

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

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11 **ENGELHARDT ET AL.: MALE ASSESSMENT OF REPRODUCTIVE STATUS**

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

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56 In mammals, in which the life span of the gametes is usually limited, the phase in which
57 copulation with a fertile male can lead to conception (fertile phase) is short-lived, lasting
58 only a few days immediately preceding and around the time of ovulation (Gomendio et al.
59 1998). In most mammalian taxa, females "advertise" this period through sexual activity
60 known as oestrus (e.g. mice, ground squirrels, Parker 1984). Catarrhine primates (Old
61 world monkeys and apes) however, differ from the general mammalian pattern in that their
62 ovarian cycles are characterised by extended follicular phases and periods of receptivity,
63 which considerably exceed the length of the fertile phase (Hrdy & Whitten 1987; Martin
64 1992; van Schaik et al. 2000).

65 Several hypotheses have been developed to explain the adaptive value of extended
66 receptivity in primates. Most commonly, it has been proposed that such a trait represents
67 part of a female strategy to conceal the timing of ovulation from males in order to prevent
68 individual males from monopolising access to females during the fertile phase, thus
69 facilitating polyandrous matings or female mate choice. Both have been proposed as

70 possible female strategies for ensuring the best male for paternity (Small 1989), avoiding
71 inbreeding or genetic incompatibilities (Tregenza & Wedell 2002; Newcomer et al. 1999;
72 Zeh & Zeh 2001) or for guaranteeing high quality fertile sperm through sperm competition
73 (Dixon 1998). Alternatively, concealed timing of ovulation could serve to confuse
74 paternity (e.g. Hrdy 1979; van Schaik et al. 2000). In this way, females could derive a
75 number of potential benefits including the enhancement of paternal care (e.g. Taub 1980),
76 improved defence through increasing the number of males per group (e.g. van Schaik &
77 Höstermann 1994; Cheney & Seyfarth 1987), or the reduction of the risk of male infanticide
78 (Hrdy 1979; van Schaik et al. 2000; Heistermann et al., 2001). These arguments need not be
79 mutually exclusive since Nunn (1999) pointed out that at least in some primate species
80 with a multi-male system, an extended period of receptivity serves a dual function of
81 enabling females to combine paternity confusion with an attempt to bias paternity towards
82 dominant males. In this way, the graded-signal hypothesis (initially generated to explain the
83 evolution of exaggerated sexual swellings; Nunn, 1999), argues that females signal the
84 probability of ovulation rather than its precise timing, thereby attracting dominant males
85 during times of high probability and mating with lower ranking males during times of low
86 (but not zero) probability (Nunn 1999, van Schaik et al. 2000). Thus, females would not
87 only reduce the risk of infanticide through paternity confusion, but would also receive
88 protection by the dominant male for the ensuing offspring (van Schaik & Kappeler 1997).
89 The key question, however, namely to what extent males are able to assess the fertile phase
90 of a female's ovarian cycle, still remains largely unanswered.

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

103 studies, preferably on primates living under natural social conditions, are needed to clarify
104 this issue.

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

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

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METHODS**Animals and study site**

The study was carried out in the Recreation Park and Nature Reserve of Pangandaran, West Java, Indonesia (7°43'S, 108°52'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.

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

Tab. 1

168 of male-female interactions during copulations and whether or not ejaculation occurred. For
 169 the playback experiments (see below), copulation calls of each study-female were recorded
 170 ad libitum (distance 1-3 m) during ejaculatory copulations with adult males using a
 171 Sennheiser ME 66 directional microphone with a Sennheiser MZW 66 windshield and a
 172 SONY TCD-D 100 Digital Audio Tape-Recorder.

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175 **Playback Protocol**

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177 Playback experiments were carried out by J.-B. P. and an Indonesian assistant from April
 178 to July 2001. Playbacks were conducted using a SONY TCD-D 100 Digital Audio Tape-
 179 Recorder and a NAGRA DSM amplifier-loudspeaker. All copulation calls were equalised
 180 to reach an average sound pressure level of 70 dB at 1 m distance using an Eagle DB 120
 181 sound level meter. The copulation call of each study-female was played back separately
 182 towards the three different test-males every second day from the early follicular phase until
 183 after the onset of detumescence of the sexual swelling. In the case of female JA, however,
 184 the experimental period ended before the onset of detumescence due to technical problems.

Tab. 2

185 Details of the number of experiments conducted per male/female dyad during the different
 186 phases of the female's ovarian cycle are given in Table 2.

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

196 The loudspeaker was hidden from the test-males in an average distance of 15m (range
 197 8-23m). Differences in the distance between the speaker and the test-males could be ruled
 198 out to have an effect on male response in a previous pilot-phase (unpublished data).

199 Playbacks were only conducted, when the study-female and higher ranking males had been
 200 out of sight already for at least two minutes and when the male was sitting and facing away
 201 from the loudspeaker either resting or self-grooming. During the experiments, test-males

202 were not engaged in consortships with any female and other cycling females were at least
203 10m away. An interval of at least half an hour was left between playbacks towards males
204 from the same group.

205 Male response towards the playback stimulus was recorded for one minute using a Canon
206 XL 1 digital video camera. Male response intensity following the onset of the playback was
207 classified into seven categories ranging from 0 to 6 as defined as follows: 0: either no
208 response or head turn; 1-6: approach the loudspeaker for at least 2m within either 51-60s
209 (1), 41-50s (2), 31-40s (3), 21-30s (4), 11-20s (5), 0-10s (6).

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

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

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226 **Behavioural observations**

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228 All females of a group were monitored for sexual activity, consortships, swelling size,
229 menstruation, pregnancy and infant birth or loss whenever in contact with the group,
230 starting at least a month prior to the experimental period. On the same days the sub-ordinate
231 males were presented the playback stimulus, study-females were followed and length of
232 consortships and sexual interactions (inspections, mating attempts, matings) were
233 continuously recorded using focal animal sampling (Altmann 1974). Monitoring of females
234 and behavioural observations were carried out by A.E. and an Indonesian assistant.
235 Observation time averaged 6.6h per experimental day. In order to determine male and

236 female rank, aggressive interactions and the display of the 'bared-teeth-face', a
237 unidirectional submissive display (van Hooff 1967), were recorded whenever occurring. A
238 consortship was called mate guarding, when the alpha-male exclusively mated with the
239 female and followed her within a distance of 5m for more than 99% of the observation
240 time. As soon as mate guarding was observed, the female was also observed for three hours
241 on days between experiments in order to control whether they were continuously
242 monopolised.

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245 **Faecal sample collection and hormone analysis**

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247 From each study-female, 2-3 g faeces were collected every second morning and stored in
248 15 ml ethanol at 4° C until hormone analysis. In the laboratory, faecal samples were
249 homogenized in their ethanolic solvent and two times extracted and the dry weight of each
250 sample determined (for details see Ziegler et al. 2000). The efficiency of the extraction
251 procedure, determined by monitoring the recovery of ³H-progesterone, was 81.5±4.9%.

252 Faecal extracts were measured for levels of immunoreactive oestrogen and progestogen
253 metabolites using enzyme-immunoassays (EIA) for total oestrogens (iE_{total}) (see Ostner &
254 Heistermann 2003) and pregnanediol-3-glucuronide (iPdG) (see Ziegler et al. 2000).
255 Sensitivity of the assays at 90% binding was 1.5 pg for iE_{total} and 20 pg for iPdG. Serial
256 dilutions of faecal extracts from the follicular and luteal phase of the ovarian cycle gave
257 displacement curves parallel to that obtained with the respective standard. Intra- and
258 interassay coefficients of variation, determined by replicate determinations of quality
259 controls, were 7.2% (N=32) and 9.9% (N=16) for iE_{total} and 3.5% (N=32) and 5.5% (N=16)
260 for iPdG.

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263 **Definition of the female's fertile phase**

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265 The faecal progestogen profiles were used to determine the presumed time of ovulation
266 and thereby the fertile phase of each ovarian cycle. In this respect, a defined rise in faecal
267 iPdG levels above a threshold of the mean + 2SD of five preceding baseline values was
268 used to indicate the onset of the post-ovulatory phase of each cycle (Carosi et al. 1999;
269 Heistermann et al. 2001). Since faecal samples were only collected every second day and

270 since in the long-tailed macaque the time lag in the excretion of progestogens into the
271 faeces was shown to vary between 24 h and 56 h (Shideler et al. 1993), it was not possible
272 to specifically determine the day of ovulation. Instead, we have determined a three day
273 “ovulation window”, comprising the period between day -2 (latest possible day of
274 ovulation) to day -4 (earliest possible day of ovulation) relative to the defined faecal iPdG
275 rise (day 0). On this basis, the fertile phase was defined as the period comprising the three
276 potential days of ovulation plus the two preceding days, since experiments have shown that
277 in long-tailed macaques matings can be fertile when occurring up to two days before
278 ovulation and on the day of ovulation, but not any later (Behboodi et al. 1991). All days
279 preceding the fertile phase will hereafter be called the pre-fertile phase and days following
280 this phase will be called post-fertile phase.

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283 **Data analysis**

284

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

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

301 In order to test for a relationship between female oestrogen profiles and male response
302 intensities, the Spearman's rank correlation coefficient was calculated for the median
303 hormone value of all five females and the respective median response intensity values of all

304 males on a given experimental day. Days were aligned to the day of maximum oestrogen
 305 level. For those cycles, during which two clear oestrogen peaks occurred, the one closer to
 306 the progesterone rise was taken as the one reflecting the presumed pre-ovulatory oestrogen
 307 peak.

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

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315 RESULTS

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317 Female reproductive cycles

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

Fig. 1

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330 Mate guarding by alpha-males

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332 During the study-cycles, all five females were mate guarded by alpha-males on 4-13
 333 consecutive days (Fig. 2). During these periods, all sub-ordinate males were excluded from
 334 access to females (inspections as well as copulations). Overall, mate guarding occurred on
 335 20 out of 25 days representing the fertile phases of the females' cycles. A comparison of
 336 conception versus non-conception cycles, however, revealed that alpha-males more often
 337 monopolised females during their fertile phases when these were associated with

Fig. 2

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

Fig. 3

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

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353

354 Playback experiment

355

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

Fig. 4

364 During non-conception cycles, males responded most strongly to the stimulus during
 365 the pre-fertile phase (Permutation test: $t=2.67$, $N=6$, $K=3$, $P<0.05$; Fig. 4b). In contrast,
 366 during conception cycles, male responses were significantly stronger within the fertile
 367 phase than compared with both, the pre-fertile (A) and the post-fertile phase (B) (Friedman
 368 test: $\chi^2=12$, $N=9$, $K=3$, $P<0.003$; Wilcoxon matched-pairs signed-ranks test: $T_A=3$, $P_A=0.01$,
 369 $T_B=0.5$, $P_B<0.005$; Fig. 4c).

Fig. 5

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

372 aligned to the day of maximum oestrogen value (Spearman rank correlation: $r_s=0.866$, $N=8$,
373 $P<0.01$; Fig. 5).

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DISCUSSION

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

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

406 It is thus reasonable to assume that male long-tailed macaques use one or a combination
407 of characters to assess a female's cycle stage. Our results indicate that at least some of the
408 cues males appear to rely on are oestrogen related. Sexual swellings, female sexual
409 behaviour and vaginal secretion of volatile fatty acids, which are considered to function as
410 sex-pheromones in macaques, have been shown to be under oestrogen control, and
411 moreover, are known to increase male sexual motivation in macaques (Zumpe & Michael
412 1985; Zimmer et al. 2003; Michael & Keverne 1968, 1970; Michael & Zumpe 1993). This
413 may also apply to female copulation calls, which have recently been proposed to advertise
414 female reproductive status at least in Barbary macaques (Semple & McComb 2000).
415 Variation in female swellings, copulation calls and female sexual behaviour can easily be
416 recognised, even from a distance, giving all males, including those which do not have direct
417 access to the female, the opportunity to monitor her reproductive status. However, since the
418 perception of vaginally secreted pheromones might be restricted to males that are able to
419 inspect the female's genitalia, information gathering might still differ between dominant
420 and subordinate males. The precision with which each of the cues mentioned assigns the
421 fertile phase in free-ranging long-tailed macaques and to what extent males use them still
422 needs to be clarified.

423 Given the close temporal relationship between the occurrence of elevated oestrogen levels
424 during the late follicular phase and ovulation (e.g. Dukelow & Brüggemann 1979),
425 oestrogen related cues would indeed be potentially good indicators of the female's fertile
426 phase, at least for cycles in which the pattern of oestrogen secretion is normal. As also
427 shown in the present study, irregularities in oestrogen secretion, however, may mislead
428 males in their assessment of the female's cycle stage. In both study cycles in which males
429 wrongly assessed the fertile phase, endocrine irregularities occurred, with maximum
430 oestrogen values being measured outside the defined fertile phase, suggesting a de-coupling
431 of the normal temporal relationship between ovulation and luteinisation (i.e. pre-ovulatory
432 luteinisation of the follicle). Physiological irregularities seem to be the most likely
433 explanation for the absence of conception in these cycles, since in both females several
434 copulations with ejaculations could be observed during the fertile phase. Strikingly, in
435 female AEB male response and alpha-male mate guarding was temporarily related to the
436 period of elevated oestrogen values. By relying on oestrogen related cues, the alpha-male as
437 well as the sub-ordinate males wrongly assessed what has been a fertile phase by (our
438 definition, though the cycle itself was apparently not fertile. However, it remains unclear
439 why subordinate males stopped reacting to the playback call before the onset of the fertile

440 phase during the cycle of female JA. In this cycle, maximum interest of sub-ordinate males
441 coincided with the onset of mate guarding by the alpha-male, but unlike the dominant male,
442 who still showed interest into the female during her fertile phase, the subordinate males did
443 not. This early decline in subordinate male interest in the female is difficult to understand,
444 in view of the fact that the same males correctly assessed the fertile phase during the cycle
445 of female CA, but might be connected to the unusually long duration of mate guarding by
446 the dominant male.

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

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

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

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

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604 aggressive behavior of macaques. *American Journal of Primatology*, **38**, 233-261.
- 605

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

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

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

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

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

637

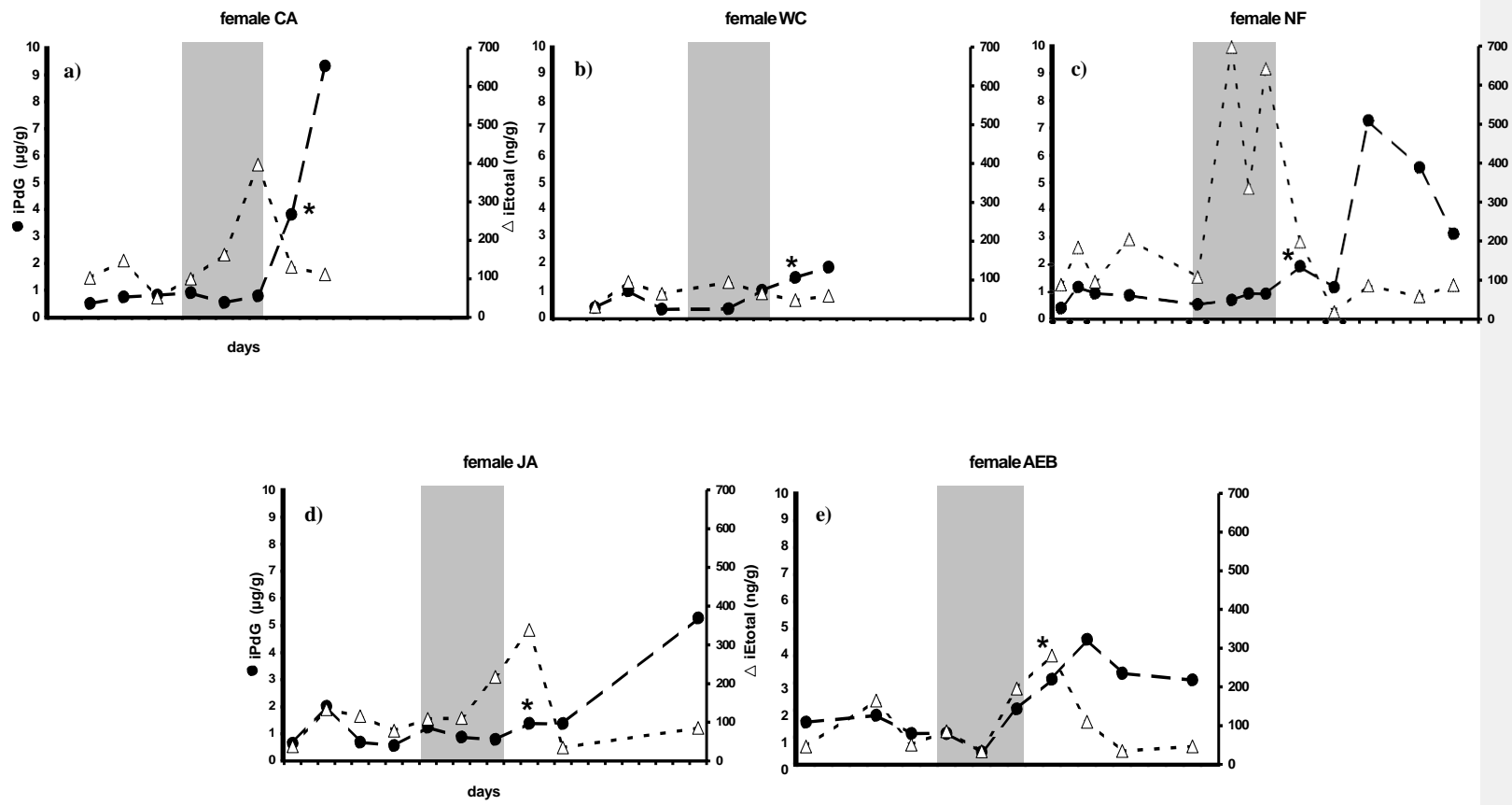


Fig. 1

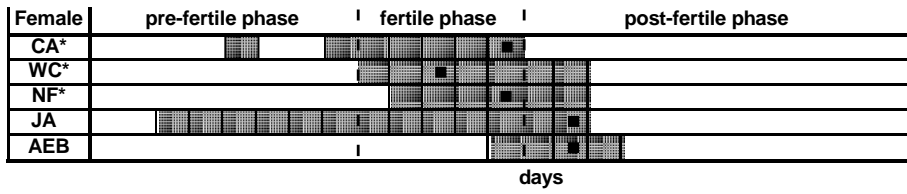


Fig. 2

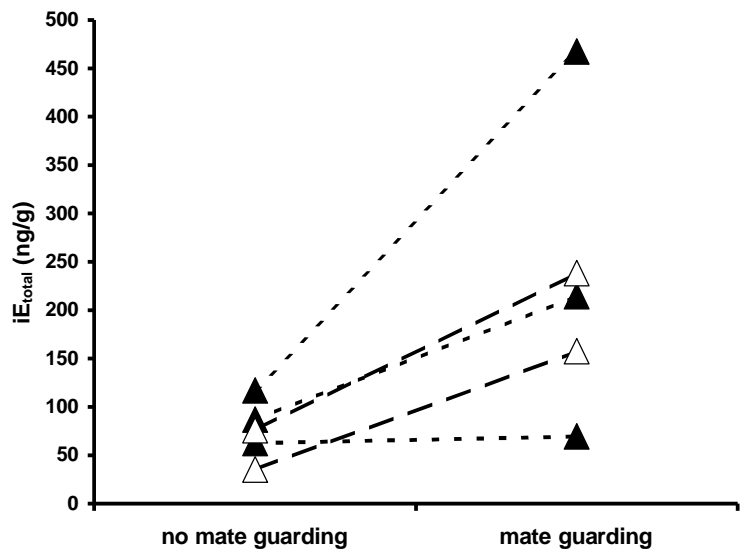


Fig. 3

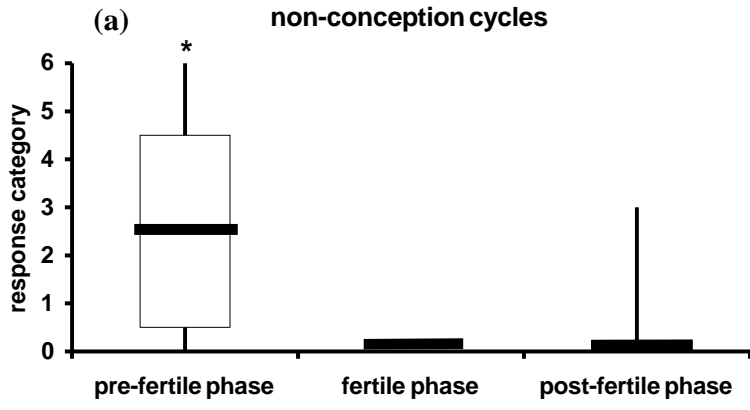
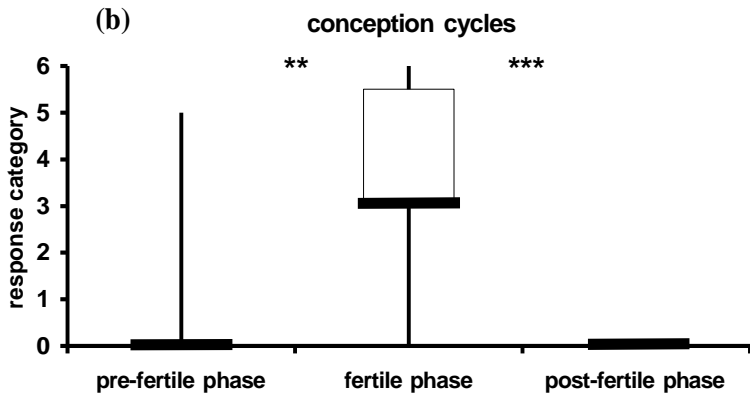


Fig. 4



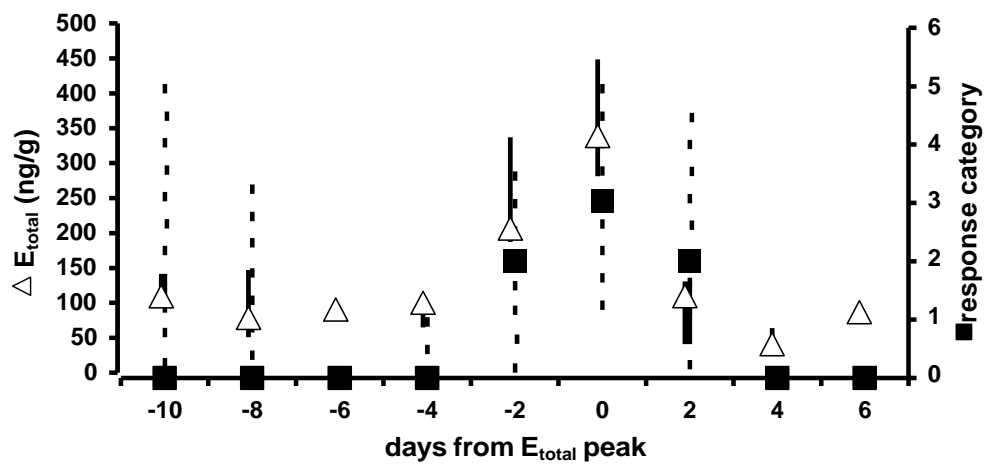


Fig. 5

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	Adult females	Cycling females	Study-female	Rank	Reproductivestatus	Conception
A	5	7	1	CA	4	First cycle after death of three month old infant	Yes
				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

Study-female	Test-male	Rank	Experiments per cycle phase		
			Pre-fertilephase	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.