilitating mating with several males – but (ii) most attractive to males during the fertile phase – increasing the probability of being mate-guarded by the highest-ranking male available during that period specifically. The classic example of a signal proposed to have evolved to serve this function is the exaggerated anogenital swelling that females of several primate species gradually develop in response to rising oestrogens levels during the follicular phase of the ovarian cycle (“graded-signal hypothesis”; Nunn, 1999; Zinner et al., 2004).

To date, most studies testing whether ovulation is concealed or not in anthropoid primates have been conducted in species in which females develop exaggerated sexual swellings, and this work has shown that top-ranking males time mate-guarding with the maximal swelling size and the period of fertility in several species (e.g. yellow baboons, *Papio cynocephalus*: Gesquiere et al., 2007; Barbary macaques, *Macaca sylvanus*: Heistermann et al., 2008; olive baboons, *P. anubis*: Higham et al., 2009; crested macaques, *M. nigra*: Higham et al., in press) allowing them to secure a large proportion of paternity (e.g. Alberts et al., 2006; but see: Brauch et al., 2008). However this pattern may not be universal (e.g. chimpanzees, *Pan troglodytes*: Deschner et al., 2004; bonobos, *P. paniscus*: Reichert et al., 2002) and particularly different in species lacking exaggerated swellings. Data from these latter species are still scarce to date and those that do exist show mixed support. Whereas males appear to

be able to time mate-guarding and/or mating effort in regard to the fertile phase in wild long-tailed (*M. fascicularis*; Engelhardt et al., 2004; 2005; 2006), and captive Japanese macaques (*M. fuscata*; Garcia et al., 2009), at least to a certain extent, they seem to lack this ability in wild Assamese macaques (*M. assamensis*; Fürtbauer et al. 2010; 2011), Hanuman langurs (*Semnopithecus entellus*; Heistermann et al., 2001b), and Tibetan macaques (*M. thibetana*; Li et al., 2005). Collectively, these results suggest that there might be wider inter-specific variation in the extent to which primate females give away information regarding the exact timing of the fertile phase than previously thought. This highlights the need to conduct more studies of this nature in different species, especially those without exaggerated swellings, to identify the context under which the timing of fertility is revealed or concealed in primate females.

In this respect, rhesus macaques (*M. mulatta*) are an interesting species to test whether high-ranking males can properly time their mate-guarding attempts with the fertile phase. In this species, adult females do not develop the exaggerated swelling typical for cercopithecines, which is thought to be due to a secondary loss during their evolution (Dixon, 1983; 1998; Nunn, 1999). In contrast, adult females undergo a cyclical change in facial coloration, an oestrogens-dependent feature that contains information about the timing of the fertile phase (Dubuc et al., 2009; Higham et al., 2010). However, a recent experimental study conducted in free-ranging rhesus macaques showed that while males were more attracted to images of faces collected in the fertile phase than to those collected earlier in the cycle, they were equally attracted to images collected during the fertile phase and those collected later in the cycle, suggesting that they were not able to perceive the difference in facial colour between these two phases (Higham et al., 2012). Moreover, only males who were socially familiar with the females were able to differentiate those images (Higham et al. 2012). Thus, facial coloration might be a less precise and/or conspicuous signal of the fertile phase than those exhibited by some other species, such as exaggerated sexual swellings. Rhesus macaque males may however have access to other, more reliable sexual cues under oestrogen control. Indeed, captive studies have shown that female proceptive behaviors are under control of oestrogens (Zehr et al. 1998) and thus are good predictors of fertility (Zehr et al. 2000), suggesting that female behaviours might be used as a cue in this species. It remains to be seen whether males are able to time mate-guarding with the fertile phase in this anthropoid species.

In rhesus macaques, male mate-guarding takes place through long-consortships in which a male coordinates his movements and activities with that of a receptive female for up to several consecutive days, with or without female cooperation (see Carpenter, 1942; Lindburg, 1983; Manson, 1997). On the male's part, this behaviour is costly not only because it translates into significant weight loss and energy expenditure (Higham et al., 2011; see also Alberts et al., 1996; Bercovitch and Nürnberg, 1996; Bercovitch, 1997; Matsubara, 2003), but also because it prevents to monitor or mate-guard other group females (e.g. Altmann, 1962; van Schaik et al, 1999). The little work conducted on this topic so far leads to believe that top-ranking males' consortships is not limited to the fertile phase in rhesus macaques (Carpenter, 1942; Chapais, 1983; Lindburg, 1983; Small, 1990). However, given that these studies were not in a position to estimate the timing of ovulation objectively (e.g. birth date: Small 1990; female mating activity: Chapais, 1983; Lindburg, 1983), a study investigating this question using objective means to assess the timing of the fertile phase (i.e. hormonal analysis) is needed to establish whether males are able to correctly time their mate-guarding effort with the fertile phase in this species.

Another interesting aspect of rhesus macaques is that they have a strict breeding seasonality (Brockman and van Schaik, 2005; Carpenter, 1942; Lindburg, 1983; Hoffmann et al., 2008). In this context, several females can be sexually receptive at the same time, preventing the

alpha male to mate-guard them all (Altmann, 1962). In order to optimize their time, energy, and, ultimately, reproductive success, alpha males are therefore expected to time their mating effort precisely with the maximal period of fertility if they are able to detect the fertile phase (Nunn, 1999). Overlap in females sexual activity might also lead to some females being mate-guarded by males other than the alpha male (priority-of-access model; Altmann, 1962) or else, not being mate-guarded at all (e.g. long-tailed macaques: Engelhardt et al., 2006). However, a relatively low reproductive success of top-ranking males is frequently reported for this species (e.g. Berard et al., 1993; Smith, 1994), lower than predicted based both on the degree of synchronisation of females' sexual activity and on their mating skew (Dubuc et al., 2011). As such, it appears possible that top-ranking males may not successfully monopolise females when the probability of fertilisation is at the highest in this species.

In this study, we investigated whether high-ranking rhesus macaque males timed their mate-guarding effort with female fertile phases in such a way as to secure fertilisation. We collected behavioural, hormonal and genetic paternity data during two consecutive mating seasons in one social group of the free-ranging population of Cayo Santiago, Puerto Rico. Long-consortships are typically formed in this population (e.g. Carpenter, 1942; Berard et al., 1994; Manson, 1997), which gave us the opportunity to investigate whether or not males time this costly behaviour with the fertile phase. In a previous study, we have shown that intermediate- and low-ranking males sired a significant share of the infants born in the group during the study period (Dubuc et al. 2011). Here, we examine the hypothesis that this is because top-ranking males did not time their mate-guarding effort with the fertile phase. We addressed four main questions: (1) how dominance rank influences male mate-guarding behaviour, (2) whether mate-guarding is timed with a female's maximal probability of fertilisation, (3) whether the degree of overlap between females' period of sexual activity influences the mate-guarding pattern, and (4) if mate-guarding was an efficient tactic allowing males to successfully secure fertilization in this population. This integrated approach will allow us to test whether, even though female rhesus macaques are sexually receptive for an extended period of time, they are still more likely to be mate-guarded by top-ranking males during the fertile phase, increasing the probability of paternity of those males.

METHODS

Study site and subjects

The study was conducted during two consecutive mating seasons (end of March to mid-September) in 2006 and 2007 on one troop (group V) of the provisioned free-ranging population of rhesus macaques living on Cayo Santiago. This 16-ha island off the coast of Puerto Rico is operated by the Caribbean Primate Research Center (CPRC; Rawlins and Kessler, 1986 for details on population). At the time of the study, the population was divided in six naturally formed heterosexual troops that can range freely throughout the island, which allows inter-group encounters, male dispersal between groups, as well as isolation from other group members (e.g., to sneak copulations). During the study, group V was composed of 21-23 adult parous females (6-24 years old), 4-9 sub-adult nulliparous females (3-5 years old), 17-25 adult males (> 4 years old), and 39-41 immature individuals across years, which appears to fall in the range of group size encountered in natural habitat in this species (reviewed in Fooden, 2000; Ménard, 2004). In the studied group, the group sex ratio ranged from 0.63 to 0.70 males per females (or 1.42 to 1.63 females per male). Based on an average of 4 sexually active females per day (Dubuc et al., 2011), the operational sex ratio was of 0.21 females per males (or 1 female per 4.75 males).

Behavioural and hormonal data collection was limited to adult parous females (i.e. excluded nulliparous females). Analyses were limited to conception cycles because male attraction towards females can vary between conceptive and non-conceptive cycles (e.g. Engelhardt et al., 2007). A total of 33 conception cycles from 20 females were detected during the study, 31 of these conceptions resulted in the birth of an offspring (17 in 2006, 14 in 2007), while two ended with a miscarriage (see details below). Of those 33 conception cycles, the timing of the fertile phase was hormonally established for 15 cycles from 14 females (5 in 2006, 10 in 2007). These cycles were used to examine the temporal relationship between high-ranking males' mating effort and the timing of females' fertile phase. Paternity could be reliably determined for 28 infants from 19 females (16 for 2006, 12 for 2007); three infants died before they could be sampled. The corresponding 28 conception cycles were used to evaluate the success of the mate-guarding tactic in terms of probability of fertilisation, with a special attention towards the 11 conception cycles from 10 females (4 for 2006, 7 for 2007) for which data on both the timing of the fertile phase and the identity of the sire were available. The investigation was approved by the IACUC of the University of Puerto Rico, Medical Sciences Campus (protocol No. 9400106).

Behavioural data collection

Behavioural data were collected from 7:00 to 14:30 (opening hours of the site), 5-6 days a week, for a total of 1045 contact hours with the group (498h in 2006, 547h in 2007). All occurrence of mating activity (mount, mount series, ejaculations), presence of sperm plugs in females' vagina and instances of male-female proximity (including following pattern) were recorded (Altmann, 1974; Martin and Bateson, 1986) for all parous females. Continuous data in 30-min focal protocols were also collected on receptive females every 1-3 days. In order to determine the male dominance hierarchy, male-male agonistic interactions (bites, chase, rush towards, threats, stare, displacement, flee, fear grin, spontaneous retreat, avoid) were noted *ad libitum* throughout the study (2006-2007) and during continuous sampling on males between August and September 2006 (Altmann, 1974; Martin and Bateson, 1986). Sexually receptive females and their consortship partners were identified daily by CD (see definitions below). In addition, the study group was observed by field assistants and other experienced observers in such a way that, at the peak of the mating season, 4-6 people monitored sexual activity in the group every day and shared *ad libitum* observations. These observations were combined to assess consortships' length of (see below).

Definitions of sexual receptivity and consortship

We used a very stringent definition of sexual receptivity, excluding the period of the ovarian cycle during which females were receptive and attractive to males, but with no witness of sexual activity. A female was considered *sexually receptive* if she was seen involved in a mating series or with a visible sperm plug (Dixon, 1998). Only mounts with penetration and pelvic thrusts that did not occur in a context of social tension and conflicts were considered. Since rhesus macaques are multiple-mounters, two observed mounts were considered part of the same mount series if they took place 30 minutes apart from each other, unless an ejaculation pause or a new sperm plug was observed (based on mating series lasting 1-56 minutes; Manson, 1996). Sexual receptivity follows a cyclic pattern in rhesus macaques. We therefore defined *receptive periods* for each cycle to be the period in which mating activity occurred surrounded by periods lacking mating activity. The receptive period was regarded as continuous if no more than 2 days were without observed mating activity in-between mating periods.

A male and a receptive female were considered *consortship partners* if they were seen sitting in close proximity (< 2m when in the group, < 5m when isolated from the rest of the group)

and synchronizing movements (i.e. follow; see van Noordwijk 1985) for at least 90 min (based on average sexual association length of 88 min: Berard et al., 1994), even when they were not seen mating. Duration was established based on the number of consecutive 30-minute blocs during which the pair were systematically seen together. Consortships were defined as *long-consortships* if the female was consorted (i) by the same partner for at least one whole day, (ii) proximity/movement synchronisation of the two partners was observed continuously (80% of observation time) and (iii) was maintained by the male (approach, follow, groom), with or without female cooperation. Cases in which proximity was completely maintained by the female were excluded because there was no evidence for males being attracted by the female.

Rhesus females often have more than one consortship partner per period of sexual receptivity (Carpenter, 1942; Loy, 1971). As such, we established a *consortship period*, which is defined as the consecutive days during which the female was systematically consorted, regardless of partner identity and the length of the association. This period does not include the days of the receptive period during which a female was in consortship only for a portion of the day in order to be sure to include only days of maximal attractiveness to males. We identified the *main consortship partner* as the male with whom the female was associated with for the longest long-consortship (i.e. highest number of days), i.e. forming the *main consortship*.

Male dominance hierarchy

Rhesus males' dominance relationships are rather stable over time, with new immigrants entering the group at the bottom of the hierarchy and queuing for dominance with rare direct dyadic aggressions (Berard, 1999; Bercovitch, 1992). During the study, all adult and sub-adult males who synchronized their movements with group V (i.e. including peripheral males) at some point during the two-year study period were considered as members of the group (30 in 2006, 27 in 2007), leading to a total of 37 different males across years. However, given that only central males consorted with females, analyses were limited to core male members of the group, i.e. the 19 males who were already in the group at the beginning of the study and/or those who resided in the group during both mating seasons.

A total of 426 agonistic dyadic interactions were recorded between those males across the two years (average number of interactions per male: 44.8, range: 20-82). We used the program SOCPROG (<http://myweb.dal.ca/hwhitehe/social.htm>) to generate the rank order, using the de Vries's "I&SI" method minimizing inconsistencies, and test for linearity, using the corrected landau index, h' , accounting for unknown relationships (de Vries, 1995, 1998; Whitehead, 2009a, b). A linear hierarchical order was generated (see Dubuc et al., 2011). Males from the top-third of the dominance hierarchy were referred to as high-ranking males. All males included in this category had resided in the group long enough to cover at least one entire mating season (including natal males: 2006: 3.7 ± 1.4 years, 2007: 3.3 ± 1.5 ; excluding natal males: 2006: 3.7 ± 1.5 , 2007: 3.0 ± 1.4). It should be noted that the alpha male was outranked by the beta male towards the end of the second mating season and that the reversed rank was used for the two conceptive cycles that took place after the overthrow.

Hormonal analysis

We collected faecal samples directly after defecation and discarded those that were contaminated with urine. The faecal boluses were homogenized and an aliquot of 0.5-2 g was placed in individual polypropylene tubes. The samples were kept on ice until return to the field station at the end of the observation day, where they were stored at -20°C . Samples were shipped on dry ice to the endocrine laboratory of the German Primate Center for hormone analysis. Samples were lyophilized, extracted with 3 ml 80% methanol and

measured for concentrations of progestogens and oestrogens using a validated microtiter plate enzyme immunoassay (EIA) for the measurement of pregnanediol glucuronide (PdG) and estrogen conjugates (E1C), major faecal metabolite of progesterone and oestrogens, respectively. These assays are described in detail by Heistermann et al. (1995), and have both been successfully used for monitoring female reproductive status and the timing of ovulation in macaque species (Fujita et al., 2001; Heistermann et al., 2001a; Shideler et al., 1993), including rhesus macaques (Dubuc et al., 2009). Sensitivity of the assays at 90% binding was 12.5 pg for PdG and 1.0 pg for E1C. Interassay coefficients of variation determined from quality controls were 10.6% (high, N=37) and 14.9% (low, N=37) for PdG, and 11.4% (high, N=27) and 14.6% (low, N=27) for E1C. Intraassay coefficients of variation were 7.2% (high, N=16) and 9.4% (low, N=16) for PdG and 5.3% (high, N=16) and 7.7% (low, N=16) for E1C.

Determination of the fertile phase and of the conceptive cycles

We used the patterns of faecal progesterone metabolites for the determination of the timing of ovulation, assessment of the conceptive cycles and of the fertile phase. We considered ovulation to have occurred when PdG concentrations rose above a threshold of the mean plus 2 standard deviations of 3-5 preceding baseline values, and maintained at this level for at least 3 consecutive samples (Heistermann et al., 2001a; Jeffcoate, 1983). Taking into account a time lag between secretion of progesterone into the blood and excretion of its metabolites into the faeces in macaques of 1-2 days (24-56 h; Shideler et al., 1993), we defined the *ovulation window* as days -2/-3 days relative to the defined PdG rise (cf. Brauch et al., 2007; Engelhardt et al., 2004; Heistermann et al., 2001a). A time lag of 1-2 days in faecal hormone excretion in the rhesus macaques of the Cayo Santiago population is supported by an average lag time of 41 hours (range: 24-70 h; N= 11 individuals) found for cortisol metabolite excretion in response to capture stress (Heistermann, unpublished data).

To account for sperm life span in the female tract (Behboodi et al., 1991; Wilcox et al., 1995; see also Czaja et al., 1975; Zehr et al., 1998 for rhesus macaques) and the gap in sample collection, we defined a 5-day *fertile phase* including the 2-day ovulation window and the three preceding days (Days -4 to -6 relative to the PdG rise). This method has been used to estimate the timing of the fertile phase non-invasively in primates (e.g. long-tailed macaques: Engelhardt et al., 2004; baboons: Higham et al., 2008; Assamese macaques: Fürtbauer et al., 2010; chimpanzees: Deschner et al., 2004). We referred to the 5 days before (Days -7 to -11) and the 5 days after the fertile phase (Days -1 to 3) as *pre-* and *post-fertile phases*, respectively. Details on how the days were plotted for statistical analyses are provided below (Behavioral data analysis).

We considered that the fertile phase could be established with sufficient reliability if the gap between the progestogen rise and the previous sample comprised three days or less (see Dubuc et al. 2009). In the extreme cases, this imprecision in the method may have led us to estimate days of the luteal phase as being part of the fertile phase. The fertile phase was established for 15 cycles: 11 conceptive cycles for which paternity was known, 2 conceptive cycles for which the infant died before being sampled and 2 miscarriages.

Conceptive cycles were identified as ovarian cycles in which levels of progesterone remained elevated for at least 30 days. We were able to use the hormonal profiles to identify 27 conceptive cycles, including 2 miscarriages. For the remaining 6 conceptive cycles, conception was identified by backdating from the date of birth (CPRC database) considering an average gestation length of 166.5 days (Silk et al., 1993).

Determination of paternity

Genetic paternity analysis was carried out by LM using the genotypic data previously available (methods detailed in Nürnberg et al., 1998), in addition to newly generated data for group-V individuals. Description on genetic analysis performed can also be found in Dubuc et al. (2011). Biological samples (hair, blood and tissue samples) were collected during the annual trapping season (mid-January to mid-March) 2007-2009 and shipped to the molecular genetics laboratory of the Max-Planck Institute for Evolutionary Biology for analysis. All infants born during the study period and still alive during trapping were sampled, as well as all mothers and potential sires for which DNA was lacking or insufficient beforehand. Males of the entire population older than 1250 days of age (based on youngest age at reproduction; Bercovitch et al., 2003) and present on the island at least 200 days before the actual birth of a given infant (i.e. longer than maximal pregnancy length; Silk et al., 1993) were considered as potential sires. A total of 98% of potential sires in the population were considered in the analysis, including all males who resided in group V at some point during the study period.

Genomic DNA was extracted using the DNEasy Blood & Tissue kit (Qiagen Inc., Valencia, CA, U.S.A.) and amplified at 12 to 15 autosomal microsatellite loci ($\text{mean} \pm \text{SD} = 13.5 \pm 1.5$). Paternity was determined for all 28 infants for which a sample was available using a combination of exclusion and likelihood analyses. All paternity assignment was supported at the 95% confidence level by the maximum likelihood method calculated by CERVUS 3.0 (Kalinowski et al., 2007). In 24 cases, all males but one (assigned sire) were excluded on at least two loci. In three other cases, the sire was assigned with zero mismatches with the offspring, but at least one potential sire could only be excluded at one locus. For the remaining case, the assigned sire had a single genetic mismatch with the offspring, but we are confident about the assignment because all other sampled males were excluded at 3 or more loci (see Dubuc et al., 2011).

Details on paternity distribution in relation to male dominance rank can be found in Dubuc et al. (2011).

Behavioural data analysis

We used the Spearman correlation coefficient to test for correlation between male dominance rank and the number of consortships formed. The alpha male was assigned rank 1, the beta male was assigned rank 2, and so on.

Two methods were used to determine whether mate-guarding was timed with maximal probability of fertilisation. Firstly, we compared the distribution of both (i) the overall consortship period, and (ii) the main consortship (see definitions above) between the three phases (pre-fertile, fertile and post-fertile) among females. We used general linear mixed models (GLMMs) (*F*-statistic) to compare the proportion of days females were consorted (response factor) between the three phases (categorical fixed factor). “Female ID” was included as a random factor in all the analyses, and “male ID” was included as a random factor in the analyses considering the main consortship. When applicable, we used the post-hoc pair-wise comparison LSD test to locate the existing variation.

Secondly, we tested whether females who had more than one consortship partner were associated with their highest-ranking consortship partner during the fertile phase, as one would expect if males have information concerning the timing of the fertile phase. Specifically, we tested the prediction that high-ranking males would consort females during the fertile phase and that consort partners would be lower in rank the further they would consort the female before or after this period, i.e. parabolic curve. Because we predicted males to be attracted to females the entire fertile phase, the value “0” (i.e. the expected

turning point of the parabolic curve) was attributed to the mid-point of the fertile phase (i.e. Day -4 relative to the PgG rise) in the analyses. In order to test for a linear relationship, Days -3 and -5 were assigned the value '1', Days -6 and -2, the value '2', Days -7 and -1, the value '3', and so on (e.g. Dubuc et al., 2009; Higham et al., 2008). All short and long-consortships formed during the cycles under study were included into the analyses. We used GLMMs fit by Laplace approximation for Poisson distributed data (Z statistic) to test for a relationship between the rank of the females' consortship partner (response factor) and the day of the cycle (continuous fixed factor), with both "female ID" and "male ID" included as random factors.

We then investigated whether the degree of overlap between females' period of sexual receptivity during the fertile phase influenced the probability of females to be consorted during their fertile phase. The *degree of overlap* was calculated as the average daily number of females who were sexually receptive at the same time as the female during the studied cycle. We used GLMMs (F statistic) to examine whether the degree of overlap (response factor) was different between females who were consorted during the majority of their fertile phase (i.e. 75 %) as compared to those who were not (categorical fixed factor), including "Female ID" as a random factor in the analyses. Moreover, to further investigate whether the degree of overlap may have influenced the distribution of consortship throughout the ovarian cycle, we compared the degree of overlap (response factor) between the three phases (categorical fixed factor), using the same statistical approach. When applicable, we used the post-hoc pair-wise comparison LSD test to locate the existing variation.

Finally, we evaluated the success of mate-guarding in terms of reproductive success. We established the proportion of infants that were sired by one of their mother's long-consortship partner during the mother's conceptive cycle, and the proportion that were sired by the main consortship partner. Moreover, we compared the rank of the sire with the average rank of the other consortship partners of a given female. We used GLMMs, setting male rank as the response variable and sire/partner as the categorical fixed variable, including both "female ID" and "sire ID" as random factors.

GLMMs fit with Laplace approximation for Poisson distributed data were performed with the R v. 2.6.0 statistical package for Windows (R Development Core Team, 2010) using the library "lme4" (Bates and Maechler, 2010). All other analyses were conducted in PAWS 18.0 (SPSS Inc.). "Mating season" and "cycle number" were dropped from all GLMMs analyses because they did not significantly contribute to total variation. The level of significance was set at $p < 0.05$ for all statistical tests. Average measures are presented as mean $\pm$ SD.

RESULTS

Male dominance rank and long-consortships

Long-consortships (i.e. 1 day) were only formed by males ranked 1-7 (i.e. the highest ranking males), and mainly by the three top-ranking males, all of which were familiar with the females. Females were seen in a long-consortship with the alpha male in only 21.4% of conception cycles ($N=28$). In 71.4% of the cycles, females formed a long-consortship with at least one of the three top ranking males. There is a significant relationship between the dominance rank of males and the number of females they formed long-consortships with across all males (Spearman ranked-test: $r_s = -0.841$, $p < 0.001$), as well as among the 7 highest-ranking males who formed long-consortships ($r_s = -0.775$, $p = 0.041$) (Figure 1). Females' main consortship partner was the highest-ranking out of all consortship partners they had in all but one case, and all had resided in the group for at least three years (2006: 4.0 ± 1.6 ; 2007: 3.6 ± 1.3).

Timing of mate-guarding with the maximal probability of fertility

As shown in Figure 2, both assays were highly successful in yielding the typical patterns of estrogen and progesterone during the ovarian cycle in rhesus macaques, from which timing of ovulation and the fertile phase could be reliably deduced.

In the studied cycles, female receptive periods lasted on average 10.6 ± 3.1 days ($N=33$ cycles, Figure 3). All but one female were mate-guarded for at least one day of their conceptive cycle (96.4%), and females usually had 1 to 3 long-consortship partners (mean: 1.26 ± 0.59 partners). Overall consortship periods lasted on average 7.0 ± 3.3 consecutive days. Long-consortships lasted 3.8 ± 3.0 days and main consortships, 4.6 ± 3.0 days (range: 1-9.5). Consortships were thus long enough to potentially cover the entire fertile phase.

In all but one case, the consortship period and the main consortship included at least one day of the fertile phase (Table I), and usually covered the ovulation window (Figure 3) ($N=15$ cycles). During the fertile phase, consortships lasted on average 3.2 ± 1.5 days (with 1.4 ± 0.66 consortship partners), while main consortships lasted for 2.5 ± 1.4 days, thus covering only half of this period. As a result, (i) only half of the fertile phase was covered by consortships (mean: $59.4 \pm 8.0\%$), (ii) the entire fertile phase was covered by the consortship period only for a small proportion of the females (26.6%) and (iii) usually less than half of the consortship period (mean: $48.2 \pm 4.8\%$) took place during that phase (Table I, Figure 3). Another large proportion of the consortship period was spent during the post-fertile phase (mean: $45.3 \pm 6.8\%$) and only a minor proportion of it took place during the pre-fertile phase (mean: $6.5 \pm 4.0\%$) (Table I, Figure 3). A similar pattern is obtained if only the main consortship is considered (Table I, Figure 3).

Accordingly, while the proportion of days of mate-guarding was significantly smaller during the pre-fertile phase than during the fertile phase (with a similar tendency when compared with the post-fertile phase), long-consortships were usually evenly distributed between the fertile and the post-fertile phases, showing no significant statistical difference between these two phases (GLMMs: $F=4.433$, $p=0.022$; fertile vs. pre-fertile: $p=0.007$; fertile vs. post-fertile: $p=0.368$; pre-fertile vs. post-fertile: $p=0.056$). This pattern is especially pronounced when the main consortship is considered ($F=12.749$, $p<0.001$; fertile vs. pre-fertile: $p<0.001$; fertile vs. post-fertile: $p=0.792$; pre-fertile vs. post-fertile: $p<0.001$). Given that the error in the method used may have led us to consider some days of the luteal phase as being in the fertile phase, the observation that the consortship period is often shifted towards the luteal phase is conservative. Furthermore, in 60% of the cases, females were still in consortship with their main consortship partner on the day of the PdG rise in the faeces, a day in which fertilisation was impossible.

A similar slight shift towards the post-fertile phase is also detected when inspecting the gradual change in male consortship partners' dominance rank over the course of the female cycle (Figure 4). Consortship partners were higher in rank (i.e. closer to 1) with progressing cycle and males of highest rank consorted the female at the end of the fertile phase/the beginning of the post-fertile phase. Hereafter, rank of male consort partners gradually decreased again (i.e. partner's relative rank increased). The turning point of the male partners' rank curve was thus on Day 0/1 rather than on Day -2, contrary to what was set in the Methods. As such, although there is no correlation between the day of the cycle and the relative rank if Day -2 is used as turning point (GLMMs; $Z=0.372$, $p=0.70995$), there is a significant association if Day 0 is used instead ($Z=2.066$, $p=0.0389$).

Degree of overlap

The average degree of overlap in receptivity during the fertile phase of the cycles under study was 4.7 ± 1.5 females (range: 1-7; Figure 5). There was no difference in the degree of

overlap during the fertile phase between the females who were consorted during 75 % of the fertile phase and those that were consorted for a shorter proportion of this phase (GLMMs: consortship period: $F=0.976$, $p=0.366$; main consortship: $F=0.234$, $p=0.637$). Moreover, there is no difference between the three phases in the number of females who were sexually receptive at the same time as the focal females (GLMMs: $F=1.809$, $p=0.182$) (e.g. Figure 5), although there is a non-significant tendency for having more females receptive in the post-fertile phase than in the pre-fertile phase (fertile vs. pre-fertile: $p=0.661$; fertile vs. post-fertile: $p=0.178$; pre-fertile vs. post-fertile: $p=0.079$).

Consortship and probability of fertilisation

Of the 28 infants for which paternity is known, only 39.3% were sired by one of their mother's long-consortship partners, with only 32.1% of the infants sired by the main consortship partner. Similarly, only 36.3% of the eleven long-consortships formed during the fertile phase led to conception. Looking at the results from a male perspective, of the 35 long-consortships formed with females during their conception cycle, only 31.4% resulted in successful fertilisation.

Only males ranked 1 to 4 successfully sired infants using the long consortship tactic. In general, sires were significantly lower ranking than was the average of males forming long-consortships with a female (GLMMs: $F=9.751$, $N=28$ cycles, $p=0.003$). However, if only the infants sired by long-consortship partners are considered, the sire was significantly higher-ranking than other males consorting the mother ($F=8.331$, $N=8$ cycles, $p=0.024$).

Interestingly, each year, the most successful sire (males ranked 3; different IDs; identified in Dubuc et al., 2011) used this tactic more successfully than other high-ranking males. These males formed long-consortships more often than other males (Figure 1), and 63 % of these led to conception. Moreover, although long-consortships by these males still did not cover the entire fertile phase (Figure 3), 75% of the consortships that included at least one day of the fertile phase led to conception (3 in 2006, 4 in 2007).

DISCUSSION

In this study, we examined the timing of male mate-guarding in relation to the period in which copulation can lead to fertilisation in conceptive ovarian cycles of free-ranging female rhesus macaques. Our results show that only high-ranking males mate-guarded females over the course of the cycles. Although mate-guarding was not randomly distributed throughout the ovarian cycle (e.g. it usually included the most likely days of ovulation) and was long enough to potentially cover the entire period during conception, females were in fact rarely mate-guarded throughout the entire course of their fertile phase. As a result, mate-guarding accounted for only about a third of all conceptions, with other fertilisations attributed to a non-mate guarding tactic performed by partners lower in rank than those mate-guarding the female.

The relatively low success of the mate-guarding tactic observed in our study echoes the results obtained in another study conducted on the same population. Berard and colleagues (1994) observed that males sired far fewer infants than one would have expected based on the amount of time invested into mate-guarding. Our study extends these earlier results by suggesting that the incomplete and imprecise mate-guarding of females during the fertile phase of conception cycles might contribute for this discrepancy between investment into mate guarding and return in terms of infants sired. Moreover, our findings provide an explanation for the relatively low reproductive skew in favour of high-ranking males generally reported for this species (Berard et al., 1993; Bercovitch and Nürnberg, 1997; Dubuc et al., 2011; Smith, 1994; Widdig et al., 2004), a skew that is lower than what one

would expect based on mating success (see Dubuc et al., 2011). This might explain why, in contrast to other primate species (e.g. yellow baboons: Alberts et al., 2006; long-tailed macaques: Engelhardt et al., 2006), alternative tactics such as sneak and opportunistic copulations appear to play an important role in shaping paternity distribution in rhesus macaques (Berard et al., 1994; Bercovitch, 1992; Dubuc et al., 2011).

Although mate guarding did not seem to guarantee fertilisation in our study, it may still have been the most efficient reproductive tactic used by rhesus males. Since consortship formation is the best predictor of mating rate in this species (Higham and Maestripieri, 2010) and the timing of ovulation generally appears to be honestly signalled (Higham et al., 2010), a male who mate-guards a female during the period when ovulation is most likely might have a reproductive advantage over other consortship partners if he (i) successfully prevents other males from mating with the female during that period, (ii) is able to mate several times with her, and (iii) produces high quality sperm. This idea is supported by the observation that each year, the most successful sire of our group fertilised a large number of females with whom he formed long consortships, even when the entire fertile phase was not covered. However, mate-guarding is not the sole efficient tactic available in this species. Indeed, in our study, males who were never seen mate-guarding females (i.e. dominance rank < 7) account for 32% of all fertilisations. Moreover, 35% of the fertilisations attributed to high-ranking males took place without mate-guarding, including the most successful sire (20%). Similar results were obtained in Berard et al. (1994). Consequently, perhaps the best way for males of this species to optimise their reproductive success is to combine mate-guarding with alternative tactics such as mating opportunistically with females who are not mate-guarded or sneak copulations. Given that the sex-ratio of the study group was less skewed than in most natural groups, perhaps top-ranking males' reproductive success is further decreased than it would be in a natural context. Studies conducted in wild and unprovisioned groups will be needed to accurately assess the efficiency in terms of reproductive output of mate-guarding in this species.

Interestingly, incomplete mate-guarding of females during fertile phases was not due to males' restricting it to short periods, but rather resulted from a shift of this period into the luteal phase of the cycle: while males rarely started mate-guarding early enough in the cycle to cover the entire fertile phase, they often continued this behaviour for up to a few days after the end of the fertile phase when conception was no longer possible. This observation is in contrast to what has been observed in some other primate species where the timing of mate-guarding was well aligned with the occurrence of the fertile phase and rapidly ending afterwards (e.g. long-tailed macaques: Engelhardt et al., 2004, 2005; yellow baboons: Gesquiere et al., 2007; crested macaques: Engelhardt et al., under review). Furthermore, while the turning point in males' mating effort occurred at the end of the fertile phase in rhesus macaques, it appears to happen in the middle of the fertile phase in other species (olive baboons: Higham et al., 2009; crested macaques: Engelhardt et al., under review). This shift in male's investment in the fertile phase might not be unique to rhesus macaques, however. For instance, a similar significant prolongation of males' mating effort within the post-fertile phase has been reported for wild chimpanzees, a species in which maximum sexual swelling size is maintained few days in the early luteal phase (Deschner et al., 2004).

Although the method we used introduced an error in the estimation of the timing of the fertile phase, this is unlikely to solely explain our results. Indeed, the fact that females were regularly mate-guarded on the day when a rise in progesterone levels were detected in the faeces shows that the female could not be fertile on that day. Similarly, our results are unlikely to be the sole consequence of the high degree of overlap between females' period of sexual activity in the population. Indeed, there were usually four females sexually active at a time, and as such, one of the seven high-ranking males who used the mate-guarding tactic

should have usually been available to mate-guard a female at the beginning of her fertile phase. Moreover, there was no difference in the degree of overlap between females who were mate-guarded the entire fertile phase and those who were not, and no difference in overlap between females' fertile and post-fertile phases. Another possible confounding factor may be that top-ranking males might have difficulties to mate-guard fertile females in a context where there is a high number of males in the group as it may be the case in the studied population. Although we cannot rule-out this possibility, it is not supported by the low level of harassment and levelling coalitions against higher-ranking males observed in this species (e.g. Bercovitch 1992, Higham and Maestripieri 2010). While a high degree of overlap in females' sexual activity and/or a high number of competitors might have accounted for why males often started mate-guarding females relatively late in the fertile phase, it does not explain why they prolonged mate-guarding into the luteal phase, an observation in contrast to what one would expect if males optimise their time and energy effort in a context of strict seasonality (see Nunn 1999).

There are different, non-mutually exclusive explanations for why mate-guarding was shifted towards the luteal phase in our study. One likely explanation for the observed temporal shift in male mate-guarding might be that male rhesus macaques receive limited information regarding the exact timing of the fertile phase. Indeed, our observation echoes the results obtained in an experimental study which showed that, while rhesus macaque males were more attracted to images of female faces collected during the ovulation window than to those collected during the pre-fertile phase, they were equally attracted to images collected during the fertile period and later in the post-fertile phase (Higham et al., 2012). As such, our results further supports the view that sexual signals emitted by rhesus macaque females are hard to interpret by males in comparison to other anthropoid primates. Another possible explanation is that male-female associations in the luteal phase are not a form of mate-guarding, but a consequence of the bond built up during the fertile phase. Consortship partners show a high degree of social tolerance towards each other with a high level of proximity, grooming and mating (e.g. Dubuc et al., in press). Maintenance of consortship could be adaptive if it allows solidifying the bond, facilitating the resumption of consortship in the female's subsequent proceptive period or strengthening the bond with potential offsprings' mother. Detailed behavioural studies combining sexual signals and cues, as well as detailed behavioural data will be needed to examine why long-consortships are often prolong in the luteal phase in this population of rhesus macaques.

Our finding that high-ranking males did not successfully mate-guard females during the entire fertile phase, preventing them to secure a large portion of fertilisation, is in contradiction to what is predicted by the female-dilemma hypothesis (van Schaik et al., 1999, 2004). While the pattern of mate-guarding observed in our study might still have allowed to maintain a high paternity confidence of high ranking males at the behavioural level, it appears to have facilitated fertilisation by lower ranking males since high-ranking males did not mate-guard during the entire fertile phase. Therefore, our results strengthen the view that in this species, female strategies play a crucial role in determining mating and paternity distribution among males in comparison to other anthropoid primate species, and that females do not necessarily prefer dominant males (e.g. Chapais, 1983; Manson 1992; 1997; Berard, 1999). The reason for this may be that rhesus females face a different dilemma than the one usually expected in primates: while the female-dilemma hypothesis assumes that the highest-ranking males are the best quality males and thus most attractive to females, this does not seem to systematically be the case in rhesus macaques (Berard, 1999; see also: Dubuc et al., 2011). In such a context, females may use different strategies than those predicted by the female-dilemma hypothesis, leading to the evolution of sexual signals and/or reproductive tactics (e.g. resistance to mate-guarding) that increases their behavioural freedom to exert mate choice and/or promiscuous mating during the period of fertility (see

Dubuc et al., 2011). This idea is supported by the observation that among some catarrhine primate species in which female signalling appears to confuse males in their ability to tune mating effort with the fertile phase (chimpanzees: Deschner et al., 2004; Assamese macaques: Fürtbauer et al., 2010, 2011; bonobos: Reichert et al., 2002), male dominance is not always based on individual attributes alone, but also on collective power resulting from coalition formation with allies and/or with female close relatives (Boesch, 2009; Furuichi, 1997; Mitani, 2009; Schülke et al., 2010; Surbeck et al., 2010).

In conclusion, our findings support the view that there is wide inter-specific variation in the interplay of female sexual signals and the efficacy of male mate-guarding effort in primate multi-male groups. More studies examining how the timing of high ranking males' mate-guarding effort with the fertile phase influences paternity outcome and how female strategies contribute to this pattern will be needed to fully understand under which conditions females may reveal or conceal the precise timing of the fertile phase to males and how this ultimately affects male reproductive success within multi-male multi-female primate groups.

Acknowledgments

We thank the CRPC for permission to conduct this study on Cayo Santiago, and CPRC employees. Particular thank goes to Véronique Martel, Julie Cascio, Camille Guillier, Edith Hovington, Giulia Sirianni, Isabelle Benoît, Claude Richer, and François Bourgault, as well as Juliet Alla, Charles MacIntyre, Doreen Langos and Akie Yanagi for assisting in data and sample collection; Lauren Brent, Amanda Accamando, Richard MacFarland, and Maria Rahkovskaya for sharing valuable observations; and Andrea Heistermann, Jutta Hagedorn, Sven Schreiter and Sara Hermann, for laboratory assistance. Linda Vigilant kindly provided laboratory access, and Michael Krawczak and Olaf Junge, to FINDSIRE software. We also thank Lauren Brent for sharing valuable observations, samples, and conversations, Bernard Chapais and Ines Fürtbauer for fruitful discussions. We are grateful to Geoff Gallice and two anonymous reviewers for helpful comments on previous versions of the manuscript. This project was funded by SSHRC (to CD), Université de Montréal (to CD), DFG (grant No. WI 1808/3-1 to AW; and grant No. EN 719/2-1 to AE), NSERC (to Bernard Chapais) and the German Initiative for Excellence. The population of Cayo Santiago was supported by the Medical Sciences Campus of the University of Puerto Rico and the National Center for Research Resources (NCRR), a component of the NIH (NCRR grant P40RR003640 award to the CPRC). The content of this publication are solely the responsibility of the authors and do not necessarily represent the official views of NCRR or NIH.

REFERENCES

- Alberts SC, Altmann J, Wilson ML. Mate guarding constrains foraging activity of male baboons. *Animal Behaviour*. 1996; 51:1269–1277.
- Alberts SC, Buchan JC, Altmann J. Sexual selection in wild baboons: from mating opportunities to paternity success. *Animal Behaviour*. 2006; 72:1177–1196.
- Altmann J. Observational study of behaviour: sampling methods. *Behaviour*. 1974; 49:227–267. [PubMed: 4597405]
- Altmann SA. A field study of the sociobiology of rhesus monkeys, *Macaca mulatta*. *Annals of the New York Academy of Sciences*. 1962; 102:338–435. [PubMed: 14012344]
- Bates, D.; Maechler, M. lme4: Linear mixed-effects models using S4 classes. 2010. R package version 0.999375-34
- Behboodi E, Katz DF, Samuels SJ, Tell L, Hendrickx AG, Lasley BL. The use of a urinary estrone conjugates assay for detection of optimal mating time in the cynomolgus macaque (*Macaca fascicularis*). *Journal of Medical Primatology*. 1991; 20:229–234. [PubMed: 1920378]
- Berard JD, Nuernberg P, Epplen JT, Schmidtke J. Male rank, reproductive behavior, and reproductive success in free-ranging rhesus macaques. *Primates*. 1993; 34:481–489.
- Berard JD, Nuernberg P, Epplen JT, Schmidtke J. Alternative reproductive tactics and reproductive success in male rhesus macaques. *Behaviour*. 1994; 129:177–201.

- Berard JD. A four-year study of the association between male dominance rank, residency status, and reproductive activity in rhesus macaques (*Macaca mulatta*). *Primates*. 1999; 40:159–175. [PubMed: 23179538]
- Bercovitch, FB. Sperm competition, reproductive tactics, and paternity in savanna baboons and rhesus macaques. In: Martin, RD.; Dixon, AF.; Wickings, EJ., editors. *Paternity in Primates: Genetic Tests and Theories*. Karger; Basel: 1992. p. 225-237.
- Bercovitch FB. Reproductive strategies of rhesus macaques. *Primates*. 1997; 38:247–263.
- Bercovitch FB, Nürnberg P. Socioendocrine and morphological correlates of paternity in rhesus macaques (*Macaca mulatta*). *Journal of Reproduction and Fertility*. 1996; 107:59–68. [PubMed: 8699435]
- Bercovitch FB, Nürnberg P. Genetic determination of paternity and variation in male reproductive success in two populations of rhesus macaques. *Electrophoresis*. 1997; 18:1701–1705. [PubMed: 9378148]
- Bercovitch FB, Widdig A, Trefilov A, Kessler MJ, Berard JD, Schmidtke J, Nuernberg P, Krawczak M. A longitudinal study of age-specific reproductive output and body condition among male rhesus macaques, *Macaca mulatta*. *Naturwissenschaften*. 2003; 90:309–312. [PubMed: 12883773]
- Boesch, C. *The real chimpanzee: sex strategies in the forest*. Cambridge University Press; Cambridge: 2009.
- Brauch K, Pfefferle D, Hodges K, Möhle U, Fischer J, Heistermann M. Female sexual behavior and sexual swelling size as potential cues for males to discern the female fertile phase in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. *Hormones and Behavior*. 2007; 52:375–383. [PubMed: 17644098]
- Brauch K, Hodges K, Engelhardt A, Fuhrmann K, Shaw E, Heistermann M. Sex-specific reproductive behaviours and paternity in free-ranging Barbary macaques (*Macaca sylvanus*). *Behavioral Ecology and Sociobiology*. 2008; 62:1453–1466.
- Brockman, DK.; van Schaik, C. Seasonality and reproductive function. In: Brockman, DK.; van Schaik, CP., editors. *Seasonality in Primates: Studies of Living and Extinct Human and Non-human Primates*. Cambridge University Press; New York: 2005. p. 269-305.
- Carpenter CR. Sexual behaviour of free ranging rhesus monkeys (*Macaca mulatta*). I. Specimens, procedures and behavioral characteristics of estrus. *Journal of Comparative Psychology*. 1942; 33:113–142.
- Chapais B. Reproductive activity in relation to male dominance and the likelihood of ovulation in rhesus monkeys. *Behavioral Ecology and Sociobiology*. 1983; 12:215–228.
- Clutton-Brock TH. Mammalian mating systems. *Proceedings of the Royal Society of London B*. 1989; 236:339–372.
- Czaja JA, Eisele SG, Goy RW. Cyclical changes in the sexual skin of female rhesus: relationships to mating behavior and successful artificial insemination. *Federation Proceedings*. 1975; 34:1680–1684. [PubMed: 1132527]
- de Vries H. An improved test of linearity in dominance hierarchies containing unknown or tied relationships. *Animal Behaviour*. 1995; 50:1375–1389.
- de Vries H. Finding a dominance order most consistent with a linear hierarchy: a new procedure and review. *Animal Behaviour*. 1998; 55:827–843. [PubMed: 9632471]
- Deschner T, Heistermann M, Hodges K, Boesch C. Female sexual swelling size, timing of ovulation and male behavior in wild West African chimpanzees. *Hormones and Behavior*. 2004; 46:204–215. [PubMed: 15256310]
- Dixon AF. Observations on the evolution and behavioral significance of 'sexual skin' in female primates. *Advances in the Study of Behavior*. 1983; 13:63–106.
- Dixon, AF. *Primate Sexuality: Comparative Studies of Prosimians, Monkeys, Apes, and Human Beings*. Oxford University Press; Oxford: 1998.
- Dubuc C, Brent LJN, Accamando AK, Gerald MS, MacLarnon A, Semple S, Heistermann M, Engelhardt A. Sexual skin color contains information about the timing of the fertile phase in free-ranging *Macaca mulatta*. *International Journal of Primatology*. 2009; 30:777–789.

- Dubuc C, Muniz L, Heistermann M, Engelhardt A, Widdig A. Testing the priority-of-access model in a seasonally breeding primate species. *Behavioral Ecology and Sociobiology*. 2011; 65:1615–1627. [PubMed: 21874084]
- Dubuc C, Hughes KD, Cascio J, Santos L. Social tolerance in a despotic primate: co-feeding between consortship partners in rhesus macaques. *American Journal of Physical Anthropology*. In press.
- Engelhardt A, Pfeifer J-B, Heistermann M, Niemitz C, van Hooff JARAM, Hodges JK. Assessment of female reproductive status by male longtailed macaques, *Macaca fascicularis*, under natural conditions. *Animal Behaviour*. 2004; 67:915–924.
- Engelhardt A, Hodges JK, Niemitz C, Heistermann M. Female sexual behaviour, but not sex skin swelling, reliably indicates the timing of the fertile phase in wild long-tailed macaques (*Macaca fascicularis*). *Hormones and Behavior*. 2005; 47:195–204. [PubMed: 15664023]
- Engelhardt A, Heistermann M, Hodges JK, Nuernberg P, Niemitz C. Determinants of male reproductive success in wild long-tailed macaques (*Macaca fascicularis*) -- male monopolisation, female mate choice or post-copulatory mechanisms? *Behavioral Ecology and Sociobiology*. 2006; 59:740–752.
- Engelhardt A, Hodges JK, Heistermann M. Post-conception mating in wild long-tailed macaques (*Macaca fascicularis*): characterization, endocrine correlates and functional significance. *Hormones and Behavior*. 2007; 51:3–10. [PubMed: 16889780]
- Fooden J. Systematic review of the rhesus macaques, *Macaca mulatta* (Zimmermann, 1780). *Fieldiana: Zoology*. 2000; 96:1–180.
- Fujita S, Mitsunaga F, Sugiura H, Shimizu K. Measurement of urinary and fecal steroid metabolites during the ovarian cycle in captive and wild Japanese macaques, *Macaca fuscata*. *American Journal of Primatology*. 2001; 53:167–176. [PubMed: 11283977]
- Fürtbauer I, Schülke O, Heistermann M, Ostner J. Reproductive and life history parameters in wild female Assamese macaques (*Macaca assamensis*). *International Journal of Primatology*. 2010; 31:501–517. [PubMed: 20651906]
- Fürtbauer I, Heistermann M, Schülke O, Ostner J. Concealed fertility and extended female sexuality in a non-human primate (*Macaca assamensis*). *PLoS ONE*. 2011; 6(8):e23105. [PubMed: 21853074]
- Furuichi T. Agonistic interactions and matrifocal dominance rank of wild bonobos (*Pan paniscus*) at Wamba, Zaire. *International Journal of Primatology*. 1997; 18:855–875.
- Garcia C, Shimizu K, Huffman M. Relationship between sexual interactions and the timing of the fertile phase in captive female Japanese macaques (*Macaca fuscata*). *American Journal of Primatology*. 2009; 71:1–12. [PubMed: 18850586]
- Gesquiere LR, Wango EO, Alberts SC, Altmann J. Mechanisms of sexual selection: sexual swellings and estrogen concentrations as fertility indicators and cues for male consort decisions in wild baboons. *Hormones and Behavior*. 2007; 51:114–125. [PubMed: 17027007]
- Heistermann M, Finke M, Hodges JK. Assessment of female reproductive status in captive housed Hanuman langurs (*Presbytis entellus*) by measurement of urinary and fecal steroid excretion patterns. *American Journal of Primatology*. 1995; 37:275–284.
- Heistermann M, Uhrigshardt J, Husung A, Kaumanns A, Hodges JK. Measurement of faecal steroid metabolites in the lion-tailed macaque (*Macaca silenus*): A non-invasive tool for assessing female ovarian function. *Primate Report*. 2001a; 59:27–42.
- Heistermann M, Ziegler T, van Schaik CP, Launhardt K, Winkler P, Hodges JK. Loss of oestrus, concealed ovulation and paternity confusion in free-ranging Hanuman langurs. *Proceedings of the Royal Society of London Series B. Biological Sciences*. 2001b; 268:2445–2451.
- Heistermann M, Brauch K, Moehle U, Pfefferle D, Dittami J, Hodges K. Female ovarian cycle phase affects the timing of male sexual activity in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. *American Journal of Primatology*. 2008; 70:44–53. [PubMed: 17577208]
- Higham JP, Maestripieri D. Revolutionary coalitions in male rhesus macaques. *Behaviour*. 2010; 147:1889–1908.
- Higham JP, MacLarnon AM, Ross C, Heistermann M, Semple S. Baboon sexual swellings: Information content of size and color. *Hormones and Behavior*. 2008; 53:452–462. [PubMed: 18206889]

- Higham JP, Semple S, MacLarnon A, Heistermann M, Ross C. Female reproductive signaling, and male mating behavior, in the olive baboon. *Hormones and Behavior*. 2009; 55:60–67. [PubMed: 18786539]
- Higham JP, Brent LNJ, Dubuc C, Accamando AK, Engelhardt A, Gerald MS, Heistermann M, Stevens M. Colour signal information content and the eye of the beholder: a case study in the rhesus macaque. *Behavioral Ecology*. 2010; 21:739–746. [PubMed: 22475874]
- Higham JP, Heistermann M, Maestripieri D. The energetics of male-male endurance rivalry in free-ranging rhesus macaques. *Animal Behaviour*. 2011; 81:1001–1007.
- Higham JP, Hughes KD, Santos LR, Dubuc C, Brent LNJ, Maestripieri DM, Heistermann M, Stevens M. Familiarity affects the assessment of female facial signals of fertility by free-ranging male rhesus macaques. *Proceedings of the Royal Society B: Biological Sciences*. 2012; 27:3452–3458.
- Higham JP, Heistermann M, Saggau C, Agil M, Perwitasari- Farajallah D, Engelhardt A. Sexual signalling in female crested macaques and the evolution of primate fertility signals. *BMC Evolutionary Biology*. In press.
- Hoffman CL, Ruiz-Lambides AV, Davila E, Maldonado E, Gerald MS, Maestripieri D. Sex differences in survival costs of reproduction in a promiscuous primate. *Behavioral Ecology and Sociobiology*. 2008; 62:1711–1718.
- Hrdy, SB.; Whitten, PL. Patterning of sexual activity. In: Smuts, BB.; Cheney, DL.; Seyfarth, RM.; Wrangham, RW.; Struhsaker, TT., editors. *Primate Societies*. University of Chicago Press; Chicago: 1987. p. 370-384.
- Isvaran K, Clutton Brock T.H. Ecological correlates of extra-group paternity in mammals. *Proceedings of the Royal Society of London Series B*. 2007; 274:219–224. [PubMed: 17148250]
- Jeffcoate, SL. Use of rapid hormone assays in the prediction of ovulation. In: Jeffcoate, SL., editor. *Ovulation: Methods for Its Prediction and Detection*. Wiley; Chichester: 1983. p. 67-82.
- Kalinowski ST, Taper ML, Marshall TC. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*. 2007; 16:1099–1106. [PubMed: 17305863]
- Li JH, Yin HB, Wang QS. Seasonality of reproduction and sexual activity in female Tibetan macaques (*Macaca thibetana*) at Huangshan, China. *Acta Zoologica Sinica*. 2005; 51:365–375.
- Lindburg, DG. Mating behavior and estrus in the Indian rhesus monkey. In: Seth, PK., editor. *Perspectives in primate biology*. Today & Tomorrow's Printers and Publishers; New Delhi: 1983. p. 45-61.
- Loy J. Estrous behavior of free-ranging rhesus monkeys (*Macaca mulatta*). *Primates*. 1971; 12:1–31.
- Manson JH. Measuring female mate choice in Cayo Santiago rhesus macaques. *Animal Behaviour*. 1992; 44:405–416.
- Manson JH. Male dominance and mount series duration in Cayo Santiago rhesus macaques. *Animal Behaviour*. 1996; 51:1219–1231.
- Manson JH. Primate consortships: a critical review. *Current Anthropology*. 1997; 38:353–374.
- Martin, P.; Bateson, P. *Measuring Behaviour: an introductory guide*. Cambridge University Press; Cambridge: 1986.
- Martin, RD. Female cycles in relation to paternity in primate societies. In: Martin, RD.; Dixon, AF.; Wickings, EJ., editors. *Paternity in primates: genetic tests and theories*. Karger; Basel: 1992. p. 238-274.
- Matsubara M. Costs of mate guarding and opportunistic mating among wild male Japanese macaques. *International Journal of Primatology*. 2003; 24:1057–1075.
- Ménard, N. Do ecological factors explain variation in social organization?. In: Thierry, B.; Singh, M.; Kaumanns, W., editors. *Macaque Societies: A Model for the Study of Social Organization*. Cambridge University Press; New York: 2004. p. 237-262.
- Mitani JC. Cooperation and competition in chimpanzees: current understanding and future challenges. *Evolution and Human Behavior*. 2009; 18:215–227.
- Nürnberg P, Sauermann U, Kayser M, Lanfer C, Manns E, Widdig A, Berard J, Bercovitch FB, Kessler M, Schmidtke J, Krawczak M. Paternity assessment in rhesus macaques (*Macaca mulatta*): multilocus DNA fingerprinting and PCR marker typing. *American Journal of Primatology*. 1998; 44:1–18. [PubMed: 9444319]

- Nunn CL. The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. *Animal Behaviour*. 1999; 58:229–246. [PubMed: 10458874]
- R Development Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; Vienna: 2010.
- Rawlins, RG.; Kessler, MJ. The history of the Cayo Santiago colony. In: Rawlins, RG.; Kessler, MJ., editors. *The Cayo Santiago Macaques: History, Behavior and Biology*. State University of New York Press; Albany: 1986. p. 13-45.
- Reichert KE, Heistermann M, Hodges JK, Boesch C, Hohmann G. What females tell males about their reproductive status: are morphological and behavioural cues reliable signals of ovulation in bonobos (*Pan paniscus*)? *Ethology*. 2002; 108:583–600.
- Schülke O, Bhagavatula J, Vigilant L, Ostner J. Social bonds enhance reproductive success in male macaques. *Current Biology*. 2010; 20:2207–2210. [PubMed: 21093261]
- Shideler SE, Ortuero AM, Moran FM, Moorman EA, Lasley BL. Simple extraction and enzyme immunoassays for estrogen and progesterone metabolites in the feces of *Macaca fascicularis* during non-conceptive and conceptive ovarian cycles. *Biology of Reproduction*. 1993; 48:1290–1298. [PubMed: 8318583]
- Silk J, Short J, Roberts J, Kusnitz J. Gestation length in rhesus macaques (*Macaca mulatta*). *International Journal of Primatology*. 1993; 14:95–104.
- Sillén-Tullberg B, Møller AP. The relationship between concealed ovulation and mating systems in anthropoid primates: a phylogenetic analysis. *The American Naturalist*. 1993; 141:1–25.
- Small MF. Consortships and conceptions in captive rhesus macaques (*Macaca mulatta*). *Primates*. 1990; 31:339–350.
- Smith DG. Male dominance and reproductive success in a captive group of rhesus macaques (*Macaca mulatta*). *Behaviour*. 1994; 129:225–242.
- Surbeck M, Mundry R, Hohmann G. Mothers matter! Maternal support, dominance status and mating success in male bonobos (*Pan paniscus*). *Proceedings of the Royal Society of London Series B. Biological Sciences*. 2010 doi: 10.1098/rspb.2010.1572.
- Thornton, JE.; Finn, PD. Estrus. In: Knobil, E.; Neill, JD., editors. *Encyclopedia of Reproduction*. Vol. Vol 2. Academic Press; San Diego: 1998. p. 136-141.
- van Noordwijk MA. Sexual behaviour of Sumatran long-tailed macaques (*Macaca fascicularis*). *Zeitschrift für Tierpsychologie*. 1985; 70:277–296.
- van Schaik, CP.; van Noordwijk, MA.; Nunn, CL. Sex and social evolution in primates. In: Lee, PC., editor. *Comparative Primate Socioecology*. Cambridge University Press; Cambridge: 1999. p. 204-240.
- van Schaik, CP.; Hodges, JK.; Nunn, CL. Paternity confusion and the ovarian cycles of female primates. In: van Schaik, CP.; Janson, CH., editors. *Infanticide by males and its implications*. Cambridge University Press; Cambridge: 2000. p. 361-387.
- van Schaik, CP.; Pradhan, GP.; van Noordwijk, MA. Mating conflict in primates: infanticide, sexual harassment and female sexuality. In: Kappeler, PM.; van Schaik, CP., editors. *Sexual Selection in Primates: New and Comparative Perspectives*. Cambridge University Press; Cambridge: 2004. p. 131-150.
- Whitehead H. Programs for analyzing social structure. *SOCPROG Users' Manual*. 2009a
- Whitehead H. *SOCPROG programs: analyzing animal social structures*. *Behavioral Ecology and Sociobiology*. 2009b; 63:765–778.
- Widdig A, Bercovitch FB, Streich WJ, Sauermaun U, Nuernberg P, Krawczak M. A longitudinal analysis of reproductive skew in male rhesus macaques. *Proceedings of the Royal Society of London*. 2004; B271:819–826.
- Wilcox AJ, Weinberg CR, Baird DD. Timing of sexual intercourse in relation to ovulation. *New England Journal of Medicine*. 1995; 333:1517–1522.
- Zehr JL, Maesriperi D, Wallen K. Estradiol increases female sexual initiation independent of male responsiveness in rhesus monkeys. *Hormones and Behavior*. 1998; 33:95–103. [PubMed: 9647935]

- Zehr JL, Tannenbaum PL, Jones B, Wallen K. Peak occurrence of female sexual initiation predicts day of conception in rhesus monkeys (*Macaca mulatta*). *Reproduction, Fertility, and Development*. 2000; 12:397–404.
- Zinner, DP.; van Schaik, CP.; Nunn, CL.; Kappeler, PM. Sexual selection and exaggerated sexual swellings of female primates. In: Kappeler, PM.; van Schaik, CP., editors. *Sexual Selection in Primates: New and Comparative Perspectives*. Cambridge University Press; Cambridge: 2004. p. 71-89.

HIGHLIGHTS

- > In anthropoid primate females, the fertile phase of the cycle may be concealed.
- > Males need to time their investment into mate-guarding to this period.
- > In rhesus macaques, mate-guarding is not precisely timed with the fertile phase.
- > Consequently, only a third of paternities could be attributed to this tactic.
- > Female rhesus macaques might have some control over their own reproduction.

Figure 1. Mean number of consortships formed with conceptive adult females in relation to male dominance during the two-year study period. Rank 1 was assigned to the highest-ranking male (alpha male).

Figure 2. Composite hormonal profile of the 15 ovarian cycles included in this study. The estimated fertile phase is framed (See Methods for details). Black line: PdG (pregnanediol glucuronide); dashed line: E1C (estrogen conjugates). Values represent means + SEM.

1

Figure 3.

Distribution of receptive periods (pale gray) and of the consortship periods (dark gray) throughout the conceptive cycles under study. Note that, by definition, consorted females were considered sexually receptive. The estimated fertile phase is framed and the two days of the estimated ovulation window are numbered '-3' and '-2'. The consortship period is the period during which a female was systematically consorted, regardless of partner identity; the main consortship partner is the male with whom the female was associated with for the longest period. Days in which no females were sexually receptive are not shown, and days where no behavioural data were collected are indicated by '-'. The ID of the main consortship partner is indicated in the figure; the ID is in parenthesis when only a short consortship was formed (i.e. did not cover the entire day). The female and sire ID are also indicated on the left. Note that the ID of the alpha male is 51A, and that the most successful sire's ID was 44E for the first year, and 12E, for the second year (identified in Dubuc et al., 2011 based on all conceptions).

Figure 4.

Variation of the dominance rank of the females' male partners during the conceptive cycles under study. The fertile phase is framed. Relative rank is presented (i.e. absolute value of the difference with the highest-ranking partner's rank +1; see Method). Only females who had more than one partner are included, and only days for which at least 2 data points were available are presented; the exact number of data points used on each day is indicated on the graph. Data are presented as mean $\pm$ SEM. This figure shows that the shifting point of the parabolic curve is on the last day of the fertile phase and the following day.

Figure 5.

Example of the degree of synchrony during the 10 cycles for which we have precise enough hormonal data from the 2007 mating season (second year). All of those cycles took place in two months, between April 21th and June 20th. The receptive periods of the focal cycles used in this study are hatches and the fertile phase is indicated in black, while the receptive period of other adult females are shown in pale gray. Females are classified in alphanumerical order. This figure illustrates that the degree of synchrony was not higher during the fertile phase than at other time of the receptive period.

Table I

Descriptive statistics of the distribution of consortships between the three phases.

		Pre-fertile	Fertile	Post-fertile
Consortship period				
Female	1 day ^a	20.0 %	93.3 %	86.7 %
	Phase ^b	0 %	26.6 %	20.0 %
Consortship ^c		6.5 ± 4.0 %	48.2 ± 4.8 %	45.3 ± 6.8 %
Phase d		7.6 ± 4.1 %	59.4 ± 8.0 %	50.1 ± 9.0 %
Main Consortship				
Female	1 day ^a	6.7 %	93.3 %	86.7 %
	Phase ^b	0 %	20.0 %	20.0 %
Consortship ^c		3.3 ± 3.3 %	47.8 ± 6.6 %	48.9 ± 7.4 %
Phase ^d		2.7 ± 2.7 %	48.9 ± 8.2 %	48.4 ± 9.1 %

^aPercentage of Females in Consortship during at least one day of the Phase^bPercentage of Females in Consortship during the entire Phase^cPercentage of the Consortship taking place during the Phase^dPercentage of the Phase covered by Consortship