

1 Sex-specific reproductive behaviours and paternity in free-ranging Barbary
2 macaques (*Macaca sylvanus*).

3

4 Katrin Brauch^{1,2}, Keith Hodges¹, Antje Engelhardt¹, Kerstin Fuhrmann¹, Eric Shaw³ and
5 Michael Heistermann¹

6

7 ¹Department of Reproductive Biology, German Primate Centre, Goettingen, Germany;

8 ²Department of Behavioural Biology, University of Muenster, Germany and ³Gibraltar

9 Ornithological and Natural History Society, Gibraltar Natural History Field Centre, Upper

10 Rock Nature Reserve, Gibraltar

11

12

13

14

15

16

17

18

19

20 Corresponding author: Dr. Michael Heistermann, Department of Reproductive Biology,

21 German Primate Center, Kellnerweg 4, 37077 Goettingen, Germany

22 Tel.:++49-551-3851290; Fax: ++49-551-3851288; e-mail:mheiste@gwdg.de

23

24 **Abstract**

25 In a wide variety of species, male reproductive success is determined by contest for
26 access to females. Among multi-male primate groups, however, factors in addition to male
27 competitive ability may also influence paternity outcome although their exact nature and force
28 is still largely unclear. Here, we have investigated in a group of free-ranging Barbary
29 macaques whether paternity is determined on the pre- or post-copulatory level and how male
30 competitive ability and female direct mate choice during the female fertile phase are related to
31 male reproductive success. Behavioural observations were combined with faecal hormone
32 analysis for timing of the fertile phase (13 cycles, 8 females) and genetic paternity analysis
33 (n=12). During the fertile phase, complete monopolisation of females did not occur. Females
34 were consorted for only 49% of observation time and all females had ejaculatory copulations
35 with several males. Thus, in all cases paternity was determined on the post-copulatory level.
36 More than 80% of infants were sired by high-ranking males and this reproductive skew was
37 related to both, male competitive ability and female direct mate choice as high-ranking males
38 spent more time in consort with females than low-ranking males and females solicited
39 copulations mainly from dominant males. Since most ejaculatory copulations were female-
40 initiated, female direct mate choice appeared to have the highest impact on male reproductive
41 success. However, female preference was not directly translated into paternity since fathers
42 were not preferred over non-fathers in terms of solicitation, consortship and mating
43 behaviour. Collectively, our data show that in the Barbary macaque both sexes significantly
44 influence male mating success, but that sperm of several males generally compete within the
45 female reproductive tract and that therefore paternity is determined by mechanisms operating
46 at the post-copulatory level.

47

48 **Key words:** Faecal hormone analysis, Paternity, Male reproductive success, Female mate
49 choice, Post-copulatory mechanisms

50

51 **Introduction**

52 Sexual selection theory posits that in order to maximize their reproductive success,
53 males should compete among themselves for access to as many fertile females as possible,
54 whereas females, due to their substantially higher degree of investment into offspring, should
55 be more selective (Darwin 1871; Trivers 1972; Andersson 1994). As a consequence, in group-
56 living mammals mating and reproductive success differs greatly among same-sexed
57 individuals, particularly males (e.g. fallow buck, *Dama dama*: McElligott and Hayden 2000;
58 African wild dogs, *Lycaon pictus*: Creel et al. 1997; rhesus macaque, *Macaca mulatta*:
59 Widdig et al. 2004; mandrill, *Mandrillus sphinx*: Setchell et al. 2005).

60 Whether a male is successful in fathering an infant can be determined on two different
61 levels. The first one is the pre-copulatory level, where mating opportunities between the sexes
62 are influenced by, for instance, male-male competition or female choice. Paternity is
63 determined on the pre-copulatory level if mating of a fertile female is restricted to a single
64 male, either due to monopolisation of access by the male or to direct female mate choice. On
65 the other hand, paternity is determined on the post-copulatory level when females mate with
66 several males during their fertile period so that sperm of the different males compete for
67 fertilisation within the female reproductive tract (i.e. sperm competition; Parker 1998;
68 Birkhead and Møller 1998; Birkhead 2000). The outcome of sperm competition can, however,
69 be influenced by pre- and post-copulatory behaviour, e.g. by variables such as mating
70 frequency, establishment of consortships or female mating preferences, as well as by selective
71 uptake or rejection of ejaculated sperm of certain males within the female tract (cryptic
72 female choice; reviewed in Eberhard 1996; Reeder 2003).

73 One of the factors affecting mating pattern, and thus often also paternity outcome, is
74 male dominance rank. High-ranking males usually gain a mating advantage over low ranking
75 males (Dewsbury 1982; African elephant, *Loxodonta africana*: Poole 1989; Cowlshaw and

76 Dunbar, 1991; fallow buck: Moore et al. 1995; McElligott and Hayden 2000; sheep, *Ovis*
77 *aries*: Preston et al. 2001), which often results in higher reproductive success for dominant
78 individuals compared to subordinates (Dewsbury 1982; Ellis 1995 for review). In several
79 species of primates, however, subordinate (and extra-group) males sire a significant number
80 of offspring (rhesus macaque: Berard et al. 1993; Hanuman langur, *Semnopithecus entellus*:
81 Launhardt et al. 2001; Japanese macaque, *Macaca fuscata*: Soltis et al. 2001), which indicates
82 that within this taxon, male dominance rank explains only part of the variation in male mating
83 and reproductive success. This is presumably related to the fact that successful
84 monopolization of fertile females is often difficult to achieve because i) unlike other mammal
85 species, anthropoid primates show an extended period of receptivity (Hrdy and Whitten 1987;
86 van Schaik et al. 2000, 2004) in which the timing of ovulation is usually unpredictable and
87 sometimes concealed from males (Nunn, 1999; Hanuman langur: Heistermann et al. 2001)
88 and ii) male monopolization ability also depends on factors other than rank, such as number
89 of females and degree of synchrony of female ovarian cycles (rhesus macaque: Altmann
90 1962; Nunn 1999; van Noordwijk and van Schaik et al. 2004; chimpanzee, *Pan troglodytes*:
91 Boesch et al. 2006), number of rival males present (mandrill: Setchell et al. 2005; *ibid*:
92 Boesch et al. 2006; Kutsukake and Nunn, 2006), costs of mate-guarding (olive baboon, *Papio*
93 *anubis*: Bercovitch 1983; Alberts et al. 1996) or the degree to which females resist or
94 cooperate with males during mating (Manson 1992; savanna baboon, *Papio cynocephalus*:
95 Bercovitch 1995; Japanese macaque: Soltis 2004). Thus, in addition to male-male contest and
96 male monopolization, other strategies, such as those pursued by the female, have become
97 increasingly recognized in recent years as potentially important determinants of paternity
98 outcome in primates (Barbary macaque, *Macaca sylvanus*: Taub 1980; Small 1989; Dunbar
99 and Cowlshaw 1992; brown capuchin monkey, *Cebus apella*: Janson 1984; patas monkey:
100 *Erythrocebus patas*: Chism and Rogers 1997; Japanese macaque: Soltis et al. 2000, 2001).

101 For many years it was thought that females preferentially restrict mating to a single
102 male (most likely the dominant one) in order to receive “good genes” for their offspring
103 (female mate choice: Small 1989) and avoid the costs of mating with multiple males (e.g.
104 reduced feeding time and increased male aggression: Japanese macaque: Matsubara and
105 Sprague 2004; risk of sexually transmitted disease: Nunn and Altizer 2004; risk of sexual
106 coercion: chimpanzee: Muller et al. 2007). Now, however, there is increasing evidence that
107 females living in multi male multi female groups actually prefer to mate with several males
108 (e.g. long-tailed macaque, *Macaca fascicularis*: Nikitopoulos et al. 2005; Engelhardt et al.
109 2006). Potential benefits for polyandrous mating would be avoiding inbreeding or genetic
110 incompatibilities (Tregenza and Wedell 2002; Newcomer et al. 1999; Zeh and Zeh 2001) or
111 ensuring high quality sperm through sperm competition (Dixson 1998). Alternatively, by
112 polyandrous mating, females may confuse paternity (e.g. Hrdy 1979; van Schaik et al. 2000;
113 Wolff and Macdonald 2004) and in this way possibly increase paternal care or protection of
114 their offspring (e.g. Barbary macaque: Taub 1980, van Schaik and Höstermann 1994; vervet
115 monkey, *Cercopithecus aethiops*: Cheney and Seyfarth 1987; Hanuman langur: Borries et al.,
116 1999), and/or reduce the risk of male infanticide (Hrdy 1979; Hrdy and Whitten 1987; van
117 Schaik and Kappeler 1997; van Schaik et al. 2000). As argued by the graded-signal
118 hypothesis to explain anogenital swelling (Nunn, 1999), females use sexual signals to
119 advertise the probability of ovulation (but not its precise timing), thereby attracting dominant
120 males during times of high ovulation probability and mating with lower ranking males during
121 times of low probability (Nunn 1999, van Schaik et al. 2000). However, despite mounting
122 evidence that females actually pursue their own reproductive strategies (brown capuchin
123 monkey: Janson 1984; Japanese macaque: Huffman 1987; ringtailed lemur, *Lemur catta*:
124 Pereira and Weiss 1991; Hanuman langur: Heistermann et al. 2001; rhesus macaque: Waitt et
125 al. 2003; chimpanzee: Stumpf and Boesch 2006), empirical data showing the degree to which
126 paternity is determined on the pre- or post-copulatory level, to what extent female mate choice

127 and male monopolization influence paternity and what role female sexual signals play in this
128 respect are still limited.

129 In the present study we examine how male monopolisation and female mate choice
130 during the female fertile phase (i.e. the period in which mating can lead to conception) relates
131 to male reproductive success in free-ranging Barbary macaques (*Macaca sylvanus*). Like
132 other macaques, the Barbary macaque lives in multimale-multifemale groups with a
133 promiscuous mating system in which females copulate with many males throughout much of
134 their ovarian cycle (Taub 1980; Small 1990; Küster and Paul 1992). Although males consort
135 females during periods of mating, extended consortships and mate guarding as often seen in
136 other macaques (e.g. lion-tailed macaque, *Macaca silenus*: Kumar and Kurup 1985; Tonkean
137 macaque, *Macaca tonkeana*: Aujard 1998; long-tailed macaque: Engelhardt et al. 2006) have
138 not been described in the study species (Küster and Paul 1988; 1992). Furthermore, unlike
139 most other macaque species, reproduction in the Barbary macaque is highly seasonal, with
140 mating restricted to a 3-4 month period (Taub 1980; Küster and Paul 1984; Ménard and Vallet
141 1996), and a relatively high degree of female ovarian cycle synchrony (Möhle et al. 2005). All
142 these characteristics suggest that, in contrast to other macaques (e.g. *ibid*: Engelhardt et al.
143 2006; *ibid*: Aujard et al. 1998; *ibid*: Kumar and Kurup 1985) and other catarrhine primates
144 (e.g. mandrill: Setchell et al. 2005), monopolisation of fertile female Barbary macaques by
145 males is extremely difficult to achieve and that consequently, females have more options to
146 pursue their own reproductive interests and to influence paternity outcome. On the other hand,
147 we have recently shown that female Barbary macaques advertise the timing of their fertile
148 phase through changes in sexual swelling appearance and that males probably use this female
149 signal to concentrate their mating efforts to the period when conception is most likely (Brauch
150 et al. 2007; Heistermann et al. 2008). Providing information to males about timing of the
151 fertile phase should theoretically facilitate male mate guarding behaviour, which in turn
152 reduces female options to mate with multiple or sub-ordinate males (van Schaik et al. 2000).

153 To what extent this is indeed the case in the Barbary macaque, particularly during the period
154 of the cycle when copulation can actually lead to conception is, however, unclear. Using an
155 integrated approach in which we have combined detailed behavioural observations with faecal
156 hormone measurements for assessing the female fertile phase and faecal microsatellite
157 analysis for genetic paternity determination, our specific aims were to investigate i) how
158 paternity is distributed over competing males, ii) to what extent male consortship and
159 monopolisation behaviour occurs during the female fertile phase and how males vary in this
160 respect and also in their mating success, iii) to what extent female mate choice occurs during
161 the female fertile phase and which males are preferred by females and iv) how consortship
162 behaviour, mating success, and female mate choice relates to male reproductive success.

163

164 **Methods**

165 **Animals and study site**

166 The study was conducted on the “Middle Hill” group of Barbary macaques inhabiting
167 a home range of about 25 ha on the Upper Rock, Gibraltar. The core area is military property
168 and therefore not accessible to the general public. Natural food resources form an important
169 part of the macaques` diet, but daily supplementary feeding (fruit, vegetables, seeds and also
170 fresh water) is provided.

171 Data were collected during the mating seasons (October till February) 2003/2004 and
172 2004/2005. The size of the group varied between 45 and 50 animals of different age classes,
173 including 14-17 adult females (ages 6 to 22 years) and 4 to 6 adult males (ages 5 to 18 years).
174 In the first season 6 adult males were in the group (3 young adult males, 2 males of prime age
175 and one old adult male), while in the second season one of the young adult males (had left the
176 group) and one of the prime adult males (had died) were no longer present. The young adult
177 males were natal males, whereas the older males had immigrated into the group. All males
178 lived in the group since several years before onset of the study.

179 All animals were individually known and well habituated to the presence of human
180 observers. Due to logistic constraints and the fact that several females were synchronized in
181 their ovarian activity (see Results), data could not be collected from all adult females of the
182 group. Instead, we collected behavioural data and faecal samples for hormone analyses from a
183 subset of 8 focal females. The females selected were all multiparous and covered all rank and
184 age classes (Table 1), thus providing a representative sub-sample of the group of females.

185

186 **Behavioural observations**

187 Behavioural data were collected using *focal animal sampling* and *continuous*
188 *recording* (Altmann 1974; Martin and Bateson 1993) during attempted daily follows of
189 female subjects as previously described in detail in Brauch et al. (2007). Due to bad weather
190 conditions and the nature of the habitat (steep rocky area), animals were sometimes not
191 observable and data are restricted to, on average, 5-6 days per week per female. In each
192 season, focal observations were carried out by following each female for up to 4 hours during
193 the period of maximum anogenital swelling, the period of the female ovarian cycle which
194 encompasses the fertile phase (Möhle et al. 2005; Brauch et al. 2007). Females were observed
195 in a random order and data collected were evenly distributed over the different times of day.
196 In the second season, behavioural data were collected from two observers. Comparison of
197 observations conducted in parallel to assess inter-observer reliability yielded a high degree of
198 inter-observer agreement, with values exceeding 90% in all comparisons.

199 The occurrence of the following sexual behaviours was recorded: female presents
200 anogenital area to male (“presenting”), female actively solicits copulation from a male
201 (“active solicitation, see Brauch et al. 2007), female refuses a male’s genital inspection or
202 copulation attempt (“refusal”) and copulations (indicated by intromission). Copulations were
203 divided into non-ejaculatory and ejaculatory copulations, the latter being indicated by the
204 presence of the typical ejaculatory pause (Küster and Paul 1984). Identity of the individual

205 initiating an ejaculatory copulation was also recorded. In this respect, all ejaculatory
206 copulations that followed after a female showed soliciting behaviour (presenting, active
207 solicitation) to the male were considered to be female-initiated (except those cases in which
208 the solicitation behaviour was shown directly after a male's approach), while all ejaculatory
209 copulations that followed directly after a male had approached a female or where a male
210 mounted a female when in consort without being solicited by the female were considered to
211 be male-initiated. Additionally, we recorded the number and duration of consortships.
212 According to Berard et al. (1993) we defined a consortship as occurring when there was i) a
213 close social proximity between male and female (<5m when sitting; <10m when walking) and
214 ii) copulatory behaviour between the consort partners. Consorts were only counted as such,
215 when they were lasting at least 5 minutes. A consort was considered to have ended if the
216 female mated with another male, or if the male or female moved more than 10 m away and
217 the consort partner did not follow within the first 10 minutes. Identity of the individual
218 initiating, maintaining or finishing the consort was also recorded (see Heistermann et al.,
219 2008).

220

221 **Assessment of male rank**

222 We determined the rank position of individual males by creating an agonistic interaction
223 matrix based on displacement events (i.e. one animal retreats from an approaching opponent)
224 and the outcome of these dyadic interactions (Altmann 1974). Submissive displays in
225 response to aggressive behaviours were recorded whenever they were observed, and were
226 additionally used in the analysis.

227

228 **Faecal sample collection, hormone analysis and definition of the fertile phase**

229 On average, from each focal female 3.8 ± 1.4 faecal samples (range 2-6) were
230 collected each week as described previously (Möhle et al. 2005). Samples were extracted

231 twice according to the method reported by Ziegler et al. (2000). After extraction, extracts
232 were analyzed for concentrations of immunoreactive oestrogen (total, E_t) and progesterone
233 metabolites (5α -reduced 20-oxo pregnanes, 5-P-3OH) using validated enzyme immunoassays
234 previously shown to accurately reflect female ovarian function in the Barbary macaque
235 (Möhle et al. 2005, Heistermann et al. 2008). Sensitivities of the assays at 90% binding were
236 1.9 pg for E_t and 39 pg for 5-P-3OH. For both assays, intra- and inter-assay coefficients of
237 variation, calculated from replicate determinations of high and low value quality controls,
238 were <10% and <18%, respectively.

239 Across the two mating seasons, hormone profiles were obtained from a total of 16
240 cycles (8 females). Analysis of behavioural data was, however, restricted to those cycles in
241 which the frequency of faecal sample collection was sufficiently high (sample gap not greater
242 than two days prior to the faecal progestogen rise, see below) to allow estimation of the day of
243 ovulation with an acceptable degree of precision (see Heistermann et al. 2008). Thus, finally
244 13 cycles entered the analysis. Ovulatory cycles could clearly be distinguished from non-
245 ovulatory post-conception “cycles” based on differences in hormone profiles (Möhle et al.,
246 2005).

247 As described previously (Heistermann et al. 2008), for each cycle a defined rise in
248 faecal 5-P-3OH levels above a threshold value (2 standard deviations above the preceding
249 mean baseline level) was used to determine the most likely day of ovulation and thereby to
250 define the fertile phase. Based on the time lag between ovulation and the postovulatory rise in
251 faecal progestogen excretion of 2-3 days in macaques (Shideler et al. 1993), we determined
252 for each cycle a two-day window (days -2/-3 relative to the defined rise; see Heistermann et
253 al. 2008) in which ovulation was most likely. The fertile phase was then defined as the period
254 comprising these two days plus three preceding days in order to account for sperm life span in
255 the female reproductive tract (Behboodi et al. 1991; Wilcox et al. 1995).

256

257 **Genetic paternity analysis**

258 For paternity analysis, faecal samples were collected from all mothers and infants
259 (n=12) that were sired during the observation period and from all sexually mature males in the
260 group. 3-5 g of fresh faeces was collected and placed in a tube containing 15 ml of absolute
261 ethanol. Samples were then stored at ambient temperatures until extraction.

262 Prior to our paternity determination tests, we validated the method of genotyping from
263 faecal samples by analyzing matched blood and faecal samples from 10 animals of the
264 Gibraltar Barbary macaque population. DNA from faeces and blood was extracted using the
265 QIAamp[®] DNA Stool Mini Kit and the QIAamp[®] DNA Mini Kit (Quiagen, Hilden,
266 Germany), respectively, following the instructions given in the manual. After extraction,
267 DNA was stored at -20°C until further processing. Initially, we tested 26 different
268 microsatellite loci (di- and tetra-nucleotide) with an amplification length ranging from 97 bp
269 to 280 bp for their suitability to detect different alleles. The primers were originally designed
270 for the amplification of microsatellite loci in humans, but have been successfully applied for
271 genotyping in a number of Old World monkeys, including macaques (Nürnberg et al. 1998;
272 von Segesser et al. 1999; Lathuillière et al. 2001; Engelhardt et al. 2006). Because of the high
273 degree of relatedness and the relatively low genetic variability in the Gibraltar population
274 (Modolo et al. 2005), only 11 loci proved to be sufficiently polymorphic to allow paternity
275 analysis. Therefore, for all subsequent tests as well as for paternity analysis, the following
276 primers were used: dinucleotides: D6S311 (Altmann et al. 1996), D7S503, D11S925
277 (Altmann et al. 1996, von Segesser et al. 1999, Lathuillière et al. 2001), D14S255 (Nürnberg
278 et al. 1998); tetranucleotides: D1S584, D3S1768, D6S501 (Engelhardt et al. 2006) D5S1457
279 (Bayes et al. 2000), D7S2204 (Vigilant et al. 2001), D8S1106 (Kümmerli and Martin 2005),
280 D10S1432 (Chambers et al. 2004, Marvan et al. 2006). For each microsatellite locus, the
281 forward primer was labelled with fluorescent FAM, while the reverse remained unlabeled.
282 PCR reactions were performed in a total volume of 10µl by using the Eppendorf Mastercycler

283 gradient (Eppendorf, Hamburg, Germany). The PCR master mix contained 1x buffer, 1.5 mM
284 $MgCl_2$, 0.2 mM dNTP, 2 pM of each primer (forward and reverse), 0.5 U Hot Master Taq
285 DNA Polymerase (Eppendorf, Hamburg, Germany) and 8 μ l DNA extract for faecal samples
286 and 2 μ l DNA extract for blood samples. PCR was conducted as follows: after initial
287 denaturation for 3 min at 94°C, amplification consisted of 35 cycles (94°C for 30s, 58°C
288 respectively 60°C for primer D7S503 and D14S255 for 30s and 70°C for 30s) and a final
289 extension (70°C for 3 min). Genotyping was performed on a denaturing 4.5% polyacrylamide
290 gel on an ABI Prism 377 DNA sequencer (Applied Biosystems), with analysis using
291 GENESCAN software (Applied Biosystems).

292 We used the multiple-tube approach (Taberlet et al. 1996, 1999) for individual
293 genotyping from faecal samples in order to overcome false genotyping. If possible, we used
294 two different samples for each individual, but at least three different DNA extracts. Alleles
295 had to be confirmed twice each in three independent PCRs (Engelhardt et al. 2006). In the
296 case that a third allele emerged, PCR was repeated, and the two most frequent alleles were
297 considered as the “true” alleles, when they were confirmed at least four times each in
298 independent PCR products from at least two different extracts (Engelhardt et al 2006).
299 Homozygosity was confirmed when a single allele occurred in six independent PCRs. If a
300 second allele occurred, PCR was repeated, and homozygosity was confirmed if the second
301 allele occurred only once in nine independent PCRs from at least two different extracts. For
302 blood samples, heterozygosity was confirmed when the two alleles occurred in two
303 independent PCRs and homozygosity when one allele occurred in three independent PCRs.

304 To test the reliability of our genotyping results from faecal samples, we assessed the
305 agreement in allele patterns between the matched blood and faecal samples of the 10 animals.
306 Out of 108 possible genotypes, 102 were identical between blood and faecal samples. From
307 the 6 inconsistent samples, one additional allele was found in faeces in 5 cases and in one case
308 allelic drop out was seen in the faecal sample. Overall, the agreement between genotyping

309 from faeces and blood was 94.4%, indicating a high degree of reliability of our faecal
310 genotyping method.

311 For assignment of paternity, all infants, except one (an infant that could not be
312 genotyped for one locus) could be genotyped for all loci. For one locus, a mother could not be
313 genotyped; in this case we excluded this locus from the analyses. According to a number of
314 other studies (e.g. Kümmerli and Martin 2005; Setchell et al. 2005; Engelhardt et al. 2006),
315 we determined paternity on the basis of a maximum likelihood method via the program
316 CERVUS 2.0 (Tristan Marshall, Edinburgh, UK; Marshall et al. 1998) with confidence levels
317 of >80% (relaxed confidence) and >95% (strict confidence; Marshall et al., 1998).

318

319 **Data analysis**

320 All behavioural data (frequencies, durations) presented here exclusively refer to the
321 defined fertile phases of the observed ovarian cycles, as effects of male dominance rank and
322 female mate choice should have the highest impact on paternity outcome during times when
323 conception is most likely. In order to account for potentially limited observation times during
324 single fertile phases in certain individuals, for each female we combined the behavioural data
325 collected during the 2-3 fertile phases monitored and averaged them for statistical analyses.
326 There was no evidence from our data that the behavioural pattern in the fertile phases of the
327 two non-conception cycles differed from those in the conception cycles (see for example
328 Figure 1)

329 For examining the influence of male competitive ability on paternity determination,
330 we used male rank as a proxy measure. In this respect, males were classified into “high-
331 ranking” and “low-ranking” males. This was done because the sociometric matrix of
332 displacement and agonistic interactions used for determining an individual’s rank position
333 indicated that in each season the actual ranks between two high- and two low-ranking males
334 were undecided as agonistic interactions occurred almost equally in both directions (see also

335 Küster and Paul 1988; 1992). High-ranking males (top half of the hierarchy) could, however,
336 be unambiguously differentiated from low-ranking males (bottom half of the hierarchy).

337 We used the Binomial test to test whether the observed distribution of paternities
338 between males of the two rank classes differed from an expected 50% to 50% distribution.
339 For this analysis, 11 of the 12 paternities assigned could be used because in one case an infant
340 was sired by an extra-group male, the identity of which was unknown. For examining the
341 influence of rank on male mating frequency and on the number and total and mean duration of
342 consortships, we compared the frequency of total and ejaculatory copulations and number and
343 duration of consortships shown by high-ranking males with those shown by low-ranking
344 males using the Wilcoxon signed-rank test.

345 Female sexual behaviours which facilitate or prevent mating with certain males (e.g.
346 sexual presentation, refusals) are considered to be most likely part of female direct mate
347 choice (Small 1994; Setchell 2005). To test whether females exerted any mate choice
348 according to male rank, we tested for differences in frequencies of female sexual behaviours
349 (presenting, active solicitation, refusals) shown towards the two rank classes of males using
350 the Wilcoxon signed-rank test. We included only those presentations and solicitations into the
351 analysis, which were clearly female-initiated, i.e. occurred after the female had approached
352 the male. We tested which sex is more responsible for initiating an ejaculatory copulation by
353 comparing per female the numbers of ejaculatory matings initiated by either of the two sexes
354 using the Wilcoxon signed-ranks test.

355 Furthermore, we examined whether the assigned fathers of infants had more and
356 longer consortships, more copulations (total and ejaculatory), received more solicitations
357 (presentation and active solicitations) or received fewer refusals than non-fathers. For this,
358 according to Engelhardt et al. (2006), we averaged the frequencies of the respective
359 behaviours shown towards all non-fathers (providing an “average non-father”) and compared
360 these to those frequencies shown towards the likely father using the Wilcoxon signed-ranks

361 test. Since behavioural data were not available from all 11 mothers of group-sired infants, and
362 since we excluded those conception cycles from analysis in which observation time may
363 potentially have been too limited, this analysis was restricted to 7 conception cycles.

364 All statistical tests were conducted with SPSS 14.0 for Windows and used two-tailed
365 probabilities. The level of significance was set at $\alpha < 0.05$

366

367 **Results**

368 Of the 13 cycles for which the fertile phase could be reliably determined and
369 behavioural data were available, 11 resulted in conception. In both seasons, the fertile phases
370 of the study females were restricted to the months of December and January, with >80%
371 being confined to one month (December). As a result of this high degree of seasonality,
372 approximately one third (2003/2004: 34.6%; 2004/2005: 33.4%) of days on which females
373 were in their fertile phase overlapped with each other. Given that our study was restricted to
374 about 50% of sexually mature females in the group, it is likely that the values on degree of
375 fertile phase overlap reported above are, however, substantially underestimated.

376

377 **Male rank and paternity outcome**

378 Paternity assignments could be made on a >95% level of likelihood for 8 of the 12
379 determinations, while the remaining 4 paternity assignments had a confidence level of >80%
380 (Table 2). 11 offspring were sired by males belonging to the study group, while one infant
381 was sired by an extra-group male (Table 2) of unknown identity. Although not statistically
382 significant, paternity was biased towards high-ranking males which sired >80% (9/11) of
383 offspring (Binomial test: $p = 0.066$; Table 2)

384

385 **Consortship and male mating success in relation to male rank class**

386 Figure 1a shows the proportion of observation time females spent in consort with
 387 males and number of consort partners. Females were engaged in consortships during all fertile
 388 phases, but with varying duration, number and identity of partners. On average, females spent
 389 48.6% (range 17.9-93.8%) of the observation time in consortship with a minimum of two
 390 consort partners (Figure 1a). Complete monopolization (in consort with a single male for
 391 >95% of observation time, c.f. Engelhardt et al. 2006) of focal females, irrespective of their
 392 rank, never occurred (see Fig. 1a). There was, however, a significant relationship between
 393 male rank class and the occurrence of consortships. As shown in Figure 1b, high-ranking
 394 males were three times more often in consort with females than low-ranking males (Wilcoxon
 395 signed rank test: $Z=-1.75$, $N=8$, $p=0.080$) and consortships lasted significantly longer with
 396 high-ranking compared to low-ranking males, both in terms of total and average duration
 397 (Wilcoxon signed rank test: total duration, $Z=-2.10$, $N=8$, $p=0.036$; average duration, $Z=-2.10$,
 398 $N=8$, $p=0.036$; Figure 1 b).

399 On average, during observation times females mated with 2.9 males (range 2-5) during
 400 their fertile phase, with rates of copulations (total and ejaculatory) being biased towards high-
 401 ranking males. Specifically, females mated 4 times more often with high-ranking than with
 402 low-ranking males (high-ranking: 1.10 ± 0.216 vs. low-ranking: 0.27 ± 0.11 , Wilcoxon signed-
 403 ranks test: $Z=-1.89$, $N=8$, $p=0.058$) and also received significantly higher frequencies of
 404 ejaculations from high-ranking than low-ranking males (high-ranking: 0.88 ± 0.16 vs. low-
 405 ranking: 0.16 ± 0.06 Wilcoxon-signed-ranks test: $Z=-2.25$, $N=8$, $p=0.025$).

406

407 **Female sexual behaviour in relation to male rank class**

408 On average, females solicited copulations from 3.5 males (range: 1-6 males) during
 409 their fertile phase. As shown in Figure 2, female sexual behaviour was clearly biased
 410 according to male rank class, with significantly higher frequencies of presentation and active

411 solicitation directed to the class of high-ranking males (Wilcoxon signed-ranks test:
412 presenting: $Z=-2.24$, $N=8$, $p=0.025$; active solicitation: $Z=-2.20$, $N=8$, $p=0.028$). Females
413 rarely refused male sexual approaches (inspection and mounting attempts), but did so more
414 often towards low-ranking than high-ranking males (Figure 2), although the difference was
415 not significant (Wilcoxon signed-ranks test: $Z=-1.36$, $N=8$, $p=0.176$). Females were also
416 mainly responsible for initiating ejaculatory copulations, which they did so in more than two
417 thirds of the observed cases (67.3% compared to 32.7% initiated by males; Wilcoxon signed-
418 ranks test: $Z=-2.54$, $N=8$, $p=0.011$).

419

420 **Paternity and male and female sexual behaviour**

421 Having shown that consortships, male mating success and female sexual behaviours
422 were all biased towards high-ranking males and that males of high rank had a higher
423 reproductive success, we tested for differences in behavioural patterns between fathers and
424 non-fathers. Figure 3 demonstrates the duration of time spent in consortship and the
425 frequencies of male ejaculatory copulations and female solicitations (presenting and active
426 solicitation combined) shown by or towards the assigned fathers compared to those shown by
427 or towards non-father males. Although in a few individual cases the likely fathers consorted
428 more often and for a longer duration with the mother, ejaculated at higher frequencies and
429 received more solicitations from the mother, overall none of the behavioural parameters tested
430 was significantly related to paternity outcome. Females were neither more often in consort
431 with the likely father (Wilcoxon signed-ranks test: $Z=-1.02$, $N=7$, $p=0.31$) nor was duration of
432 consortship with the father longer than with other males (Wilcoxon signed- ranks test: $Z=-$
433 0.85 , $N=7$, $p=0.40$; Fig 3a). Furthermore, fathers neither copulated more often (Wilcoxon
434 signed-ranks test: $Z=-0.85$, $N=7$, $p=0.39$), nor had more ejaculatory copulations with mothers
435 (Wilcoxon signed-ranks test: $Z= -0.43$, $N=7$, $p=0.67$) than other males of the group (Fig. 3b).
436 Finally, there was no significant difference between solicitation rates shown by mothers

437 towards the likely fathers compared to those shown towards non-fathers (Wilcoxon signed-
438 ranks test: $Z=-0.67$, $N=7$, $p=0.50$, Fig. 3c)

439

440 **Discussion**

441 Since in this study, monopolization of females by a single male did not occur and
442 females mated with multiple males during the fertile phase, our results indicate that paternity
443 in Barbary macaques is largely determined at the post-copulatory level (i.e. within the female
444 reproductive tract). Paternity was biased towards high-ranking males partly due to their highly
445 competitive ability in consorting females, but also to female direct mate choice. However,
446 males that sired infants seemed not to be preferred over those that did not in terms of female
447 solicitation, consortship and mating, suggesting that these pre-copulatory behaviours were not
448 directly transferred into reproductive success.

449 One of the main mechanisms by which male primates maximise their reproductive
450 success is to monopolize access to fertile females, i.e. to consort them and outcompete other
451 males during the fertile phase of the female's ovarian cycle (chimpanzee: Hasegawa and
452 Hiraiwa Hasegawa 1990; mandrill: Setchell et al. 2005; long-tailed macaque: Engelhardt et al.
453 2006). In this way, dominant males strive to prevent the situation in which their sperm needs
454 to compete with that of other males for fertilization within the female reproductive tract.
455 Although in Barbary macaques (Heistermann et al. 2008), as in macaques in general (bonnet
456 macaque, *Macaca radiata*: Glick 1980; long-tailed macaque: Engelhardt et al. 2006), it is the
457 males that are mainly responsible for maintaining consortships, in our study group females
458 were consorted by males during their fertile phase for only 49% of the observation time.
459 Furthermore, females were consorted by and mated with several males (see also Taub 1980)
460 during their fertile phase. Thus, in all cases, sperm of different males competed within the
461 female reproductive tract and paternity was most likely determined at the post-copulatory
462 level.

463 The reason why dominant males did not monopolize females, even though they are
464 able to discern the fertile phase from the pattern of female anogenital swelling (Brauch et al.
465 2007; Heistermann et al. 2008), is not clear. Models of reproductive skew provide possible
466 explanations. According to the concession model (Clutton-Brock 1998; Johnstone 2000),
467 high-ranking males should not completely monopolize access to and reproduction with
468 females when the presence of subordinates increases the dominant's fitness benefits. To date,
469 however, there is no clear support for this model in primates (Kutsukake and Nunn 2006) and,
470 to our knowledge, there are no empirical data showing that high-ranking Barbary macaque
471 males benefit from the presence of a number of potential rival males. Since Barbary macaque
472 males provide extensive care for infants (Paul et al. 1992), it might be that by sharing
473 paternity with subordinate males, dominant males gain fitness advantages through enhanced
474 paternal care of the infants they sired. Similarly, dominant males may potentially benefit from
475 sharing reproduction with subordinate males if the presence of the latter helps to protect
476 infants and prevent group takeovers by attacks from outside males (e.g. Hanuman langur:
477 Borries et al. 1999) or reduces predation risk (van Schaik and Hörstermann, 1994).

478 A further alternative which our present data support, is the limited control model (Cant
479 1998; Clutton-Brock 1998; Reeve et al. 1998), which assumes that dominant males are unable
480 to completely control access to fertile females due to factors such as alternative male mating
481 strategies, female cycle synchrony or female choice. The present data showing a considerable
482 overlap in fertile phases indicates a high degree of female cycle synchrony in the Barbary
483 macaque. Under such conditions, it is difficult for a male to effectively monopolize more than
484 one female at a time during her fertile phase (rhesus macaque: Altmann 1962; Emlen and
485 Oring 1977; Ims, 1988; Paul 1997; Say et al. 2001; mouse lemur, *Microcebus murinus*: Eberle
486 and Kappeler 2002) and this would explain why female monopolization by dominant males
487 did not occur even though females preferred high-ranking males as mating partners (see
488 below). Irrespective of the specific reasons for the lack of male monopolization, our data

489 clearly suggest that monopolization of fertile females as a result of male contest is not an
490 important determinant of male reproductive success in the Barbary macaque, a conclusion
491 which would be in line with general predictions about factors determining paternity under
492 conditions of limited male control (Altmann 1962; van Noordwijk and van Schaik et al.
493 2004). In this respect, Barbary macaques differ markedly from less seasonally breeding
494 anthropoid primate species, such as long-tailed macaques (Engelhardt et al. 2006) and
495 mandrills (Setchell et al. 2005) in which monopolization of fertile females is a highly
496 effective male strategy to maximise male reproductive success.

497 Although dominant males did not completely monopolise access to fertile females, our
498 data clearly show that high-ranking males sired the majority of offspring, thus confirming the
499 previous findings of Paul et al. (1993) that male reproductive success in the Barbary macaque
500 is rank related. In contrast, a more recent study by Kümmerli and Martin (2005) suggested
501 male age to be more important than rank in influencing mating outcome. Since in our study
502 higher-ranking males were also considerably older, any potential age-related effects on male
503 reproductive success as reported by Kümmerli and Martin (2005) and Küster et al. (1995)
504 cannot be excluded. Given that age and rank are closely related in the Barbary macaque (Paul
505 1989), as in other macaque species (Sprague 1998 for review), it is in any case difficult to
506 disentangle the effect of rank and age on male reproductive output.

507 The mechanisms underlying paternity distribution in our study seem to be multiple.
508 Despite the inability of males to monopolize females, their competitive ability still may have
509 influenced reproductive success. Since high-ranking males consorted females more often and
510 for a longer duration during the peri-ovulatory period than low-ranking individuals, their
511 chances of mating were higher and this has been reported for other species (mandrill: Setchell
512 et al. 2005; baboon: Weingrill et al. 2000, 2003). Furthermore, our observation that low-
513 ranking males terminated consortships more often as a result of being disturbed by more

514 dominant males (unpublished data) also supports the contention that male competitive ability
515 is at least partly responsible for the rank-related bias in consortship pattern.

516 Other mechanisms, however, seem to be involved and one of these is female direct
517 mate choice. In our study, females initiated the majority of copulations that led to ejaculation
518 whilst, at the same time, soliciting copulations mainly from high-ranking males (see also
519 Küster and Paul 1992). As a consequence, high-ranking males had a higher mating success
520 and thus were provided with a better chance of fertilising the female (see below). Female
521 mating preference for males signalling their physical superiority (e.g. dominance status)
522 appears to be widespread among primates (brown capuchin monkey: Janson 1984; squirrel
523 monkey, *Saimiri oerstedii*: Boinski 1987; Small 1989; Japanese macaque: Soltis et al. 1999;
524 Thomas langur, *Presbytis thomasi*: Steenbeek 2000; mandrill: Setchell 2005), although this is
525 by no means universal (Manson 1992; Soltis et al. 2001). Since the subordinate males in our
526 study group were, however, natal males, it seems also possible that female preference for
527 high-ranking males might have been related to avoidance of sexual interactions in situations
528 of increased familiarity (Küster et al. 1994). However, continuous co-residence alone appears
529 to be insufficient for mating avoidance in the Barbary macaque (Küster et al. 1994). In any
530 case, the freedom of mate choice puts female Barbary macaques into an optimal situation. On
531 one hand, they are able to promote paternity in preferred males, whilst still confusing it
532 through mating polyandrously and receiving the potential benefits of promiscuity (reviewed
533 in Wolff and Macdonald, 2004). Whether female preference is based on male rank only is,
534 however, not clear since factors such as age (Barbary macaque: Paul et al. 1989; Kümmerli
535 and Martin 2005), friendship with certain males (baboon: Smuts, 1985) or degree of
536 dissimilarity of MHC alleles (Grob et al. 1998; Penn and Potts 1999; rhesus macaque: Widdig
537 et al. 2004; mouse lemur: Schwensow et al. 2008) may also be involved.

538 The observed difference in mating success between high- and low-ranking males
539 corresponded closely with reproductive success since high-ranking males sired nearly 4 times

540 more offspring than low-ranking males. Nevertheless, since females were not in consort and
541 did not copulate more often with the assigned father of their infant than with non-father
542 males, our results also suggest that male mating success was not directly translated into
543 individual reproductive success. Behavioural data, however, could not be collected during the
544 entire fertile phase (see Methods), and we may have missed copulations which occurred with
545 the fathers. Nevertheless, since individual observation time was spread over different days of
546 the fertile phase and equally distributed over the daily activity phase of the animals, we can
547 reasonably assume that the copulatory pattern we observed is representative for the entire
548 fertile phase. Interestingly, a similar discrepancy between number of copulations and
549 paternity has also been described for the long-tailed macaque (Engelhardt et al. 2006). Thus,
550 we propose that actual paternity in the Barbary macaque is most likely determined through
551 individual advantages operating at the post-copulatory level such as differences in sperm
552 number, vitality and/or viability, together with cryptic female choice (Eberhard 1996; Reeder
553 2003). Although our study design did not allow further investigation into the character of
554 these potential mechanisms and the degree to which they may have affected paternity, the
555 potential for them to exist in primates is high (Harcourt 1995; Harcourt et al. 1996; Birkhead
556 and Kappeler, 2004; Engelhardt et al. 2006).

557 Collectively, our data indicate that paternity in the Barbary macaque is principally
558 determined at the post-copulatory level. Pre-copulatory mechanisms clearly also operate in
559 the form of male consortship ability and female mate choice. Our present data, although
560 inconclusive tend to suggest that the female component in this respect is the more influential.
561 The mechanisms by which paternity is determined at the post-copulatory level remain to be
562 investigated.

563

564

565

566 Acknowledgements

567 This study was conducted as part of the Gibraltar Barbary macaque project (GBMP).
568 We express our gratitude to Dr. John Cortes of the Gibraltar Ornithological and Natural
569 History Society, and Marc Pizarro of the Gibraltar Veterinary Clinic for their support and
570 cooperation. We also thank Brian Gomilla for his help in collection of behavioural data and
571 Nina Stobbe, Dana Pfefferle and Brian Gomilla for their help in collecting faecal samples, Dr.
572 Eckhard Heymann and Dr. Dietmar Zinner are acknowledged for help with the statistics. We
573 are grateful to Dr. N. Menard for her advice on the methodology of microsatellite analysis and
574 suitability of genetic markers and Dr. L. Modolo for providing access to blood samples used
575 for the genetic validation tests. Dana Pfefferle, Andrea Heistermann and Jutta Hagedorn
576 provided support in hormone analyses and Dr. C. Roos and Dr. M. Eberle provided advice in
577 paternity analysis. We thank the Royal Air Force Gibraltar for permission to enter the study
578 area. The study was conducted completely non-invasively and under the permission of
579 GOHNS, the authority responsible for any research on the Gibraltar Barbary macaques. We
580 adhered to the Guidelines of the Use of Animals in Research, the legal requirements of
581 Gibraltar, and the guidelines of the involved institutes. The study was supported by a grant
582 (GRK 289/3-03) from the Deutsche Forschungsgemeinschaft (DFG).

583

584 References

- 585 Alberts SC, Altmann J, Wilson ML (1996) Mate guarding constrains foraging activity of male
586 baboons. *Anim Behav* 51: 1269-1277
- 587 Altmann J (1974) Observational study of behavior: sampling methods. *Behaviour* 49: 227-267
- 588 Altmann J, Alberts SC, Haines SA, Durbach J, Muruthi P, Coote T, Geffen E, Cheesman DJ,
589 Mututua RS, Saiyalel SN, Wayne RK, Lacy RC, Brudford MW (1996) *Behavior*

- 590 predicts genetic structure in a wild primate group. Proc Natl Acad Sci USA 93:5797-
591 5801
- 592 Altmann SA (1962) A field study of the socio-biology of rhesus monkeys, *Macaca mulatta*.
593 Ann NY Acad Sci 102:277-285
- 594 Andersson M (1994) Sexual Selection. Princeton University Press, Princeton, New Jersey
- 595 Aujard F, Heistermann M, Thierry B, Hodges JK (1998) Functional significance of
596 behavioral, morphological, and endocrine correlates across the ovarian cycle in
597 semifree ranging female Tonkean macaques. Am J Primatol 46:285-309
- 598 Bayes MK, Smith KL, Alberts SC, Altmann J, Bruford MW (2000) Testing the reliability of
599 microsatellite typing from faecal DNA in the savannah baboon. Cons Gen 1:173-
600 176
- 601 Behboodi E, Katz DF, Samuels SJ, Tell L, Hendrickx AG, Lasley BL (1991) The use of a
602 urinary estrone conjugates assay for detection of optimal mating time in the
603 cynomolgus macaque (*Macaca fascicularis*). J Med Primatol 20:229-234
- 604 Berard JD, Nurnberg P, Epplen JT, Schmidtke J (1993) Male rank, reproductive behavior, and
605 reproductive success in free-ranging rhesus macaques. Primates 34:481-489
- 606 Bercovitch FB (1983) Time budgets and consortships in olive baboons (*Papio anubis*). Folia
607 Primatol 41:180-190
- 608 Bercovitch FB (1995) Female cooperation, consortship maintenance and male mating success
609 in savanna baboons. Anim Behav 50:137-149
- 610 Birkhead TR (2000) Promiscuity: an evolutionary history of sperm competition and sexual
611 conflict. Faber and Faber, London
- 612 Birkhead TR, Kappeler PM (2004) Post-copulatory sexual selection in birds and primates In:
613 Kappeler PM, van Schaik CP (eds) Sexual selection in primates: new and
614 comparative aspects. Cambridge University, Cambridge, pp151-171

- 615 Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic Press,
616 London
- 617 Boesch C, Kohou G, Néné H, Vigilant L (2006) Male competition and paternity in wild
618 chimpanzees of the Taï Forest. *Am J Phys Anthropol* 130:103-115
- 619 Boinski S (1987) Mating patterns in squirrel monkeys (*Samiri oerstedii*): Implications for
620 seasonal sexual dimorphisms. *Behav Ecol Sociobiol* 21:13-21
- 621 Borries, C, Launhardt, K, Epplen, C, Epplen, JT, Winkler, P (1999) Males as infant protectors
622 in Hanuman langurs (*Presbytis entellus*) living in multimale groups – defence pattern,
623 paternity and sexual behaviour. *Behav Ecol Sociobiol* 46:350-356
- 624 Brauch K, Pfefferle D, Hodges K, Möhle U, Fischer J, Heistermann M (2007) Female sexual
625 behavior and sexual swelling size as potential cues for males to discern the female
626 fertile phase in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. *Horm*
627 *Behav* 52:375-383
- 628 Cant MA (1998) A model for the evolution of reproductive skew without reproductive
629 suppression. *Anim Behav* 55:163-169
- 630 Chambers KE, Reichard UH, Möller A, Nowak K, Vigilant L (2004) Cross-species
631 amplification of human microsatellite markers using noninvasive samples from white-
632 handed gibbons (*Hylobates lar*). *Am J Primatol* 64:19-27
- 633 Cheney DL, Seyfarth RM (1987) The influence of intergroup competition on the survival and
634 reproduction of female vervet monkeys. *Behav Ecol Sociobiol* 21:375-386
- 635 Chism J, Rogers W (1997) Male competition, mating success and female choice in a
636 seasonally breeding primate. *Ethology* 103:109-126
- 637 Clutton-Brock TH (1998) Reproductive skew concessions and limited control. *TREE* 13:288-
638 292
- 639 Cowlishaw G, Dunbar RIM (1991) Dominance rank and mating success in male primates.
640 *Anim Behav* 41:1045-1056

- 641 Creel SR, Mills MGL, Monfort SL (1997) Rank and reproduction in cooperatively breeding
642 African wild dogs: behavioural and endocrine correlates. *Behav Ecol* 8:298-306
- 643 Darwin C (1871) *The descent of man and the selection in relation to sex*. John Murray,
644 London
- 645 Dewsbury DA (1982) Ejaculate cost and male choice. *Am. Nat* 119:601-610
- 646 Dixson AF (1998) *Primate Sexuality*. Oxford University Press, Oxford
- 647 Dunbar RIM, Cowlshaw G (1992) Mating success in male primates: Dominance rank, sperm
648 competition and alternative strategies. *Anim Beh* 44:1171-1173
- 649 Eberhard WG (1996) *Female control: sexual selection by cryptic female choice*. Princeton
650 University Press, Princeton
- 651 Eberle M, Kappeler PM (2002) Mouse lemurs in space and time: a test of the socioecological
652 model. *Behav Ecol Sociobiol* 51:131-139
- 653 Ellis L (1995) Dominance and reproductive success among non-human animals: a cross-
654 species comparison. *Ethol Sociobiol* 16:257-333
- 655 Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems.
656 *Science* 197:215-223
- 657 Engelhardt A, Heistermann M, Hodges JK, Nürnberg P, Niemitz C (2006) Determinants of
658 male reproductive success in wild long-tailed macaques (*Macaca fascicularis*) - male
659 monopolisation, female mate choice or post-copulatory mechanisms? *Behav Ecol*
660 *Sociobiol* 59:740-752
- 661 Glick BB (1980) Ontogenetic and psychobiological aspects of the mating activities of male
662 *Macaca radiata*. In: Lindburg DG (ed) *The macaques: studies in ecology, behaviour and*
663 *evolution*. Van Nostrand Reinhold, New York, pp345-369
- 664 Grob B, Knapp LA, Martin RD, Anzenberger G (1998) The major histocompatibility complex
665 and mate choice: inbreeding avoidance and selection of good genes. *Exp Clin*
666 *Immunogen* 15:119-129

- 667 Harcourt AH (1995) Sexual selection in primates: what are male genitalia good for? *Evol*
668 *Anthropol* 4:121-129
- 669 Harcourt AH, Purvis A, Liles L (1996) Sperm competition: mating system, not breeding
670 season, affects testes size of primates. *Funct Ecol* 9:468-476
- 671 Hasegawa T, Hiraiwa-Hasegawa M (1990) Sperm competition and mating behaviour. In:
672 Nishida T (ed) *The chimpanzees of the Mahale mountains: sexual and life history*
673 *strategies*. University of Tokyo Press, Tokyo, pp115-132
- 674 Heistermann M, Ziegler T, van Schaik CP, Launhardt K, Winkler P, Hodges JK (2001) Loss
675 of oestrus, concealed ovulation and paternity confusion in free-ranging Hanuman
676 langurs. *Proc R Soc Lond B* 268:2445-2451.
- 677 Heistermann M, Brauch K, Möhle U, Pfefferle D, Dittami J, Hodges K (2008) Female ovarian
678 cycle phase affects the timing of male copulatory and other sexual behaviors in free-
679 ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. *Am J Primatol* 70:44-53
- 680 Hrdy, SB (1979) Infanticide among animals: a review, classification, and examination of the
681 implications for the reproductive strategies of females. *Ethol Sociobiol* 1:13-40
- 682 Hrdy, S.B. and Whitten, P.L. (1987) Patterning of sexual activity. In: Smuts BB, Cheney DL,
683 Seyfarth RM, Wrangham RW, Strusaker TT (eds) *Primate Societies*. University of
684 Chicago Press, Chicago, pp370-384
- 685 Huffman MA (1987) Consort intrusion and female mate choice in Japanese macaques
686 (*Macaca fuscata*). *Ethology* 75:221-234
- 687 Ims RA (1988) The potential for sexual selection in males: effect of sex ratio and spatio
688 temporal distribution of receptive females. *Evol Ecol* 3:338-352
- 689 Janson CH (1984) Female choice and mating system of the brown capuchin monkey *Cebus*
690 *apella*. *Z Tierpsychol* 65:177-200
- 691 Johnstone RA (2000) Models of reproductive skew: a review and synthesis. *Ethology* 106:5-
692 26

- 693 Kumar A, Kurup GU (1985) Sexual behaviour of the lion-tailed macaque (*Macaca silenus*).
694 In: Heltne P (ed) The Lion-tailed macaque: status and conservation. Alan R Liss, New
695 York, pp109-130
- 696 Kutsukake N, Nunn CL (2006) Comparative tests of reproductive skew in male primates: the
697 roles of demographic factors and incomplete control. Behav Ecol Sociobiol 60:695-706
- 698 Kümmerli R, Martin RD (2005) Male and female reproductive success in *Macaca sylvanus* in
699 Gibraltar: no evidence for rank dependence. Int J Primatol 26:1229-1249
- 700 Küster J, Paul A (1984) Female reproductive characteristics in semi-free ranging Barbary
701 macaques (*Macaca sylvanus* L. 1758). Folia Primatol 43:69-83.
- 702 Küster J, Paul A (1988) Rank relations of juvenile and subadult natal males of Barbary
703 macaques (*Macaca sylvanus*) at Affenberg Salem. Folia Primatol 51:33-44
- 704 Küster J, Paul A (1992) Influence of male competition and female mate choice on male
705 mating success in Barbary macaques (*Macaca sylvanus*). Behaviour 120:192-217
- 706 Küster J, Paul A, Arnemann J (1994) Kinship, familiarity and mating avoidance in Barbary
707 macaques, *Macaca sylvanus*. Anim Behav 48:1183-1194
- 708 Küster J, Paul A, Arnemann J (1995) Age-related and individual differences of reproductive
709 success in male and female Barbary macaques (*Macaca sylvanus*). Primates 36:461-476
- 710 Lathuillière M, Ménard N, Gautier-Hion A, Crouau-Roy B (2001) Testing the reliability of
711 non-invasive genetic sampling by comparing analyses of blood and fecal samples in
712 Barbary macaques (*Macaca sylvanus*). Am J Primatol 55:151-158
- 713 Launhardt K, Borries C, Hardt C, Epplen JT, Winkler P (2001) Paternity analysis of
714 alternative male reproductive routes among the langurs (*Semnopithecus entellus*) of
715 Ramnagar. Anim Behav 61:53-64
- 716 Manson JH (1992) Measuring female mate choice in Cayo Santiago rhesus macaques. Anim
717 Behav 44:405-416

- 718 Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-
719 based paternity inference in natural populations. *Mol Ecol* 7:639-655
- 720 Martin P, Bateson P (1993) *Measuring behaviour*. Cambridge University Press, Cambridge
- 721 Marvan R, Stevens JMG, Roeder AD, Mazura I, Bruford MW, de Ruiter J.R (2006) Male
722 dominance rank, mating and reproductive success in captive bonobos (*Pan paniscus*).
723 *Folia Primatol* 77:364-376
- 724 Matsubara M; Sprague DS (2004) Mating tactics in response to costs incurred by mating with
725 multiple males in wild female Japanese macaques. *Int J Primatol* 25: 901-917
- 726 McElligott AG, Hayden TJ (2000) Lifetime mating success, sexual selection and life history
727 of fallow bucks (*Dama dama*) *Behav Ecol Sociobiol* 48:203-210
- 728 Ménard N, Vallet D (1996) Demography and ecology of Barbary macaques (*Macaca*
729 *sylvanus*) in two different habitats. In: Fa JE, Lindburg DG (eds) *Evolution and*
730 *ecology of macaque societies*. Cambridge University Press, New York, pp106-131
- 731 Modolo L, Salzburger W, Martin RD (2005) Phylogeography of Barbary macaques (*Macaca*
732 *sylvanus*) and the origin of the Gibraltar colony. *Proc Natl Acad Sci USA* 102:7392-
733 7397
- 734 Moore NP, Kelly PF, Cahill JP, Hayden TJ (1995) Mating strategies and mating success of
735 fallow (*Dama dama*) bucks in a non-lekking population. *Behav Ecol Sociobiol* 36:91-
736 100
- 737 Möhle U, Heistermann M, Dittami J, Reinberg V, Hodges JK (2005) Patterns of anogenital
738 swelling size and their endocrine correlates during ovulatory cycles and early
739 pregnancy in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. *Am J*
740 *Primatol* 66:351-368
- 741 Muller MN; Kahlenberg SM; Emery Thompson M; Wrangham RW (2007) Male coercion and
742 the costs of promiscuous mating for female chimpanzees. *Proc Roy Soc Lond B*
743 274:1009-1014

- 744 Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of
745 polyandrous females. *P Natl Acad Sci USA* 94:10236-10241
- 746 Nikitopoulos E, Heistermann M, de Vries H, van Hooff JARAM, Sterck EHM (2005) A pair
747 choice test to identify female mating pattern relative to ovulation in long-tailed
748 macaques, *Macaca fascicularis*. *Anim Behav* 70:1283–1296
- 749 Nunn CL (1999) The evolution of exaggerated sexual swellings in primates and the graded-
750 signal hypothesis. *Anim Behav* 58:229-246
- 751 Nunn CL, Altizer SM (2004) Sexual selection, behaviour and sexually transmitted diseases.
752 In: Kappeler PM, van Schaik, C (eds) *Sexual selection in primates: new and*
753 *comparative perspectives*. Cambridge University Press, New York, pp 117-130
- 754 Nürnberg P, Sauer mann U, Kayser M, Lanfer C, Manz E, Widdig A, Berard J, Bercovitch FB,
755 Kessler M, Schmidtke J, Krawczak M (1998) Paternity assessment in rhesus
756 macaques (*Macaca mulatta*): multilocus DNA fingerprinting and PCR marker typing.
757 *Am J Primatol* 44:1-18
- 758 Parker GA (1998) Sperm competition and the evolution of ejaculates: towards a theory base.
759 In: T.R. Birkhead TR, Møller AP (eds) *Sperm competition and sexual selection*.
760 Academic Press, London, pp3-54
- 761 Paul A (1989) Determinants of male mating success in a large group of Barbary macaques
762 (*Macaca sylvanus*) at Affenberg Salem. *Primates* 30:461-476.
- 763 Paul A (1997) Breeding seasonality affects the association between dominance and
764 reproductive success in non-human male primates. *Folia Primatol* 68:344-349
- 765 Paul A, Küster J, Arnemann J (1992) DNA fingerprinting reveals that infant care by male
766 Barbary macaques (*Macaca sylvanus*) is not parental investment. *Folia Primatol* 58:93-
767 98

- 768 Paul A, Küster J, Timme A, Arnemann J (1993) The association between rank, mating effort,
769 and reproductive success in male Barbary Macaques (*Macaca sylvanus*). Primates
770 34:491-502
- 771 Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility
772 complex genes. Am Nat 153:145-164
- 773 Pereira ME, Weiss ML (1991) Female mate choice, male migration, and the threat of
774 infanticide in ringtailed lemurs. Behav Ecol Sociobiol 28:141-152
- 775 Poole JH (1989) Mate guarding, reproductive success and female choice in African elephants.
776 Anim Beh 37:842-849
- 777 Preston BT, Stevenson IR, Pemberton JM, Wilson K (2001) Dominant rams lose out by
778 sperm depletion. Nature 409:681-682
- 779 Reeder DM (2003) The potential for cryptic female choice in primates: behavioural,
780 anatomical and physiological considerations. In: Jones CB (ed) Sexual selection and
781 reproductive competition in primates: new perspectives and directions. American
782 Society of Primatologists, Norman, pp255-303
- 783 Reeve HK, Emlen ST, Keller L (1998) Reproductive sharing in animal societies: reproductive
784 incentives or incomplete control by dominant breeders? Behav Ecol 9:267-278
- 785 Say L, Pontier D, Natoli E (2001) Influence of oestrus synchronization on male reproductive
786 success in the domestic cat (*Felis catus* L.). Proc R Soc Lond B 268:1049-1053
- 787 Schwensow N, Eberle M, Sommer S (2008) Compatibility counts: MHC-associated mate
788 choice in a wild promiscuous primate. Proc R Soc B 275:555-564
- 789 Setchell JM (2005) Do female mandrills prefer brightly colored males? Int J Primatol 26:715-
790 735
- 791 Setchell JM, Charpentier M, Wickings J (2005) Mate guarding and paternity in mandrills:
792 factors influencing alpha male monopoly. Anim Behav 70:1105-1120

- 793 Shideler SE, Shackleton CHL, Moran FM, Stauffer P, Lohstroh PN, Lasley BL (1993)
794 Enzyme immunoassays for ovarian steroid metabolites in the urine of *Macaca*
795 *fascicularis*. J Med Primatol 22:301-312
- 796 Small MF (1989) Female choice in nonhuman primates. Ybk Phys Anthropol 32:103-127
- 797 Small MF (1990) Promiscuity in Barbary Macaques (*Macaca sylvanus*). Am J Primatol
798 20:267-282.
- 799 Small MF (1994) When Mr. Right is too much effort. New Scientist 143:30-33
- 800 Smuts BB (1985) Sex and friendships in baboons. Aldine, New York
- 801 Soltis J (2004) Mating systems. In: Thierry B, Singh M, Kaumanns W (eds) Macaque
802 societies: a model for the study of social organization. Cambridge University Press,
803 Cambridge, pp135-151
- 804 Soltis J, Mitsunaga F, Shimizu K, Yanagihara Y, Nozaki M (1999) Female mating strategy in
805 an enclosed group of Japanese macaques. Am J Primatol 47: 263-278
- 806 Soltis J, Thomsen R, Matsubayashi K, Takenaka O (2000) Male infanticide by resident males
807 and female counter-strategies in wild Japanese macaques (*Macaca fuscata*). Behav Ecol
808 Sociobiol 48:195-202
- 809 Soltis J, Thomsen R, Takenaka O (2001) The interaction of male and female reproductive
810 strategies and paternity in wild Japanese macaques, *Macaca fuscata*. Anim Behav
811 62:485-494
- 812 Sprague DS (1998) Age, dominance rank, natal status, and tenure among male macaques. Am
813 J Phys Anthropol 105:511-521
- 814 Steenbeek R (2000) Infanticide by male and female choice in wild Thomas langurs. In: van
815 Schaik CP, Janson C (eds) Infanticide by males and its implications. Cambridge
816 University Press, Cambridge, pp153-177
- 817 Stumpf RM, Boesch C (2006) The efficacy of female choice in chimpanzees of the Tai
818 Forest, Cote d'Ivoire. Behav Ecol Sociobiol 60:749-765

- 819 Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap.
820 TREE 14:323-327
- 821 Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J
822 (1996) Reliable genotyping of samples with very low DNA quantities using PCR.
823 Nucleic Acids Res 24:3189-3194
- 824 Taub DM (1980) Female choice and mating strategies among wild Barbary macaques
825 (*Macaca sylvanus L.*) In: Lindburg DG (ed) The Macaques: studies in ecology,
826 behaviour and evolution. Van Nostrand Reinhold Co., New York, pp287-344
- 827 Tregenza T, Wedell N (2002) Polyandrous females avoid costs of inbreeding. Nature 415:71–
828 73
- 829 Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual
830 selection and the decent of man. Aldine, Chicago, pp136-179
- 831 van Noordwijk MA, van Schaik CP (2004) Sexual selection and the careers of primate males:
832 paternity concentration, dominance-acquisition tactics and transfer decisions. In:
833 Kappeler PM, van Schaik CP (eds) Sexual selection in primates: new and comparative
834 aspects. Cambridge University, Cambridge, pp208-229
- 835 van Schaik CP, Hodges JK, Nunn CL (2000) Paternity confusion and the ovarian cycles of
836 female primates. In: van Schaik CP, Janson C (eds) Infanticide by males and its
837 implications. Cambridge University Press, Cambridge, pp361-387
- 838 Van Schaik C.P., Höstermann M. (1994) Predation risk and the number of adult males in a
839 primate group: a comparative test. Behav Ecol Sociobiol 35:261–272
- 840 Van Schaik CP, Kappeler PM (1997) Infanticide risk and the evolution of male-female
841 association in primates. Proc Roy Soc Lond B 264: 1687-1694
- 842 van Schaik CP, Pradhan GR, van Noordwijk MA (2004) Mating conflict in primates:
843 infanticide, sexual harassment and female sexuality. In: Kappeler PM, van Schaik CP

- 844 (eds) Sexual selection in primates: new and comparative aspects. Cambridge University
845 Press, Cambridge, pp131-150
- 846 Vigilant L, Hofreiter M, Siedel H, Boesch C (2001) Paternity and relatedness in wild
847 chimpanzee communities. *Proc Natl Acad Sci USA* 98:12890-12895
- 848 von Segesser F, Ménard N, Gaci B, Martin RD (1999) Genetic differentiation within and
849 between isolated Algerian subpopulations of Barbary macaques (*Macaca sylvanus*):
850 evidence from microsatellites. *Mol Ecol* 8:433-442
- 851 Waitt C, Little AC, Wolfensohn S, Honess P, Brown AP, Buchanan-Smith HM, Perrett DI
852 (2003) Evidence from rhesus macaques suggests that male coloration plays a role in
853 female primate mate choice. *Proc R Soc Lond B* 270:144-146
- 854 Weingrill T, Lycett JE, Henz SP (2000) Consortship and mating success in chacma baboons
855 (*Papio cynocephalus ursinus*). *Ethology* 106:1033-1044
- 856 Weingrill T, Lycett JE, Barrett L, Hill RA, Henzi SP (2003) Male consortship behaviour in
857 chacma baboons: the role of demographic factors and female conceptive probabilities.
858 *Behaviour* 140:405-427
- 859 Widdig A, Bercovitch FB, Streich WJ, Saueremann U, Nürnberg P, Krawczak M (2004) A
860 longitudinal analysis of reproductive skew in male rhesus macaques. *Proc R Soc Lond B*
861 271:819-826
- 862 Wilcox AJ, Weinberg CR, Baird DD (1995) Timing of sexual intercourse in relation to
863 ovulation. *New Engl J Med* 333:1517-1522
- 864 Wolff JO, Macdonald DW (2004) Promiscuous females protect their offspring. *TREE* 19:127-
865 134
- 866 Zeh JA, Zeh DW (2001) Reproductive mode and the genetic benefits of polyandry. *Anim*
867 *Behav* 61:1051-1063

868 Ziegler T, Hodges JK, Winkler P and Heistermann M (2000) Hormonal correlates of
869 reproductive seasonality in wild female hanuman langurs (*Presbytes entellus*). Am J
870 Primatol 51:119-134

For Review Only

871 **Figure legends:**

872

873 **Figure 1.** (a) Proportion of total observation time females spent in consortship with different
874 males (black, light and dark grey bars represent high-ranking males, hatched bars represent
875 low-ranking males; numbers refer to the number of consort partners a female had during the
876 observation time) and (b) frequency, total duration and average duration of consortships
877 females had with high-ranking and low-ranking males. Values represent mean \pm SEM.
878 Asterisks indicate non-conception cycles. Statistics: Wilcoxon signed-rank test (for details see
879 text).

880

881

882 **Figure 2.** Frequencies of presenting, active solicitation, and refusals of females towards high-
883 and low-ranking males. Values represent mean \pm SEM (n=8 females). Statistics: Wilcoxon
884 signed-rank test (for details see text).

885

886

887 **Figure 3.** (a) Duration of consortships, (b) frequencies of ejaculatory copulations and (c)
888 frequencies of solicitations by mothers with the likely father (black bar) and with other males
889 (open bars). For statistics see text.

890 **Table 1.** Demographic data of study females

891

| Study female | Rank | Age at first observation | Hours of observation during the fertile phase |
|--------------|------|--------------------------|---|
| Pixie | 1 | ~21 | 11.6 |
| Ren | 2 | 10 | 5.7 |
| Jutta | 3 | 8 | 23.0 |
| Jasmin | 4 | 6 | 20.1 |
| Gallia | 6 | 11 | 6.5 |
| Sadie | 7 | 7 | 7.6 |
| Fanny | 9 | 7 | 22.5 |
| Sunblest | 12 | ~14 | 8.0 |

892

893

For Review Only

894 **Table 2.** Results of genetic paternity analysis for 12 mother-infant pairs

| Mother-infant pair | Number of genotyped loci | Likelihood of paternity ^a | ID of father | Rank class of likely father |
|--------------------|--------------------------|--------------------------------------|--------------|-----------------------------|
| Pixie - Inf.04 | 11 | >95% | Thoran | high |
| Ren - Inf.05 | 10 | - ^b | not known | not known ^a |
| Jutta - Inf.04 | 11 | >95% | Faustino | high |
| Jutta - Inf.05 | 11 | >95% | Thoran | high |
| Jasmin - Inf.04 | 11 | >95% | Faustino | high |
| Gallia - Inf.04 | 11 | >80% | Thoran | high |
| Sadie - Inf.04 | 11 | >95% | Faustino | high |
| Sadie - Inf.05 | 11 | >80% | Che | low |
| Fanny - Inf.04 | 11 | >95% | Gregory | low |
| Fanny - Inf.05 | 11 | >95% | Mephisto | high |
| Sunblest - Inf.04 | 11 | >80% | Thoran | high |
| Sunblest - Inf.05 | 11 | >95% | Mephisto | high |

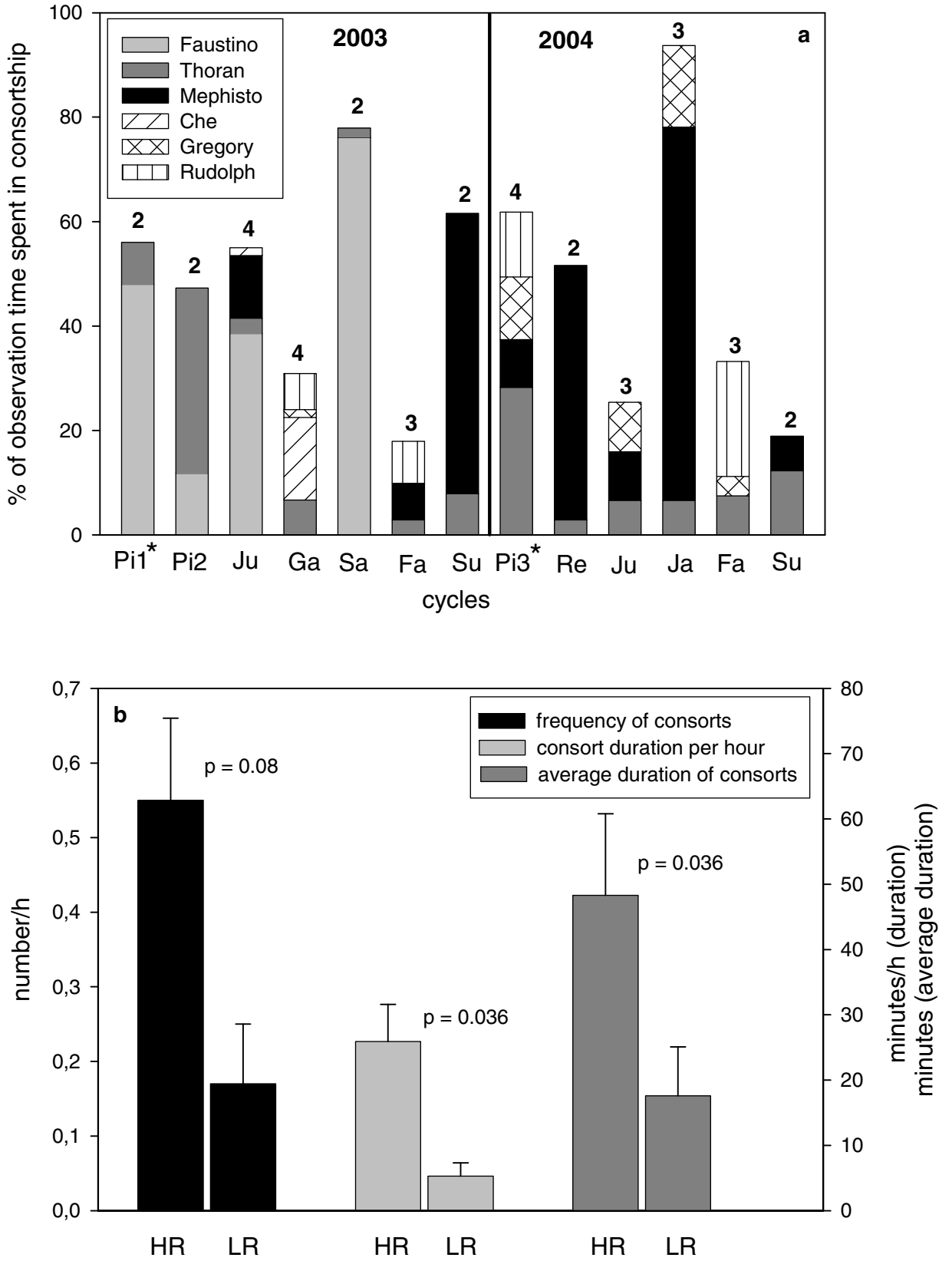
895

896 ^a as determined by Cervus 2.0 (see methods)897 ^b infant sired by extra-group male

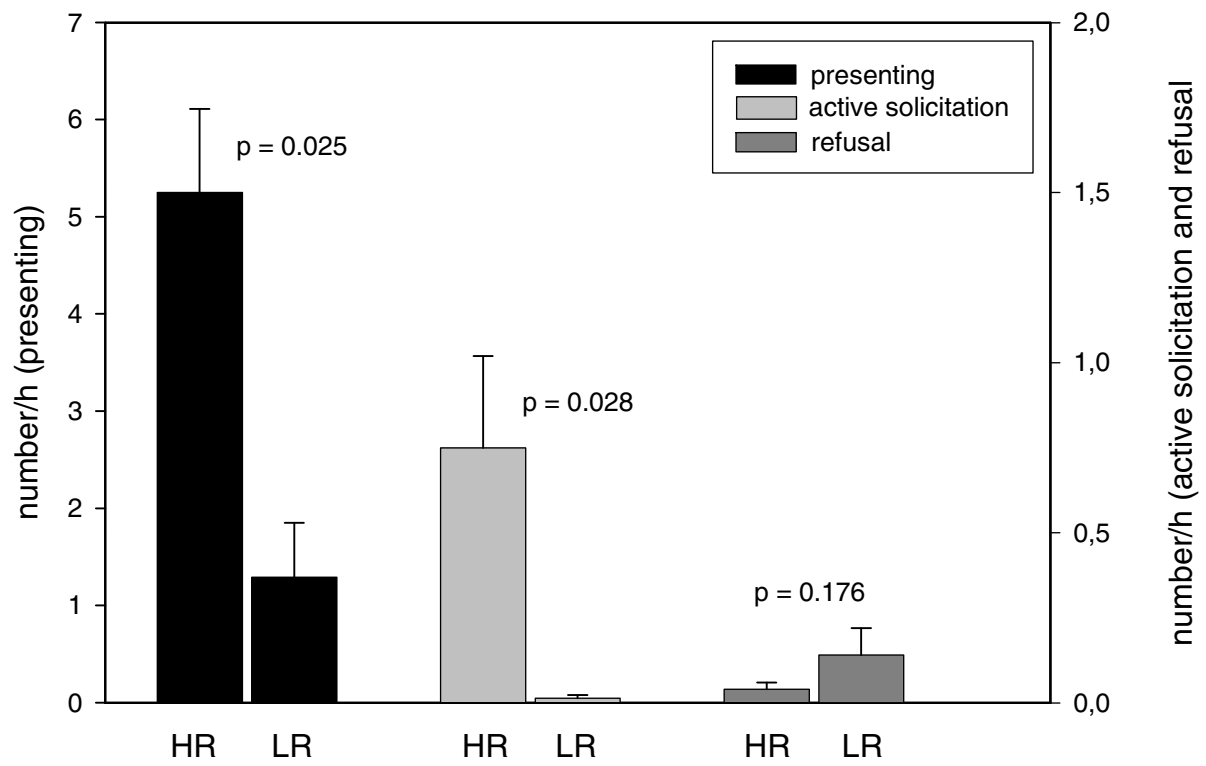
898

899

Figure 1



901 Figure 2



902

View Only

Figure 3

