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

3  
4 **ANTJE ENGELHARDT\*†, JAN-BOJE PFEIFER\*, MICHAEL HEISTERMANN†,**  
5 **CARSTEN NIEMITZ\*, JAN A.R.A.M. VAN HOOFF‡ & J. KEITH HODGES†**

6  
7 \* Human Biology and Anthropology, Freie Universität Berlin

8 †Department of Reproductive Biology, German Primate Centre

9 ‡Behavioural Biology, Utrecht University

10  
11 **ENGELHARDT ET AL.: MALE ASSESSMENT OF REPRODUCTIVE STATUS**

12  
13  
14 **Addresses:**

15 Human Biology and Anthropology, Free University of Berlin, Albrecht-Thaer-Weg 6, 14 195  
16 Berlin, Germany

17 Department of Reproductive Biology, German Primate Centre, Kellnerweg 4, 37077  
18 Göttingen, Germany

19 Behavioural Biology, Utrecht University, PO Box 80086, 35088 TB, Utrecht, The  
20 Netherlands

21  
22  
23 **Corresponding address:**

24 Antje Engelhardt

25 Department of Reproductive Biology

26 German Primate Centre

27 Kellnerweg 4

28 37077 Göttingen

29 Germany

30 Phone: 0551 - 3851 202

31 Fax: 0551 - 3851 288

32 e-mail: [aengelhardt@dpz.gwdg.de](mailto:aengelhardt@dpz.gwdg.de)

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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.

53

54

55

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 (Dixson 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 & Zumpe 1993,  
94 Zumpe & 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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133

134 **METHODS**

135  
136 **Animals and study site**

137  
138 The study was carried out in the Recreation Park and Nature Reserve of Pangandaran,  
139 West Java, Indonesia (7°43'S, 108°52'E), which consists of mixed primary and secondary  
140 evergreen rain forest. For a detailed description of the study area and its climate see Kool  
141 (1993). Long-tailed macaques show a moderate degree of seasonality in reproduction with  
142 the birth season lasting for about six months (Kavanagh & Laursen 1984; van Schaik & van  
143 Noordwijk 1985), and births in the study area peak in January and February (Engelhardt &  
144 Kusay 2002). The animals involved in this study belonged to four groups, each containing  
145 4-8 adult males and 5-12 adult females, and spent most of their time in the Recreation Park  
146 where visitors fed them occasionally. All animals were habituated and individually known.

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

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

156  
157  
158 **Female copulation calls**

159  
160 Although copulatory vocalisations of female long-tailed macaques are sequences of  
161 distinct calls, they are referred to collectively as “copulation calls” in the literature (e.g.  
162 Deputte & Goustard 1980; Semple 2001). We will continue to employ the term “copulation  
163 call” for the functional unit of calls uttered by female long-tailed macaques during  
164 copulations. Sexually active female long-tailed macaques mate frequently during the day  
165 and utter copulation calls in 80% of all matings (van Noordwijk 1985). In a variability  
166 factors analysis, Deputte & Goustard (1980) found inter-individual differences in call  
167 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.

173

174

### 175 **Playback Protocol**

176

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.

224

225

## 226 **Behavioural observations**

227

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.

243

244

#### 245 **Faecal sample collection and hormone analysis**

246

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 <sup>3</sup>H-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<sub>total</sub>) (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<sub>total</sub> 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<sub>total</sub> and 3.5% (N=32) and 5.5% (N=16)  
260 for iPdG.

261

262

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

264

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.

281

282

### 283 **Data analysis**

284

285 For comparison of female oestrogen levels on days with and without  $\alpha$ -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.

313

314

## 315 RESULTS

316

### 317 Female reproductive cycles

318

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

328

329

### 330 Mate guarding by alpha-males

331

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).

352

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).

374

375

376

## DISCUSSION

377

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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483

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- 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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618

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.  $P=0.05$

622

623

624

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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632

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

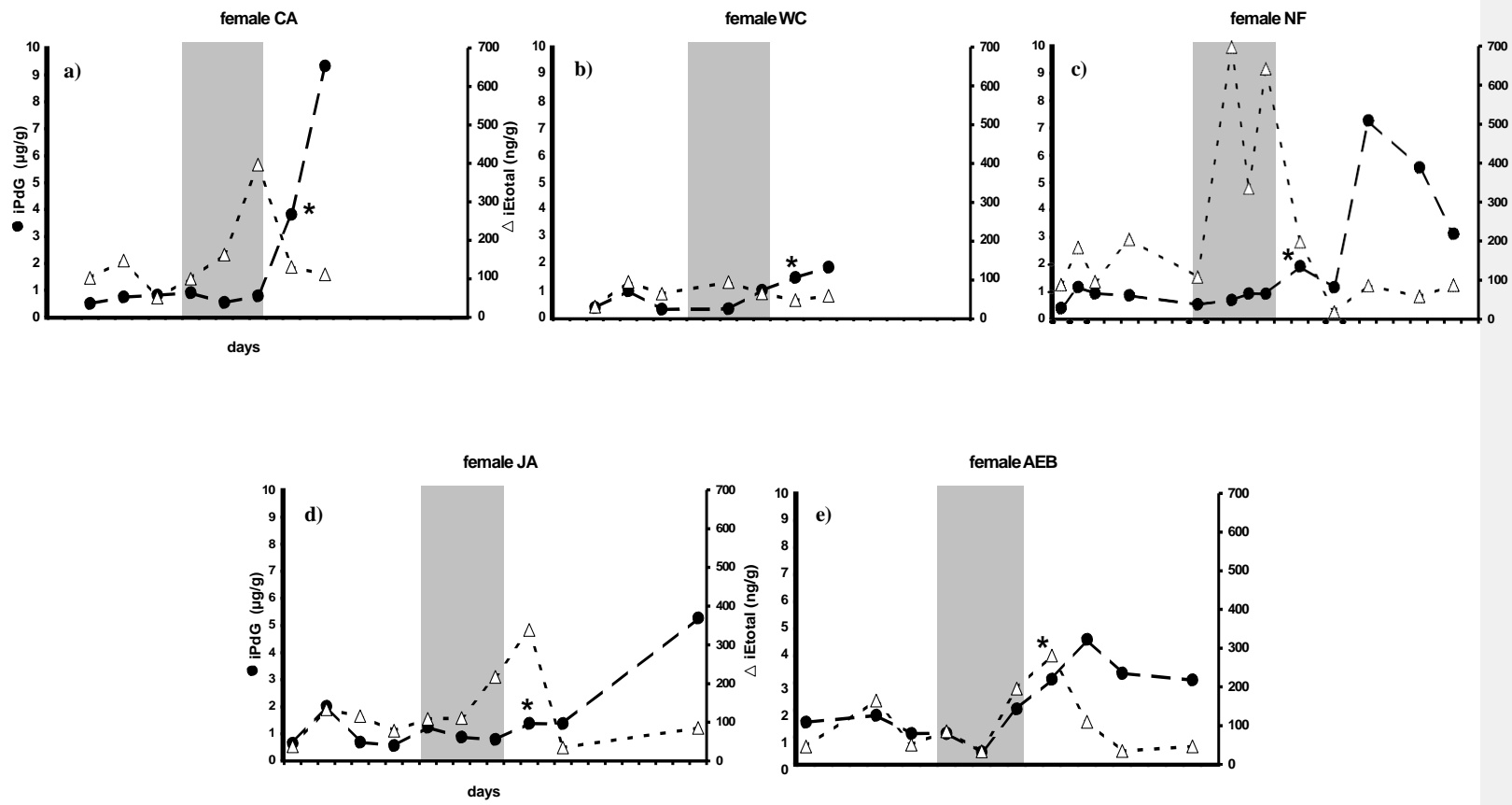


Fig. 1

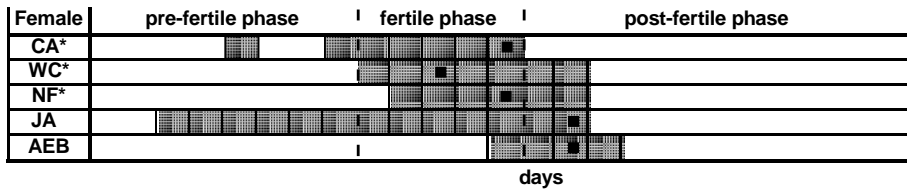


Fig. 2

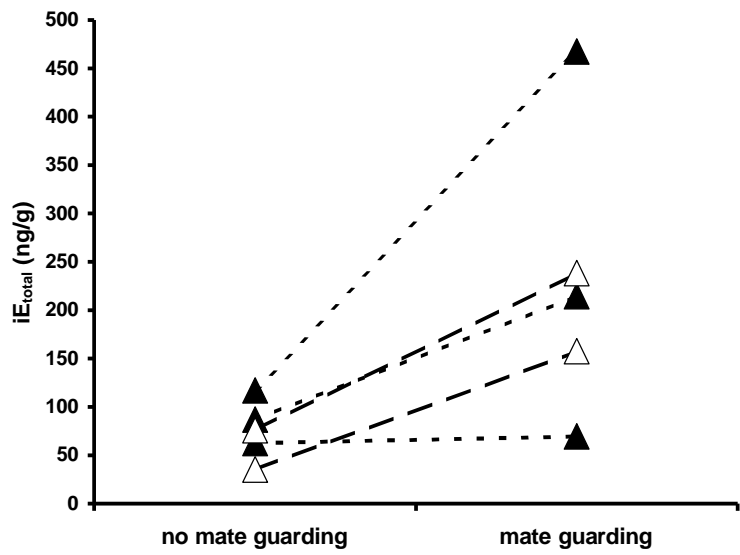


Fig. 3

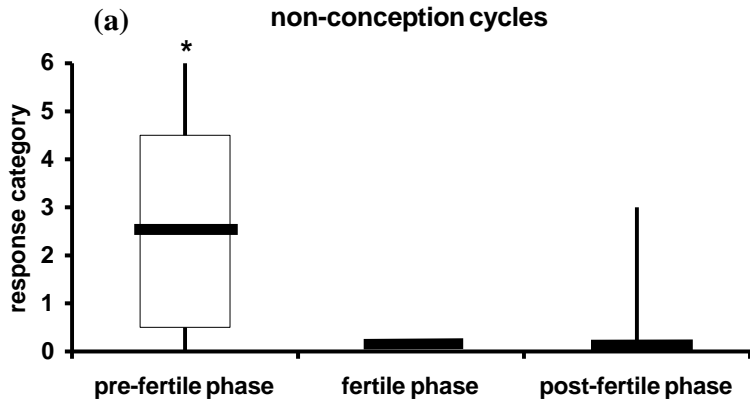
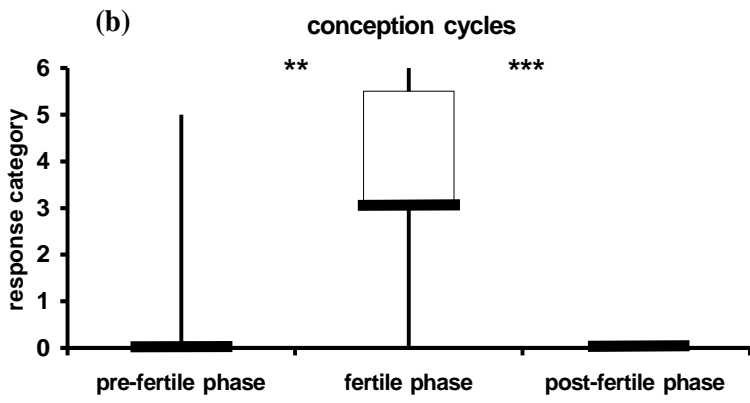


Fig. 4





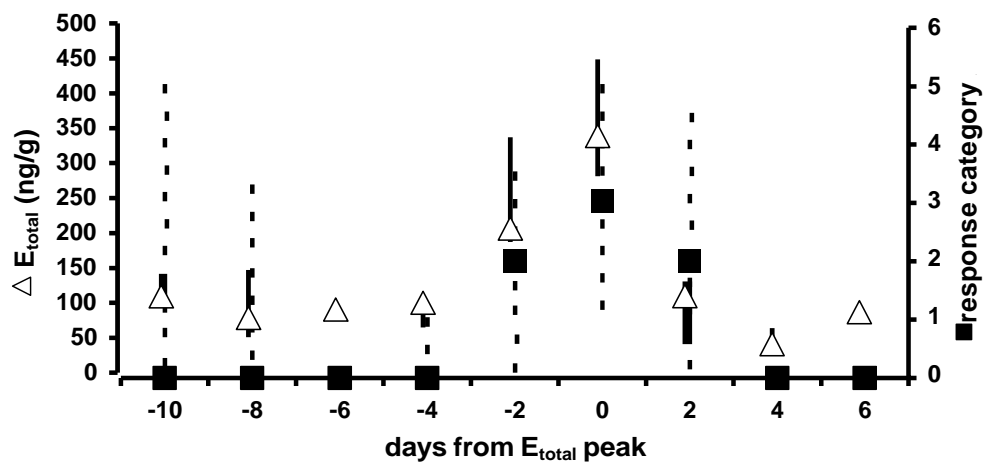


Fig. 5

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.