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

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Abstract

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

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

Introduction

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

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

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

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

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

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

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

Methods

Animals and study site

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

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

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

Behavioural observations

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

The occurrence of the following sexual behaviours was recorded: female presents anogenital area to male (“presenting”), female actively solicits copulation from a male (“active solicitation, see Brauch et al. 2007), female refuses a male’s genital inspection or copulation attempt (“refusal”) and copulations (indicated by intromission). Copulations were divided into non-ejaculatory and ejaculatory copulations, the latter being indicated by the presence of the typical ejaculatory pause (Küster and Paul 1984). Identity of the individual initiating an ejaculatory copulation was also recorded. In this respect, all ejaculatory copulations that followed after a female showed soliciting behaviour (presenting, active solicitation) to the male were considered to be female-initiated (except those cases in which the solicitation behaviour was shown directly after a male's approach), while all ejaculatory copulations that followed directly after a male had approached a female or where a male mounted a female when in consort without being solicited by the female were considered to be male-initiated. Additionally, we recorded the number and duration of consortships. According to Berard et al. (1993) we defined a consortship as occurring when there was i) a close social proximity between male and female (<5m when sitting; <10m when walking) and ii) copulatory behaviour between the consort partners. Consorts were only counted as such, when they were lasting at least 5 minutes. A consort was considered to have ended if the female mated with another male, or if the male or female moved more than 10 m away and the consort partner did not follow within the first 10 minutes. Identity of the individual initiating, maintaining or finishing the consort was also recorded (see Heistermann et al., 2008).

Assessment of male rank

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

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

On average, from each focal female 3.8 ± 1.4 faecal samples (range 2-6) were collected each week as described previously (Möhle et al. 2005). Samples were extracted twice according to the method reported by Ziegler et al. (2000). After extraction, extracts were analyzed for concentrations of immunoreactive oestrogen (total, E_t) and progesterone metabolites (5α-reduced 20-oxo pregnanes, 5-P-3OH) using validated enzyme immunoassays previously shown to accurately reflect female ovarian function in the Barbary macaque (Möhle et al. 2005, Heistermann et al. 2008). Sensitivities of the assays at 90% binding were 1.9 pg for E_t and 39 pg for 5-P-3OH. For both assays, intra- and inter-assay coefficients of variation, calculated from replicate determinations of high and low value quality controls, were <10% and <18%, respectively.

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

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

Genetic paternity analysis

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

Prior to our paternity determination tests, we validated the method of genotyping from faecal samples by analyzing matched blood and faecal samples from 10 animals of the Gibraltar Barbary macaque population. DNA from faeces and blood was extracted using the QIAamp ® DNA Stool Mini Kit and the QIAamp ® DNA Mini Kit (Quiagen, Hilden, Germany), respectively, following the instructions given in the manual. After extraction, DNA was stored at -20°C until further processing. Initially, we tested 26 different microsatellite loci (di- and tetra-nucleotide) with an amplification length ranging from 97 bp to 280 bp for their suitability to detect different alleles. The primers were originally designed for the amplification of microsatellite loci in humans, but have been successfully applied for genotyping in a number of Old World monkeys, including macaques (Nürnberg et al. 1998; von Segesser et al. 1999; Lathuillière et al. 2001; Engelhardt et al. 2006). Because of the high degree of relatedness and the relatively low genetic variability in the Gibraltar population (Modolo et al. 2005), only 11 loci proved to be sufficiently polymorphic to allow paternity analysis. Therefore, for all subsequent tests as well as for paternity analysis, the following primers were used: dinucleotides: D6S311 (Altmann et al. 1996), D7S503, D11S925 (Altmann et al. 1996, von Segesser et al. 1999, Lathuillière et al. 2001), D14S255 (Nürnberg et al. 1998); tetranucleotides: D1S584, D3S1768, D6S501 (Engelhardt et al. 2006) D5S1457 (Bayes et al. 2000), D7S2204 (Vigilant et al. 2001), D8S1106 (Kümmerli and Martin 2005), D10S1432 (Chambers et al. 2004, Marvan et al. 2006). For each microsatellite locus, the forward primer was labelled with fluorescent FAM, while the reverse remained unlabeled. PCR reactions were performed in a total volume of 10µl by using the Eppendorf Mastercycler gradient (Eppendorf, Hamburg, Germany). The PCR master mix contained 1x buffer, 1.5 mM $MgCL_2$, 0.2 mM dNTP, 2 pM of each primer (forward and reverse), 0.5 U Hot Master Taq DNA Polymerase (Eppendorf, Hamburg, Germany) and 8 µl DNA extract for faecal samples and 2 µl DNA extract for blood samples. PCR was conducted as follows: after initial denaturation for 3 min at 94°C, amplification consisted of 35 cycles (94°C for 30s, 58°C respectively 60°C for primer D7S503 and D14S255 for 30s and 70°C for 30s) and a final extension (70°C for 3 min). Genotyping was performed on a denaturing 4.5% polyacrylamide gel on an ABI Prism 377 DNA sequencer (Applied Biosystems), with analysis using GENESCAN software (Applied Biosystems).

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

To test the reliability of our genotyping results from faecal samples, we assessed the agreement in allele patterns between the matched blood and faecal samples of the 10 animals. Out of 108 possible genotypes, 102 were identical between blood and faecal samples. From the 6 inconsistent samples, one additional allele was found in faeces in 5 cases and in one case allelic drop out was seen in the faecal sample. Overall, the agreement between genotyping from faeces and blood was 94.4%, indicating a high degree of reliability of our faecal genotyping method.

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

Data analysis

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

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

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

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

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

Furthermore, we examined whether the assigned fathers of infants had more and longer consortships, more copulations (total and ejaculatory), received more solicitations (presentation and active solicitations) or received fewer refusals than non-fathers. For this, according to Engelhardt et al. (2006), we averaged the frequencies of the respective behaviours shown towards all non-fathers (providing an “average non-father”) and compared these to those frequencies shown towards the likely father using the Wilcoxon signed-ranks test. Since behavioural data were not available from all 11 mothers of group-sired infants, and since we excluded those conception cycles from analysis in which observation time may potentially have been too limited, this analysis was restricted to 7 conception cycles.

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

Results

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

Male rank and paternity outcome

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

Consortship and male mating success in relation to male rank class

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

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

Female sexual behaviour in relation to male rank class

On average, females solicited copulations from 3.5 males (range: 1-6 males) during their fertile phase. As shown in Figure 2, female sexual behaviour was clearly biased according to male rank class, with significantly higher frequencies of presentation and active solicitation directed to the class of high-ranking males (Wilcoxon signed–ranks test: presenting: Z=-2.24, N=8, p=0.025; active solicitation: Z=-2.20, N=8, p=0.028). Females rarely refused male sexual approaches (inspection and mounting attempts), but did so more often towards low-ranking than high-ranking males (Figure 2), although the difference was not significant (Wilcoxon signed-ranks test: Z=-1.36, N=8, p=0.176). Females were also mainly responsible for initiating ejaculatory copulations, which they did so in more than two thirds of the observed cases (67.3% compared to 32.7% initiated by males; Wilcoxon signed-ranks test: Z=-2.54, N=8, p=0.011).

Paternity and male and female sexual behaviour

Having shown that consortships, male mating success and female sexual behaviours were all biased towards high-ranking males and that males of high rank had a higher reproductive success, we tested for differences in behavioural patterns between fathers and non-fathers. Figure 3 demonstrates the duration of time spent in consortship and the frequencies of male ejaculatory copulations and female solicitations (presenting and active solicitation combined) shown by or towards the assigned fathers compared to those shown by or towards non-father males. Although in a few individual cases the likely fathers consorted more often and for a longer duration with the mother, ejaculated at higher frequencies and received more solicitations from the mother, overall none of the behavioural parameters tested was significantly related to paternity outcome. Females were neither more often in consort with the likely father (Wilcoxon signed-ranks test: Z=-1.02, N=7, p=0.31) nor was duration of consortship with the father longer than with other males (Wilcoxon signed- ranks test: Z=-0.85, N=7, p=0.40; Fig 3a). Furthermore, fathers neither copulated more often (Wilcoxon signed-ranks test: Z=-0.85, N=7, p=0.39), nor had more ejaculatory copulations with mothers (Wilcoxon signed-ranks test: Z= -0.43, N=7, p=0.67) than other males of the group (Fig. 3b). Finally, there was no significant difference between solicitation rates shown by mothers towards the likely fathers compared to those shown towards non-fathers (Wilcoxon signed-ranks test: Z=-0.67, N=7, p=0.50, Fig. 3c)

Discussion

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

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

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

A further alternative which our present data support, is the limited control model (Cant 1998; Clutton-Brock 1998; Reeve et al. 1998), which assumes that dominant males are unable to completely control access to fertile females due to factors such as alternative male mating strategies, female cycle synchrony or female choice. The present data showing a considerable overlap in fertile phases indicates a high degree of female cycle synchrony in the Barbary macaque. Under such conditions, it is difficult for a male to effectively monopolize more than one female at a time during her fertile phase (rhesus macaque: Altmann 1962; Emlen and Oring 1977; Ims, 1988; Paul 1997; Say et al. 2001; mouse lemur, *Microcebus murinus*: Eberle and Kappeler 2002) and this would explain why female monopolization by dominant males did not occur even though females preferred high-ranking males as mating partners (see below). Irrespective of the specific reasons for the lack of male monopolization, our data clearly suggest that monopolization of fertile females as a result of male contest is not an important determinant of male reproductive success in the Barbary macaque, a conclusion which would be in line with general predictions about factors determining paternity under conditions of limited male control (Altmann 1962; van Noordwijk and van Schaik et al. 2004). In this respect, Barbary macaques differ markedly from less seasonally breeding anthropoid primate species, such as long-tailed macaques (Engelhardt et al. 2006) and mandrills (Setchell et al. 2005) in which monopolization of fertile females is a highly effective male strategy to maximise male reproductive success.

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

The mechanisms underlying paternity distribution in our study seem to be multiple. Despite the inability of males to monopolize females, their competitive ability still may have influenced reproductive success. Since high-ranking males consorted females more often and for a longer duration during the peri-ovulatory period than low-ranking individuals, their chances of mating were higher and this has been reported for other species (mandrill: Setchell et al. 2005; baboon: Weingrill et al. 2000, 2003). Furthermore, our observation that low-ranking males terminated consortships more often as a result of being disturbed by more dominant males (unpublished data) also supports the contention that male competitive ability is at least partly responsible for the rank-related bias in consortship pattern.

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

The observed difference in mating success between high- and low-ranking males corresponded closely with reproductive success since high-ranking males sired nearly 4 times more offspring than low-ranking males. Nevertheless, since females were not in consort and did not copulate more often with the assigned father of their infant than with non-father males, our results also suggest that male mating success was not directly translated into individual reproductive success. Behavioural data, however, could not be collected during the entire fertile phase (see Methods), and we may have missed copulations which occurred with the fathers. Nevertheless, since individual observation time was spread over different days of the fertile phase and equally distributed over the daily activity phase of the animals, we can reasonably assume that the copulatory pattern we observed is representative for the entire fertile phase. Interestingly, a similar discrepancy between number of copulations and paternity has also been described for the long-tailed macaque (Engelhardt et al. 2006). Thus, we propose that actual paternity in the Barbary macaque is most likely determined through individual advantages operating at the post-copulatory level such as differences in sperm number, vitality and/or viability, together with cryptic female choice (Eberhard 1996; Reeder 2003). Although our study design did not allow further investigation into the character of these potential mechanisms and the degree to which they may have affected paternity, the potential for them to exist in primates is high (Harcourt 1995; Harcourt et al.1996; Birkhead and Kappeler, 2004; Engelhardt et al. 2006).

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

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References

Alberts SC, Altmann J, Wilson ML (1996) Mate guarding constrains foraging activity of male baboons. Anim Behav 51: 1269-1277

Altmann J (1974) Observational study of behavior: sampling methods. Behaviour 49: 227-267

Altmann J, Alberts SC, Haines SA, Durbach J, Muruthi P, Coote T, Geffen E, Cheesman DJ, Mututua RS, Saiyalel SN, Wayne RK, Lacy RC, Brudford MW (1996) Behavior predicts genetic structure in a wild primate group. Proc Natl Acad Sci USA 93:5797-5801

Altmann SA (1962) A field study of the socio-biology of rhesus monkeys, *Macaca mulatta.* Ann NY Acad Sci 102:277-285

Andersson M (1994) Sexual Selection. Princeton University Press, Princeton, New Jersey

Aujard F, Heistermann M, Thierry B, Hodges JK (1998) Functional significance of behavioral, morphological, and endocrine correlates across the ovarian cycle in semifree ranging female Tonkean macaques. Am J Primatol 46:285-309

Bayes MK, Smith KL, Alberts SC, Altmann J, Bruford MW (2000) Testing the reliability of microsatellite typing from faecal DANN in the savannah baboon. Cons Gen 1:173-176

Behboodi E, Katz DF, Samuels SJ, Tell L, Hendrickx AG, Lasley BL (1991) The use of a urinary estrone conjugates assay for detection of optimal mating time in the cynomolgus macaque (*Macaca fascicularis*). J Med Primatol 20:229-234

Berard JD, Nurnberg P, Epplen JT, Schmidtke J (1993) Male rank, reproductive behavior, and reproductive success in free-ranging rhesus macaques. Primates 34:481-489

Bercovitch FB (1983) Time budgets and consortships in olive baboons (*Papio anubis*). Folia Primatol 41:180-190

Bercovitch FB (1995) Female cooperation, consortship maintenance and male mating success in savanna baboons. Anim Behav 50:137-149

Birkhead TR (2000) Promiscuity: an evolutionary history of sperm competition and sexual conflict. Faber and Faber, London

Birkhead TR, Kappeler PM (2004) Post-copulatory sexual selection in birds and primates In: Kappeler PM, van Schaik CP (eds) Sexual selection in primates: new and comparative aspects. Cambridge University, Cambridge, pp151-171

Birkhead TR, Møller AP (1998) Sperm competition and sexual selection. Academic Press, London

Boesch C, Kohou G, Néné H, Vigilant L (2006) Male competition and paternity in wild chimpanzees of the Taï Forest. Am J Phys Anthropol 130:103-115

Boinski S (1987) Mating patterns in squirrel monkeys (*Samiri oerstedii*): Implications for seasonal sexual dimorphisms. Behav Ecol Sociobiol 21:13-21

Borries, C, Launhardt, K, Epplen, C, Epplen, JT, Winkler, P (1999) Males as infant protectors in Hanuman langurs (*Presbytis entellus*) living in multimale groups – defence pattern, paternity and sexual behaviour. Behav Ecol Sociobiol 46:350-356

Brauch K, Pfefferle D, Hodges K, Möhle U, Fischer J, Heistermann M (2007) Female sexual behavior and sexual swelling size as potential cues for males to discern the female fertile phase in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. Horm Behav 52:375-383

Cant MA (1998) A model for the evolution of reproductive skew without reproductive suppression. Anim Behav 55:163-169

Chambers KE, Reichard UH, Möller A, Nowak K, Vigilant L (2004) Cross-species amplification of human microsatellite markers using noninvasive samples from white-handed gibbons (*Hylobates lar*). Am J Primatol 64:19-27

Cheney DL, Seyfarth RM (1987) The influence of intergroup competition on the survival and reproduction of female vervet monkeys. Behav Ecol Sociobiol 21:375-386

Chism J, Rogers W (1997) Male competition, mating success and female choice in a seasonally breeding primate. Ethology 103:109-126

Clutton-Brock TH (1998) Reproductive skew concessions and limited control. TREE 13:288-292

Cowlishaw G, Dunbar RIM (1991) Dominance rank and mating success in male primates. Anim Behav 41:1045-1056

Creel SR, Mills MGL, Monfort SL (1997) Rank and reproduction in cooperatively breeding African wild dogs: behavioural and endocrine correlates. Behav Ecol 8:298-306

Darwin C (1871) The descent of man and the selection in relation to sex. John Murray, London

Dewsbury DA (1982) Ejaculate cost and male choice. Am. Nat 119:601-610

Dixson AF (1998) Primate Sexuality. Oxford University Press, Oxford

Dunbar RIM, Cowlishaw G (1992) Mating success in male primates: Dominance rank, sperm competition and alternative strategies. Anim Beh 44:1171-1173

Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton

Eberle M, Kappeler PM (2002) Mouse lemurs in space and time: a test of the socioecological model. Behav Ecol Sociobiol 51:131-139

Ellis L (1995) Dominance and reproductive success among non-human animals: a cross-species comparison. Ethol Sociobiol 16:257-333

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. Science 197:215-223

Engelhardt A, Heistermann M, Hodges JK, Nürnberg P, Niemitz C (2006) Determinants of male reproductive success in wild long-tailed macaques (*Macaca fascicularis*) - male monopolisation, female mate choice or post-copulatory mechanisms? Behav Ecol Sociobiol 59:740-752

Glick BB (1980) Ontogenetic and psychobiological aspects of the mating activities of male *Macaca radiat*a. In: Lindburg DG (ed) The macaques: studies in ecology, behaviour and evolution. Van Nostrand Reinhold, New York, pp345-369

Grob B, Knapp LA, Martin RD, Anzenberger G (1998) The major histocompatibility complex and mate choice: inbreeding avoidance and selection of good genes. Exp Clin Immunogen 15:119-129

Harcourt AH (1995) Sexual selection in primates: what are male genitalia good for? Evol Anthropol 4:121-129

Harcourt AH, Purvis A, Liles L (1996) Sperm competition: mating system, not breeding season, affects testes size of primates. Funct Ecol 9:468-476

Hasegawa T. Hiraiwa-Hasegawa M (1990) Sperm competition and mating behaviour. In: Nishida T (ed) The chimpanzees of the Mahale mountains: sexual and life history strategies. University of Tokyo Press, Tokyo, pp115-132

Heistermann M, Ziegler T, van Schaik CP, Launhardt K, Winkler P, Hodges JK (2001) Loss of oestrus, concealed ovulation and paternity confusion in free-ranging Hanuman langurs. Proc R Soc Lond B 268:2445-2451.

Heistermann M, Brauch K, Möhle U, Pfefferle D, Dittami J, Hodges K (2008) Female ovarian cycle phase affects the timing of male copulatory and other sexual behaviors in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. Am J Primatol 70:44-53

Hrdy, SB (1979) Infanticide among animals: a review, classification, and examination of the implications for the reproductive strategies of females. Ethol Sociobiol 1:13-40

Hrdy, S.B. and Whitten, P.L. (1987) Patterning of sexual activity. In: Smuts BB, Cheney DL, Seyfarth RM, Wrangham RW, Strusaker TT (eds) Primate Societies. University of Chicago Press, Chicago, pp370-384

Huffman MA (1987) Consort intrusion and female mate choice in Japanese macaques (*Macaca fuscata*). Ethology 75:221-234

Ims RA (1988) The potential for sexual selection in males: effect of sex ratio and spatio temporal distribution of receptive females. Evol Ecol 3:338-352

Janson CH (1984) Female choice and mating system of the brown capuchin monkey *Cebus apella*. Z Tierpsychol 65:177-200

Johnstone RA (2000) Models of reproductive skew: a review and synthesis. Ethology 106:5-26

Kumar A, Kurup GU (1985) Sexual behaviour of the lion-tailed macaque (*Macaca silenus*). In: Heltne P (ed) The Lion-tailed macaque: status and conservation. Alan R Liss, New York, pp109-130

Kutsukake N, Nunn CL (2006) Comparative tests of reproductive skew in male primates: the roles of demographic factors and incomplete control. Behav Ecol Sociobiol 60:695-706

Kümmerli R, Martin RD (2005) Male and female reproductive success in *Macaca sylvanus* in Gibraltar: no evidence for rank dependence. Int J Primatol 26:1229-1249

Küster J, Paul A (1984) Female reproductive characteristics in semi-free ranging Barbary macaques (*Macaca sylvanus* L. 1758). Folia Primatol 43:69-83.

Küster J, Paul A (1988) Rank relations of juvenile and subadult natal males of Barbary macaques (*Macaca sylvanus*) at Affenberg Salem. Folia Primatol 51:33-44

Küster J, Paul A (1992) Influence of male competition and female mate choice on male mating success in Barbary macaques (*Macaca sylvanus*). Behaviour 120:192-217

Küster J, Paul A, Arnemann J (1994) Kinship, familiarity and mating avoidance in Barbary macaques, *Macaca sylvanus*. Anim Behav 48:1183-1194

Küster J, Paul A, Arnemann J (1995) Age-related and individual differences of reproductive success in male and female Barbary macaques (*Macaca sylvanus*). Primates 36:461-476

Lathuillière M, Ménard N, Gautier-Hion A, Crouau-Roy B (2001) Testing the reliability of non-invasive genetic sampling by comparing analyses of blood and fecal samples in Barbary macaques (*Macaca sylvanus*). Am J Primatol 55:151-158

Launhardt K, Borries C, Hardt C, Epplen JT, Winkler P (2001) Paternity analysis of alternative male reproductive routes among the langurs (*Semnopithecus entellus*) of Ramnagar. Anim Behav 61:53–64

Manson JH (1992) Measuring female mate choice in Cayo Santiago rhesus macaques. Anim Behav 44:405-416

Marshall TC, Slate J, Kruuk LEB, Pemberton JM (1998) Statistical confidence for likelihood-based paternity inference in natural populations. Mol Ecol 7:639-655

Martin P, Bateson P (1993) Measuring behaviour. Cambridge University Press, Cambridge

Marvan R, Stevens JMG, Roeder AD, Mazura I, Bruford MW, de Ruiter J.R (2006) Male dominance rank, mating and reproductive success in captive bonobos (*Pan paniscus*). Folia Primatol 77:364-376

Matsubara M; Sprague DS (2004) Mating tactics in response to costs incurred by mating with multiple males in wild female Japanese macaques. Int J Primatol 25: 901-917

McElligott AG, Hayden TJ (2000) Lifetime mating success, sexual selection and life history of fallow bucks (*Dama dama*) Behav Ecol Sociobiol 48:203-210

Ménard N, Vallet D (1996) Demography and ecology of Barbary macaques (*Macaca sylvanus*) in two different habitats. In: Fa JE, Lindburg DG (eds) Evolution and ecology of macaque societies. Cambridge University Press, New York, pp106-131

Modolo L, Salzburger W, Martin RD (2005) Phylogeography of Barbary macaques (*Macaca sylvanus*) and the origin of the Gibraltar colony. Proc Natl Acad Sci USA 102:7392-7397

Moore NP, Kelly PF, Cahill JP, Hayden TJ (1995) Mating strategies and mating success of fallow (*Dama dama*) bucks in a non-lekking population. Behav Ecol Sociobiol 36:91-100

Möhle U, Heistermann M, Dittami J, Reinberg V, Hodges JK (2005) Patterns of anogenital swelling size and their endocrine correlates during ovulatory cycles and early pregnancy in free-ranging Barbary macaques (*Macaca sylvanus*) of Gibraltar. Am J Primatol 66:351-368

Muller MN; Kahlenberg SM; Emery Thompson M; Wrangham RW (2007) Male coercion and the costs of promiscuous mating for female chimpanzees. Proc Roy Soc Lond B 274:1009-1014

Newcomer SD, Zeh JA, Zeh DW (1999) Genetic benefits enhance the reproductive success of polyandrous females. P Natl Acad Sci USA 94:10236-10241

Nikitopoulos E, Heistermann M, de Vries H, van Hooff JARAM, Sterck EHM (2005) A pair choice test to identify female mating pattern relative to ovulation in long-tailed macaques, *Macaca fascicularis*. Anim Behav 70:1283–1296

Nunn CL (1999) The evolution of exaggerated sexual swellings in primates and the graded-signal hypothesis. Anim Behav 58:229-246

Nunn CL, Altizer SM (2004) Sexual selection, behaviour and sexually transmitted diseases. In: Kappeler PM, van Schaik, C (eds) Sexual selection in primates: new and comparative perspectives. Cambridge University Press, New York, pp 117-130

Nürnberg P, Sauermann U, Kayser M, Lanfer C, Manz E, Widdig A, Berard J, Bercovitch FB, Kessler M, Schmidtke J, Krawczak M (1998) Paternity assessment in rhesus macaques (*Macaca mulatta*): multilocus DNA fingerprinting and PCR marker typing. Am J Primatol 44:1-18

Parker GA (1998) Sperm competition and the evolution of ejaculates: towards a theory base. In: T.R. Birkhead TR, Møller AP (eds) Sperm competition and sexual selection. Academic Press, London, pp3-54

Paul A (1989) Determinants of male mating success in a large group of Barbary macaques (*Macaca sylvanus*) at Affenberg Salem. Primates 30:461-476.

Paul A (1997) Breeding seasonality affects the association between dominance and reproductive success in non-human male primates. Folia Primatol 68:344-349

Paul A, Küster J, Arnemann J (1992) DNA fingerprinting reveals that infant care by male Barbary macaques (*Macaca sylvanus*) is not parental investment. Folia Primatol 58:93-98

Paul A, Küster J, Timme A, Arnemann J (1993) The association between rank, mating effort, and reproductive success in male Barbary Macaques (*Macaca sylvanus*). Primates 34:491-502

Penn DJ, Potts WK (1999) The evolution of mating preferences and major histocompatibility complex genes. Am Nat 153:145-164

Pereira ME, Weiss ML (1991) Female mate choice, male migration, and the threat of infanticide in ringtailed lemurs. Behav Ecol Sociobiol 28:141-152

Poole JH (1989) Mate guarding, reproductive success and female choice in African elephants. Anim Beh 37:842-849

Preston BT, Stevenson IR, Pemberton JM, Wilson K (2001) Dominant rams lose out by sperm depletion. Nature 409**:**681-682

Reeder DM (2003) The potential for cryptic female choice in primates: behavioural, anatomical and physiological considerations. In: Jones CB (ed) Sexual selection and reproductive competition in primates: new perspectives and directions. American Society of Primatologists, Norman, pp255-303

Reeve HK, Emlen ST, Keller L (1998) Reproductive sharing in animal societies: reproductive incentives or incomplete control by dominant breeders? Behav Ecol 9:267-278

Say L, Pontier D, Natoli E (2001) Influence of oestrus synchronization on male reproductive success in the domestic cat (*Felis catus* L.). Proc R Soc Lond B 268:1049-1053

Schwensow N, Eberle M, Sommer S (2008) Compatibility counts: MHC-associated mate choice in a wild promiscuous primate. Proc R Soc B 275:555-564

Setchell JM (2005) Do female mandrills prefer brightly colored males? Int J Primatol 26:715-735

Setchell JM, Charpentier M, Wickings J (2005) Mate guarding and paternity in mandrills: factors influencing alpha male monopoly. Anim Behav 70:1105-1120

Shideler SE, Shackleton CHL, Moran FM, Stauffer P, Lohstroh PN, Lasley BL (1993) Enzyme immunoassays for ovarian steroid metabolites in the urine of *Macaca fascicularis*. J Med Primatol 22:301-312

Small MF (1989) Female choice in nonhuman primates. Ybk Phys Anthropol 32:103-127

Small MF (1990) Promiscuity in Barbary Macaques (*Macaca sylvanus*). Am J Primatol 20:267-282.

Small MF (1994) When Mr. Right is too much effort. New Scientist 143:30-33

Smuts BB (1985) Sex and friendships in baboons. Aldine, New York

Soltis J (2004) Mating systems. In: Thierry B, Singh M, Kaumanns W (eds) Macaque societies: a model for the study of social organization. Cambridge University Press, Cambridge, pp135-151

Soltis J, Mitsunaga F, Shimizu K, Yanagihara Y, Nozaki M (1999) Female mating strategy in an enclosed group of Japanese macaques. Am J Primatol 47: 263-278

Soltis J, Thomsen R, Matsubayashi K, Takenaka O (2000) Male infanticide by resident males and female counter-strategies in wild Japanese macaques (*Macaca fuscata*). Behav Ecol Sociobiol 48:195-202

Soltis J, Thomsen R, Takenaka O (2001) The interaction of male and female reproductive strategies and paternity in wild Japanese macaques, *Macaca fuscata*. Anim Behav 62:485-494

Sprague DS (1998) Age, dominance rank, natal status, and tenure among male macaques. Am J Phys Anthropol 105:511-521

Steenbeek R (2000) Infanticide by male and female choice in wild Thomas langurs. In: van Schaik CP, Janson C (eds) Infanticide by males and its implications. Cambridge University Press, Cambridge, pp153-177

Stumpf RM, Boesch C (2006) The efficacy of female choice in chimpanzees of the Tai Forest, Cote dÌvoire. Behav Ecol Sociobiol 60:749-765

Taberlet P, Waits LP, Luikart G (1999) Noninvasive genetic sampling: look before you leap. TREE 14:323-327

Taberlet P, Griffin S, Goossens B, Questiau S, Manceau V, Escaravage N, Waits LP, Bouvet J (1996) Reliable genotyping of samples with very low DNA quantities using PCR. Nucleic Acids Res 24:3189-3194

Taub DM (1980) Female choice and mating strategies among wild Barbary macaques (*Macaca sylvanus L.*) In: Lindburg DG (ed) The Macaques: studies in ecology, behaviour and evolution. Van Nostrand Reinhold Co., New York, pp287-344

Tregenza T, Wedell N (2002) Polyandrous females avoid costs of inbreeding. Nature 415:71–73

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed) Sexual selection and the decent of man. Aldine, Chicago, pp136-179

van Noordwijk MA, van Schaik CP (2004) Sexual selection and the careers of primate males: paternity concentration, dominance-acquisition tactics and transfer decisions. In: Kappeler PM, van Schaik CP (eds) Sexual selection in primates: new and comparative aspects. Cambridge University, Cambridge, pp208-229

van Schaik CP, Hodges JK, Nunn CL (2000) Paternity confusion and the ovarian cycles of female primates. In: van Schaik CP, Janson C (eds) Infanticide by males and its implications. Cambridge University Press, Cambridge, pp361-387

Van Schaik C.P., Höstermann M. (1994) Predation risk and the number of adult males in a primate group: a comparative test. Behav Ecol Sociobiol 35:261–272

Van Schaik CP, Kappeler PM (1997) Infanticide risk and the evolution of male-female association in primates. Proc Roy Soc Lond B 264: 1687-1694

van Schaik CP, Pradhan GR, van Noordwijk MA (2004) Mating conflict in primates: infanticide, sexual harassment and female sexuality. In: Kappeler PM, van Schaik CP (eds) Sexual selection in primates: new and comparative aspects. Cambridge University Press, Cambridge, pp131-150

Vigilant L, Hofreiter M, Siedel H, Boesch C (2001) Paternity and relatedness in wild chimpanzee communities. Proc Natl Acad Sci USA 98:12890-12895

von Segesser F, Ménard N, Gaci B, Martin RD (1999) Genetic differentiation within and between isolated Algerian subpopulations of Barbary macaques (*Macaca sylvanus*): evidence from microsatellites. Mol Ecol 8:433-442

Waitt C, Little AC, Wolfensohn S, Honess P, Brown AP, Buchanan-Smith HM, Perrett DI (2003) Evidence from rhesus macaques suggests that male coloration plays a role in female primate mate choice. Proc R Soc Lond B 270:144-146

Weingrill T, Lycett JE, Henz SP (2000) Consortship and mating success in chacma baboons (*Papio cynocephalus ursinus*). Ethology 106:1033-1044

Weingrill T, Lycett JE, Barrett L, Hill RA, Henzi SP (2003) Male consortship behaviour in chacma baboons: the role of demographic factors and female conceptive probabilities. Behaviour 140:405-427

Widdig A, Bercovitch FB, Streich WJ, Sauermann U, Nürnberg P, Krawczak M (2004) A longitudinal analysis of reproductive skew in male rhesus macaques. Proc R Soc Lond B 271:819-826

Wilcox AJ, Weinberg CR, Baird DD (1995) Timing of sexual intercourse in relation to ovulation. New Engl J Med 333:1517-1522

Wolff JO, Macdonald DW (2004) Promiscuous females protect their offspring. TREE 19:127-134

Zeh JA, Zeh DW (2001) Reproductive mode and the genetic benefits of polyandry. Anim Behav 61:1051-1063

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

Figure legends:

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

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

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

Table 1. Demographic data of study females

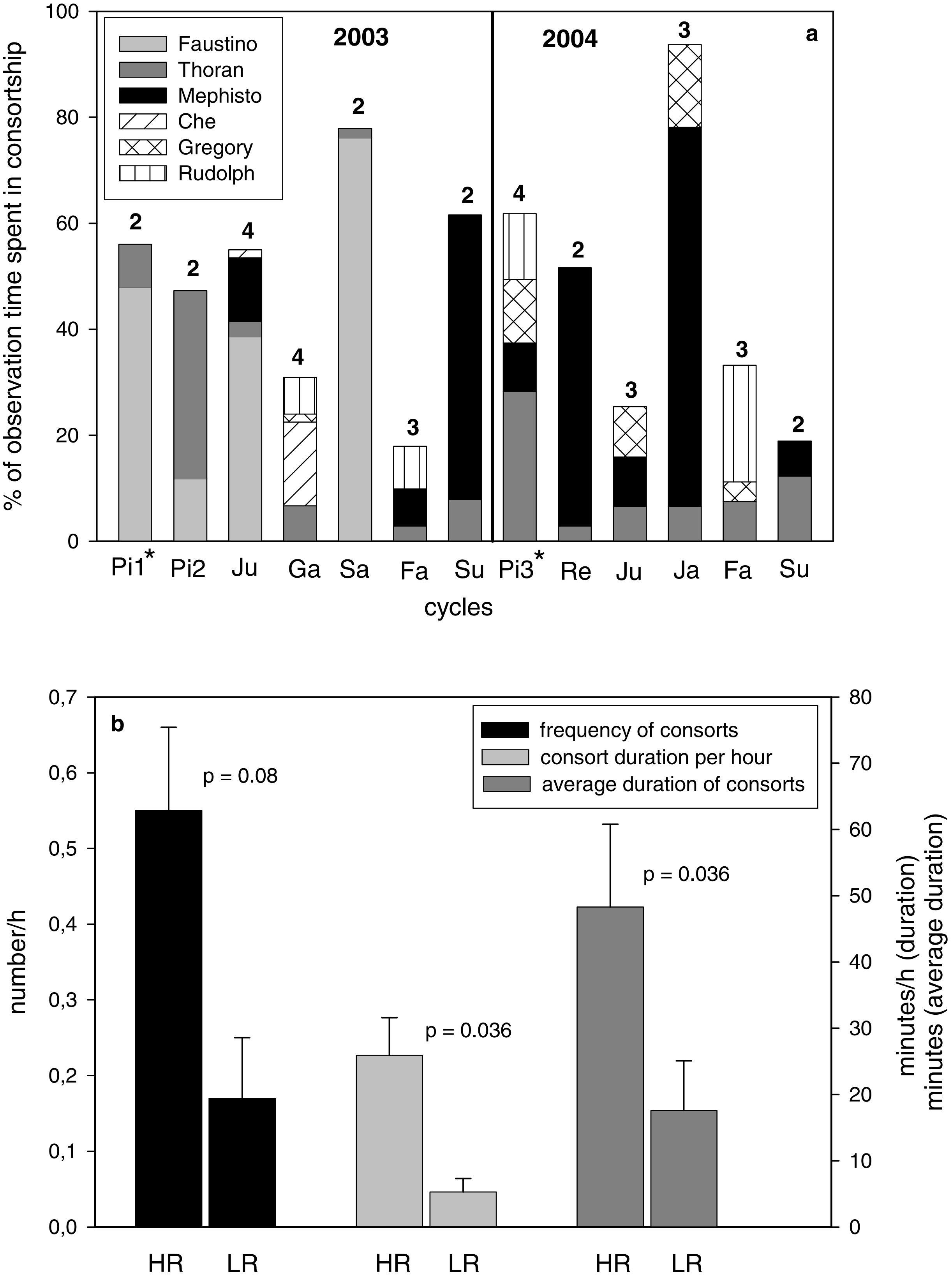
Study female	Rank	Age at first observation	Hours of observation during the fertile phase
Pixie	1	~21	11.6
Ren	2	10	5.7
Jutta	3	8	23.0
Jasmin	4	6	20.1
Gallia	6	11	6.5
Sadie	7	7	7.6
Fanny	9	7	22.5
Sunblest	12	~14	8.0

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

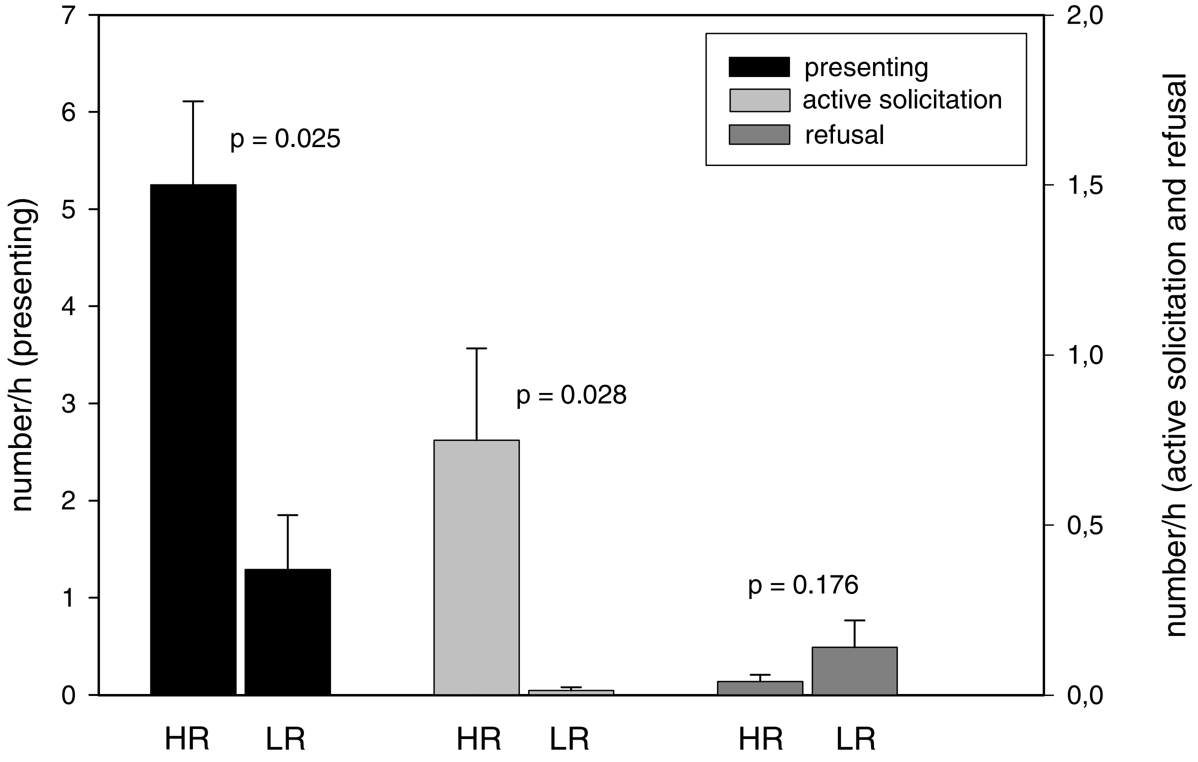
Mother-infant pair	Number of genotyped loci	Likelihood of paternity[a]	ID of father	Rank class of likely father
Pixie - Inf.04	11	>95%	Thoran	high
Ren - Inf.05	10	-[b]	not known	not known[a]
Jutta - Inf.04	11	>95%	Faustino	high
Jutta - Inf.05	11	>95%	Thoran	high
Jasmin - Inf.04	11	>95%	Faustino	high
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

[a] as determined by Cervus 2.0 (see methods)
[b] infant sired by extra-group male

Figure 1

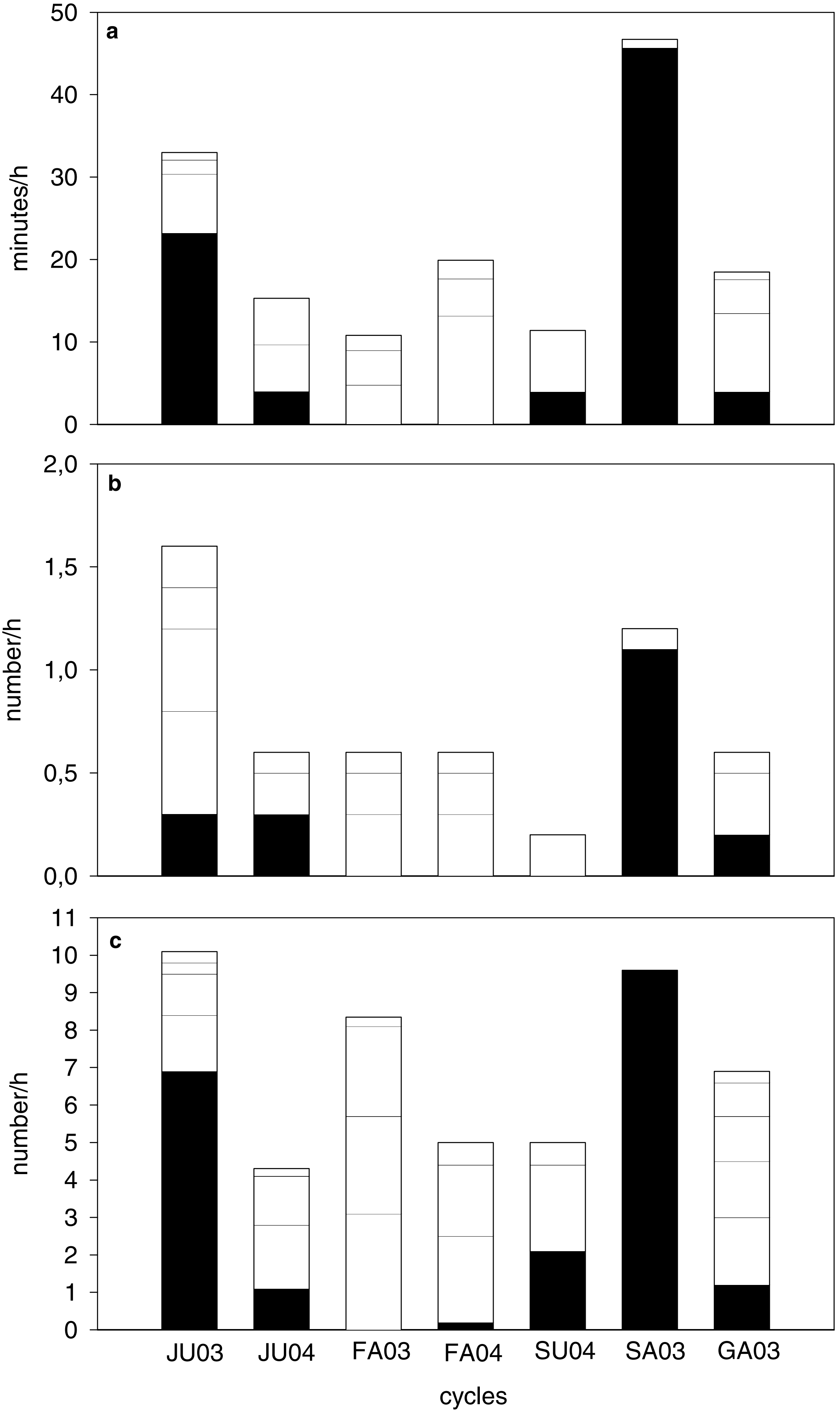

100
80
60
40
20
0
% of observation time spent in consortship
Faustino
Thoran
Mephisto
Che
Gregory
Rudolph
2003
2004
a
2
2
4
4
2
3
2
4
2
3
3
3
2
Pi1*
Pi2
Ju
Ga
Sa
Fa
Su
Pi3*
Re
Ju
Ja
Fa
Su
cycles
b
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0,0
number/h
80
70
60
50
40
30
20
10
0
minutes/h (duration)
minutes (average duration)
frequency of consorts
consort duration per hour
average duration of consorts
p = 0.08
p = 0.036
p = 0.036
HR
LR
HR
LR
HR
LR

901 Figure 2



902

Figure 3


a
minutes/h
0
10
20
30
40
50
b
number/h
0,0
0,5
1,0
1,5
2,0
c
number/h
0
1
2
3
4
5
6
7
8
9
10
11
JU03
JU04
FA03
FA04
SU04
SA03
GA03
cycles

903