Assessment of female reproductive status by male long-tailed macaques (*Macaca fascicularis*) under natural conditions

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Recent theories on primate sexual selection have paid increasing attention to the importance of reproductive strategies of females living in multi-male groups. However, the extent to which females are able to effectively conceal or advertise the time of ovulation as part of these strategies remains unclear. Few studies have investigated the ability of males to discern female reproductive status under natural conditions and none has taken differences in male rank (and thus ability to gain access to females and/or cues) into account. In this study, we tested male assessment of a female's fertile phase under natural conditions in long-tailed macaques (*Macaca fascicularis*). We used timing of mate guarding by dominant males and the response of sub-ordinate males towards the repeated playback of a female copulation call to measure male interest in females throughout the ovarian cycle. Relating the degree of male interest to female reproductive status as determined non-invasively by faecal hormone analysis, we demonstrate that interest in females shown by both dominant and sub-ordinate males is strongest during and around the fertile phase. Our data also indicate that males were better able to recognize the fertile period in conception versus non-conception cycles. Further, our finding of a strong positive relationship between male interest and female oestrogen levels in all cycles indicates that at least some of the cues used by males to assess female reproductive status are oestrogen-related.

In mammals, in which the life span of the gametes is usually limited, the phase in which copulation with a fertile male can lead to conception (fertile phase) is short-lived, lasting only a few days immediately preceding and around the time of ovulation (Gomendio et al. 1998). In most mammalian taxa, females "advertise" this period through sexual activity known as oestrus (e.g. mice, ground squirrels, Parker 1984). Catarrhine primates (Old world monkeys and apes) however, differ from the general mammalian pattern in that their ovarian cycles are characterised by extended follicular phases and periods of receptivity, which considerably exceed the length of the fertile phase (Hrdy & Whitten 1987; Martin 1992; van Schaik et al. 2000).

Several hypotheses have been developed to explain the adaptive value of extended receptivity in primates. Most commonly, it has been proposed that such a trait represents part of a female strategy to conceal the timing of ovulation from males in order to prevent individual males from monopolising access to females during the fertile phase, thus facilitating polyandrous matings or female mate choice. Both have been proposed as possible female strategies for ensuring the best male for paternity (Small 1989), avoiding inbreeding or genetic incompatibilities (Tregenza & Wedell 2002; Newcomer et al. 1999; Zeh & Zeh 2001) or for guaranteeing high quality fertile sperm through sperm competition (Dixson 1998). Alternatively, concealed timing of ovulation could serve to confuse paternity (e.g. Hrdy 1979; van Schaik et al. 2000). In this way, females could derive a number of potential benefits including the enhancement of paternal care (e.g. Taub 1980), improved defence through increasing the number of males per group (e.g. van Schaik & Höstermann 1994; Cheney & Seyfarth 1987), or the reduction of the risk of male infanticide (Hrdy 1979; van Schaik et al. 2000; Heistermann et al., 2001). These arguments need not be mutually exclusive since Nunn (1999) pointed out that at least in some primate species with a multi-male system, an extended period of receptivity serves a dual function of enabling females to combine paternity confusion with an attempt to bias paternity towards dominant males. In this way, the graded-signal hypothesis (initially generated to explain the evolution of exaggerated sexual swellings; Nunn, 1999), argues that females signal the probability of ovulation rather than its precise timing, thereby attracting dominant males during times of high probability and mating with lower ranking males during times of low (but not zero) probability (Nunn 1999, van Schaik et al. 2000). Thus, females would not only reduce the risk of infanticide through paternity confusion, but would also receive protection by the dominant male for the ensuing offspring (van Schaik & Kappeler 1997). The key question, however, namely to what extent males are able to assess the fertile phase of a female’s ovarian cycle, still remains largely unanswered.

Earlier studies, investigating macaque sexual behaviour in captivity, mostly in pair-test situations, described a relationship between the frequency of male sexual activity and female gonadal hormones, especially oestrogens (for a review, see Michael & Zumpe 1993, Zumpe & Michael 1996, see also Aujard et al. 1998), suggesting that male macaques might indeed be able to assess the time of ovulation. However, since sexual motivation in primates is strongly influenced by social context (Wallen 2001), it is not clear how applicable these results are to group living primates in natural settings. In the only study so far on a truly wild primate population, Heistermann et al. (2001) showed that in Hanuman langurs (*Semnopithecus entellus*) living in multi-male groups, males appear to be unable to discern the time of ovulation, although dominant males monopolise access to females during certain periods of the cycle. Thus, the limited data so far yield a contrasting picture with regard to the extent to which timing of ovulation is recognised by males. Further studies, preferably on primates living under natural social conditions, are needed to clarify this issue.

The aim of the present study was therefore to determine the ability of male long-tailed macaques living under natural conditions to recognise a female's fertile phase. In free-ranging long-tailed macaques, sexually active females mate with almost all adult male group members, but also form long-lasting consortships, mostly with dominant males (van Noordwijk 1985). Males differ in their ability to obtain access to females according to their rank, but whether the observed reproductive skew in favour of dominant males is a product of female choice or whether it reflects male ability to monopolise females during the fertile phase is not clear (de Ruiter et al. 1994).

In the present study, male interest in females was determined in relation to the stage of the ovarian cycle, using two protocols, which differed according to the dominance status of the males and thus their ability to get access to females. Firstly, interest of dominant males was examined by monitoring mate-guarding behaviour. Since mate guarding is a potentially costly activity in terms of energetics and risk of injury (Alberts et al. 1996), it should be limited to those days on which a successful mating could lead to conception. Subordinate males on the other hand, generally have limited opportunities for mate guarding, being restricted or excluded by higher-ranking males. Here, we measured interest of subordinate males by means of a playback experiment in order to provoke a response that should reflect their interest in the female. The stimulus, given repeatedly throughout the ovarian cycle of the female, was a female copulation call, since it is well known that these vocalisations can be individually recognised and attract male attention in a number of catarrhine primates (e.g. *Macaca sylvanus*: Semple 1998; *Papio cynocephalus cynocephalus*: Semple 2001). Combining hormone analysis with behavioural observations and a playback-experiment, our specific objectives were to investigate male ability to discern the fertile period of the female ovarian cycle in free-ranging long-tailed macaques by relating i) timing of mate guarding by alpha-males and ii) subordinate male response intensities towards a playback stimulus (female copulation call) to the female´s fertile phase as defined by faecal progestin profiles.

METHODS

Animals and study site

The study was carried out in the Recreation Park and Nature Reserve of Pangandaran, West Java, Indonesia (7º43′S, 108º52′E), which consists of mixed primary and secondary evergreen rain forest. For a detailed description of the study area and its climate see Kool (1993). Long-tailed macaques show a moderate degree of seasonality in reproduction with the birth season lasting for about six months (Kavanagh & Laursen 1984; van Schaik & van Noordwijk 1985), and births in the study area peak in January and February (Engelhardt & Kusay 2002). The animals involved in this study belonged to four groups, each containing 4-8 adult males and 5-12 adult females, and spent most of their time in the Recreation Park where visitors fed them occasionally. All animals were habituated and individually known.

All study-females (CA, JA, AEB, WC, NF) were multiparous and had been monitored for at least a month before the experiments started. Further details on group composition, study-female rank and status are presented in Table 1.

Tab. 1

For each female, three adult non-dominant male group members served as test-males during the playback experiment (see below). Only males younger than the alpha-male (thereby still holding the potential to reach alpha-rank position in the future) and of these the highest-ranking ones were chosen as test-males, since these were expected to have the „highest degree of freedom“ in terms of responding to the stimulus. Male/female dyads and dominance rank of the test-males are presented in Table 2.

Female copulation calls

Although copulatory vocalisations of female long-tailed macaques are sequences of distinct calls, they are referred to collectively as “copulation calls” in the literature (e.g. Deputte & Goustard 1980; Semple 2001). We will continue to employ the term “copulation call” for the functional unit of calls uttered by female long-tailed macaques during copulations. Sexually active female long-tailed macaques mate frequently during the day and utter copulation calls in 80% of all matings (van Noordwijk 1985). In a variability factors analysis, Deputte & Goustard (1980) found inter-individual differences in call duration and structure, as well as strong intra-individual differences according to the nature of male-female interactions during copulations and whether or not ejaculation occurred. For the playback experiments (see below), copulation calls of each study-female were recorded ad libitum (distance 1-3 m) during ejaculatory copulations with adult males using a Sennheiser ME 66 directional microphone with a Sennheiser MZW 66 windshield and a SONY TCD-D 100 Digital Audio Tape-Recorder.

Playback Protocol

Playback experiments were carried out by J.-B. P. and an Indonesian assistant from April to July 2001. Playbacks were conducted using a SONY TCD-D 100 Digital Audio Tape-Recorder and a NAGRA DSM amplifier-loudspeaker. All copulation calls were equalised to reach an average sound pressure level of 70 dB at 1 m distance using an Eagle DB 120 sound level meter. The copulation call of each study-female was played back separately towards the three different test-males every second day from the early follicular phase until after the onset of detumescence of the sexual swelling. In the case of female JA, however, the experimental period ended before the onset of detumescence due to technical problems. Details of the number of experiments conducted per male/female dyad during the different phases of the female´s ovarian cycle are given in Table 2.

Tab. 2

The same copulation call was used from each female as playback stimulus throughout the whole experimental period. The stimulus was kept constant in order to ensure that the information content of the signal remained the same throughout the duration of the experimental period. Only those copulation calls, which were uttered at the beginning of a female’s reproductive cycle, were used in the experiment, since studies in Barbary macaques indicate that males normally show little interest in these calls compared to those made at later stages of the cycle (Semple & McComb 2000). Thus, it was reasoned that any increase in response to this type of call during the playback experiment should reflect changes in male motivation independent of the stimulus itself.

The loudspeaker was hidden from the test-males in an average distance of 15m (range 8-23m). Differences in the distance between the speaker and the test-males could be ruled out to have an effect on male response in a previous pilot-phase (unpublished data). Playbacks were only conducted, when the study-female and higher ranking males had been out of sight already for at least two minutes and when the male was sitting and facing away from the loudspeaker either resting or self-grooming. During the experiments, test-males were not engaged in consortships with any female and other cycling females were at least 10m away. An interval of at least half an hour was left between playbacks towards males from the same group.

Male response towards the playback stimulus was recorded for one minute using a Canon XL 1 digital video camera. Male response intensity following the onset of the playback was classified into seven categories ranging from 0 to 6 as defined as follows: 0: either no response or head turn; 1-6: approach the loudspeaker for at least 2m within either 51-60s (1), 41-50s (2), 31-40s (3), 21-30s (4), 11-20s (5), 0-10s (6).

In order to avoid habituation towards the experimental situation, we regularly conducted so-called mock trials in which the speaker and the camera were set up, but no stimulus was presented. Furthermore, we controlled for habituation towards the playback stimulus by conducting a second experiment on the day that the test-male stopped responding. This was done by presenting the test male with another copulation call (from the same female), which was also recorded during an ejaculatory copulation in the early follicular phase, but which differed from the test stimulus in terms of sonographic structure and duration (visualised with Avisoft SASLab Pro). At least half an hour was left between the playback of the repeated stimulus and the control stimulus. On no occasion did any male approach the speaker when presented with the control stimulus.

Due to the time-consuming nature of the playback experiment itself, the need to monitor consortships continously once established and the time needed to search and locate each of the four study groups, experiments for the different females were performed consecutively, not in parallel.

Behavioural observations

All females of a group were monitored for sexual activity, consortships, swelling size, menstruation, pregnancy and infant birth or loss whenever in contact with the group, starting at least a month prior to the experimental period. On the same days the sub-ordinate males were presented the playback stimulus, study-females were followed and length of consortships and sexual interactions (inspections, mating attempts, matings) were continuously recorded using focal animal sampling (Altmann 1974). Monitoring of females and behavioural observations were carried out by A.E. and an Indonesian assistant. Observation time averaged 6.6h per experimental day. In order to determine male and female rank, aggressive interactions and the display of the 'bared-teeth-face', a unidirectional submissive display (van Hooff 1967), were recorded whenever occurring. A consortship was called mate guarding, when the alpha-male exclusively mated with the female and followed her within a distance of 5m for more than 99% of the observation time. As soon as mate guarding was observed, the female was also observed for three hours on days between experiments in order to control whether they were continuously monopolised.

Faecal sample collection and hormone analysis

From each study-female, 2-3 g faeces were collected every second morning and stored in 15 ml ethanol at 4° C until hormone analysis. In the laboratory, faecal samples were homogenized in their ethanolic solvent and two times extracted and the dry weight of each sample determined (for details see Ziegler et al. 2000). The efficiency of the extraction procedure, determined by monitoring the recovery of ^{3}H-progesterone, was 81.5±4.9%.

Faecal extracts were measured for levels of immunoreactive oestrogen and progestogen metabolites using enzyme-immunoassays (EIA) for total oestrogens (iE_{total}) (see Ostner & Heistermann 2003) and pregnanediol-3-glucuronide (iPdG) (see Ziegler et al. 2000). Sensitivity of the assays at 90% binding was 1.5 pg for iE_{total} and 20 pg for iPdG. Serial dilutions of faecal extracts from the follicular and luteal phase of the ovarian cycle gave displacement curves parallel to that obtained with the respective standard. Intra- and interassay coefficients of variation, determined by replicate determinations of quality controls, were 7.2% (N=32) and 9,9% (N=16) for iE_{total} and 3.5% (N=32) and 5.5% (N=16) for iPdG.

Definition of the female's fertile phase

The faecal progestogen profiles were used to determine the presumed time of ovulation and thereby the fertile phase of each ovarian cycle. In this respect, a defined rise in faecal iPdG levels above a threshold of the mean + 2SD of five preceding baseline values was used to indicate the onset of the post-ovulatory phase of each cycle (Carosi et al. 1999; Heistermann et al. 2001). Since faecal samples were only collected every second day and since in the long-tailed macaque the time lag in the excretion of progestogens into the faeces was shown to vary between 24 h and 56 h (Shideler et al. 1993), it was not possible to specifically determine the day of ovulation. Instead, we have determined a three day "ovulation window", comprising the period between day –2 (latest possible day of ovulation) to day –4 (earliest possible day of ovulation) relative to the defined faecal iPdG rise (day 0). On this basis, the fertile phase was defined as the period comprising the three potential days of ovulation plus the two preceding days, since experiments have shown that in long-tailed macaques matings can be fertile when occurring up to two days before ovulation and on the day of ovulation, but not any later (Behboodi et al. 1991). All days preceding the fertile phase will hereafter be called the pre-fertile phase and days following this phase will be called post-fertile phase.

Data analysis

For comparison of female oestrogen levels on days with and without α-male mate guarding, median oestrogen levels were calculated for each female and day-category and tested for a significant difference among the two categories using the Wilcoxon matched-pairs signed-ranks test.

For the analysis of male response intensities during the playback experiment, video recordings were analysed on a frame-by-frame basis directly in the camera by A.E., who was not present when the playbacks were conducted. In order to test male response intensities during the fertile phase against the pre-fertile and post-fertile phase, the median response intensity was calculated for each male and phase starting from day –10 and lasting till day +6 with day 0 being the last day of the fertile phase. As the experimental period during the cycle of female JA was interrupted, no median response intensity could be calculated for her post-fertile phase. Therefore, a permutation test for related samples with missing values was used (see Mundry 1999) to compare the median male response intensity of all cycle phases during non-conception cycles. The Friedman test was conducted for conception cycles, since here no values were missing, with post-hoc comparisons using the Wilcoxon matched-pairs signed-ranks test.

In order to test for a relationship between female oestrogen profiles and male response intensities, the Spearman's rank correlation coefficient was calculated for the median hormone value of all five females and the respective median response intensity values of all males on a given experimental day. Days were aligned to the day of maximum oestrogen level. For those cycles, during which two clear oestrogen peaks occurred, the one closer to the progestogen rise was taken as the one reflecting the presumed pre-ovulatory oestrogen peak.

All statistics followed Bortz et al. (1990). As there is already substantial evidence from laboratory studies for a positive relationship between male sexual motivation and female oestrogen levels in long-tailed macaques (Zumpe & Michael 1985), a one-tailed test was conducted whenever female oestrogen values were compared with measurements of male interest into the female.

RESULTS

Female reproductive cycles

Profiles of immunoreactive oestrogen and progestogen metabolites in relation to the fertile phase of each female are shown in Figure 1. Oestrogen levels differed inter-individually and a clear oestrogen peak was detected in four of the five females. It is likely that we missed the oestrogen peak in female WC due to our sample collection procedure or because there was a gap of three days within the sample series (Fig.1b). In the three females (CA, NF, WC) that conceived during the study period and gave birth to an infant, maximum oestrogen values were measured within the fertile phase (Fig. 1a-c). The two other females (AEB, JA) showed highest oestrogen values two days after the end of the fertile phase (Fig. 1d-e).

Fig. 1

Mate guarding by alpha-males

During the study-cycles, all five females were mate guarded by alpha-males on 4-13 consecutive days (Fig. 2). During these periods, all sub-ordinate males were excluded from access to females (inspections as well as copulations). Overall, mate guarding occurred on 20 out of 25 days representing the fertile phases of the females’ cycles. A comparison of conception versus non-conception cycles, however, revealed that alpha-males more often monopolised females during their fertile phases when these were associated with

Fig. 2

conception. During conception cycles, mate guarding covered 14 out of 15 days (93%) ascribed to the fertile phase, whereas during non-conception cycles the figure was six out of ten (60%). It should be noted that mate guarding during one of these cycles (female AEB) occurred almost exclusively outside the fertile phase. Efficiency of mate guarding (proportion of days invested into mate guarding that coincided with the fertile phase) also varied according to cycle type: during conception cycles, 14 of the 20 days (70%) alpha-males invested into mate guarding overlapped with the fertile phase, while during non-conception cycles, the figure was only six out of 17 days (35%).

Comparing oestrogen profiles to periods of mate guarding, we found a strong relationship between the two variables: although periods of mate guarding coincided with fertile phases in only four of five females, they included the day of maximum oestrogen levels in all cases (Fig. 2). Furthermore, taking all cycles together, mean oestrogen levels were significantly higher during periods of mate guarding than during other stages of the cycles (Wilcoxon matched-pairs signed-ranks test: $T=0$, $N=5$, $P=0.05$; Fig. 3).

Fig. 3

Playback experiment

In 14 of the 15 male/female test-pairs, male response to the stimulus varied during the course of the playback period. Response intensities ranged from ignoring the stimulus to approaching the speaker immediately after being presented with the stimulus. During the three conception cycles, 75% of all approaches ($N=24$) occurred during the fertile phase. In contrast, during the non-concepetion cycles, test-males either stopped approaching the speaker before the onset of the fertile phase (JA) or showed strongest response towards the copulation call mostly during the post-fertile phase coinciding with the time of elevated post-fertile oestrogen levels (AEB).

During non-conception cycles, males responded most strongly to the stimulus during the pre-fertile phase (Permutation test: $t=2.67$, $N=6$, $K=3$, $P<0.05$; Fig. 4b). In contrast, during conception cycles, male responses were significantly stronger within the fertile phase than compared with both, the pre-fertile (A) and the post-fertile phase (B) (Friedman test: $\chi^2=12$, $N=9$, $K=3$, $P<0.003$; Wilcoxon matched-pairs signed-ranks test: $T_A=3$, $P_A=0.01$, $T_B=0.5$, $P_B<0.005$; Fig. 4c).

Fig. 4

Taking all male/female pairs together, median response intensities showed a highly significant positive correlation with median female oestrogen levels, when variables were

Fig. 5

aligned to the day of maximum oestrogen value (Spearman rank correlation: r_s=0.866, N=8, P<0.01; Fig. 5).

DISCUSSION

The results of this study on free-ranging long-tailed macaques indicate that interest in females shown both by dominant and sub-ordinate males is strongest during and around the fertile phase of the ovarian cycle. Our data also indicate that males of both social statuses were better able to recognize the fertile period in conception versus non-conception cycles. Further, our finding of a strong positive relationship between male interest and female oestrogen levels in all cycles indicates that at least some of the cues used by males to assess female reproductive status are oestrogen-related.

Our findings that male free-living long-tailed macaques are able to discern the fertile phase are in accordance with former studies on macaques in captivity, in which a close temporal relationship between mating frequency and timing of ovulation was reported (Michael & Zumpe 1993, Zumpe & Michael 1996, Aujard et al. 1998). The degree with which male long-taile macaques recognise female reproductive status, however, is at present not clear. In the study reported here, the fertile phase extended over a period of five days and additionally, mate guarding by dominant males usually exceeded this interval. Thus with the information at hand, it is probably safer to say that male long-tailed macaques are able to discern periods with higher (fertile phase) and lower (non-fertile phase) probability of ovulation, rather than to pinpoint the day of ovulation. Nevertheless, even this ability exceeds that of Hanuman langurs (*Semnopithecus entellus*), the only other primate species for which such data in a free-ranging population are available. In the langur, patterns of female monopolisation by dominant males and male copulatory response to female solicitations provided no indication that males were able to recognise the fertile phase at all. (Heistermann et al. 2001). Although the reasons underlying these species differences are not fully understood, a number of factors may provide at least a partial explanation. Sexual swellings, copulation calls and pheromones have all been shown to play an important role as potential cues of female reproductive status in macaques (Zinner et al. 2003; Semple & McComb 2000; Michael & Keverne 1968, 1970). In contrast, sexual swellings and copulation calls are absent in Hanuman langurs and to date no evidence for the use of pheromonal cues in this species exists.

It is thus reasonable to assume that male long-tailed macaques use one or a combination of characters to assess a female's cycle stage. Our results indicate that at least some of the cues males appear to rely on are oestrogen related. Sexual swellings, female sexual behaviour and vaginal secretion of volatile fatty acids, which are considered to function as sex-pheromones in macaques, have been shown to be under oestrogen control, and moreover, are known to increase male sexual motivation in macaques (Zumpe & Michael 1985; Zinner et al. 2003; Michael & Keverne 1968, 1970; Michael & Zumpe 1993). This may also apply to female copulation calls, which have recently been proposed to advertise female reproductive status at least in Barbary macaques (Semple & McComb 2000). Variation in female swellings, copulation calls and female sexual behaviour can easily be recognised, even from a distance, giving all males, including those which do not have direct access to the female, the opportunity to monitor her reproductive status. However, since the perception of vaginally secreted pheromones might be restricted to males that are able to inspect the female's genitalia, information gathering might still differ between dominant and subordinate males. The precision with which each of the cues mentioned assigns the fertile phase in free-ranging long-tailed macaques and to what extent males use them still needs to be clarified.

Given the close temporal relationship between the occurrence of elevated oestrogen levels during the late follicular phase and ovulation (e.g. Dukelow & Brüggemann 1979), oestrogen related cues would indeed be potentially good indicators of the female's fertile phase, at least for cycles in which the pattern of oestrogen secretion is normal. As also shown in the present study, irregularities in oestrogen secretion, however, may mislead males in their assessment of the female's cycle stage. In both study cycles in which males wrongly assessed the fertile phase, endocrine irregularities occurred, with maximum oestrogen values being measured outside the defined fertile phase, suggesting a de-coupling of the normal temporal relationship between ovulation and luteinisation (i.e. pre-ovulatory luteinisation of the follicle). Physiological irregularities seem to be the most likely explanation for the absence of conception in these cycles, since in both females several copulations with ejaculations could be observed during the fertile phase. Strikingly, in female AEB male response and alpha-male mate guarding was temporarily related to the period of elevated oestrogen values. By relying on oestrogen related cues, the alpha-male as well as the sub-ordinate males wrongly assessed what has been a fertile phase by (our) definition, though the cycle itself was apparently not fertile. However, it remains unclear why subordinate males stopped reacting to the playback call before the onset of the fertile phase during the cycle of female JA. In this cycle, maximum interest of sub-ordinate males coincided with the onset of mate guarding by the alpha-male, but unlike the dominant male, who still showed interest into the female during her fertile phase, the subordinate males did not. This early decline in subordinate male interest in the female is difficult to understand, in view of the fact that the same males correctly assessed the fertile phase during the cycle of female CA, but might be connected to the unusually long duration of mate guarding by the dominant male.

Our finding that dominant male long-tailed macaques monopolise females during their fertile phases is novel. Although paternity data are not available, it is very likely that the alpha-males fathered the infants during the study period since during times of mate guarding, they were with the females for more than 99% of the observation time, exclusively mated with them and followed the females into the sleeping tree, where they were still together at dawn. Thus, male ability to assess a female's fertile phase and adjustment of his mate guarding behaviour accordingly, would clearly explain the high reproductive skew found in male long-tailed macaques (de Ruiter et al. 1994). According to the findings in this study, it is reasonable to assume that this is, at least in part, related to the fact that females assign the probability of occurrence of ovulation thereby biasing paternity to the dominant male.

Since our results indicate that not only dominant but also sub-ordinate males (which are usually prevented from mating with a female during her fertile phase) are able to recognise the fertile phase, all adult males should be able to assess their probability of paternity. According to Hrdy (1979), Hrdy & Whitten (1987) and van Schaik et al. (2000) this would lead to an enhanced risk of infanticide, since any sub-ordinate male subsequently achieving dominance status would be potentially infanticidal. However, given the reatively low degree of accuracy in actually pinpointing the time of ovulation, Nunn's (1999) graded-signal hypothesis would predict that even males mating with females exclusively outside the fertile phase should not be able to exclude the possibility of fathering an infant, and this, in contrast, would lower the risk of infanticide. The extent to which male long-tailed macaques are indeed able to assess their probability of paternity and how this affects the risk of infanticide, however, could not be determined in this study. Infanticide has been reported for long-tailed macaques in the wild (de Ruiter 1994), but events are rather rare.

Taken together, the data obtained in this study show that free-ranging male long-tailed macaques are able to discern the fertile phase, particularly in cycles leading to conception. The degree of precision in determining the time of ovulation however is not known.

Oestrogen related cues appear to be involved in the process of female reproductive assessment by males, but the exact nature of these cues and how males use them under natural conditions remains to be clarified. Further studies addressing these issues should improve our understanding not only of the proximate mechanisms underlying reproductive strategies, but also of how sexual selection in general has contributed to evolutionary processes within the catarrhine primate taxon.

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Figure 1. Faecal progestin (black circles) and oestrogen profiles (white triangles) of the five test-females (a-e). The fertile phase (shaded areas) has been deduced by identifying the day on which faecal progestin level is increased above a defined threshold value (marked by asterisks). For details see the method section in the text.

Figure 2. Days on which the dominant male guarded a female (hatched area) in relationship to the female’s fertile phase. Asterisks mark conception cycles and black squares indicate days with maximum oestrogen levels.

Figure 3. Median faecal oestrogen values in all study-females (N=5) on days when they were mate-guarded by the dominant male, compared to days, on which they were not. Black triangles indicate conception cycles, white triangles non-conception cycles. $P=0.05$

Figure 4. Comparison of male response intensities towards the playback stimulus during the pre-fertile, fertile and post-fertile phase for **a)** male/female pairs during non-conception cycles (N=6) and **b)** male/female pairs during conception cycles (N=9). The figures show the median, the first and third quartile, as well as the range of male response intensities for each phase. $^{*}P<0.05$, $^{**}P=0.01$, $^{***}P<0.005$

Figure 5. Relationship between the median male response intensity (black squares) for all male/female pairs (N=15) and median female oestrogen level (white triangle) in all study-females (N=5) on a given day. Days were aligned to the day of maximum oestrogen level (day 0). The figure shows the median as well as the first and third quartile. $r_s=0.866$, $P<0.01$

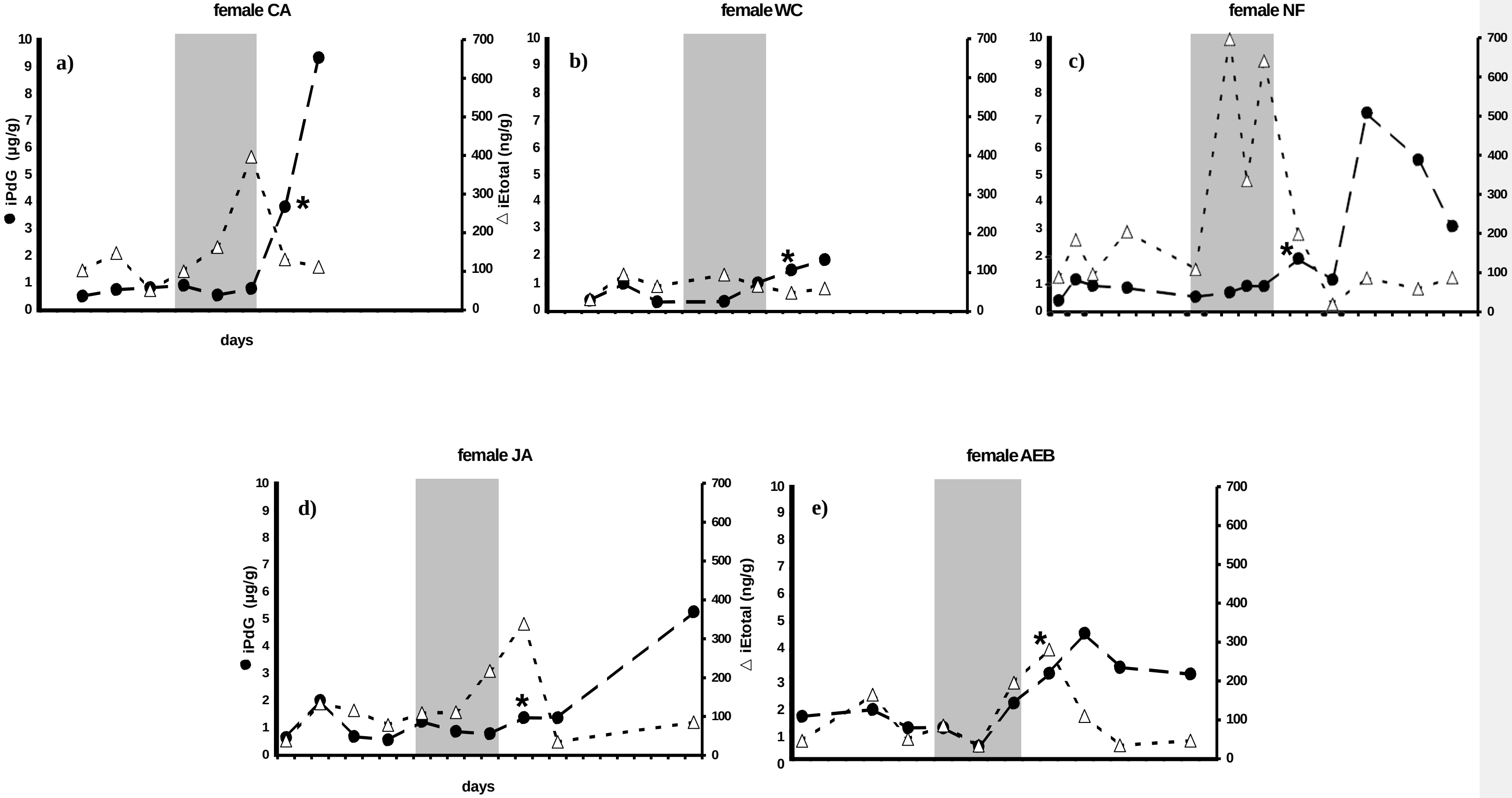

female CA
a)
female WC
b)
female NF
c)
female JA
d)
female AEB
e)
● iPdG (µg/g)
△ iEtotal (ng/g)
days
*
0
1
2
3
4
5
6
7
8
9
10
0
100
200
300
400
500
600
700

Fig. 1

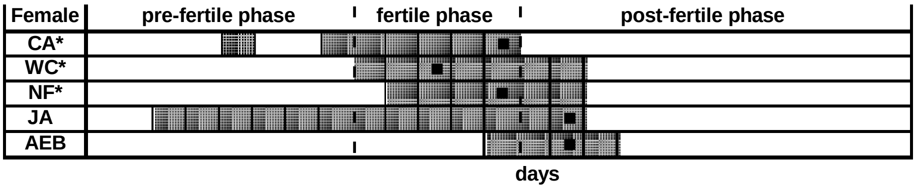

Female
pre-fertile phase
fertile phase
post-fertile phase
CA*
WC*
NF*
JA
AEB
days

Fig. 2

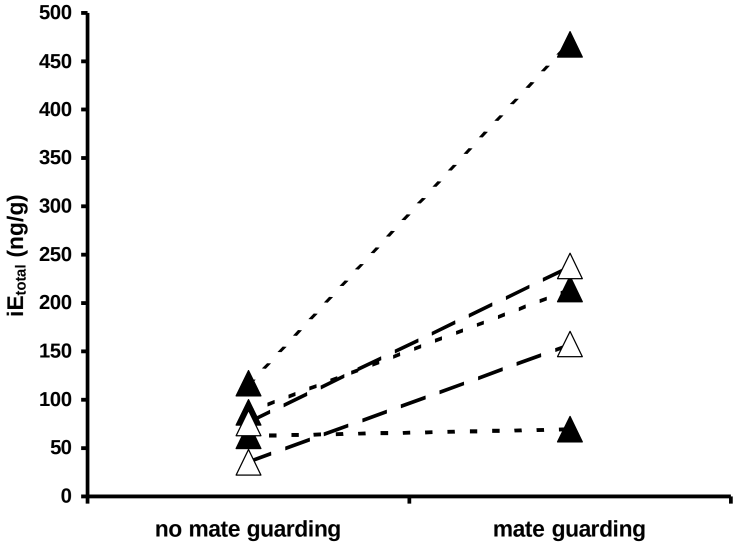

500
450
400
350
300
250
200
150
100
50
0
iE_total (ng/g)
no mate guarding
mate guarding

Fig. 3

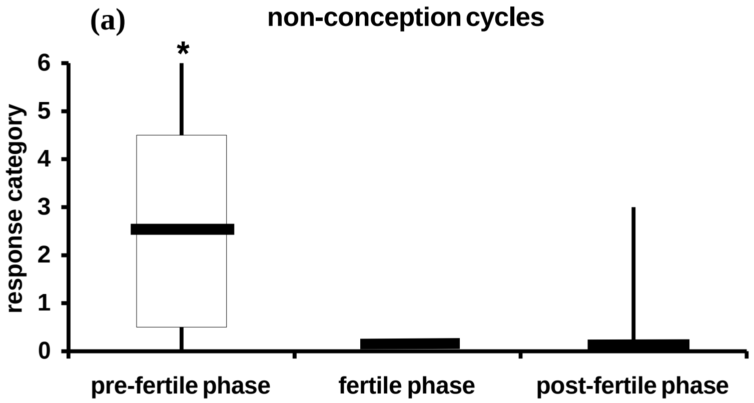
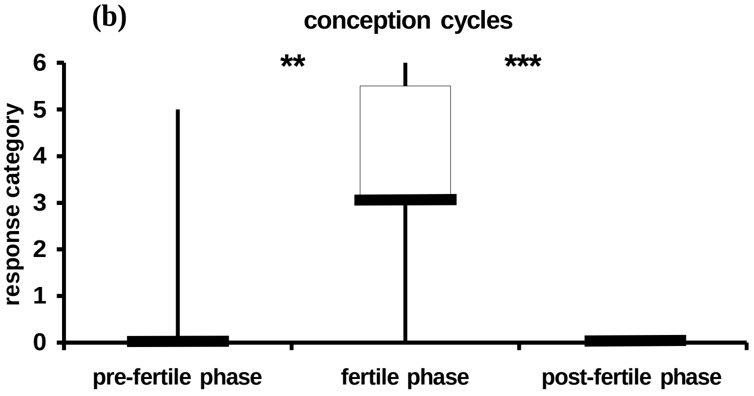

(a)
non-conception cycles
*
6
5
4
3
2
1
0
response category
pre-fertile phase
fertile phase
post-fertile phase

Fig. 4


(b)
conception cycles
**
***
6
5
4
3
2
1
0
response category
pre-fertile phase
fertile phase
post-fertile phase

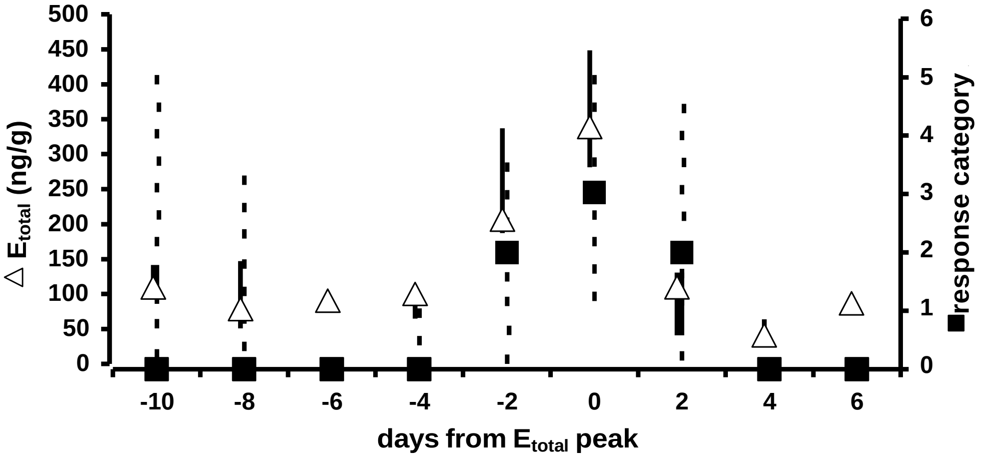

500
450
400
350
300
250
200
150
100
50
0
△ E_{total} (ng/g)
6
5
4
3
2
1
0
■ response category
-10
-8
-6
-4
-2
0
2
4
6
days from E_{total} peak

Fig. 5

Table 1. Study-groups, number of adult males and females per group, number of cycling females per experimental period, dominance rank and reproductive status of the five study-females at the onset and termination of the respective experimental periods

Group	Adult males	Adullt females	Cycling females	Study-female	Rank	Reproductive status	Conception
A	5	7	1	CA	4	First cycle after death of three month old infant	Yes
			2*	JA	5	Menstruating after previous cycle	No
B	7	5	2**	AEB	1	Menstruating after previous cycle	No
C	8	9	1	WC	4	Menstruating after previous cycle	Yes
F	4	12	1	NF	10	First cycle after death of two month old infant	Yes

* The second cycling female was already very old.
** The second cycling female was still nulliparous.

Table 2. Study-females, test-males, male rank and number of experiments conducted per female/male dyad during the pre-fertile, fertile and post-fertile phase of the respective study cycles

			Experiments per cycle phase		
Study-female	*Test-male*	*Rank*	Pre-fertile phase	Fertile phase	Post-fertile phase
CA	*TA*	*2*	3	3	3
	BA	*3*	3	3	3
	FA	*4*	3	3	3
JA	*TA*	*2*	3	3	0
	BA	*3*	3	3	0
	FA	*4*	3	3	0
AEB	*BB*	*3**	3	3	3
	YB	*4**	3	3	3
	CB	*5**	3	3	3
WC	*TC*	*4***	1	3	3
	DC	*5***	1	3	3
	JC	*6***	1	2	3
NF	*WF*	*2*	3	2	3
	BF	*3*	3	2	3
	SF	*4*	3	2	3

* The former alpha-male held dominance rank 2.

** The former alpha-male and another old male held dominance ranks 2 and 3.