THE SIGNIFICANCE OF MALE AND FEMALE REPRODUCTIVE STRATEGIES FOR MALE REPRODUCTIVE SUCCESS IN WILD LONGTAILED MACAQUES

(*MACACA FASCICULARIS*)

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For Malin and Sandra – and future generations

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CONTENTS

Preface

When intending to study aspects of sexual selection, there are many difficulties when choosing primates as model species: limited sample sizes due to small groups, difficulties to measure lifetime reproductive success due to longevity, a slow reproductive rate and ethical constraints on experimental manipulations limit primatological studies and generalization of results deriving from these. This probably explains why there still remains a big gap of knowledge concerning basic questions of primate sexual selection, whereas a number of extensive studies in this field have been conducted on other species by ornithologists, ichthyologists and entomologists (reviewed in Andersson 1994).

Nevertheless, since primates share a unique mixture of specific characteristics not found in other mammalian taxa and, moreover, since our own species is part of this taxon, it is sensible to take the challenge to study sexual selection in primates. In particular, the deviation from the general mammalian pattern of oestrus, i.e. the close temporal relation between female willingness to mate (behavioural receptivity) and female ability to conceive (physiological receptivity), in anthropoid primates, suggests that sexual selection may act differently on this phylogenetic group, raising particular attention of primatologists and anthropologists (e.g. Alexander & Noonan 1979; Burley 1979; Hrdy 1979; Manson 1986; Andelman 1987; Schröder 1993; Heistermann et al. 2001). The observation that female behavioural receptivity extends well over the period during which conception can actually take place, has led to numerous speculations about the ultimate causes and consequences of this phenomenon, which I will present in more detail later in the introduction. From a practical point of view, this peculiar pattern has made it difficult to study reproductive processes, and thus aspects of sexual selection, in wild groups, because the period of highest fertility within a female ovarian cycle is no longer indicated by female sexual activity in anthropoid primates. Most investigations, therefore, have for a long time either been carried out on captive primates using invasive techniques for the determination of female reproductive status, or, when conducted in the wild, been limited to behavioural observations due to a lack of appropriate methods to examine other parameters. Though useful, results obtained under artificial conditions are not necessarily transferable to natural situations and explanations of evolutionary processes can hardly be bases on these. However, in most studies on free-living populations, information on physiological status of female primates could not be gained, so that the timing of reproductive events, such as ovulation and conception, could only be inferred by assumptions. As blood sampling for genetic paternity analysis was not practical under natural conditions either, male mating success was used as a measure of male reproductive success, although both variables need not necessarily be related to each other (Alberts et al. 2003). Hence, results of studies on free-living anthropoid orimates, too, must be assessed with great care.

Recent developments of noninvasive endocrine and genetic analyses, however, have offered new and exciting opportunities to achieve more reliable data on reproductive processes even under natural conditions. The development of genetic paternity based on the use of small amounts of hypervariable DNA (microsatellites) from faecal samples nowadays allows to reliably determine paternity in wild populations and has already been used successfully in recent field studies across all primate taxa (e.g. Constable et al. 1995; *Chlorocebus aethiops*: Newman et al. 2002; *Macaca fuscata*: Inoue & Takenata 1993, Soltis et al. 2001; *Macaca mulatta*: Nürnberg et al. 1998; *Macaca sinica*: Keane et al. 1997; *Papio hamadryas*: Yamane et al. 2003; *Pan paniscus*: Gerloff et al. 1995, 1999; *Pan troglodytes*: Vigilant et al. 2001; *Pongo pygmaeus*: Immel et al. 1999, Utami et al. 2002; *Trachypithecus entellus*: Launhardt et al. 2001). Hormone analyses from faecal samples even make it possible to distinguish the component phases of a female's ovarian cycle, particularly, ovulation and conception (Shideler et al. 1993; Whitten & Russell 1996; Heistermann et al. 1993, 1995, 1996). Recent investigations have clearly shown that faecal samples provide a valuable source for endocrine assessment of both female and male reproductive status in free-living primate populations (e.g. Wasser 1996; Brockman et al. 1998; Strier et al. 1999; Ziegler et al. 1998; Kraus et al. 1999, Heistermann et al. 2001, Deschner et al. 2003).

The aim of my thesis was, therefore, to combine detailed behavioural observations with modern non-invasive techniques of endocrine and genetic analysis in order to investigate male and female reproductive strategies and the factors determining paternity in multimale primate groups, using the long-tailed macaque (*Macaca fascicularis*) as the model species. Although it is clear that a short-term study on a single species cannot illuminate all aspects of this topic, the information obtained in this thesis will hopefully add to a better understanding of the proximate mechanisms regulating male reproductive success in primate groups with a multimale mating system and, as such, help to extend our knowledge about the effect of sexual selection on primate social systems.

1

Reproductive strategies and the 'struggle of the sexes' in primate multimale groups: an introduction

Evolution and the 'Struggle of the Sexes'

The theory of sexual selection is nowadays commonly invoked to explain primate behaviour and evolution, although details of the proximate mechanisms involved remain poorly understood. Darwin already noted that females are apt to be the choosy sex and males the competitive sex due to differences in offspring investment. Although Fisher's (1930) 'runaway model' and Zahavi's (1975) 'handicap principle' strongly supported the idea of sexual selection for female mate preference, primatologists have for a long time seen female primates as the 'passive' sex, and males as actively determining reproductive events. Just recently, the importance of female reproductive strategies has become increasingly recognized, but still, little is known about the adaptive value of female reproductive strategies and their interaction with male reproductive strategies (Chism & Rogers 1997; Janson 1984; Small 1989; Taub 1980; Vasey 1998; Small 1989; Sterck et al. 1997). Sexual selection acts differently on males and females as females, especially in mammals, allocate a great proportion of their lifetime to parental effort through pregnancy and lactation, which limits the number of offspring a female mammal can produce in her lifetime. Males, on the other hand, produce lots of small, 'cheap' microgametes, predisposing them to maximize the number of successful matings. Thus, while males should compete for access to fertile females, females should ensure a high reproductive rate and survival of their offspring and, therefore, should be choosy in with whome they mate (Darwin 1871; Trivers 1972). As a consequence, reproductive strategies of the genders may even counteract each other and lead to inter-sexual conflict over reproduction (e.g. Trivers 1972; Smuts & Smuts 1993; Clutton-Brock & Parker 1995; Gowaty 1996; Nunn & van Schaik 2000).

Male and Female Sexual Strategies in Multimale Primate Groups

According to sexual selection theory, contest competition among males for access to mating partners should lead to polygynous socio-sexual systems where sexual dimorphism in body weight and canine size clearly exists (Mitani et al. 1996; Plavcan & van Schaik 1997) and in which the strongest male gains exclusive access to fertile females (priority of access to fertile females model; Altmann 1962; see also Fedigan 1983). However, a lot of primate species are organized in multimale groups. In these groups, males form dominance hierarchies, which often seem to be the most important factor influencing a male‘s reproductive success (e.g. *Cercocebus torquatus atys*: Gust et al. 1998; *Macaca fascicularis*: de Ruiter et al. 1994; *Macaca sylvanus*: Paul & Kuester 1996; *Mandrillus sphinx*: Dixson et. al. 1993; *Papio cynocephalus*: Altmann et al. 1996; *Presbytis entellus*: Launhardt et al. 2001). This, however, is not always true and in some primate species, significant numbers of offspring are fathered by nondominant or extra-group males (e.g. *Macaca fuscata*: Inoue et al. 1993; Soltis et al. 2001; *Macaca mulatta*: Berard et al. 1993, 1994; Widdig et al. 2004; *Papio cynocephalus*: Alberts et al. 2003). The proximate mechanisms underlying these variations in reproductive success of dominant males in multimale primate groups still remain largely unknown.

A factor that seems to affect monopolizability of females by dominant males is cycle synchrony, because a single male can hardly monopolize more than one female at a time (Paul 1997; Nunn 1999a). Furthermore, subordinate males may form coalitions in order to break up consortships between high-ranking males and fertile females (e.g. *Papio cynocephalus*: Packer 1977; Noë & Sluijter 1990; Bercovitch 1995) or extra-group males might try to sneak copulations without notice of other males (e.g. *Macaca fuscata*: Soltis et al. 2001). Female monopolizability may also depend on female cooperation. The significance of female cooperation for male reproductive success, however, is still under discussion (Bercovitch 1995).

Even when fertile females are not monopolized by dominant males, males in general may still coerce matings (reviewed in Smuts & Smuts 1993) and thus restrict female freedom to display their own mating preferences. On the other hand, females that are able to resist mating attempts gain freedom to exert their own reproductive strategies. Small (1989) has suggested that females should actively choose the male with the ‘best genes’ to father their offspring in order to improve the offspring’s genetic strength (‘direct female choice’). On the other hand, females may benefit from mating polyandrously (reviewed in Wolff & Macdonald 2004), thus avoiding inbreeding or genetic incompatibilities (e.g. Tregenza & Wedell 2002;

Newcomer et al. 1999; reviewed in Zeh & Zeh 2001) or receiving high quality fertile sperm through sperm competition (Dixson 1998), since competition between spermatozoa of rival males will occur when females mate with more than one male (Parker 1970). Females may benefit from sperm competition if sperm quality is positively related to offspring fitness (Parker 1992) or if sons inherit the competitive ability of sperm from their fathers (Harvey and Bennet 1985). The observation that primate males living in multimale groups have higher testis to body weight ratios compared to those living in one-male groups or in monogamous mating systems has been interpreted as a result of sperm competition (Harcourt et al. 1981).

Apart from genetic benefits, other motives could drive females to mate with many males, for example, the attempt to tighten males to the group, as the number of males present in a group can affect the predation rate (Van Schaik & Höstermann 1994; Stanford 1998), and superiority in intergroup encounters (*Cercopithecus aethiops*: Cheney & Seyfarth 1987; *Macaca fuscata*: Kawanaka 1973). Furthermore, in several primate species, males kill infants of females with which they have previously not mated before (*Alouatta spec.*: reviewed in Crockett 2003; *Papio cynocephalus*: reviewed in Palombit 2003; *Trachypithecus entellus*: reviewed in Borries & König 2000; *Macaca fuscata*: Soltis et al. 2000). Though the adaptive value of infanticidal behaviour has not yet been fully established (Niemitz 1997), there is increasing evidence that males benefit from killing dependant infants of which they are not the father by mating with the mother soon after the killing, because these females usually become receptive again more quickly than lactating females (Hrdy 1974; Borries et al. 1999; van Schaik et al. 2000). For the female, infanticide poses a great cost, as the time and energy she has already invested in her former offspring is lost. Consequently, polyandrous mating may be a counterstrategy against infanticide, because it leads to paternity confusion (Hausfater & Hrdy 1984). Another female counterstrategy is proposed by the bodyguard hypothesis, which suggests that females mate preferentially with strong males who in future protect the ensuing infant (Mesnick 1997). This might have been the origin of the so-called ‘friendships’ observed in savannah baboons (Smuts 1985, 1987).

Thus, it seems that females would benefit from both concentrating matings to a single, strong male and from mating polyandrously. A more recent hypothesis proposes a way in which females are able to bias paternity towards strong males and, at the same time, reduce the risk of infanticide by paternity confusion. The graded-signal hypothesis (Nunn 1999b) suggests that females display a graded signal which indicates the probability of ovulation, but not its precise timing. In this way, females could mate polyandrously with subordinate males when the signal is low, as is the probability to conceive, and on the other hand attract dominant males when the signals have reached their maximum, i.e. when probability of conception is high. Since the probability of conception is low but not zero during the period in which subordinate males mate with females, all mating partners are potential fathers of the ensuing offspring and, thus, should avoid infanticide.

In contrast to the example given above, female mate choice is not always obvious: female participation in male-male aggressive interactions might be a form of female choice (indirect female choice; Smuts 1987), as it can influence group membership and, in doing so, can determine will be available as mates. Furthermore, a variety of mechanisms might allow females to be choosy after copulation (female cryptic choice; reviewed in Reeder 2003): females may discard or destroy sperm of unfavoured males, hinder sperm transport to fertilization sites, choose among sperm that have reached the egg or abort zygotes.

Thus, male and female reproductive strategies can either occur on the precopulatory level (male contest competition, male coercion, sneaking copulations, female direct or indirect choice) or on the postcopulatory level (sperm competition, cryptic female choice). On which level individual male success to fertilize a female is determined and to what extent the different reproductive strategies are involved remains unclear so far. The crucial period within an ovarian cycle, in which the adaptive value of precopulatory behaviours as well as the potential for postcopulatory mechanisms is determined, is the fertile phase.

The Fertile Phase and its Potential Cues

Timing of copulations is suggested to be a more important factor for male reproductive success than the number of copulations with a certain female, as the period within an ovarian cycle, in which a copulation can lead to conception (fertile phase), is limited to a few days around the time of ovulation (Gomendio et al. 1998). Since matings outside the fertile phase do not lead to conception, the ability to recognize this period is clearly important for males and can significantly reduce male reproductive costs. On the other hand, female masking of the fertile phase, so that males do not know when to monopolize or coerce females, increases female freedom to pursue their own reproductive strategies (see above). Since female sexual activity is well extended over the fertile period (reviewed in van Schaik et al. 2000), for a long time it was unclear whether the fertile phase was concealed from primate males. Just recently, a study on Hanuman langurs carried out under natural conditions showed that timing of ovulation is concealed from males in this species (Heistermann et al. 2001) and that the dominant male lost over 80% of paternities to low-ranking males, because he did not adjust mate guarding to the time around ovulation. However, time of ovulation seems not to be concealed from male anthropoid primates in general, since captive male macaques are most attracted by females around the time of ovulation (reviewed in Michael & Zumpe 1993; Zumpe & Michael 1996; see also Aujard et al. 1998). Whether these results can be applied to natural situations, however, is still unknown, because studies, which have tested the ability of male macaques to discern the fertile phase, are still missing for wild groups.

There are different mechanisms by which male primates could recognize a female's reproductive state. Given the fact that the fertile phase coincides with a period of elevated ovarian oestrogen levels, those female traits that closely correlate with oestrogens carry the potential to indicate the fertile phase in particular. Certain volatile aliphatic acids, for example, which have been identified in the vaginal discharge of many different primate species (reviewed in Hrdy & Whitten 1987), change in concentration according to changes in female oestrogen levels and enhance male interest, so that they have been interpreted as sex pheromones (Curtis et al. 1971; Michael & Keverne 1968, 1970; Michael et al. 1971, 1977; but see Goldfoot et al. 1978; Goldfoot 1981). Whether males make use of these cues under natural conditions, however, still needs to be investigated.

A possible visible sign of female reproductive status is the sexual swelling of skin, which occurs mainly in catarrhine primate species living in multimale groups (reviewed in Hrdy & Whitten 1987). Swelling size changes in accordance to female oestrogen and progesterone levels (e.g. Parkes & Zuckerman 1931; Gillman 1940; Carlisle et al. 1981; Ozasa & Gould 1982; Onouchi & Kato 1983), with male sexual motivation being highest when the skin is fully tumescent (Bielert & van der Walt 1982; Girolami & Bielert 1987; Bielert et al. 1989; Dixson 1998; Deschner et al. in press). Different hypotheses have been proposed to explain the specific function of sexual swellings (reviewed in Zinner et al. 2004), including those, which predict that the swelling size reliably indicates the fertile phase (reliable-indicator hypothesis, Hamilton 1984), but although in some primate species sexual swellings are a good approximation for the female periovulatory phase (e.g. *Macaca nigra*, *Papio cynocephalus*; for a review see Nunn 1999b), they do not always reliably indicate this period (see Nunn 1999b; see also Reichert et al. 2002; Deschner 2003). Another model, the graded-signal hypothesis (Nunn 1999b), proposes that graded swellings serve to enable females to mate polyandrously during periods with low probability of conception and to bias paternity towards dominant males by mating with these males during periods of high probability of conception (see above). To date, the specific functions of sexual swellings (reviewed in Zinner et al. 2004) still remain unclear and it seems that they may differ among different species.

Despite olfactory and visual cues, auditory cues may play a role in male assessment of the fertile phase. Female primates often either display copulation calls, i.e. calls that are given during copulation (e.g. *Papio cynocephalus*: Semple 2001; *Macaca sylvanus*: Semple 1998; reviewed in Nikitopoulos 2003), or oestrus calls, i.e. calls that are emitted only during times when females are sexually active, but which are not restricted to copulations (e.g. *Macaca tonkeana*: Aujard et al. 1998; *Theropithecus gelada*: Moos-Heilen & Sossinka 1990). Both types of calls are known to attract male attention, but the specific functions of these calls still remain unknown. Numerous hypotheses have been proposed so far (reviewed in Nikitopoulos 2003), including the suggestion that they serve to signal the fertile phase of an ovarian cycle (alpha male notification hypothesis; Henzi 1996; Semple 1998). Female oestrogen levels have been related to call frequencies (oestrus calls in *Macaca tonkeana*: Aujard et al. 1998) and to call pattern (copulation calls in *Macaca sylvanus*: Semple & McComb 2001), but more investigations are needed to clarify how useful both types of calls are for male assessment of the timing of a female's fertile phase.

A fourth potential indicator of the fertile phase is female behaviour. Although females are sexually active throughout almost the entire ovarian cycle, observations of macaques in captivity have shown that the frequencies of certain sexual behaviours vary in the course of the menstrual cycle according to female sex hormones (Zumpe & Michael 1983, 1985). In captivity at least, these behaviours seem to be a good predictor of the day of conception (Zehr et al. 2000), but evidence from the wild is still lacking.

Longtailed Macaques as a Model for Investigating Reproductive Strategies and their Adaptive Value in Multimale Primate Groups

Longtailed macaques are widely distributed over South East Asia, ranging from Burma to the Philippines and Indonesia. Their habitat comprises primary lowland forest up to 2,000 metres, but also secondary forest, mangroves, swamps, rubber plantations and temple sites (Lekagul & Mc Neely 1977; Crocket & Wilson 1980; Fittinghoff & Lindburg 1980; Wolfheim 1983; Wheatley 1989). In primary forests, longtailed macaques are mostly arborial and frugivorous (Wheatley 1980; van Schaik & van Noordwijk 1988; Sterck 1995), but they may spend more time on the ground in other habitats, sometimes feeding on anthropogenic food (Wheatley 1988, 1989; Lucas & Corlett 1991). They live in multimale multifemale groups, which range from 10 to 48 individuals and have an average sex ratio of 1:2.5 (Rowe 1996). Females are philopatric and form the core of a social group. Among females as well as among males, dominance hierarchies exist, which are in the case of females inherited from the mother (van Noordwijk & van Schaik 1987; Melnick & Pearl 1987; de Ruiter et al. 1994). Since the given characteristics are similar to those of most macaques and also to those of other catarrhine primates living in multimale groups, longtailed macaques represent a good model to investigate the interactions between male and female mating strategies and their significance for paternity determination.

In a longitudinal study over several years in three groups on wild longtailed macaques, de Ruiter et al. (1994) demonstrated that infants are almost exclusively sired by alpha and beta males. Whether this high-reproductive skew was determined on the pre- or postcopulatory level and which reproductive strategies usually were involved, could, however, not be clarified in this study. Observations conducted on longtailed macaques so far do not allow to draw final conclusions about the significance of specific reproductive strategies in maximizing male reproductive success, because species characteristics show an inconsistent picture. A high degree of sexual dimorphism in body weight and canine size (Plavcan & van Schaik 1997) leading to male dominance over females (Angst 1975), a moderate degree of seasonality in reproduction with the mating season and the birth season lasting for about six months each (Kavanagh & Laursen 1984; van Schaik & van Noordwijk 1985) and the observation that males form consortships with fertile females (van Noordwijk 1985) suggest male monopolization to prevail in this species. On the other hand, a high testis: body weight ratio and a high number of viable sperm in male ejaculate (Schrod 2002) suggest a high degree of sperm competition.

Female specific characteristics do also not allow consistent conclusions: female longtailed macaques show conspicuous sexual swellings, which have been interpreted as a female attempt to attract dominant males during the fertile phase (Nunn 1999b), but they have been observed to mate with males of all rank during the fertile phase in a pair choice experiment (Nikitopoulos 2003). Furthermore, in contrast to other primates, swelling size declines with female age in longtailed macaques, and swelling pattern seems not to be related to a female's hormonal cycle (van Noordwijk 1985).

Thus, whether in longtailed macaques, paternity is determined on the pre- or postcopulatory level, and to what extend the stated reproductive strategies play a role in this regard, remains unclear. More investigations are therefore needed in order to determine the proximate mechanisms regulating male reproductive success in this species.

1.3 The Thesis

The overall aim of this thesis is to examine the factors leading to paternity in a wild group of longtailed macaques in order to contribute to a better understanding of the proximate mechanisms regulating male reproductive success in primate multimale groups – and as such helping to extend our knowledge of primate sexual selection. In order to achieve this, behavioural data collection was combined with a playback experiment and with modern noninvasive techniques of endocrinological and genetic analysis from faecal samples to provide an objective assessment of female reproductive status and paternity, respectively. The study took place on two different sites. Most of the behavioural and hormonal data in addition to all genetic data were collected on a group of longtailed macaques living in an undisturbed primary forest in the Gunung Leuser National Park, North Sumatra, Indonesia. The playback experiment was conducted in a mixed primary and secondary forest, the Recreation Park and Nature Reserve of Pangandaran, West Java, Indonesia, where macaques spent most of their time on the ground, thus facilitating playback experiments.

The aim of the playback experiment, which is presented in Chapter 2, was to find out whether male longtailed macaques are able to recognize the fertile phase of an ovarian cycle under natural conditions. The ability to recognize the fertile phase is important for dominant males to successfully monopolize access to fertile females. By using a female copulation call as playback stimulus changes in interest of subordinate males in the course of this female's cycle were assessed by repeatedly playing back the call and recording male response. At the same time, mate guarding by the group's dominant male was monitored. The intensity of male response and of mate guarding during the fertile phase was compared to that of other stages of the cycle. In addition, periods of mate guarding and male response intensities were related to female oestrogen levels in order to test whether cues used by males for the assessment of the fertile phase were oestrogen-related.

After it turned out that male longtailed macaques are able to recognize the fertile phase, two potential cues, female sexual swellings and female behaviour, were tested for their reliability in indicating the fertile phase. The size of swellings as well as the frequency of specific female behaviours during the fertile phase were compared to that of other stages of the cycle and related to female sex hormone patterns. A report on this study is given in Chapter 3.

The main topic of this thesis, the study of the proximate factors leading to paternity in wild groups of longtailed macaques, is presented in Chapter 4. First, the distribution of fertile phases over the mating period was examined in order to assess constrains on male monopolization deriving from synchrony in the fertile phases. Subsequently, male and female socio-sexual behaviour and the occurrence of consortships during the fertile phase were analysed in order to 1. assess the degree of mate guarding, 2. female opportunity for mate choice, 3. female sexual preferences and 4. the number of copulation partners per female. Since it was not possible to investigate processes occurring in the female reproductive tract, only the potential for postcopulatory mechanisms to take place could be assessed. Paternity outcome was genetically determined and compared to the proportion of copulations likely fathers had with mothers compared to those of other males during the fertile phase.

In Chapter 5, I finally discuss the findings of my thesis on the background presented in Chapter 1 and make suggestions for further investigations.

2

Assessment of female feproductive status by male longtailed macaques (*Macaca fascicularis*) under natural conditions

with J.-B. Pfeifer, M. Heistermann, C. Niemitz, J.A.R.A.M. van Hooff, J.-K. Hodges

Abstract

Recent theories on primate sexual selection have paid increasing attention to the importance of reproductive strategies of females living in multimale groups. However, the extent to which females are able effectively to conceal or advertise the time of ovulation as part of these strategies remains unclear. Few studies have investigated the ability of males to discern female reproductive status under natural conditions, and none has taken into account differences in male rank, and thus ability to gain access to females or cues. We tested male assessment of a female's fertile phase under natural conditions in longtailed macaques. We used timing of mate guarding by dominant males and the response of subordinate males towards the repeated playback of a female copulation call to measure male interest in females throughout the ovarian cycle. Relating the degree of male interest to female reproductive status, as determined noninvasively by faecal hormone analysis, we found that interest in females shown by both dominant and subordinate males is strongest during and around the fertile phase. Our results also indicate that males were better able to recognize the fertile period in conception than in nonconception cycles. Furthermore, our finding of a strong positive relation between male interest and female oestrogen levels in all cycles indicates that at least some of the cues used by males to assess female reproductive status are oestrogen related.

Introduction

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

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

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

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

We determined male interest in females in relation to the stage of the ovarian cycle using two protocols, which differed according to the dominance status of the males and thus their ability to get access to females. We examined interest of dominant males by monitoring mate-guarding behaviour. Mate guarding is a potentially costly activity in terms of energetics and risk of injury (Alberts et al. 1996), so it should be limited to those days on which a successful mating could lead to conception.

Subordinate males on the other hand, generally have limited opportunities for mate guarding, being restricted or excluded by higher-ranking males. We therefore also measured interest of subordinate males with a playback experiment to provoke a response that should reflect their interest in the female. The stimulus, given repeatedly throughout the ovarian cycle of the female, was a female copulation call, because these vocalizations can be individually recognized and attract male attention in a number of catarrhine primates (e.g. Barbary macaque, *Macaca sylvanus*: Semple 1998; yellow baboon, *Papio cynocephalus cynocephalus*: Semple 2001). Combining hormone analysis with behavioural observations and a playback experiment, our objectives were to investigate male ability to discern the fertile period of the female ovarian cycle in free-ranging longtailed macaques by relating (1) timing of mate guarding by alpha males and (2) subordinate male response intensities towards a playback stimulus (female copulation call) to the female's fertile phase, as defined by faecal progestin profiles.

Methods

Animals and Study Site

The study was carried out in the Recreation Park and Nature Reserve of Pangandaran, West Java, Indonesia (7º43'S, 108º52'E), which consists of mixed primary and secondary evergreen rain forest. For a detailed description of the study area and its climate, see Kool (1993). Longtailed macaques show a moderate degree of seasonality in reproduction, with the birth season lasting for about 6 months (Kavanagh & Laursen 1984; van Schaik & van Noordwijk 1985), and births in the study area peak in January and February (Engelhardt & Kusay 2002). The subjects belonged to four groups, each containing four to eight adult males and 5–12 adult females, and spent most of their time in the Recreation Park where visitors fed them occasionally. All animals were habituated and individually known.

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

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

* Second cycling female very old.
† Second cycling female nulliparous.

All study females (CA, JA, AEB, WC, NF) were multiparous and had been monitored for at least a month before the experiments started (Table 1). For each female, three adult nondominant male group members served as test males during the playback experiment (Table 2). Only males younger than the alpha male (thereby still having the potential to reach alpha rank position in the future), and of these, the highest-ranking ones were chosen as test-males, because these were expected to have the highest variability in terms of responding to the stimulus.

Table 2. Study-groups, number of adult males per groupand dominance rank of test males

Group	Adult males	Test male	Rank
A	5	TA	2
		BA	3
		FA	4
B	7*	BB	3
		YB	4
		CB	5
C	8†	TC	4
		DC	5
		JC	6
F	4	WF	2
		BF	3
		SF	4

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

Female Copulation Calls

Although copulatory vocalizations of female longtailed macaques are sequences of distinct calls, they are referred to collectively as ‘copulation calls’ in the literature (e.g. Deputte & Goustard 1980; Semple 2001). We use this term for the functional unit of calls uttered by female longtailed macaques during copulations. Sexually active female longtailed macaques mate frequently during the day and utter copulation calls in 80% of all matings (van Noordwijk 1985). In a variability factors analysis, Deputte & Goustard (1980) found inter-individual differences in call duration and structure, as well as strong intraindividual differences according to the nature of male–female interactions during copulations and whether ejaculation occurred. For the playback experiments, copulation calls of each study female were recorded ad libitum at 1–3 m during ejaculatory copulations with adult males, using a Sennheiser ME 66 directional microphone with a Sennheiser MZW 66 windshield and a SONY TCD-D 100 Digital Audio Tape Recorder.

Playback Protocol

Playback experiments were conducted by J.-B.P. and an Indonesian assistant from April to July 2001. Playbacks were conducted with a SONY TCD-D 100 Digital Audio Tape Recorder and a NAGRA DSM amplifier–loudspeaker. All copulation calls were equalized to reach an average sound pressure level of 70 dB at 1 m using an Eagle DB 120 sound level meter. The copulation call of each study female was played back separately towards the three test males every second day from the early follicular phase until after the onset of detumescence of the sexual swelling (Table 3). In the case of female JA, however, the experimental period ended before the onset of detumescence because of technical problems.

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

Table 3. Number of experiments conducted per female/male dyad during the prefertile, fertile and postfertile phase of the respective study cycles

		Phase		
Study female	Test male	Prefertile	Fertile	Postfertile
CA	TA	3	3	3
	BA	3	3	3
	FA	3	3	3
JA	TA	3	3	0
	BA	3	3	0
	FA	3	3	0
AEB	BB	3	3	3
	YB	3	3	3
	CB	3	3	3
WC	TC	1	3	3
	DC	1	3	3
	JC	1	2	3
NF	WF	3	2	3
	BF	3	2	3
	SF	3	2	3

The loudspeaker was hidden from the test males at an average distance of 15 m (range 8-23 m). A pilot phase indicated that differences in the distance between the speaker and the test males had no effect on male response (J.-B. Pfeifer & A. Engelhardt, unpublished data). Playbacks were conducted only when the study female and higher-ranking males had been out of sight already for at least 2 min and when the male was sitting and facing away from the loudspeaker either resting or self grooming. During the experiments, test males were not engaged in consortships with any female, and other cycling females were at least 10 m away. An interval of at least 30 min was left between playbacks towards males from the same group.

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

To avoid habituation towards the experimental situation, we regularly conducted mock trials, in which the speaker and the camera were set up, but no stimulus was presented. Furthermore, we controlled for habituation towards the playback stimulus by conducting a second experiment on the day that the test male stopped responding. This was done by presenting the test male with another copulation call from the same female, which had also been recorded during an ejaculatory copulation in the early follicular phase, but which differed from the test stimulus in its sonographic structure and duration (visualized with Avisoft SASLab Pro, version 3.92, Raimund Specht Berlin, Germany). At least 30 min was left between the playback of the repeated stimulus and the control stimulus. On no occasion did any male approach the speaker when presented with the control stimulus.

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

Behavioural Observations

All females of a group were monitored for sexual activity, consortships, swelling size, menstruation, pregnancy and infant birth or loss whenever in contact with the group, starting at least a month before the experimental period. On the same days that the subordinate males were presented the playback stimulus, study females were followed and length of consortships and sexual interactions (inspections, mating attempts, matings) were continuously recorded using focal animal sampling (Altmann 1974). Monitoring of females and behavioural observations were carried out by A.E and an Indonesian assistant. Observation time averaged 6.6 h per experimental day. To determine male and female rank, aggressive interactions and the display of the 'bared-teeth-face', a unidirectional submissive display (van Hooff 1967), were recorded whenever they occurred. A consortship was called mate guarding, when the alpha male exclusively mated with the female and followed her within 5 m for more than 99% of the observation time. As soon as mate guarding was observed, the female was also observed for 3 h on days between experiments to confirm whether they were continuously monopolized.

Faecal Sample Collection and Hormone Analysis

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

Faecal extracts were measured for levels of immunoreactive oestrogen and progestogen metabolites using enzyme immunoassays (EIA) for total oestrogens (iE_{total}; Ostner & Heistermann 2002) and pregnanediol-3-glucuronide (iPdG) (Ziegler et al. 2000). Sensitivity of the assays at 90% binding was 1.5 pg for iE_{total} and 20 pg for iPdG. Serial dilutions of faecal extracts from the follicular and luteal phases of the ovarian cycle gave displacement curves parallel to that obtained with the respective standard. Intra- and interassay coefficients of variation, determined by replicate determinations of quality controls, were 7.2% (n = 32) and 9,9% (n = 16) for iE_{total} and 3.5% (n = 32) and 5.5% (n = 16) for iPdG.

Definition of the Female's Fertile Phase

The faecal progestogen profiles were used to determine the presumed time of ovulation and thereby the fertile phase of each ovarian cycle. In this respect, a defined rise in faecal iPdG levels above a threshold of the mean plus two standard deviations of five preceding baseline values was used to indicate the onset of the postovulatory phase of each cycle (Carosi et al. 1999; Heistermann et al. 2001). Faecal samples were collected only every second day, and in the longtailed macaque, the time lag in the excretion of progestogens into the faeces has varied between 24 and 56 h (Shideler et al. 1993). Therefore, it was not possible to determine specifically the day of ovulation. Instead, we determined a 3-day 'ovulation window', comprising the period between day –2 (latest possible day of ovulation) to day –4 (earliest possible day of ovulation) relative to the defined faecal iPdG rise (day 0). On this basis, the fertile phase was defined as the period comprising the three potential days of ovulation plus the two preceding days, because experiments in longtailed macaques have shown that matings can be fertile when occurring up to 2 days before ovulation and on the day of ovulation, but not later (Behboodi et al. 1991). All days preceding the fertile phase are hereafter called the prefertile phase and days following this phase the postfertile phase.

Data Analysis

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

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

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

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

Results

Female Reproductive Cycles

Figure 1 shows profiles of immunoreactive oestrogen and progestogen metabolites in relation to the fertile phase of each female. Oestrogen levels differed between females and a

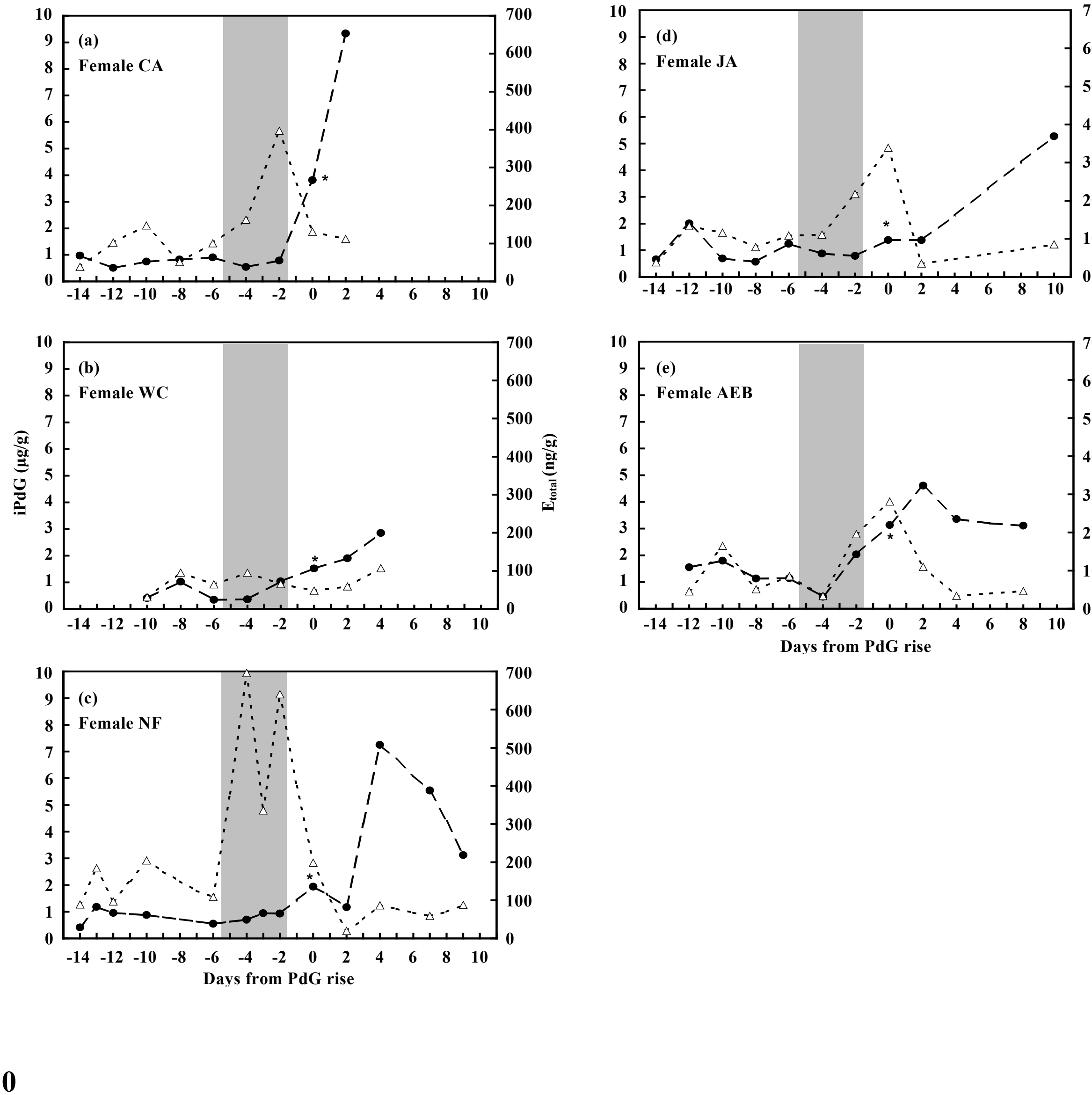

Figure 1. (a–e) Faecal progestin (•) and oestrogen profiles (∆) of the five test females. The fertile phase (shaded areas) was deduced by identifying the day on which faecal progestin level is increased above a defined threshold value (asterisks).

clear oestrogen peak was detected in four of them. It is likely that we missed the oestrogen peak in female WC as a result of our sample collection procedure or because there was a gap of 3 days within the sample series (Fig. 1b). In the three females (CA, NF, WC) that conceived during the study period and gave birth to an infant, maximum oestrogen values were measured within the fertile phase (Fig. 1a–c). The other two females (AEB, JA) showed highest oestrogen values 2 days after the fertile phase (Fig. 1d–e).

Mate Guarding by Alpha Males

During the study cycles, all five females were mate guarded by alpha males on 4–13 consecutive days (Fig. 2). During these periods, all subordinate males were excluded from access to females (inspections as well as copulations). Overall, mate guarding occurred on 20 out of 25 days (80%) representing the fertile phases of the females’ cycles. A comparison of conception versus nonconception cycles, however, revealed that alpha males more often monopolized females during their fertile phases when these were associated with conception. During conception cycles, mate guarding covered 14 out of 15 days (93%) ascribed to the fertile phase, whereas during nonconception cycles the figure was 6 out of 10 (60%). Mate guarding during one of these cycles (female AEB) occurred almost exclusively outside the fertile phase. Efficiency of mate guarding (proportion of days invested in mate guarding that coincided with the fertile phase) also varied according to cycle type: during conception cycles, 14 of the 20 days (70%) that alpha males invested in mate guarding overlapped with the fertile phase, while during nonconception cycles, the figure was only 6 of 17 days (35%).

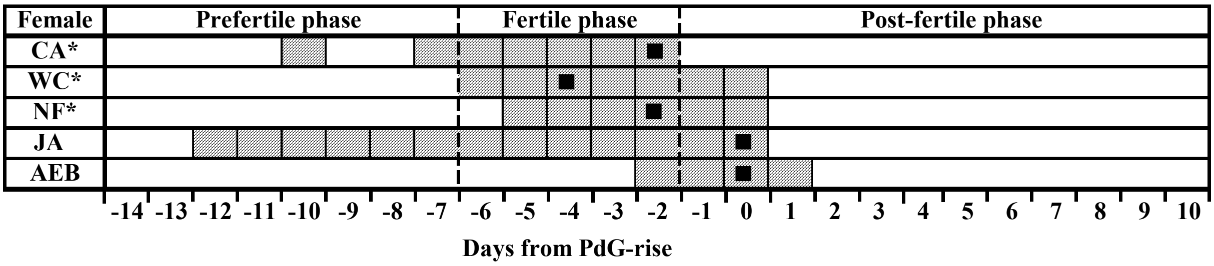

Figure 2. Days on which the dominant male guarded a female (shaded area) in relation to the female’s fertile phase. Asterisks mark conception cycles; black squares indicate days with maximum oestrogen levels.

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

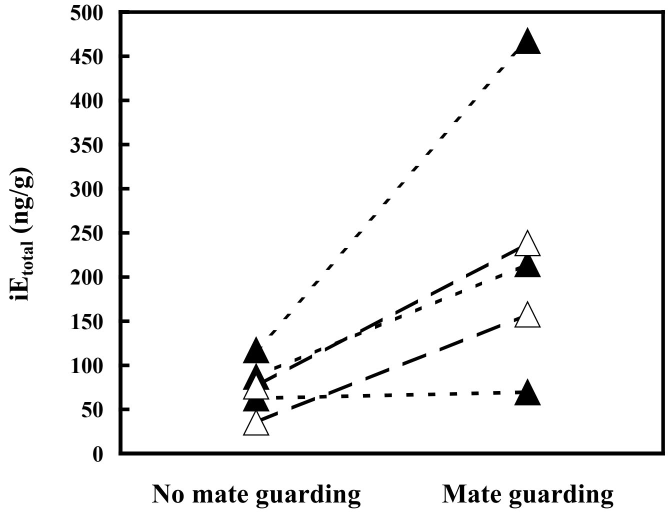

Figure 3. Median faecal oestrogen values in all study females (N = 5) on days when they were and were not mate guarded by the dominant male. ▲: Conception cycles,△: nonconception cycles.

Playback Experiment

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

During nonconception cycles, males responded most strongly to the stimulus during the prefertile phase (permutation test: $t = 2.67$, $N = 6$, $K = 3$, $P < 0.05$; Fig. 4a). In contrast, during conception cycles, male responses were significantly stronger within the fertile phase than either the prefertile (A) or the postfertile phase (B) (Friedman test: χ^2=12, N=9, K=3, P<0.003; Wilcoxon signed-ranks test: $T_A = 3$, $P_A = 0.01$, $T_B = 0.5$, $P_B < 0.005$; Fig. 4b).

Taking all male–female pairs together, median response intensities showed a highly significant positive correlation with median female oestrogen levels, when variables were aligned to the day of maximum oestrogen value (Spearman rank correlation: $r_s = 0.866$, $N = 8$, $P < 0.01$; Fig. 5).

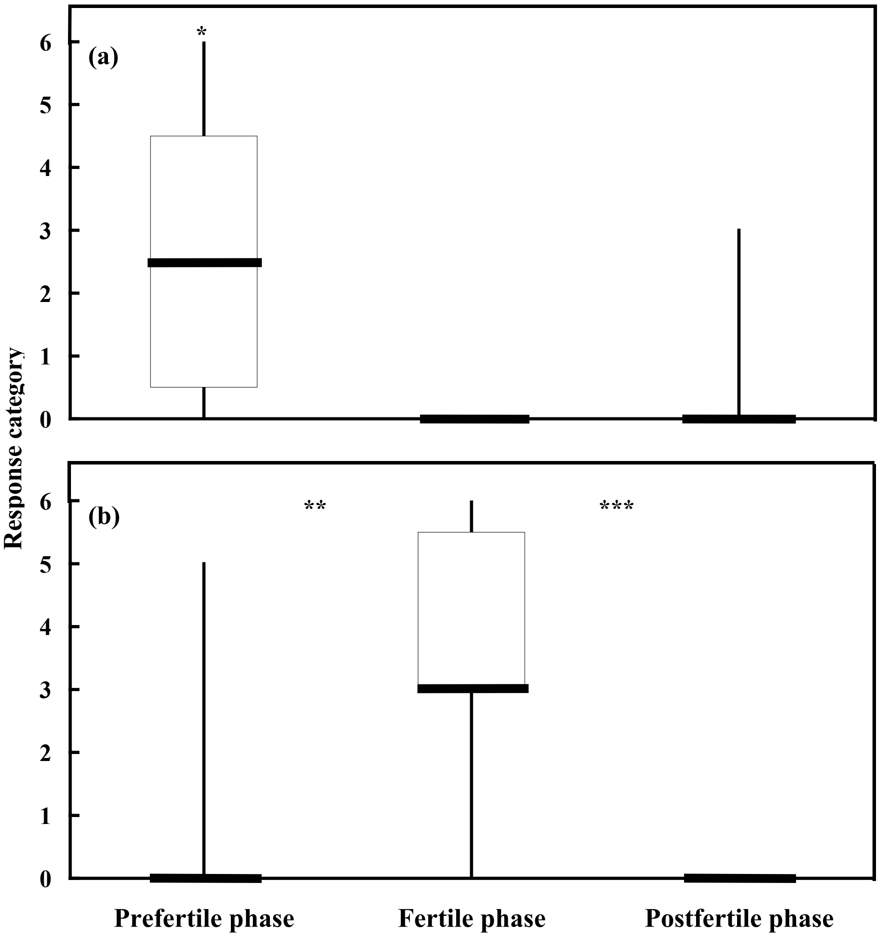

Figure 4. Comparison of male response intensities towards the playback stimulus during the prefertile, fertile and postfertile phases for male–female pairs during (a) nonconception cycles (N = 6) and (b) conception cycles (N = 9). Shown are the median, the first and third quartiles, and the range of male response intensities for each phase. (*P < 0.05, **P = 0.01, ***P < 0.005).

Discussion

The results indicate that interest in females shown by both dominant and subordinate males was strongest during and around the fertile phase of the ovarian cycle. Our results also indicate that males of both social statuses were better able to recognize the fertile period in conception than in nonconception cycles. Furthermore, our finding of a strong positive relation between male interest and female oestrogen levels in all cycles indicates that at least some of the cues used by males to assess female reproductive status are oestrogen related.

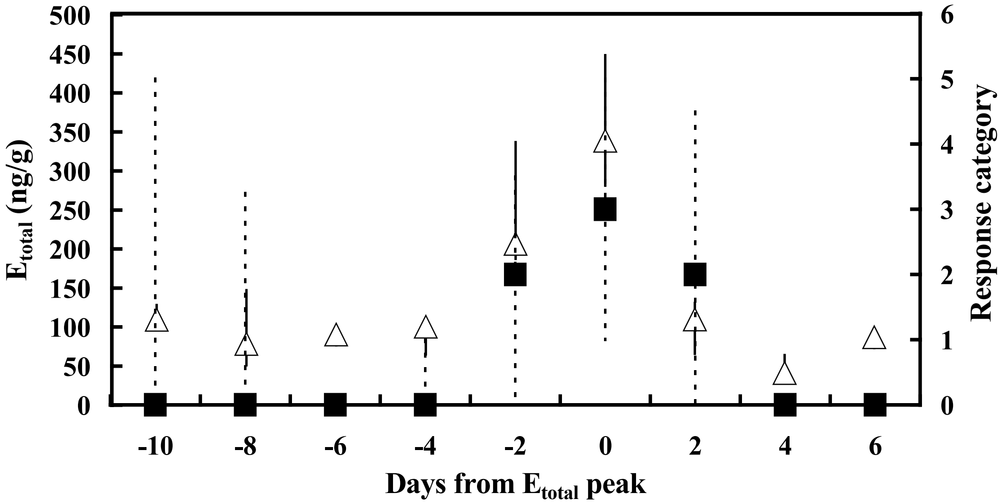

Figure 5. Relationship between the median male response intensity (■) for all male-female pairs (*N* = 15) and median female oestrogen level (△) in all study females (*N* = 5) on a given day. Days were aligned to the day of maximum oestrogen level (day 0). Shown are the median and the first and third quartile.

Our findings that male free-living longtailed macaques were able to discern the fertile phase are in accordance with former studies on macaques in captivity that reported a close temporal relation between mating frequency and timing of ovulation (Michael & Zumpe 1993; Zumpe & Michael 1996; Aujard et al. 1998). The degree with which male longtailed macaques recognize female reproductive status, however, is not clear. In our study, the fertile phase extended over 5 days, and mate guarding by dominant males usually exceeded this interval. Thus, a more conservative conclusion is that male longtailed macaques are able to discern periods with higher (fertile phase) and lower (nonfertile phase) probability of ovulation, rather than to pinpoint the day of ovulation. Nevertheless, even this ability exceeds that of Hanuman langurs, the only other primate species for which such data in a free-ranging population are available. In the langur, patterns of female monopolization by dominant males and male copulatory response to female solicitations gave no indication that males were able to recognize the fertile phase at all (Heistermann et al. 2001). Although the reasons for these species differences are not understood, several factors may provide at least a partial explanation. Sexual swellings, copulation calls and pheromones are all important as potential cues of female reproductive status in macaques (Michael & Keverne 1968, 1970; Semple & McComb 2000; Zinner et al. in press). In contrast, sexual swellings and copulation calls are absent in Hanuman langurs, and there is no evidence for the use of pheromonal cues in this species.

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

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

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

Our results indicate that not only dominant but also subordinate males, which are usually prevented from mating with a female during her fertile phase, are able to recognize the fertile phase. Therefore, all adult males should be able to assess their probability of paternity. If so, this would lead to an enhanced risk of infanticide, because any subordinate male subsequently achieving dominance status would be potentially infanticidal (Hrdy 1979; Hrdy & Whitten 1987; van Schaik et al. 2000). However, given the reatively low degree of accuracy pinpointing the time of ovulation, Nunn's (1999) graded-signal hypothesis predicts that even males mating with females exclusively outside the fertile phase should not be able to exclude the possibility of fathering an infant, and this, in contrast, would lower the risk of infanticide. However, we could not determine the extent to which male longtailed macaques in our study were able to assess their probability of paternity and how this affects the risk of infanticide. Infanticide has been reported for longtailed macaques in the wild (de Ruiter et al. 1994), but such events are rare.

Taken together, the results of our study show that free-ranging male longtailed macaques were able to discern the fertile phase, particularly in cycles leading to conception. Their degree of precision in determining the time of ovulation, however, is not known. Oestrogen-related cues appear to be involved in the process of female reproductive assessment by males, but the exact nature of these cues and how males use them under natural conditions remains to be clarified. Further studies addressing these issues should improve our understanding, not only of the proximate mechanisms underlying reproductive strategies, but also of how sexual selection in general has contributed to evolutionary processes within the catarrhine primate taxon.

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3

Female sexual behaviour, but not sexual swelling reliably indicates the timing of the fertile phase in wild longtailed macaques (*Macaca fascicularis*)

with J.-K. Hodges, C. Niemitz, M. Heistermann

Abstract

The extent to which catharrine primate males are able to discern the fertile phase during the female ovarian cycle under natural conditions is still debated. In a recent study, we showed that wild male longtailed macaques are able to detect the fertile phase, but the cues males used to assess female reproductive status remained unclear. In the present study, we tested female sexual swelling and specific female behaviours for their reliability in signalling the fertile phase as determined by measurement of faecal estrogens (E) and progestogens (P) during nine ovulatory cycles in seven free-ranging females. We found that changes in sexual swellings showed a significant positive correlation to the E:P ratio, but swelling size did not significantly differ between cycle phases. In contrast, the frequency of two of the tested female behaviours, namely initiation of sexual interactions and reaching back for the male during copulation, was not only correlated with female reproductive hormones, but was significantly elevated during the fertile phase compared to non-fertile phases of the cycle. We thus conclude that female sexual swelling does not reliably indicate the timing of the fertile phase in longtailed macaques, whereas certain female behaviours do. Since cycles differed considerably in the number of males with which females had sexual interactions as well as in the number of sexual interactions with dominant males, the signalling character of these specific female behaviours appears to be robust against inconsistencies in these social variables. Female behaviour may therefore play an important role fot the recognition of the fertile phase by male macaques under natural conditions.

Introduction

Knowing the time when copulation can lead to conception (i.e. the fertile phase), is important for males, especially those in multimale groups, since it allows them to concentrate their efforts on competing for access to females to particular stages of the ovarian cycle. Thus, recognition of the fertile phase can reduce considerably the reproductive costs for males. In most mammalian taxa, males can easily detect the fertile phase, as it is usually the short period in which females show sexual activity. Catarrhine primates, however, deviate from this general mammalian pattern in that the period of female sexual receptivity considerably exceeds the length of the fertile phase. Although it has been proposed that extended receptivity represents part of a female strategy to conceal the fertile phase from males in order to gain their own reproductive benefits (e.g. Hrdy 1979; Nunn 1999b; van Schaik et al. 2000; Heistermann et al. 2001), there is little empirical evidence showing to what extent male primates are able to discern the fertile phase, particularly in naturally reproducing groups. In a recent study on wild longtailed macaques (*Macaca fascicularis*), we demonstrated that, irrespective of rank (and therefore access to females), males were most sexually attracted by females during the fertile phase of the cycle (Engelhardt et al. 2004). From these findings we concluded that male longtailed macaques are able to discern the timing of the fertile phase under natural conditions (Engelhardt et al. 2004). Although the nature of the cues used by males to assess the timing of the fertile phase was not investigated, our finding that male interest in females was positively correlated with female oestrogen levels suggests that these cues were oestrogen-related. Given that ovulation is preceded by a period of elevated oestrogen levels, any female trait that closely correlates with ovarian estrogens, such as pheromone production, copulation calls, sexual swellings and a variety of female behaviours, could be a potentially reliable indicator of the timing and the duration of the fertile phase. Here, we investigate the usefulness of two of these potential cues, namely female sexual swelling and socio-sexual behaviour as indicators of the fertile phase in a naturally reproducing group of longtailed macaques living in the Gunung Leuser National Park, North Sumatra, Indonesia.

Sexual swellings occur in the majority of catarrhine primates living in multimale-multifemale groups (Dixson 1998). Due to the fact that the size of swellings is regulated by female oestradiol and progesterone levels (e.g. Carlisle et al. 1981; Ozasa & Gould 1982; Onouchi & Kato 1983) and that the period of maximum swelling shows a close temporal relation to the timing of ovulation in many species (e.g. *Macaca nigra*, *Papio cynocephalus*;

reviewed in Nunn 1999b), it has been proposed that swellings provide information on female reproductive state useful to males for assessing the timing of ovulation (Hamilton, 1984; Aujard et al. 1998). The assumption that males use swelling size in this way is supported by the fact that swellings increase female attractiveness and stimulate male sexual arousal (Girolami & Bielert 1987; Bielert et al. 1989) and that male mating frequencies increase with increasing swelling sizes (Reichert et al. 2002; Deschner et al., in press). Since, however, the duration of maximum swellings can be long and variable and the timing of ovulation within this period is also unpredictable (see Nunn 1999b; Reichert et al. 2002; Deschner et al. 2003), their precise value as a cue for detecting the female fertile phase remains unclear (e.g. Nunn 1999b; Deschner et al. 2003; Zinner et al. 2004) and probably differs between species.

Sexual swelling in longtailed macaques is characterised by an inflation of the tissue of the female's tail root and a red coloration of the appending skin, but data on its function in a reproductive context are sparse. In the only previous study, maximum swellings were reported to be considerably smaller in older compared to younger females and changes in swelling size did not show any clear or consistent pattern (van Noordwijk 1985). Nevertheless, copulations occurred at higher frequencies during the periods of increasing swelling size (van Noordwijk 1985), but since the relation between swelling patterns, female sex hormone levels and female reproductive state in longtailed macaques was not investigated, the reliability of sexual swellings in signalling the female fertile phase in this species remains to be determined.

In macaques, certain socio-sexual behaviours have also been reported to vary during the cycle in a way that may be useful to males in discerning the fertile phase. In captive studies, behaviours, such as female solicitations, initiation of proximity to males, grooming males etc., have all been reported to show a strong relation with female ovarian sex hormones and to increase around the time of ovulation (reviewed in Michael & Zumpe 1993, see also Zehr et al. 1998, Aujard et al. 1998). Although this suggests that certain female behaviours might reliably indicate the timing of the fertile phase, more recent data have shown that the relation between copulation frequencies and timing of ovulation can also vary markedly depending on the composition of the social group (i.e. mixed pairs, singlemale–multifemale groups, multimale–multifemale groups) (Wallen 2001). Accordingly, Wallen (2001) has proposed that among primates, social context strongly influences not only sexual behaviour itself, but also the way in which it is affected by hormones. Thus, the usefulness of behaviour for identifying female reproductive status can be questioned. In the only published study referring to this topic in macaques living under natural conditions, van Noordwijk (1985) did not find any regular changes in the frequency of copulations, but her observations were neither related to female sex hormone patterns nor to female reproductive stage (i.e. fertile phase). Therefore, the significance of female behaviour as an indicator of the fertile phase in wild macaque groups remains unclear.

In the present study, we evaluated sexual swelling and female socio-sexual behaviours as potential indicators of the fertile phase in female longtailed macaques living under natural conditions. Specifically, the size of female sexual swellings and the frequencies of selected female socio-sexual behaviours were examined in relation to the timing of the fertile phase as determined by faecal oestrogen and progestogen measurements. We also investigated whether social factors, such as harassment during sexual interactions, the number of mating partners per day and/or the number of sexual interactions with dominant males influenced the frequencies of the selected female behaviours and their reliability to signal the fertile phase.

Methods

Animals and Study Site

The study was carried out from February until October 2000 on longtailed macaques living around the Ketambe Research Station (3º41’N, 97º39’E), Gunung Leuser National Park, North Sumatra, Indonesia. The study area consists of primary lowland rainforest and has been described by Rijksen (1978) and van Schaik & Mirmanto (1985). The longtailed macaques in the area have been studied since 1979. We focused on group HA, which originated from a group (House group) that had recently split up into three different groups, HA, HB and HD (B. Putragayo, pers. comment). All group members of group HA were individually known and well habituated to observers. The group consisted of eight adult females, five adult males and several subadults/juveniles and (at the beginning of the study) two infants. All adult females served as study subject (Table 1).

The homerange of group HA overlapped with those of groups HB and HD and with that of a third group, KB. Females of group HA engaged in sexual interactions with males from all adjacent groups, which were all individually known and also well habituated to observers.

Monitoring of Sexual Swellings

In contrast to many other primates, in which sexual swellings occur around the anogenital region, female longtailed macaques exhibit a swelling on the tail root. Based on observations in a pilot study conducted immediately prior to the period of data collection,

Table 1. Dominance rank, parity and number of ovarian cycles of all females of the study group

Study female	Rank	Parity	Nonconception cycles	Conception cycles
SA	1	primiparous	2*	1
FA	2	nulliparous	0	1
KA	3	multiparous	3†	0
AA	4	multiparous	0	1
LA	5	multiparous	0	1
CA	6	multiparous	0	1
MA	7	nulliparous	0	1
IA	8	multiparous	0	0

* The first cycle showed irregular hormone patterns.
† The second cycle was anovulatory.

swellings were classified into five different categories using a drawing as reference (Fig.1). The categories ranged from 0 to 4, defined as follows: 0: no swelling; 1: slight, even swelling of the tail root; 2: prominent, even swelling; 3: prominent swelling with small lateral lumps; 4: prominent swelling with big lateral lumps. Swellings were categorised visually on a daily basis mostly by A.E. and, in a few cases, by four experienced field-assistants. Since the sexual swelling always showed a reddening, irrespective of the degree of swelling, and further colour gradation could not be distinguished objectively, coloration of the sexual swelling was not used as a criterion.

Behavioural Observations

Behavioural observations were conducted by A.E. and four experienced Indonesian field-assistants. Two to three observers followed the group at the same time. Sexual interactions were recorded using the all occurrence sampling method (Altmann 1974). For this, we noted the identity of the interacting partners and the occurrence or absence of the following behaviours: female approaches male, male approaches female, female presents anogenital region to a male ("Präsentieren", Angst 1974), female refuses sexual interaction (by moving away), male inspects female genitalia ("Inspizieren", Angst 1974), male mounts, intromission, female reaches back for the male during copulation ("Beinfassen", Angst 1974), female calls during copulation, female grooms male after copulation (either immediately or after being groomed by the male). Furthermore all agonistic interactions (threatening, chasing,

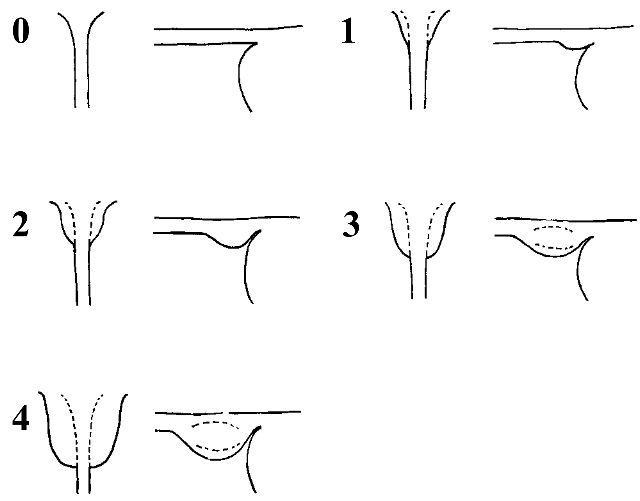

Figure 1. Swelling size categories: Swelling sizes were classified by comparison with a schematic drawing of the dorsal and lateral view of a female's tail root (see Methods for details).

biting, grabbing and pulling a fleeing female to force copulation) between the sexual partners or with a third party during sexual interactions were noted on occurrence.

In addition to the recording of sexual and agonistic interactions, each female was followed for about two hours a day (average: 1.6 h/day) and consortships were recorded with the continuous focal animal sampling method (Martin & Bateson 1993). Consortships were defined as an adult male following an adult female or vice versa within 10 m distance for at least ten consecutive minutes. Usually, both consort partners engaged in sexual interactions, but sometimes a consorting male was replaced by a higher-ranking male before he was able to closely approach the female. When consort pairs were walking away from the group, their relationship was still recorded as a consortship when being up to 20 m apart from each other.

When a female stayed in consort for ≥100 minutes, focal animal sampling was continued for the rest of the day. From this day on, the female was followed from dawn to dusk until the day males stopped consorting. When a female became an all-day focal animal, the other females were followed only every second day. In the case that two or three females simultaneously engaged in long-lasting consortships on the same day, one female was followed the whole day and the other two females were followed for only half a day. The average observation time per female was 6.1 h/day. Furthermore, the group was checked for additional consortships every 30 min. In the case of female MA, observations during the fertile phase took place only on two days because on the other days, the female was lost by the observer when moving away from the group very early in the morning.

Dominance rank of all individuals was determined by the display of the ’bared-teeth face’, a unidirectional submissive display (van Hooff 1967), and the outcome of agonistic interactions which were recorded whenever observed.

Faecal Sample Collection and Hormone Analysis

From each study female, 2–3 g faeces were collected on four to seven days per week and stored in 15 ml absolute ethanol at 4° C until hormone analysis. Prior to hormone measurements, faecal samples were twice extracted according to the method described by Ziegler et al. (2000). Faecal extracts were then measured for concentrations of immunoreactive oestrogen and progestogen metabolites using previously validated enzymeimmunoassays for the measurement of total estrogens (iEtotal) and pregnanediol-3-glucuronide (iPdG; Engelhardt et al. 2004). Both measurements have been shown to provide reliable information on female reproductive status and timing of ovulation in longtailed macaques (Shideler et al. 1993; Engelhardt et al., 2004). In the Etotal assay, an antiserum raised in a rabbit against 1,3,5(10)-estratrien-3,17β-diol-17HS-BSA (Meyer et al. 1997) was used. With 17β-estradiol as standard (100%), the antiserum showed the following cross-reactivities: estrone 200%, 17α-estradiol 119%, estriol 2% and for all other steroids tested <0.1%. The PdG EIA used an antibody raised in a rabbit against pregnanediol-3-glucuronide-BSA. Relative to PdG (100%), the antiserum showed the following cross-reactivities: 20α-hydroxyprogesterone 32%, pregnanediol 22%, 5α-pregnane-20α-ol-3-one 14%, progesterone 0.5% and <0.1% for all other steroids tested. Sensitivity of the assays at 90% binding was 1.5pg for iEtotal and 20pg for iPdG. Intra- and interassay coefficients of variation, determined by replicate determinations of high- and low-value quality controls, gave values between 2.7% and 7.5%, and 8.5% and 16.7%, respectively.

Definition of the Female’s Cycle Phases

Based on the defined postovulatory rise in iPdG levels, we determined for each cycle a two-day period in which ovulation was most likely (ovulation window) as described in Engelhardt et al. (2004). Since, in contrast to our previous study, however, in almost all cycles faecal sample collection occurred on a daily basis during the periovulatory period, the 'ovulation window' used here comprised two instead of three days. Accordingly, the fertile phase, defined as the possible days of ovulation plus the two preceding days (Engelhardt et al. 2004), covered four instead of five days. The eight days preceding the fertile phase will hereafter be called the prefertile phase and the eight days following this phase will be called the postfertile phase.

Data Analysis

Since males seem to assess a female's fertile phase correctly only during cycles with a normal hormonal pattern (Engelhardt et al. 2004), only regular, ovulatory cycles were included into the analysis. Cycles were defined as being regular when 1) a significant PdG-rise and maintenance of elevated PdG-levels for at least three consecutive days was noted and 2) the rise in PdG was preceded by an elevation in Etotal levels in the week before. Based on this definition, the first nonconception cycle of female SA (no elevation in Etotal in the week preceding the PdG rise) and the second nonconception cycle of female KA (anovulatory, no PdG rise) were excluded from analysis. Cycles from females which had infants younger than five month of age were excluded from analysis per se, because lactation is known to have a disturbing effect on cycle regularities (Ördög et al. 1998; McNeilly 2001). In the following, nonconception cycles will be numbered by occurrence and asterisks will mark conception cycles.

For analysis of swelling size in relation to female sex hormones and reproductive stage, relative measures of swelling sizes were used, because we were interested in how swelling size changed in relation to its maximum size. In this respect, the maximum swelling size category of each cycle was set to be 100%. For each day and cycle, the swelling size category of a given day was expressed as a percentage of the maximum swelling size of this cycle.

To determine changes in female sexual motivation, the frequency of female solicitations in relation to observation time on a given day were calculated. Female longtailed macaques solicit sexual interactions to males by presenting their anogenital region (see above; Zumpe & Michael 1983). Only those solicitations which occurred after the female had approached the male were included into the analysis to eliminate effects caused by differences in the frequency with which females were approached by males. The frequency of sexual interactions was calculated twice, first taking all male partners into account and secondly considering dominant males only. The definition of dominant males includes the alpha- and the beta-males of group HA as well as those of the adjacent group HB,who altogether were engaged in 83.6% of all analysed sexual interactions.

The number of male approaches refused by a female on a given day was related to the total number of male approaches. Furthermore, we related the number of copulations, in which a female reached back, the number of copulations in which a female vocalized, and the number of copulations after which a female groomed the male, on a given day to the total number of copulations performed by the female on that day.

To test for a relation between female sex hormone profiles and relative swelling sizes, time spent in consortship and the specific behaviours recorded, the days of the different cycles were aligned to the day of the faecal iPdG-rise (day 0). Only data from day –17 until day +10 relative to the day of the iPdG-rise were used for analysis. To eliminate the effect of missing values, the mean of two successive days for each cycle and parameter was taken as one data point during analysis. Then the median hormone value of all cycles and the respective median of the other parameters (swelling size, frequency of the specific behaviours) on a given day was calculated and related to the median values using the Spearman's rank correlation coefficient.

To test for differences in swelling size, time spent in consortship and frequencies of the different behavioural parameters between the three defined cycle phases, the median values were calculated for each parameter and cycle recorded during the prefertile, fertile and postfertile phase. The median values of the different cycles were then tested for significant differences between cycle phases using the Friedman test, with post hoc comparisons using the Wilcoxon signed-ranks test. All statistic tests were conducted with SPSS 11.0 for Windows and used two-tailed probabilities.

Results:

Sexual Swellings

Changes in the size of sexual swellings showed a similar pattern in all cycles: Median size increased from day –16 until day –10 relative to the day of the iPdG rise (day 0), when maximum size was reached. Swelling size started to decrease on day +4 (Fig. 2). The changes in swelling size were significantly positively correlated with the oestrogen (E): progestogen (P) ratio (Spearman rank correlation: $rs = 0.60$, $N = 14$, $P = 0.02$). The relation with individual hormones, however, (positive for E and negative for P) did not reach statistical significance (Spearman rank correlation: *rs*(E) = 0.44, *N* = 14, n.s.; *rs*(P) = -0.46, *N* = 14, n.s.; Fig. 2), even not when testing conception cycles only (Spearman rank correlation: *rs*(E) = 0.16, *N* = 14, n.s.; *rs*(P) = -0.23, *N* = 14, n.s.).

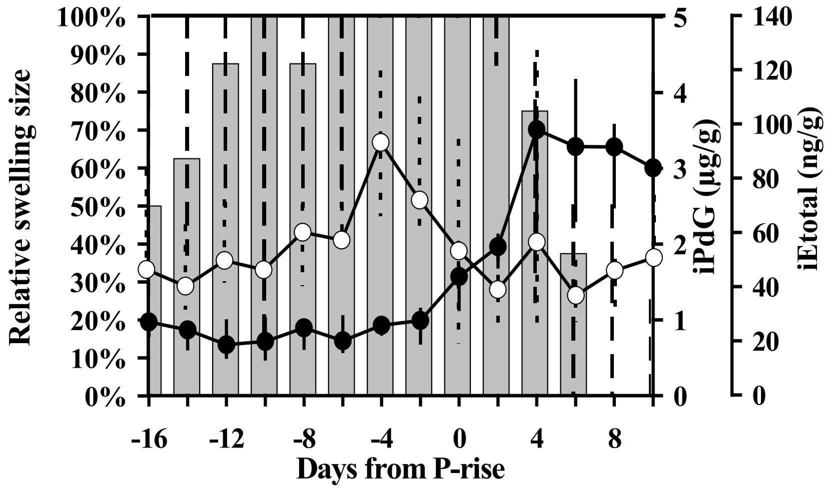

Figure 2. Relative swelling size (grey bars) of all cycles (*N* = 9) in relation to female oestrogen (○) and progestogen levels (●) on a given day (median, 1st and 3rd quartile). Days were aligned to the day of the defined rise in progestogen levels (day 0).

Individuals differed considerably in maximum swelling size attained (Fig. 3): nulli- and primiparous females had bigger swellings (maximum: category 4) than multiparous females (maximum: category 1–2). The duration of the maximum swelling period varied considerably within and between individuals (range: 5–25 days; Fig. 3), but in all cycles included the fertile phase. With an average duration of 15 days, the period of maximum swelling was almost four times the length of the defined fertile phase. As a consequence, median swelling size did not significantly differ between the prefertile, fertile and postfertile phase (Friedman test: $\chi 2$ = 4.9, *df* = 2, *N* = 9, n.s.). The same result was obtained when only conception cycles were included in the analysis (Friedman test: $\chi 2$ = 3.5, *df* = 2, *N* = 6, n.s.).

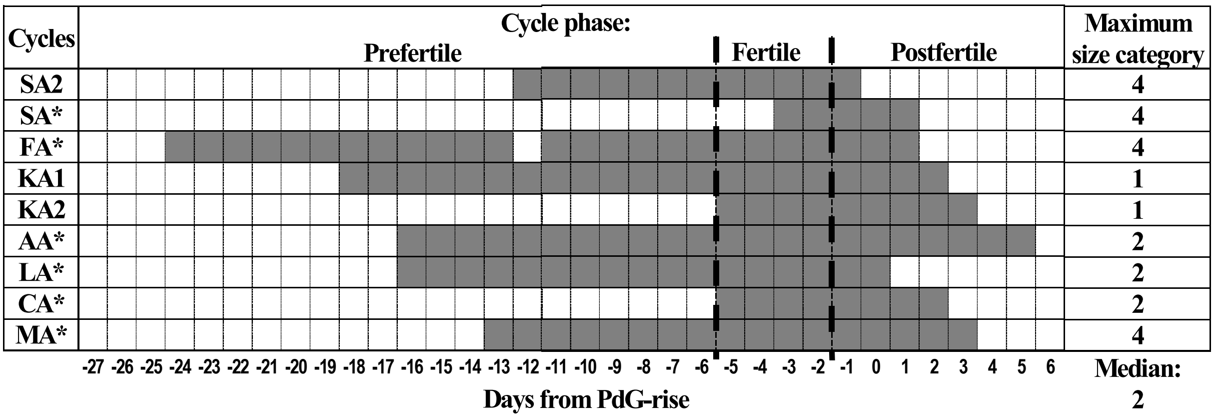

Figure 3. Maximum swelling size and temporal occurrence of the maximum swelling (shaded area) in relation to the timing of the faecal PdG rise and the fertile phase. Females are sorted by rank, nonconception cycles are numbered by occurrence, asterisks mark conception cycles. Female reproductive status is given in Table 1.

Consortships

In 87.2% of all consortships, males followed females. The time females spent in consort was positively correlated with Etotal-levels and negatively correlated with PdG-levels (Table 2). The timing and intensity of consortships varied between cycles: whereas during some cycles, females were consorted during all three cycle stages (cycle AA*, CA*, FA*, LA*, MA*, KA3), in other cycles, females had no consort partner during the prefertile (cycle SA*) or postfertile (cycle KA1, SA2) phase. All females, however, were consorted during the fertile phase (range: 30–100 % observation time). Furthermore, females spent significantly more time in consort during the fertile phase compared to either the prefertile or postfertile phase (Table 3).

Table 2. Consortships, sexual interactions and the frequency of specific female behaviours in relation to endocrine parameters

	Etotal		PdG		Etotal:PdG	
	rs	p	rs	p	rs	p
% Observation time spent in consortship	0.62	<0.02	-0.64	<0.02	0.82	<0.001
Sexual interactions/h	0.57	<0.05	-0.68	<0.01	0.81	<0.001
Female solicitations/h	0.68	<0.01	-0.54	<0.05	0.67	<0.01
% Male approaches refused by the female	0.47	n.s.	-0.28	n.s.	0.62	<0.02
% Copulations during which the female reached back	0.69	<0.01	-0.45	n.s.	0.73	<0.001
% Copulations with female copulation calls	0.45	n.s.	-0.57	<0.05	0.62	<0.02
% Copulations followed by female grooming	0.61	0.02	-0.64	<0.02	0.7	0.005

Sexual Activity and Agonistic Interactions

Altogether, 1,330 sexual interactions were recorded, of which 15.2% were female initiated. When males approached females, these approaches were refused by the female in 18.2% of all cases. The frequency of sexual interactions showed a significant positive correlation with female oestrogen and a negative correlation with female progestogen levels (Table 2). A quarter of all sexual interactions was observed during the four days of the fertile phase, when the frequency of sexual interactions per female and per day was significantly elevated compared to the two other cycle stages (Table 3).

Table 3. Differences in behavioural parameters between the prefertile, fertile and postfertile phase. The table shows mean values of all cycles

	Cycle phase:					Prefertile versus fertile phase		Postfertile versus fertile phase	
	Prefertile	Fertile	Postfertile		p	Z	p	Z	p
% Observation time spent in consortship	40.4	74.3	16.7	14.2	0.001	-2.4	<0.02	-2.7	<0.01
Sexual interactions/h	0.63	0.84	0.31	9.6	<0.01	-1.2	n.s.	-2.5	0.01
Female solicitations/h	0.12	0.19	0.09	6.1	<0.05	-2.2	<0.05	-2.3	0.02
% Male approaches refused by the female	22.9	18.5	17.8	0.8	n.s.				
% Copulations in which the female reached back	40.2	64.1	39.2	10.7	0.005	-2.7	<0.01	-2.4	<0.02
% Copulations with female copulation calls	86.8	89.2	75.8	1.5	n.s.				
% Copulations followed by female grooming	28.7	28.9	19.2	2.9	n.s.				

Females differed from each other in (A) the median number of daily male sexual partners per cycle phase (range prefertile phase: 0.04–0.36 partner/h; range fertile phase: 0.09–0.48 partner/h; range postfertile phase: 0.00–0.20 partner/h) and (B) the median number of daily sexual interactions with dominant males per phase (range prefertile phase: 0.00–0.68 sexual interactions/h; range fertile phase: 0.04–1.19 sexual interactions /h; range postfertile phase: 0.00–0.24 sexual interactions /h). The frequencies of both variables significantly decreased from the fertile to the postfertile phase (A: Friedman test: $\chi 2 = 7.4$, $df = 2$, $N = 9$, $P < 0.05$; Wilcoxon signed-ranks test: $Z = -2.2$, $P < 0.05$; B: Friedman test: $\chi 2 = 11.6$, df = 2, N = 9, $P < 0.005$; Wilcoxon signed-ranks test: $Z = -2.7$, $P<0.01$), but there were no significant differences in any of the two parameters between the fertile and prefertile phase (Wilcoxon signed-ranks test A: $Z = -0.3$, n.s.; B: $Z = -1.3$, n.s.).

Aggression during sexual interactions was rare: agonistic interactions between the sexual partners or with a third party were observed during only 4.3% of all sexual interactions. In only 3.7% of all cases in which females refused male approaches, females were subsequently punished by the male. The number of forced copulations was very low: Only 9 out of 952 observed copulations (0.9%) occurred after male aggression.

Female Behaviour

The frequency of all female behaviours investigated showed a significant positive correlation with the E:P ratio, but only soliciting, reaching back, vocalizing during copulation and grooming after copulation showed significant correlations with one of the individual hormones (Table 2).

All female behaviours occurred more frequently during the fertile phase compared to the prefertile and postfertile phase (Table 3), with the exception of female refusals of male approaches. With respect to statistical differences, however, only the frequencies of soliciting and reaching back during copulation differed significantly between the three cycle phases, with each behaviour occurring most frequently during the fertile phase although this was not true for all individual cycles (Table 3; Fig. 4a,b).

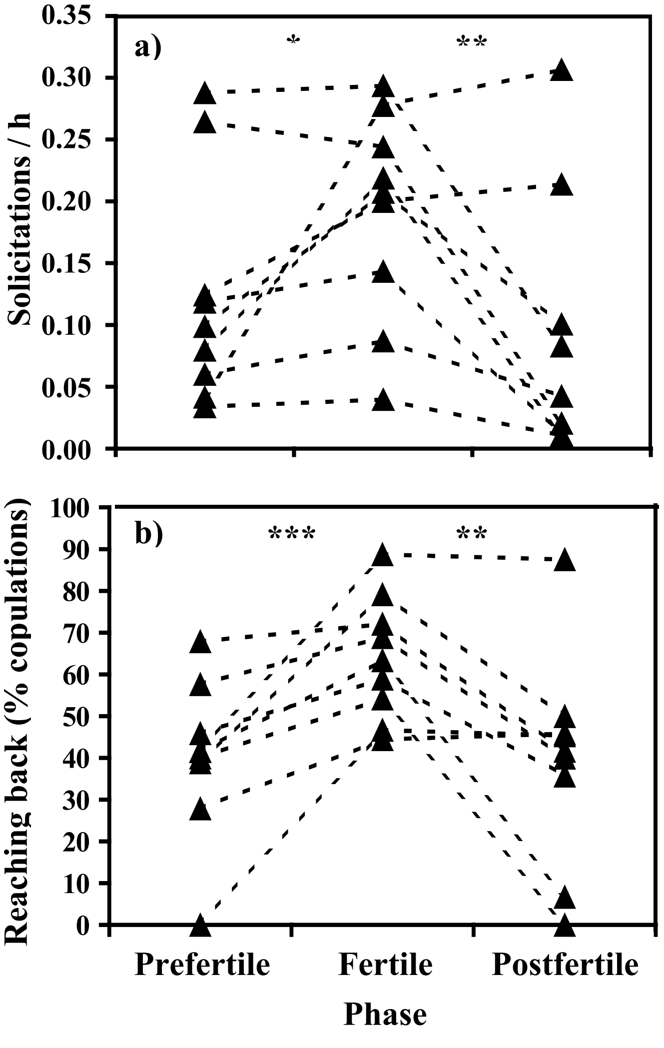

Figure 4. Median values during the prefertile, fertile and postfertile phase for a) the frequency with which females solicited sexual interactions and b) the proportion of copulations during which females reached back for the male (*$P < 0.05$; **$P \leq 0.02$; ***$P < 0.01$).

Discussion

Based on the data presented here, we conclude that female sexual behaviour, but not sexual swelling is a reliable indicator for the timing of the fertile phase in wild longtailed macaques. Specifically, it was shown that the frequencies of female solicitations and of reaching back for the male during copulation had a close temporal association with female sex hormone levels and were significantly elevated during the fertile phase. In contrast, although sexual swelling size was significantly correlated with the oestrogen:progestogen ratio, the duration of maximum swellings greatly exceeded the length of the fertile phase, and there was no statistically significant difference between swelling sizes during the eight days preceding or following the fertile phase compared to those during the fertile phase.

In the study females, changes in swelling size correlated with the oestrogen:progestogen ratio and followed a regular pattern during the ovarian cycle in both young females with big swellings and older females with small swellings. The data in longtailed macaques are thus consistent with findings from other catarrhine primate species showing that sexual swellings are regulated by female sex hormones (e.g. Carlisle et al. 1981; Ozasa & Gould, 1982; Onouchi & Kato, 1983) and show a characteristic pattern during the female ovarian cycle (e.g. *Macaca nemestrina*: Bullock et al. 1972; *Macaca tonkeana*: Thierry et al., 1996; *Papio cynocephalus*: Wildt et al. 1977; *Pan paniscus*: Heistermann et al. 1996). They differ, however, from the findings of van Noordwijk (1985), who was unable to discern a regular pattern in changes in swelling size. The most likely reason for the discrepancy in results is that the use of faecal steroid analysis made it possible to align swelling size to defined reproductive stages within the ovarian cycle, whereas van Noordwijk’s interpretation of her data was, at best, based on assumptions of female reproductive status inferred from backdating conception from the time of birth. Furthermore, for the classification of swelling size, we added an additional category for very small swellings, because older females showed only tiny, but still visible changes in swelling size.

Despite showing a regular cyclical pattern, changes in swelling size did not appear to provide reliable information about the timing of the female fertile phase. Similar to findings in common chimpanzees (*Pan troglodytes*: Deschner et al. 2003) and bonobos (*Pan paniscus*: Reichert et al. 2002), the period of maximum swelling in longtailed macaques greatly exceeded the fertile phase, and detumescence occurred well after the end of the fertile phase.

Furthermore, the period of maximum swelling size of 15 days found here for longtailed macaques is longer than that in many other catarrhine species, including those in which maximum swelling is also more closely associated with the timing of ovulation (e.g. *Macaca nigra*: Thomson et al. 1992; *Macaca sylvanus*: Möhle et al. unpublished data; *Macaca tonkeana*: Aujard et al. 2001; *Papio cynocephalus*: Wildt et al. 1977). While it appears that maximum swelling size in other macaques and baboons provides reliable information on the precise timing of ovulation, findings of a highly variable timing of ovulation within the maximum swelling period would suggest that swelling size in longtailed macaques indicates the probability of ovulation (as has been proposed for conspicuous swellings by the 'graded-signal hypothesis', see Nunn 1999b) rather than its precise timing (as generally suggested by Hamilton 1984). Furthermore, since in female longtailed macaques, swellings almost disappear with advancing age, it appears that the value of the swelling as a signalling character for female reproductive status has greater relevance for younger females than for older ones. More studies are, however, needed to clarify the functional significance of sexual swellings in longtailed macaques and why they decline with age.

In contrast to sexual swellings, two of the observed female behaviours showed a close relation to the fertile phase. The frequency with which females initiated sexual interactions and the number of copulations during which they reached back for the male, were both significantly correlated with female sex hormone levels and clearly elevated during the fertile phase. This suggests that changes in these behaviours may provide a reliable signal of the timing of the fertile phase in wild longtailed macaques. This not only confirms studies on macaques in captivity in which a strong relation between these behaviours, ovarian steroids and timing of conception has been found (Zumpe & Michael 1968; Wallen et al. 1984; Zumpe & Michael 1985; Aujard et al. 1998; Zehr et al. 1998; Zehr et al. 2000), but also show that the signalling character of these specific female behaviours is robust against social influences deriving from the complex environmental and social conditions primates face in their natural habitat. Concerning the significance of female proceptive behaviour in signalling the fertile phase, longtailed (and presumably other) macaques seem to differ from Hanuman langurs (*Trachypithecus entellus*), to our knowledge the only other Old World monkey species in which this behaviour has been studied in relation to hormonally defined timing of ovulation in a natural setting. In this species, the frequency of proceptive behaviour was not enhanced during the periovulatory period (Heistermann et al. 2001). To what extent specific female behaviour provides reliable information about the timing of the female fertile phase in other taxa of catarrhine primates or whether this phenomenon is unique to macaques needs further investigation.

While female solicitations and reaching back for the male during copulation appeared to be minimally affected by social conditions, this was apparently not the case for other female behaviours. The number of male approaches refused by the female, the frequency of female copulation calls and of grooming the male after copulation did not show any significant changes during the fertile phase when compared to other stages of the female cycle, although they were significantly correlated with the oestrogen:progestogen ratio. The most likely explanation for this is that they were more affected by parameters other than hormonal state. In contrast to female solicitations, for example, females could not decide male identity and the number and timing of sexual interactions when being approached by a male. Given that the number of male approaches and the status of approaching males differed considerably between cycles, it is not surprising that the frequency of refusals was also highly variable. The factors which influenced the occurrence of female copulation calls and female grooming after copulation are not known, but it is unlikely to be male identity, since reaching back during copulation apparently was not affected by this parameter. Furthermore, male harassment or coercion and third party harassment during sexual interactions can be ruled out in this respect, because these events rarely occurred. Possibly other factors, such as predator avoidance, might have affected the utterance of copulation calls, and in the case of grooming the male after copulation, differences in female motivation throughout the ovarian cycle might be more expressed in grooming duration than grooming frequency (see Zumpe & Michael 1983) as measured here.

The results show that not only female sexual motivation was enhanced during the fertile phase, but also male sexual interest in females. The frequency of sexual interactions and the proportion of observation time spent in consortship, events that were mainly male initiated and in the case of consortships also male-maintained, were significantly higher during the fertile phase compared to the other phases of the cycle. These data confirm our previous finding that male longtailed macaques are most attracted to females during this period, and are thus able to detect the timing of the fertile phase within an ovarian cycle (Engelhardt et al., 2004). According to the present findings that specific female behaviours are signalling the timing of the fertile phase, these behaviours might play an important role in this recognition. In this respect, consortships, especially those which last for only a few hours and thus not long enough to serve mate guarding, may be used by males to closely monitor females and their behaviour. However, although overall statistically significant, differences in the frequency of female solicitations and reaching back for the male between the fertile and the other phases of the female cycle were not always strong and, in a few cycles, did not occur. Thus, these behaviours may be reliable indicators of the timing of the fertile phase, but their usefulness as a cue for males to discern this period from other phases of the female ovarian cycle seems to be limited. It is, therefore, likely that male longtailed macaques do not rely on behavioural changes alone when assessing female reproductive status, but use a combination of behaviours and other potential indicators of the fertile phase. Additional female traits which have the potential to signal the fertile phase include volatile aliphatic acids in the vaginal discharge of female macaques, which may serve as sex pheromones (Michael & Keverne 1968, 1970; Michael et al. 1971, but see Goldfoot 1981). Furthermore, although the frequency of female copulation calls does not seem to be related to female cycle stage, changes in the acoustic pattern of these calls may well be, as a study in Barbary macaques (*Macaca sylvanus*) has indicated (Semple & McComb 2000). How these traits are related to female sex hormone concentrations in longtailed macaques under natural conditions and whether they signal the timing of the fertile phase has to be investigated in future studies.

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4

Determination of male reproductive success in wild longtailed macaques (*Macaca fascicularis*) – alpha male mate guarding, female mate choice or postcopulatory mechanisms?

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Abstract

One of the basic principles of sexual selection is that male reproductive success should be skewed towards strong males in species with anisogamous sex. Studies on primate multimale groups, however, suggest that other factors than male fighting ability might also affect male reproductive success. Since many of these studies used mating success as a measure of reproductive success (even though both variables need not always be related to each other) and since behavioural investigations focusing on a female's fertile phase, as assessed by hormone analysis, are still rare, the proximate mechanisms leading to paternity in multimale primate groups still remain largely unknown. Using a combined approach of male and female behavioural observations during the fertile phase with faecal hormone and genetic analysis for the assessment of female reproductive status and paternity, respectively, we studied the relative importance of male monopolization, female direct mate choice and postcopulatory mechanisms for paternity determination in a wild group of longtailed macaques. Our results indicate that both male monopolization and postcopulatory mechanisms have a significant influence on male reproductive success. Females influence paternity only indirectly by mating more often with high-ranking males when not mate-guarded. Alternative male reproductive strategies did not affect paternity. The degree to which postcopulatory mechanisms were exerted depended on stability of the alpha male

position and on the dominant males' interest in females. Since paternity was restricted to alpha and beta males (although females mated with up to seven males), we conclude that not only male monopolization, but also postcopulatory mechanisms operate in favour of high-ranking males in longtailed macaques, thus stabilizing the reproductive skew in this species.

Introduction

A fundamental question in primate sexual selection concerns the proximate mechanisms underlying male reproductive success in multimale groups. According to sexual selection theory, males should compete for access to receptive females, the resource that limits male reproductive success the most (Trivers 1972). Contest among males for access to mating partners, in turn, should lead to social systems in which the strongest male is able to exclude other males from mating with fertile females (priority of access to oestrus females model; Altmann 1962; see also Fedigan 1983). That monopolization of fertile females plays an important role in maximizing male reproductive success in primate multimale groups has been concluded from the observation that dominant males often sire the majority of offspring (e.g. *Cercocebus torquatus atys*: Gust et al. 1998; *Macaca sylvanus*: Paul & Kuester 1996; *Mandrillus sphinx*: Dixson et. al. 1993; *Papio cynocephalus*: Altmann et al. 1996). Complete monopolization is, however, difficult to achieve and the fact that in some primate species subordinate and extra-group males have a significant share in paternity (e.g. *Macaca fuscata*: Inoue et al. 1993; *Macaca mulatta*: Berard et al. 1993, 1994, Widdig et al. 2004) indicates that other mechanisms operate as well. Consequently, the importance of female and alternative male reproductive strategies and the need to pay more attention to the interactive aspects of male and female mating strategies have become increasingly recognized in recent years (Bercovitch 1995; Chism & Rogers 1997; Janson 1984; Small 1989; Taub 1980; Vasey 1998; Small 1989; Sterck et al. 1997).

Successful monopolization of access to fertile females depends on several factors (Fig. 1). Among these, the ability to recognize a female's fertile phase (i.e. the brief period within a female ovarian cycle when copulation can lead to conception) may, for example, significantly affect male reproductive success. Since monopolization of females is costly (Alberts et al. 1996), males benefit from discerning the fertile phase of a cycle by being able to restrict mate guarding to this period. However, male ability to recognize the fertile phase seems to vary within the catarrhine primates, being absent in some species (*Trachypithecus* *entellus*: Heistermann et al. 2001), whereas existent in others (*Macaca fascicularis*: Engelhardt et al. 2004). Another factor that may restrict monopolization of fertile females is female cycle synchrony, since a single male can hardly monopolize more than one female at a time (Paul 1997; Nunn 1999a). Furthermore, fighting ability as well as coalition-forming of subordinate males can be an important determinant of male mating and mate guarding success, as has been shown for savannah baboons (*Papio cynocephalus*; Packer 1977; Bercovitch 1995; Noë & Sluijter 1990; Alberts et al. 2003). A third variable that may influence monopolization of females is female degree of cooperation, but since in anthropoid multimale groups, males usually dominate females, the significance of female cooperation for male reproductive success is still under discussion (Bercovitch 1995).

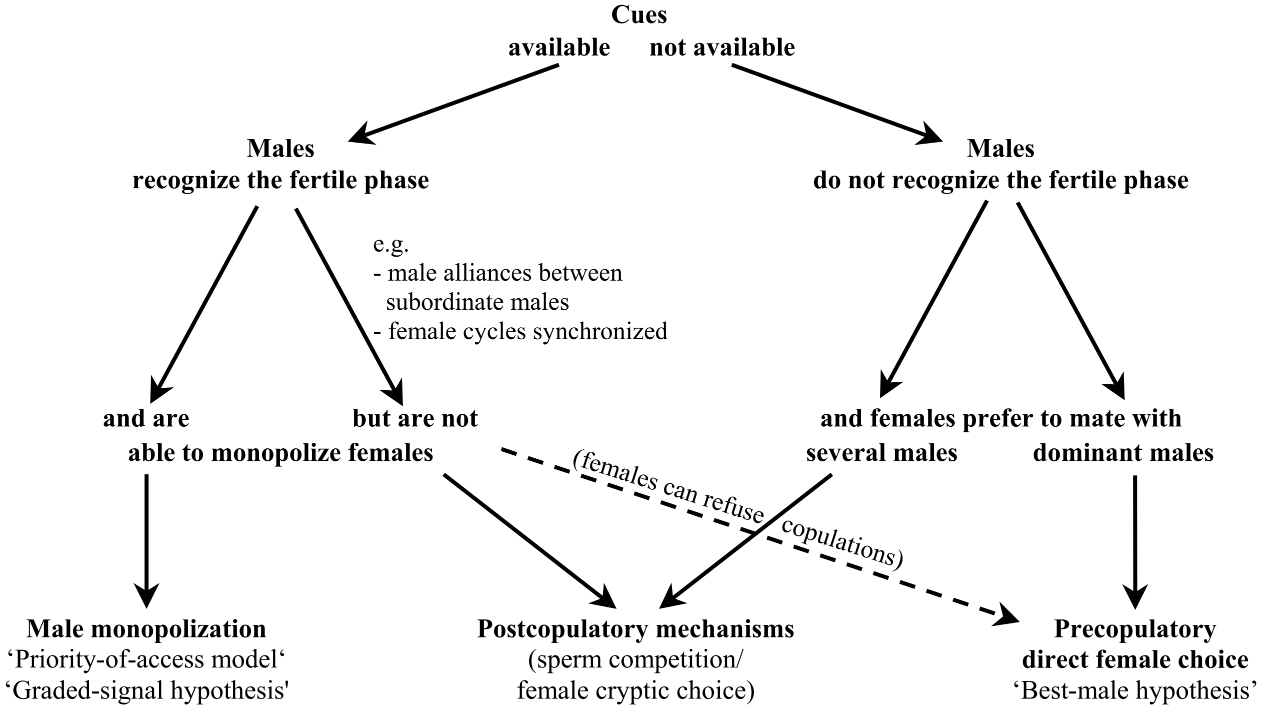

Figure 1. Mechanisms leading to male reproductive success depending on i) the availability of cues indicating the timing of the fertile phase, ii) monopolizability of females and iii) female mating preferences.

Nevertheless, female behaviour can generally be expected to affect male reproductive success due to the intersexual conflict over control of reproduction, leading to different and often opposing reproductive strategies in the gender (e.g. Trivers 1972; Smuts & Smuts 1993; Clutton-Brock & Parker 1995; Gowaty 1996; Nunn & van Schaik 2000). Two opposing behavioural strategies have been suggested for females to enhance their reproductive success: females should either try to get 'good genes' for their offspring by concentrating matings to the population's 'best male' (female direct choice; Small 1989) or they should try to mate polyandrously. Polyandrous mating can provide females with benefits (reviewed in Wolff & Macdonald 2004) by avoiding inbreeding or genetic incompatibilities (Newcomer et al. 1999; Zeh & Zeh 2001; Tregenza & Wedell 2002) or by confusing paternity, which, in turn, should reduce the risk of infanticide (Hrdy 1979; van Schaik et al. 2000; Heistermann et al. 2001) and enhance paternal care (Taub 1980). Whether females are able to mate polyandrously, however, depends on the degree of mate guarding by dominant males. The concealment of the fertile phase has, therefore, been interpreted as a female strategy to hamper mate guarding in order to get more freedom for reproductive decisions (van Schaik et al. 2000; Heistermann et al. 2001). If the probability of ovulation is only gradually and imprecisely signalled (graded-signal hypothesis; Nunn 1999b), females will be able to mate polyandrously with subordinate males during times with low (but not zero) probability of conception and to attract dominant males during times of higher probability.

If a female mates to a significant extent with more than one male during the fertile phase, paternity of the ensuing offspring will be determined at the postcopulatory level, since sperm of the different males will compete for fertilization. The number and viability of sperm a male brings into competition have, in this respect, been suggested to markedly affect his chances of siring offspring (for a review on sperm competition see Birkhead & Møller 1998). It may also be that females control which male will fertilize their egg by, for example, discarding or destroying sperm from unfavoured males (cryptic female choice; reviewed in Reeder 2003). To what extent paternity in multimale primate groups is determined on the postcopulatory level still remains largely unknown.

The aim of the present study was to investigate the relative importance of male monopolization, female direct mate choice and postcopulatory mechanisms for male reproductive success in wild longtailed macaques. In this species, dominant males appear to father the majority of offspring in the wild (de Ruiter et al. 1994), but the proximate mechanisms leading to the observed reproductive skew in favour of dominant males remain unclear. On the one hand, a pronounced sexual dimorphism in body and canine size (Plavcan & van Schaik 1992, 1997) indicating a high degree of contest competition among males and male dominance over females (Angst 1975), moderately seasonal reproduction (Kavanagh & Laursen 1984; van Schaik & van Noordwijk 1985), male ability to discern the fertile phase of a cycle (Engelhardt et al. 2004) and higher mating success in high-ranking males (van Noordwijk 1985; de Ruiter et al. 1994) all suggest that male reproductive success is mainly achieved by male monopolization in this species. On the other hand, a high testis:body weight index (Harcourt et al. 1981) and a high number of viable sperm in male ejaculates (Schrod 2002) point to a high degree of sperm competition. The female perspective is also contradictory: although female longtailed macaques display exaggerated sexual swellings that have been interpreted as a female attempt to attract dominant males during the fertile phase (Nunn 1999b), they have been observed to mate with males of all rank during this period in a pair choice experiment (Nikitopoulos 2003).

The specific aims of our project, therefore, were to assess the degree of male monopolization, female mating preferences and freedom for mate choice, and the potential for postcopulatory mechanisms to operate in a group of longtailed macaques living in the Gunung Leuser National Park, North Sumatra, Indonesia. For this, we combined detailed behavioural observations with noninvasive methods using faecal samples for i) the assessment of the fertile phase from progestogen profiles and ii) genetic paternity determination. The results achieved will contribute to a better understanding of the proximate mechanisms determining male reproductive success in primate multimale groups, and as such help to extend our knowledge about primate sexual selection.

Methods

Animals and Study Site

The study was carried out at the Ketambe Research Station (3°41'N, 97°39'E), Gunung Leuser National Park, North Sumatra, Indonesia. The research area consists of primary lowland rainforest and has been described by Rijksen (1978) and van Schaik & Mirmanto (1985). Observations and faecal sample collection occurred from February until December 2000.

The longtailed macaques of the study area have been studied since 1979 and were well habituated to observers. The study focused on a group which originated from a group (House group) that had recently split up into three different groups (B. Putragayo, pers. comment): group House Atas (HA), the focus group of this study, group House Bawah (HB) and group House Dapur (HD). At the beginning of the study, group HA consisted of eight adult females, five adult males and several subadults/juveniles and two infants. The homerange of group HA overlapped with that of groups HB and HD and further with that of a third group, group Ketambe Bawah (KB). Males from all adjacent groups and, in addition, those from a fifth group (group Antara), which had its homerange close by, but not overlapping, entered the homerange of group HA from time to time and engaged in sexual interactions with females. The alpha male of group HB (alpha male HB) joined the group HA almost daily from beginning of March 2000 until mid-April 2000 (Fig. 2). Furthermore, in the beginning of the mating season, a male who had been living solitary joined the group HA and challenged the alpha male position (challenger), but had not yet established dominance over the alpha male (alpha male HA) at the end of the study. All group members of the study group HA as well as all males that moved within the homerange of HA were individually known and habituated to observers.

Hormone Analysis and Definition of the Fertile Phase

Faecal hormone extraction and analysis conducted for assessment of female cycle stage and timing of the fertile phase has already been described in detail by Engelhardt et al. (submitted). In brief, 2–3 g faeces were collected from each adult female (Table 1) on four to seven days per week and stored in 15 ml absolute ethanol at 4°C until hormone analysis. Hormone metabolites were twice extracted from faecal samples, and extracts were measured for concentrations of immunoreactive oestrogen and progestogen metabolites using previously validated enzymeimmunoassays for the measurement of total oestrogens (iEtotal) and pregnanediol-3-glucuronide (iPdG; Engelhardt et al. 2004). Both measurements have been shown to provide reliable information on female ovarian function and timing of ovulation in longtailed macaques (Shideler et al. 1993; Engelhardt et al. 2004). In the following, non-conception cycles will be numbered by occurrence and asterisks will mark conception cycles.

Table 1. Dominance rank, parity and number of ovarian cycles of study females

Study female	Rank	Parity	Nonconception cycles	Conception cycles
SA	1	primiparous	2*	1
FA	2	nulliparous	0	1
KA	3	multiparous	3†	0
AA	4	multiparous	0	1
LA	5	multiparous	0	1
CA	6	multiparous	0	1
MA	7	nulliparous	0	1
IA	8	multiparous	0	0

* The first cycle showed irregular hormone patterns.
† The second cycle was anovulatory.

For each cycle, a defined rise in iPdG-level above a preceding baseline level was used to determine the presumed time of ovulation and to define the cycle's fertile phase as described in Engelhardt et al. (submitted). In this respect, we determined for each cycle a two-day period in which ovulation was most likely (ovulation window). This period comprised the days –2 and –3 relative to the iPdG-rise (day 0). Given that matings can be fertile when occurring up to two days before and on the day of ovulation (Behboodi et al. 1991), we defined the fertile phase to be the period comprising the two days of the ovulation window plus the two preceding days.

Two cycles differed from the other cycles: in one cycle (KA2), although female oestrogen levels were elevated and followed a normal pattern, ovulation did not occur (in the cycle prior to and after this cycle the female did ovulate, Table 1), and in the other, oestrogen levels were elevated more than a week before the iPdG rise. In both cycles, however, female sexual activity and male interest in the female was increased during the period of elevated female oestrogen levels, as it was in normal ovulatory cycles. Since earlier studies showed that both female sexual activity and female attractivity to males are related to female oestrogen levels (Engelhardt et al. submitted), and since the pattern of male and female behaviour was comparable to that during the fertile phases of ovulatory cycles, we included data from the four days surrounding the day of maximum female oestrogen values into our behavioural analyses. The four days included started on day –2 relative to the day of maximum oestrogen value (day 0), which was, on average, the third day of the fertile phase during the regular ovulatory cycles.

Genetic Paternity Analysis

Faecal samples for paternity analysis were collected from all female–infant pairs and from all 24 males that were observed moving within the homerange of group HA, irrespective of whether they had been observed to interact with females or not. From fresh faeces, 2–3 g were stored in absolute ethanol at 4°C until extraction. DNA was extracted from faecal samples using the QIAamp® DNA Stool Mini Kit (Qiagen, Hilden, Germany), following the instruction given in the manual. Extracts were stored at –20ºC until analysis and at 4ºC during the period of analysis.

In total, eleven primer pairs were used, which were originally designed for amplification of microsatellite loci in humans (D1S548, D3S1768, D6S501: Murray et al.

1995; D2S367, D5S502: Gyapay et al. 1994; D6S266, D8S271: Weissenbach et al. 1992; D8S601: Xu et al. 1996; D14S255: Kayser et al. 1996; D20S476: Sheffield et al. 1995) and Japanese macaques (*Macaca fuscata*, MFGT5: Inoue & Takenaka 1993). PCR was performed in a total volume of 10 µl by using a thermal cycler UNO (Biometra, Göttingen, Germany). The reaction mix contained 1 µl DNA extract and 1 x PCR buffer (containing 15 mM $MgCl_2$; Qiagen), 1x Q-Solution (except for D6S501; Qiagen), 200 µM of each dNTP (Boehringer, Mannheim, Germany), 0.5 µM of each primer, 0.25 units HotStarTaq Polymerase (Quiagen), 1 µg BSA, Aqua bidest. Solution for D8S271 contained additional 50 mM $MgCl_2$. The DNA was denaturated at 94°C for 3 min in an initial step followed by 37 cycles of a touch-down PCR with an annealing temperature decreasing from 56°C to 50°C (45 s/cycle) within four steps, except for D20S476, for which the annealing temperature was 61–55°C. Denaturation occurred for 30 s at 94°C and at 90°C in the final step. Elongation took place at 72°C for 60 s and for 45 s in the final step. The 5’ end of the forward primer was labelled by Cy5 for allele size determination. Each PCR included one to two control animals of known allele sizes (positive control) and a negative control. Allele size was determined using the automatic sequencer ALFexpress (Amersham Bioscience, Little Chalfont, UK) following the procedure described in Widdig (2003).

Since in some loci primers did not give optimal results, we designed *Macaca fascicularis* specific primers, which were located closer to the repetitive sequence than the human and Japanese macaque primers. For this, ligation of PCR products of the specific microsatellites into plasmid vector pCR®2.1-TOPO® was conducted with the TOPO TA Cloning®Kit (INVITROGEN, Carlsbad, USA) and colony hybridisation followed the procedures described in Takenaka et al. (1993). Plasmids containing the specific repeats were isolated from *E. coli* using the QIAprep Spin Miniprep Kit (Qiagen). Fluorescent sequencing was conducted with the Autocycle Sequence Kit Big Dye in the ABI Prism 3100 sequencer (Applied Biosystems, Foster City, USA). The selected primer sequences were then synthezised according to the selected sequences by Thermo Hybaid, Ulm, Germany (Table 2).

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

PCRs or if a second allele occurred only once in eleven independent PCRs. All infants could be genotyped for all loci. In the case that it was not possible to clearly determine two alleles for a certain locus, the locus was either omitted for the individual or all appearing alleles were taken as potential alleles of this individual.

Table 2. Sequences of the newly designed primers

Locus	Primer sequences (5'→3')
D3S1768	R: AAC TAC ATG ATT CTA GCA CA
D5S502	F: AGC CAT TAC ACT GAA TAG CGC R: TCA TTA AGC AAT GCA TAC
D6S266	F: CCA ATA TAT GAG TAA TTC TCT AAC C R: CCC TTC CTA CTA ATT ACT
D6S501	F: AAG GGC TTT ATC TGT ATA TAG T R: CCT CTC TAA TTC TCC ATT TC
D8S271	F: CTA GAT TGG AGG CTA TGT GT
D8S601	F: GCA CAG TTG GAT CTT GTG TC
D14S255	R: ATG CTC TTA GTG GTC ATT C
D20S476	R: AAA CTC AAN CCA AGC CCA GG
MFGT 5	F: TGT AGC TTC GCA CTG GGT

We compared the mother and offspring genotypes to deduce which infant allele was inherited from the father. If the mother and infant had the same two alleles, or if we were not able to genotype a mother at a certain locus, both infant alleles were considered as potential paternal alleles. Males who did not match with the deduced paternal alleles at anyone's locus were excluded from paternity. A male was considered the likely father, if (1) all other males were excluded from paternity and (2) he was compatible with the paternal alleles at least in six loci. In only one case, no male matched with the deduced paternal alleles in all genotyped loci. In this case, the only male that had only one mismatch with the paternal alleles was assumed to be the likely father. We furthermore calculated parentage likelihood using the program CERVUS 2.0 (Tristan Marshall, Edinburgh, UK; Marshall et al. 1998) including only those alleles that matched our definition for 'true alleles'.

Behavioural Observations

Behavioural observations were conducted by A.E. She was supported by four experienced Indonesian field-assistants and three students from the Universities UNSYAH and STIKK, Darussalam Bandah Aceh, Indonesia. Group HA was followed from dawn until dusk (average observation time: 11.1 h) every day by two to three observers at the same time. All adult females of group HA served as focal animals (Altmann 1974). During focal animal sampling, consortships were recorded with the continuous sampling method (Martin & Bateson 1993). Consortships were defined as an adult male following an adult female or vice versa within 8 m (when with the group) or within 20 m (when away from the group) for at least ten consecutive minutes. Usually, consort partners engaged in sexual interactions with each other, but sometimes a consorting male was replaced by a higher-ranking male before sexual interactions could take place. It was noted who initiated a consortship by approaching the partner, which of the partners followed the other and who terminated the consortship by leaving. In most consortships males followed females. It was difficult to assess female engagement in maintaining the consortship, because in most consorts males followed females and we might easily have missed subtle forms of consort maintenance by females, like slowing down movement when the male did not keep up with the female (see also van Noordwijk 1985). A female was judged to be involved in maintenance of the consortship if she stopped moving forward at least once during the consort period, looked back for the male and started to move again after the male had approached her.

When a female stayed in consort for 100 minutes or more (long consorts), focal animal sampling was continued for the rest of the day. From this day on, the female was followed from dawn to dusk until she was not consorted anymore. Sometimes two or three females engaged in long consorts simultaneously, so that only one female could be followed the whole day through. The other female/s was/were then followed for half a day. The average observation time during long consorts per female was 6.1 h/day. When more than one female was in long consort, all nonconsorting females were followed only every second day. When females were consorted by alpha males for ≥97% of the observation time, this was called alpha male mate guarding (equivalent to male monopolization), irrespective of whether the female was mate-guarded throughout this period by the same alpha male or whether she was mate-guarded alternately by alpha males of different groups. Focal animal sampling was interrupted every half hour to check the group for composition, activity, location and consortships.

Agonistic and sexual interactions occurring between an adult group member and any other individual were recorded in detail using the all-occurrence sampling method (Altmann 1974). Behavioural definitions followed Engelhardt et al. (submitted). To distinguish between female receptive and proceptive behaviour, the term ‘female solicitation’ was used only for those female presentations of the anogenital regions that occurred after the female had approached the male. In addition to agonistic interactions between group members, all agonistic interactions of males of the adjacent groups were recorded ad libitum (Altmann 1974). Dominance rank was determined by the display of the 'bared-teeth face', a unidirectional submissive display (van Hooff 1967), and with a sociometric matrix (Altmann 1974) in which the direction of aggression was entered. It was not possible to deduce the complete dominance hierarchies between males from all groups, but for each group the alpha and the beta male could be determined. The rank relation between the dominant male of group HA and the dominant male of group HB, who temporarily joined group HA (see above) was never clear. All males that held alpha or beta rank position in their groups were defined as high-ranking males. The second-ranking male of group HA, the challenger, often left the group or stayed at the periphery and only sporadically engaged in sexual interactions, so that the third-ranking male often held the functional beta position. He was therefore named beta male HA and categorized as high-ranking.

Data Analysis

All data presented here concern exclusively consortships and sexual interactions which occurred during defined fertile phases of the observed ovarian cycles (*N* = 9) and, alternatively, to the four days surrounding the oestrogen peak in the two cycles in which a fertile phase could not be determined (see above). Data for female MA was obtained only during half of the fertile phase, because on two of the four days the female moved away from the group silently very early in the morning and was lost by the observer. Female IA was excluded from analysis, because she neither showed ovarian nor sexual activity during the study period (Table 1). Cycles from females which had infants younger than five month of age were also excluded from analysis, because females did not show any sexual activity and males did not show any interest in these females during this period.

In order to test whether male dominance rank had an effect on male mating frequency, we compared the total number of copulations a female had with males of different classes (alpha males; high-ranking, nonalpha males; middle-and low-ranking males) against each other using the Friedman test, with post hoc comparison using the Wilcoxon signed-rank test.

We tested for differences between cycles with alpha male mate guarding and cycles without alpha male mate guarding in the total number of consort partners during the fertile phase, the proportion of time during the fertile phase in which females were consorted and the total number of mating partners during the fertile phase. For this, we compared the two cycle categories against each other using the Mann-Whitney-U test.

In order to find out whether the proportion of time for which females were consorted by high-ranking males, depended on female parity or cycle status, we classified females into two categories (1 = nulliparous and primiparous; 2 = multiparous) and tested these for differences in the total time spent in consort with high-ranking males using the Mann-Whitney-U test. We tested the time a female spent in consort with high-ranking males for a significant relation with different variables (number of cycle occurrence within the study period; average female oestrogen level during the fertile phase; average iEtotal: iPdG ratio and female dominance rank) using the Spearman's rank correlation coefficient. Since two females had more than one cycle during the study period, we calculated for each of these females the average proportion of time the female spent in consort with a high-ranking male for all of her cycles before testing for a relation with female dominance rank. We repeated the test twice, first using the data from conception cycles only, and second, using again mean values for females with several cycles, this time excluding female CA from analysis since she seemed to be an extreme outlier.

Finally, we tested whether likely fathers of infants had mated more often with females than expected by chance by calculating for each conception cycle the average number of copulations per male and comparing this to the total number of copulations a female had during the cycle with the likely father, using the Wilcoxon signed-ranks test. All statistic tests were conducted with SPSS 11.0 for Windows and used two-tailed probabilities.

Results

Female Reproductive Cycles

Seven of the eight study females showed ovarian activity (Table 1). In total, eleven cycles were observed, including six conception cycles, four ovulatory nonconception cycles and one anovulatory cycle. Five females conceived during their first cycle. Fertile phases were spread over seven months and did, with the exception of two cycles (cycle LA*, SA1; Fig. 2), not overlap with each other. However, eight fertile phases, including those of every conception cycle, occurred within the first 3.5 months (Fig. 2). All females who conceived gave birth to an infant after an average of 163 days (range: 159–166 days), counting from the last day of the fertile phase.

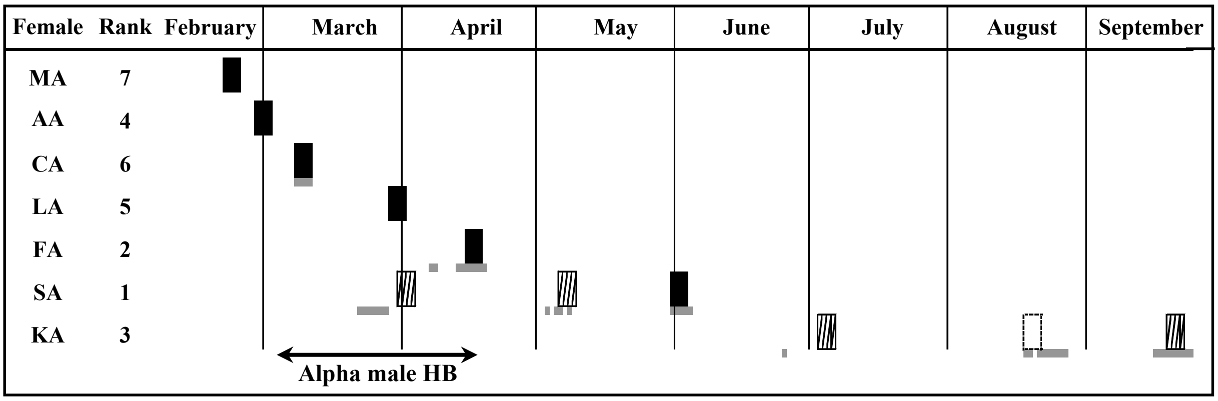

Figure 2. Distribution of the fertile phases over the study period. Black bars indicate the fertile phase of conception cycles, hatched bars those of ovulatory non-conception cycles and open bars those of anovulatory cycles. Grey lines mark days with alpha male mate guarding, and the arrow indicates the period in which the alpha male of the adjacent group HB joint group HA.

Male Rank and Copulation Rate

There was an overall significant relation between male dominance rank and copulation rate. Females copulated more often with alpha males than with high-ranking nonalpha males (A), and females copulated more often with high-ranking nonalpha males than with middle- or low-ranking males (B) (Friedman test: $\chi 2$ = 10.8, N = 10, K = 3, P = 0.005; Wilcoxon matched-pairs signed-ranks test: A: Z = –2.1, P < 0.05, B: Z = –2.4, P < 0.02; Fig. 3). The majority of sexual interactions (on average: 86.4%) was male initiated.

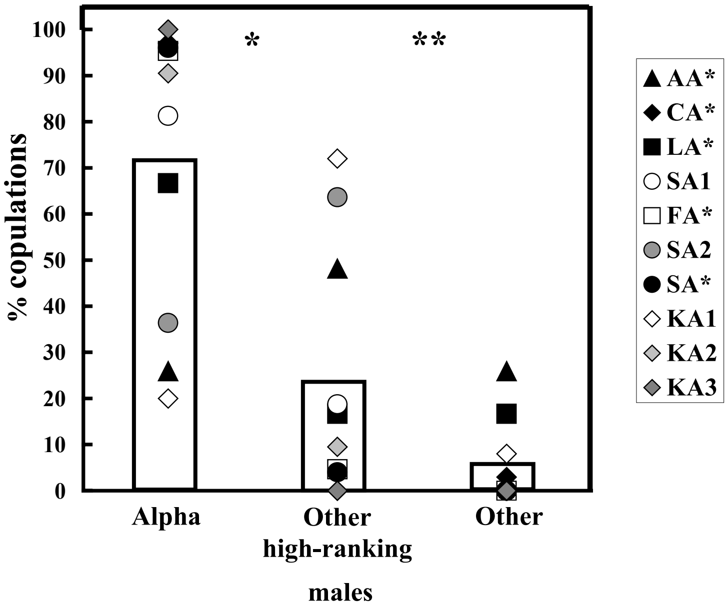

Figure 3: Proportion of copulations by females with alpha males, other high-ranking males and middle- and low-ranking males. Bars represent average valuse. (* P < 0.05; ** P < 0.02)

Consortships and Mate Guarding by Alpha Males

During all fertile phases, females were engaged in consortships, but with varying duration and varying number and identity of consort partners (Fig. 4). Cycles could be classified according to the proportion of time alpha males consorted a female during her fertile phase. In six cycles, females were consorted by alpha males for ≥97% of the observation time (alpha male mate guarding). These cycles could be classified again into those in which females were exclusively mate-guarded by alpha male HA during their fertile phases (cycle SA*, KA2, KA3) and those in which they were mate-guarded alternately by alpha male HA and alpha male HB (cycle CA*, SA1, FA*). In the remaining five cycles, alpha males consorted females on average only during 26% of the fertile phase (range: 0–56% observation time). These cycles differed from those with alpha male mate guarding in that females had significantly more consort partners (average: 4.2 partners, range: 2–8 partners; Mann-Whitney-U Test: $U = 2.5$, $N_1 = 5$, $N_2 = 6$, $P < 0.02$) and spent significantly less time in consort in general (average: 75% observation time, range: 63–94% observation time; Mann-Whitney-U Test: $U = 0$, $N_1 = 5$, $N_2 = 6$, $P < 0.01$).

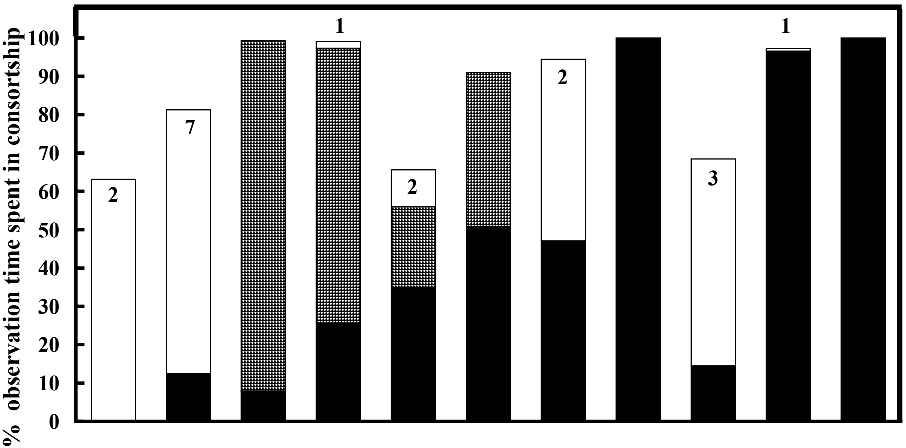

Figure 4. Proportion of observation time females spent in consortship with alpha male HA (black bars), with alpha male HB (black bars with white rhombs) and with other males (white bars). Figures refer to the number of consort partners that were not alpha males. Conception cycles are marked by asterisks, nonconception cycles are numbered by occurrence.

Consortships with High-Ranking Males and Female/Cycle Status

Whether females were mate-guarded by dominant males or not did not depend on female parity (Mann-Whitney-U Test: $U = 6$, $N_1 = 3$, $N_2 = 4$, n.s.). The proportion of time in which females were consorted by high-ranking males during the fertile phase was neither related to the order of cycle occurrence (Spearman rank correlation: $rs = 0.53$, $N = 11$, n.s.), nor to cycle oestrogen levels (Spearman rank correlation: $rs = 0.01$, $N = 11$, n.s.), nor to the average iEtotal: iPdG ratio of cycles (Spearman rank correlation: $rs = 0.19$, $N = 11$, n.s.). We could also not find any significant relation between female dominance rank and the time that females were consorted by high-ranking males (Spearman rank correlation: $rs = -0.46$, $N = 7$, n.s.), not even when testing conception cycles only (Spearman rank correlation: $rs = -0.66$, $N = 6$, n.s.). However, when we removed female CA from analysis, we found a linear, negative relation between the time females were consorted by males and female dominance rank (Spearman rank correlation: $rs = -1.00$, $N = 6$).

Cycle Type and Maintenance of Consortships

In all six fertile phases, in which females were mate-guarded by alpha males, males maintained the consortship throughout the whole consort period by following the female. The consort pair was followed by other males for on average 25.4% of the consort time (range: 0–59.3% consort time). Guarding alpha males attacked subordinate males when the latter tried to approach the female or when the female approached these males. In case that females approached other alpha males or the challenger, guarding alpha males punished the females by chasing them further than necessary to prevent contact between the female and the approached male. No alliances among subordinate males against mate-guarding alpha males were observed.

In the four fertile phases without alpha male mate guarding for which we had continuous data (cycle AA*, LA*, SA2, KA1), high-ranking males followed females, thus maintaining the consortship on average for 86.6% of the consort time (range: 66.8%–100% consort time). In the remaining time, middle- or low-ranking males followed the females, or females maintained the consortship themselves. During the observation of female MA, the female was never followed by any high-ranking male.

Cycle Type and Number of Mating Partners and Copulations

Cycles differed in the number of mating partners. During fertile phases without alpha male mate guarding, females had on average 5.2 mating partners (range: 4–7 partners) and thus significantly more mating partners than females that were guarded by alpha males (Mann-Whitney-U Test: $U = 2.5$, $N_1 = 5$, $N_2 = 6$, $P < 0.05$). However, even in cycles with alpha male mate guarding, females always had more than one mating partner during the fertile phase with one exception (female KA3 in her third cycle): two mating partners when females were consorted by alpha male HA alone and three to five mating partners when females were mate-guarded alternately by alpha male HA and HB. Nevertheless, alpha males were able to copulate the most with females: when mate-guarded by both alpha males, on average 91.2% of copulations occurred with one of these males, on average 28% with alpha male HA 63.3% with alpha male HB. During the time alpha male HA mate-guarded the female exclusively, he held 95.5% of the females' copulations.

Female Behaviour

During cycles with alpha male mate guarding, females showed clear sexual preferences for high-ranking males. In the cycles CA*, FA*, females showed a preference for alpha male HB: although they were observed to wait for alpha male HA when in consort with him, they initiated consortships with alpha male HB whenever he was around. For this, they approached alpha male HB, causing alpha male HA to give up his consortship without fighting. Both females exclusively solicited towards alpha male HB.

Female KA showed a preference for the challenger of group HA. In all three of her cycles (cycles KA1, KA2, KA3), the female approached and followed the challenger, even when mate-guarded and punished by alpha male HA. During the fertile phase of cycle KA2, female KA continued to approach the challenger for sometimes more than half an hour after nightfall, being followed and chased by alpha male HA. However, she never managed to break up her consortship with alpha male HA. Although female KA seemed to favour the challenger, she was also observed to wait for alpha male HA during the consort with him and to solicit towards alpha male and beta male HA.

Female SA also showed a preference for alpha male HB (cycle SA1) and the challenger (cycle SA2) by breaking up consortships with other males in favour of them, but she, too, was observed soliciting towards other high-ranking males.

During three cycles (CA*, FA*, SA1), females mated with beta males or low-ranking males without resistance, when they were approached without notice of the guarding alpha male or before the alpha male was able to attack them.

In cycles in which females were not mate-guarded by alpha males during the fertile phase, females also showed sexual preferences for high-ranking males. In these cycles, females approached males that were higher-ranking than the current consort partner, thus terminating the current consortship and initiating a new consortship with the approached male (N = 10). When no male was willing to follow them, females initiated and maintained a consortship themselves by following a male (MA*: 57% consort time; KA1: 26.3% consort time), who was either high-ranking or middle- or low-ranking. In general, females solicited on average 3.6 males (range: 2-6 males) and 30.8% of all solicitations were received by middle- and low-ranking males.

Table 3. Results of the paternity exclusion analysis for six mother–infant pairs and 24 males from group HA and adjacent groups

Mother–infant pair	Likely father	Number of mismatches/ genotyped loci	Likelihood of paternity
MA–VR	Beta male HB	1/9	≥ 98%
AA–CR	Beta male HA	0/10	≥ 98%
CA–XA	Alpha male HB	0/10	≥ 98%
LA–IR	Alpha male HB	0/10	≥ 98%
FA–UR	Alpha male HB	0/10	≥ 98%
SA–ER	Alpha male HA	0/10	≥ 98%

Paternity and Copulation Rate

There was a clear relation between male rank and paternity: all six infants born during the study period were sired by high-ranking males (Table 3), one by alpha male HA, three by alpha male HB and one each by beta males HA and HB. However, male copulation rate was not related to paternity: only in two of five cycles leading to the birth of an infant, the majority of copulations had occurred with the likely father (Fig. 5). Overall, fathers did not mate significantly more often with mothers than males did on average (Wilcoxon signed-ranks test: $Z = -1.5$, $N = 5$, $P < 0.05$).

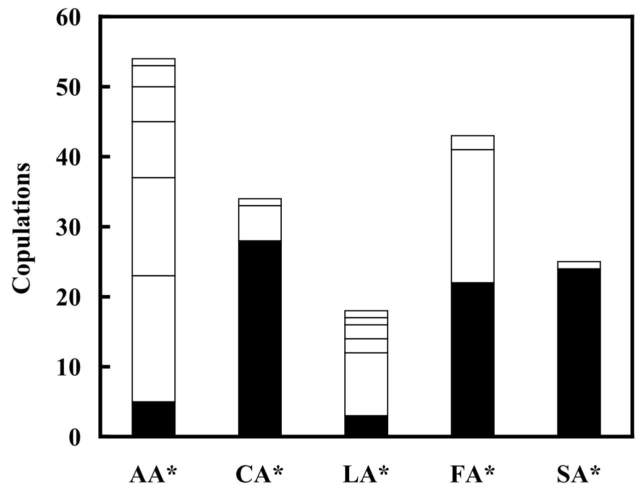

Figure 5. Number of copulations by mothers with the likely father (black bars) and with other males (open bars). Since observation of female MA was uncontinuous, her data are not presented.

Discussion

Male Dominance Rank and Reproductive Success

The results presented here confirm the finding of a prior longitudinal study on several groups of longtailed macaques of the same population as our study group that almost all infants are sired by alpha and beta males in this species (de Ruiter et al. 1994). Given that a high bias of paternity towards high-ranking males has now been observed in multiple groups of wild longtailed macaques during several years, it seems that a high reproductive skew in favour of high-ranking males is a consistent species characteristic. This finding contrasts with that of a longterm study on savannah baboons (*Papio cynocephalus*: Alberts et al. 2003), in which a high variability in reproductive skew over time and between groups was observed. Since Alberts et al. (2003) ascribed this variation to variation in fighting ability of dominant males and to the number, age and alliance formation of subordinate group mates, she concluded that variation in male reproductive skew should be a more general pattern in a wide range of mammalian species organized in multimale groups. As the present study shows, longtailed macaques do not follow this pattern. The observed differences between savannah baboons and longtailed macaques concerning variability in reproductive skew can possibly be attributed to differences in group size and group size fluctuation, because the number of competitors can be expected to influence success of dominant males during fights (and thereby reproductive success). Whereas longtailed macaque groups usually do not exceed 50 individuals in undisturbed habitats (Wolfheim 1983), savannah baboons live in groups ranging between 10 to 200 individuals (Wolfheim 1983). Thus, a relatively stable reproductive skew may be a typical characteristic of smaller multimale groups.

Alternatively, it might be that the observed variation of male reproductive skew is often overestimated when based on mating success, because, as also shown in this study, the latter is not always a predictor of reproductive success (*Macaca mulatta*: Stern & Smith 1984; *Macaca sylvanus*: Paul et al. 1993). This emphasizes the importance of using genetic paternity data for the assessment of male reproductive success in primates.

Mate Guarding by Dominant Males

This study demonstrates that the priority of access to oestrus females model (Altman 1962) can be applied to wild longtailed macaques since monopolisation of fertile females by dominant males occurs in this species. Moreover, monopolization is timed to the cycle's fertile phase, thus determining paternity (Fig. 6). Three of the six infants conceived during our study were sired by alpha males during cycles in which these males mate-guarded the mothers throughout the entire fertile phase. Copulations with other than alpha males accounted only for a minor proportion of copulations and did not affect paternity. However, two of these infants were sired during cycles, when the group's dominant male shared mate guarding and mating with the dominant male of an adjacent group, so that paternity was in these cases determined on the postcopulatory level. Sharing of mate guarding between alpha males of different groups seems to be extraordinary in longtailed macaques since it has never been described before in any of the previous longterm studies on this species (van Noordwijk 1985; van Noordwijk & van Schaik 1988, 2001; de Ruiter et al. 1994). Why it occurred is not clear, but alpha male HB may have been attracted by group HA, because he was already familiar with the females (B. Putragayo, pers. comment), and more females were cycling in this group than in his own group (A. Engelhardt, unpubl. data). It has, however, never been reported before that dominant longtailed macaque males temporarily switch groups during the mating season although longterm studies on this species do exist (van Noordwijk & van Schaik 1988, 2001; de Ruiter et al. 1994). We presume, therefore, that in a more normal situation, all three infants would have been sired by the group's dominant male.

Although mate guarding by dominant males occurred, it was not observed in all cycles. This is surprising, given the fact that fertile phases were almost not overlapping and that three cycles without mate guarding led to conception. Two basic models of reproductive skew provide possible explanations for such a phenomenon: the limited control model (Cant 1998; Clutton-Brock 1998; Reeve et al. 1998) suggests that dominant males sometimes fail to monopolize access to females due to an assortment of factors such as female cycle synchrony, alternative male reproductive strategies or female choice. This explanation may, however, not hold for our study, because mate guarding was not hampered by any external factor. Instead, males seemed to lack interest in these cycles, so that females sometimes maintained consortships themselves. According to the concession model (Vehrencamp 1983; Johnstone et al. 1999; Kokko & Johnstone 1999), high-ranking males do not always monopolize access to females provided that these males benefit from the presence of subordinate males, for example for group defence. Our observations do not show any evidence for or against the concession model, but whether dominant male longtailed macaques benefit from an increased number of male group members, which also means an increased number of competitors, is questionable. We would, therefore, alternatively suggest that in our study, males disregarded some of the cycles in order to save time and energy for fighting competitors or to regenerate depleted energy reserves. Mate guarding is time and energy consuming (Alberts et al. 1996), and males should refrain from mate guarding when energetic demands are high and the available female not preferred (Alberts et al. 2003). Strikingly, we found a relation between female dominance rank and the proportion of time a female was consorted by high-ranking males, showing that males selectively spread reproductive investment over females. That dominant males prefer high-ranking females as reproductive partner makes sense, given the fact that high-ranking female longtailed macaques have a higher lifetime reproductive success than low-ranking females (van Noordwijk & van Schaik 1999). Why the low-ranking female CA was so attractive to males, however, remains unclear.

The Potential of Postcopulatory Mechanisms for Determining Paternity

Our behavioural observations indicate for the first time in wild longtailed macaques, that polyandrous mating occurs to a nonnegligible extent within the fertile phase, opening up the opportunity for postcopulatory mechanisms to operate. Indeed, in the majority of cycles, including almost all conception cycles, females copulated with two or more males during the fertile phase. Given the fact that the situation in our study group was unusual (see above), the degree to which postcopulatory mechanisms determined paternity might have been higher in our study than normal. However, since three infants were conceived during cycles in which dominant males were not interested in the female without obvious reasons, postcopulatory mechanisms seem to be an important determinant of male reproductive success even under normal conditions.

Two factors seem to affect the potential for postcopulatory mechanisms to operate: stability of the alpha male position and male interest into females (Fig. 6). However, the fact that in our study, the group’s alpha male was able to exclude a challenger of his alpha rank position from mate guarding and mating in three cycles suggests, that instability of the alpha rank position might not always lead to postcopulatory determination of paternity.

Alternative Male Reproductive Strategies and Female Direct Mate Choice

Alternative male reproductive strategies seem to play no or at least a minor role for male reproductive success in longtailed macaques. Subordinate males often followed a consorting pair and sometimes even managed to mate with a guarded female, but none of these copulations resulted in paternity. The question however arises, why subordinate males did not form coalitions against mate-guarding males. There may be two explanations to this question. First of all, Henzi et al. (1999) argue that small groups afford fewer opportunities for coalition formation and thus weaker selection on the behavioural tendency to form social alliances that might lead to coalitions. This may also apply to longtailed macaques, in which group size and number of males within groups are small compared to other catarrhine primate multimale groups. Furthermore, male bonnet macaques (*Macaca sinica*: Silk 1993) and Barbary macaques (*Macaca sylvanus*: Widdig et al. 2000), species in which coalition formation among males is common, rarely form coalitions against higher-ranking opponents to avoid retaliation. So, the reluctance to form coalitions against males of higher status may be a general macaque specific pattern.

The other question is why females were not continuously consorted during the fertile phase when not mate-guarded by dominant males. This may be explained by the observation that females often approached and mated with males higher ranking than their current consort partner. Since mate guarding makes only sense when, in this way, other males can be excluded from copulating with the female, subordinate males might have lost interest in mate guarding, if females often turned to higher-ranking males.

Female direct mate choice also did not directly affect paternity, as female choice was either restricted by dominant males or females mated with several males, thus inciting postcopulatory mechanisms of paternity determination. Females did not even mate more often with likely fathers than with other males when not mate-guarded. They did, however, show a clear preference for high-ranking consorts and mating partners. Thus, although females did not directly try to concentrate matings to a specific male, they increased the probability for high-ranking males to father the infant. In this way, they may further indirectly affect male reproductive success by influencing male dominance rank or group membership. Female longtailed macaques often take part in male fights (pers. observation), but how effectively they can influence male status or group membership still needs to be investigated.

Determination of Male Reproductive Success in Wild Longtailed Macaques

The results presented here demonstrate that both male monopolisation and postcopulatory mechanisms contribute to male reproductive success in longtailed macaques. The degree to which one or the other factor comes into effect depends on the stability of the alpha rank position and on the ambition of dominant males to mate-guard a female (Fig. 6).

Female direct mate choice and alternative male reproductive strategies, on the other hand, seem not to directly affect paternity. Although paternity determination mostly occurred on the postcopulatory level in this study, infants were exclusively sired by alpha and beta males. Hence, it seems that in longtailed macaques, postcopulatory mechanisms act in favour of high-ranking males, thus stabilizing a high reproductive skew in favour of these males. How paternity is determined on the postcopulatory level, and to which extent sperm competition and cryptic female choice are involved, however, remains unknown. Clearly more investigations are needed for a better understanding of the processes leading to fertilisation within the female reproductive tract, and, thus, of the proximate mechanisms determining paternity in primate multimale groups.

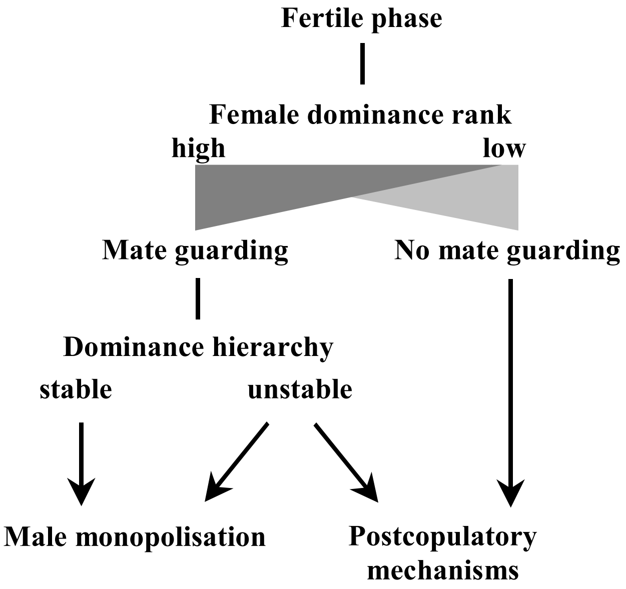

Figure 6. Factors that led to paternity determination in longtailed macaques by priority of access to oestrus females and by postcopulatory mechanisms, respectively.

Acknowledgements

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5

The significance of male and female reproductive strategies on male reproductive success in longtailed macaques: general discussion

Male Reproductive Success and Male Dominance Rank

The paternity data obtained in the present thesis confirm the finding by de Ruiter et al. (1994) that male reproductive success is positively related to male dominance rank: the majority of infants were sired by alpha males, the remaining infants by beta males. This suggests that the high reproductive skew towards dominant males is a consistent characteristic in wild longtailed macaques, which is not even modified when the alpha rank position is challenged, as was the case in the present study. The degree to which dominance rank influences male reproductive success in primate multimale groups has been much debated (reviewed in Dewsbury 1982; Fedigen 1983; Cowlishaw & Dunbar 1991; Ellis 1995). Studies on the relation between male dominance rank and mating success (as a measure of male reproductive success) showed inconsistent results even within the same species, some confirming a positive relation between these parameters (e.g. *Macaca mulatta*: Lindburg 1983; *Macaca fuscata*: Tokuda 1961; *Papio cynocephalus*: Hausfater 1975; *Pan troglodytes*: Hasegawa & Hiraiwa-Hasegawa 1990; see also Dixson 1998), others reporting that rank did not predict mating success (*Macaca mulatta*: Loy 1971; *Macaca fuscata*: Eaton 1978; *Papio cynocephalus*: Bercovitch 1986; *Pan troglodytes*: Tutin 1979). Mating success, however, represents not necessarily reproductive success, as has been demonstrated in this thesis in addition to other studies using genetic paternity data (e.g. *Macaca mulatta*: Stern & Smith 1984; *Macaca sylvanus*: Paul et al. 1993). Investigations on male reproductive success therefore need objective methods for paternity determination. The majority of investigations in which genetic data have already been used, point to a significant relation between male rank and reproductive success (e.g. *Macaca mulatta*: Widdig et al. 2004; *Mandrillus sphinx*:

Dixson et al. 1993; *Pan paniscus*: Gerloff et al. 1999; *Semnopithecus entellus*: Launhardt et al. 2001). However, further studies will be needed to clarify how consistently male rank predicts reproductive success in multimale groups of the anthropoid primate taxon.

The paternity data of this thesis are moreover important, as they not only confirm a high reproductive skew in longtailed macaques, – they also show that the reproductive skew is stable even when paternity is determined at the postcopulatory level. So far, a positive relation between male dominance rank and reproductive success has been ascribed mostly to the fighting ability of dominant males leading to advantages in mating success (e.g. Alberts et al.2003). The data presented here, however, suggest that postcopulatory mechanisms of paternity determination may also operate in favour of high-ranking males, and that the influence of male fighting ability on reproductive success may have been overestimated so far. The effect of sperm competition and cryptic female choice has already been intensively investigated in arthropods, fish and birds (reviewed in Birkhead & Møller 1992; Eberhard 1996), but studies on primates are still rare due to difficulties in methodological approaches. This study clearly shows that further investigations into the processes taking place within the female reproductive tract are needed for a better understanding of the factors affecting male reproductive success in multimale primate groups.

Reproductive Strategies of Dominant Males

The results of this thesis confirm the priority of access to fertile females model (Altmann 1962; see also Fedigan 1983), because they demonstrate that monopolization of fertile females is an evolutionary stable reproductive strategy in longtailed macaques. Mate guarding by dominant males does not only exist; it is, even timed to the fertile phase, and it leads to paternity. Although in this study, the degree to which priority of access to fertile females has led to paternity has been low, the fact that the alpha male's situation was unusual may suggest a higher effect of male monopolization on male reproductive success under more normal conditions. This assumption is supported by the observation that in the Pangandaran population of longtailed macaques, studied in behalf of this thesis, dominant males always mate-guarded fertile females exclusively during the fertile phase. Unfortunately, no paternity data is available for these males.

Mate guarding is clearly facilitated in longtailed macaques, because males are able to discern the timing of the fertile phase more or less reliably and because fertile phases are spread over the mating season almost asynchronously. The results presented here provide the first experimentally achieved evidence in an anthropoid primates for male ability to discern the fertile phase under natural conditions. Both, dominant and subordinate males, showed increased interest in females during the females' fertile phases, indicating that males of all ranks and with different degree of access to the female were able to recognize this period. However, male interest in females was not completely restricted to the fertile phase. It, therefore, seems that males are able to distinguish between periods with higher and periods with lower probability of ovulation rather than precisely recognizing the timing of the fertile phase. It may, however, also be that males extend mate guarding over the fertile phase, because the reproductive costs arising from extended mate guarding are lower than those from losing paternity in case they misjudged the beginning or end of the fertile phase. From the study itself it is not possible to clarify, which of the two reasons prevailed. Nevertheless, as our paternity data show, recognition of the fertile phase in longtailed macaques is more precise than in Hanuman langurs (Heistermann et al. 2001).

On the other hand, males were not able to discern the potential of a cycle to lead to conception, since males showed the same interest in females during conception cycles as during nonconception cycles. In chimpanzees, male interest in females increases as the number of cycles to conception decreases showing that males adjust their investment into reproduction to the probability of conception (Deschner et al. in press). The selective pressures towards a recognition of nonconception cycles may, however, be stronger on male chimpanzees than on male longtailed macaques, because, as the data on the number of cycles per female show, repeated cycling does occur in female longtailed macaques, but it is less common than in chimpanzees.

Male reproductive investment is, nevertheless, not evenly distributed over females in longtailed macaques either. High-ranking males showed a clear preference for high-ranking females, spending more time in consorting them compared to other females. Male mate choice has been reported for several primate species (Smuts 1987) and is often related to female dominance rank (reviewed in Robinson 1982; Silk & Boyd 1983; Berenstain &Wade 1983; Setchell & Kappeler 2003), which can be attributed to the fact that high-ranking females often have a higher reproductive success than low-ranking females (reviewed in Silk 1987; Dixson 1998). Lack of mate guarding in cycles of low-ranking females in the present study is, nevertheless, surprising, given the fact that male monopolization was not hampered by external factors, which would be predicted by the limited-control model of reproductive skew (Cant 1998; Clutton-Brock 1998; Reeve et al. 1998). Another explanation would be that high-ranking males share matings with subordinate males in case the latter provide benefits to the high-ranking males (concession model of reproductive skew; Vehrencamp 1983; Keller & Reeve 1994; Kokko & Johnstone 1999). So far, no study has described any benefits provided by subordinate males to dominant males in longtailed macaques, but the possibility that they provide benefits, can also not be ruled out by the available data either. A more sensible explanation would, however, be, that the energetic demands of mate guarding forced the dominant male to give up some of the cycles (Alberts et al. 2003). Alberts et al. (1996) showed that mate guarding constrains foraging activity in male savannah baboons, thus imposing considerable costs upon males. Few studies, so far, investigated the costs arising for males from mate guarding, although they may play an important role in male reproductive decision making. In the present study, however, costs of male choice were high for the dominant male, as he lost half of the paternities to other males because of his reluctance to mate-guard low-ranking females. Further investigations are needed into the trade-off between costs and benefits of mate guarding in order to understand the mechanisms of decision making in the reproductive context by males in multimale primate groups.

Reproductive Strategies of Subordinate Males

It seems that alternative male reproductive strategies have only a negligible effect on male reproductive success in longtailed macaques. Mate guarding was not successfully carried out by subordinate males, because females tended to approach and mate with males higher-ranking than their current consort partner, thus opening up the opportunity for postcopulatory mechanisms to operate. Coalition formation against high-ranking males did not occur and even when males managed to sneak copulations with mate-guarded females, this did not result in paternity. There may be two reasons why subordinate longtailed macaques did not form coalitions against dominant males which, in savannah baboons, is a common alternative reproductive strategy to break up consortships (*Papio cynocephalus*: Bercovitch 1995; Alberts et al. 2003). Firstly, the opportunity for coalition formation may be smaller in small primate groups, and, hence, also the selection on the behavioural tendency to form social alliances that might lead to coalitions weaker (Henzi et al. 1999). Secondly, the reluctance to form coalitions against males of higher status may be a general macaque specific pattern, because it is rarely observed even in those macaque species in which males form coalitions more frequently (*Macaca sinica*: Silk 1993; *Macaca sylvanus*: Widdig et al. 2000).

The fact that subordinate males sire almost no offspring in longtailed macaques raises the question whether this phenomenon increases the risk of infanticide. Infanticide has been observed in longtailed macaques (de Ruiter et al. 1994), but events are rare. Risk of infanticide should be low for those infants resulting from cycles in which paternity has been confused, because the mother mated with several males. It should, however, be higher for infants from high-ranking females, which are usually monopolized by dominant males during the fertile phase. It is not clear whether subordinate males are able to assess the probability of their own paternity of these infants, but given the fact that subordinate males are able to quite reliably discern the timing of the fertile phase, risk of infanticide should be increased for these infants. This may explain, why former alpha males tend to stay on with the group for another year after they have lost alpha rank position, if interpreting it as a form of infant protection (van Noordwijk & van Schaik 1988).

On the other hand, females may confuse paternity after conception: longtailed macaques, in addition to several other primate females living in multimale groups, start to be sexually active again during the first trimester of pregnancy. This ‘postcopulatory oestrus’, as it is called, may serve those females that have been mate-guarded around the time of conception to confuse paternity by mating with subordinate males. So far, primatologists have paid minor attention to this phenomenon, but a study on sooty mangabeys (*Cercopithecus torquatus atys*: Gordon et al. 1991) has shown that female sex hormone levels during the period of postconception oestrus were similar to those during the ovarian cycle and that males, during the postconception oestrus, started again to mate with females. I will continue my studies on longtailed macaques with comparing data on male and female sexual behaviour obtained during ovarian cycles with those obtained during the period of postconception oestrus by using female sex hormone profiles in order to assess the potential for paternity confusion after conception.

The Discernability of the Fertile Phase and Female Reproductive Strategies

There is an ongoing discussion among primatologists about the question whether, how and why female primates in multimale groups advertise or conceal the timing of ovulation (see Chapter 1). The results of this study show that female longtailed macaques possess traits that indicate the timing of the fertile phase. Certain female sexual behaviour occurs more frequently during than outside the fertile phase, thus giving males the opportunity to assess the timing of the fertile phase. It has already been observed in several studies on captive macaques that female sexual behaviour changes according to female oestrogen levels (e.g. Zumpe & Michael 1983, 1985; Zehr et al. 2000), but the present study is the first to confirm that this is also true for females living under natural conditions. The question, whether ability to discern the fertile phase is a side effect of the phenomenon that female sexual motivation increases with increasing oestrogen levels or whether the fertile phase is signalled to males, maybe even by further female traits, cannot be solved in this thesis. It may, however, be that males use a combination of cues in order to assess the timing of the fertile phase rather than exclusively relying on female behaviour. One of the female traits that may serve to signal the fertile phase, is the uttering of copulation calls (Semple & McComb 2000). Since it was possible to record female copulation calls alongside the playback experiment of this study on a regular basis, I will compare the pattern of these copulation calls to female sex hormones and to the timing of the fertile phase in the future.

Strikingly, female sexual swellings did not reliably indicate the fertile phase in longtailed macaques, but they indicated the probability of ovulation to occur somewhere within the period of maximum swelling. Maximum swelling itself is well extended beyond the fertile phase, thus providing support for the graded-signal hypothesis (Nunn 1999b). The question, however, remains, why sexuals swellings are so imprecise in indicating the fertile phase, although other female traits indicate the fertile phase more reliably, and, furthermore, why they decline with female age. Exaggerated sexual swellings in young females have been suggested to derive from female-female competition (Anderson & Bielert 1994). In some primate species, males show low sexual interest in adolescent and nulliparous females (*Macaca radiata*: Glick 1980; *Macaca fuscata*: Takahata 1982; *Pan troglodytes*: Hasegawa & Hiraiwa-Hasegawa 1983; *Papio cynocephalus*: Scott 1984; *Pongo pygmaeus*: Galdikas 1985), presumably because, in comparison to other females, these females are often less fertile (Dunbar 1987), and infant mortality for their offspring is higher (Nicolson 1987). Thus, young females require more effort than prime (or older) females to attract males. In the present study, male choice for certain females could be observed however, male preference was related to female dominance rank, not to female parity. The specific function of sexual swellings, thus, needs further clarification.

The reasons suggested so far why females should signal the fertile phase include paternity certainty for males, leading to enhanced paternal care (reliable-indicator hypothesis; Hamilton 1984), incitement of male-male competition, so that only the ‘best’ male mates with the female (best-male hypothesis; Clutton-Brock & Harvey 1976), provocation of mate guarding in order to reduce sperm competition (female choice hypothesis; Maestripieri & Roney in press) or, if the fertile phase is gradually and not precisely signalled, to reduction of the risk of infanticide (graded-signal hypothesis; Nunn 1999b, see Chapter 1). These hypotheses are, however, not mutually exclusive, but share the common prediction that by advertising the fertile phase, matings during the fertile phase will be concentrated to a single male, which would usually be the dominant male, given that cycles are not synchronized. It is not possible to conclude from the data obtained in this study which of the proposed benefits led to the development of reliable indication of the fertile phase in longtailed macaques and it may well be that it was even a combination of benefits.

In accordance with the conclusion that the fertile phase is made discernable by female longtailed macaques in order to attract dominant males, females in this study showed a clear preference for high-ranking consort and mating partners. Female direct mate choice was, however, not decisive for paternity of a specific male. This can be attributed to two circumstances: first, mate guarding by dominant males constrained female ability to approach other males, and, second, when not mate-guarded, females always mated with several males, thus exciting postcopulatory mechanisms. Females did, however, influence paternity indirectly on the postcopulatory level by mating more often with high-ranking than with middle- or low-ranking males. By mating more often with a female, males should gain an advantage in sperm competition (Birkhead & Møller 1992). Nevertheless, during those fertile phases, in which females were not mate-guarded by dominant males, females did not mate more often with the later father of their infant than with other males. It could be assumed that females were not able to refuse mating attempts, but data on agonistic interactions show that male coercion and punishment of female refusals were rare. On the contrary, the fact that females initiated sexual interactions with more than one male indicates that mating with multiple males was desired.

It has been suggested that females benefit from polyandrous mating in several ways, for example by avoiding genetic incompatibilities (e.g. Tregenza & Wedell 2002; Newcomer et al. 1999; reviewed in Zeh & Zeh 2001) or by reducing the risk of infanticide (e.g. Hausfater & Hrdy 1984; van Schaik et al. 2000; Wolff & Macdonald 2004). Furthermore, in the present study, it seemed that females wanted to be consorted, so soliciting several males may have served to lure males into a consortship. So far, it is not clear whether and how female longtailed macaques benefit from consortships. The only study so far investigating this aspect on two females did not find any benefits (van Noordwijk 1985). Nevertheless, consortships must be beneficial for females, as shows our observation as well as that of others (van Noordwijk 1985), otherwise females would not sometimes maintain consortships themselves. More investigations will be needed to clarify how females benefit from being consorted. A third explanation for polyandrous mating in female longtailed macaques might be that females do not avoid matings, because they benefit from sperm competition, for example by receiving high-quality sperm (Dixson 1998) or because they are still able to influence paternity on the postcopulatory level. Although evidence for sperm competition and female cryptic choice is still rare in primates, this phenomenon has been observed in numerous other taxa, including mammals (Birkhead & Møller 1992; Eberhard 1996). Given the long way and the difficulties sperm has to overcome from the vagina through the female's complex and often hostile reproductive tract to the oviduct, where fertilisation takes place, there seems to be a high potential for postcopulatory mechanisms to act on sperm even in primates.

Females may, furthermore, indirectly influence paternity by intervening in male fights, in this way influencing male dominance rank or group affiliation and, thus, the composition of males available as mates (Smuts 1987). Female longtailed macaques have been observed taking part in male fights (pers. observation), and it is known for other primates that females are able to manipulate male group affiliation (*Macaca sylvanus*: Witt et al. 1981; *Pan troglodytes*: Beerlage & Wrangham 1996; *Papio anubis*: Smuts 1985; *Theropithecus gelada*: Mori & Dunbar 1985). The extent to which female longtailed macaques can effectively influence male status or group membership still needs to be investigated.

Male Paternity Determination in Primate Multimale Groups

This thesis shows that a high reproductive skew in favour of high-ranking males is a consistent characteristic of wild longtailed macaques, resulting from male monopolization of fertile females during the fertile phase on the one hand, and from postcopulatory mechanisms operating in favour of high-ranking males on the other. These findings explain why there is a pronounced sex dimorphism in body and canine size in longtailed macaques (Plavcan & van Schaik 1992, 1997) in addition to a high testis:body weight index (Harcourt 1981) and a high number of viable sperm in male ejaculate (Schrod 2002). Female direct mate choice and alternative male reproductive strategies do not directly affect male reproductive success. Females do, however, indirectly manipulate males by advertising the timing of the fertile phase and in this way often inciting mate guarding by dominant males. When not mate-guarded, females show a preference for high-ranking males, which is nevertheless not restricted to a single male. Females may further influence paternity indirectly through manipulating male dominance rank or group membership. There is a high potential for females to influence paternity on the postcopulatory level. Whether cryptic female choice is an adaptive female reproductive strategy will have to be verified in future investigations.

My findings can, of course, not be applied to all multimale primate groups in general. They should, however, apply to those primate species that are organized in relatively small multimale groups, in which males are able to recognize the fertile phase. Unfortunately, the ability of males to discern the fertile phase has so far not been extensively tested in the wild, but behavioural observations suggest that it may be common among the macaques (*Macaca maurus*: Matsumura 1993; *Macaca mulatta*: Zumpe & Michael 1983; *Macaca tonkeana*: Aujard et al. 1998), among the baboons (*Papio cynocephalus*: Hendrickx & Kramer 1969; Wildt et al. 1977; Shaikh et al. 1982) and possibly also, to a certain extent, in chimpanzees (*Pan troglodytes*: Deschner et al. in press). Nevertheless, as the findings of this thesis show, paternity may to a significant extent be determined on the postcopulatory level, having a greater affect on male reproductive success than expected so far. Thus, more investigations in the future will be needed, assessing the potential for postcopulatory mechanisms to operate and studying processes occurring within the female reproductive tract, in order to get a comprehensive understanding of the proximate mechanisms determining male reproductive success in multimale primate groups.

Finally, both the fact that paternity in this study was overall not related to mating success, and the finding that the interval between the last day of the fertile phase and infant birth (often used for the assessment of the fertile phase) varied for a period twice as long as the fertile phase itself, strongly point out the need for objective measurements of infant paternity and female reproductive status in primate studies addressing aspects of reproduction.

Summary

In this thesis, a combination of modern, noninvasive techniques for hormone and genetic analyses from faecal samples in addition to detailed behavioural observations was used in order to study the proximate mechanisms leading to paternity in multimale primate groups under natural conditions. For this, the longtailed macaque was used as a model. Since the mechanisms of paternity determination operate during the fertile phase of ovarian cycles, most of the analyses focused on this part of the female cycle. In particular, 1. the significance of monopolization of fertile females by dominant males and of female direct mate choice on male reproductive success and 2. the potential for postcopulatory mechanisms to operate on paternity determination were assessed. Starting point was the question whether and by which cues males are able to recognize a female's fertile phase. Results demonstrate that male longtailed macaques are able to discern the fertile phase of an ovarian cycle under natural conditions, and that this ability is held by both dominant and subordinate males. Furthermore, results showed that the assessment of the fertile phase by males appears to depend on oestrogen-related cues. When testing the reliability of oestrogen-dependent female sexual swellings and specific female behaviours to indicate the fertile phase, female sexual swellings turned out to be no reliable indicator of the timing of the fertile phase although ovulation always occurs within maximum swelling. On the other hand, certain female behaviours, namely female solicitations of sexual interactions and reaching back for the male during copulation, occurred more frequently during the fertile phase than outside this period, thus providing information that males could use for the assessment of female reproductive status. Paternity data confirmed the finding by de Ruiter et al. (1994), that paternity in wild longtailed macaques is skewed towards high-ranking males. During the fertile phases, females were either mate-guarded by dominant males or, when not monopolized, they mated with several males. It can, therefore, be concluded that in wild longtailed macaques, male monopolization is important for male reproductive success. It is facilitated in this species, because female cycles are not synchronized and because males are able to discern the fertile phase. Male monopolization, however, is not the only factor determining paternity in longtailed macaques. A considerable number of paternities was determined at the postcopulatory level. Postcopulatory mechanisms, thus, may play a more important role for male reproductive success in this species than previously thought. Furthermore they seem to operate in favour of high-ranking males. The degree to which postcopulatory mechanisms operate depends on two factors: instability in the alpha rank position and lack of interest of dominant males in low-ranking females. On the other hand, female direct mate choice and alternative male reproductive strategies did not directly affect paternity. Female mate choice was either restricted by dominant males or, when females were not monopolized, spread over several males. Reproductive strategies of subordinate males, such as coalition formation against high-ranking males and sneaking copulations, did either not occur or not affect paternity in this study. Finally, the fact that in our study, the interval between the last day of the fertile phase and infant birth, which is often used for the assessment of the fertile phase, varied for a period twice as long as the fertile phase itself, demonstrates that this method is not reliable enough to determine the fertile phase. In addition, the statistical evidence that male mating success, which is often used as a measure of male reproductive success in primate field studies, is not necessarily related to paternity outcome in longtailed macaques, further emphasizes the importance of using genetic analyses for paternity assessments when studying primate reproductive processes in the wild.

Zusammenfassung

In dieser Studie werden die proximaten Mechanismen untersucht, die unter natürlichen Bedingungen zum Reproduktionserfolg von Primatenmännchen in Mehrmännchengruppen führen. Hierzu wurden detaillierte Verhaltensbeobachtungen an Javaneraffen durchgeführt und mit modernen, nicht-invasiven Methoden der Hormon- und genetischen Analyse aus Kotproben kombiniert. Da die Befruchtung eines Weibchens, also der Moment, in dem die Vaterschaft festgelegt wird, während der fertilen Phase eines ovariellen Zyklus erfolgt, konzentrierten sich die meisten Analysen auf diesen Zeitraum. Ziel meiner Arbeit war es, 1. die relative Bedeutung der Monopolisierung fertiler Weibchen durch dominante Männchen und die der direkten weiblichen Partnerwahl auf den männlichen Reproduktionserfolg zu untersuchen, sowie 2. festzustellen, ob und in welchem Umfang die Mechanismen, die zum männlichen Reproduktionserfolg führen, erst nach erfolgter Kopulation einsetzen. Im ersten Teil der Studie habe ich untersucht, in welchem Maß und woran männliche Javaneraffen die fertile Phase von Weibchen erkennen. Die Ergebnisse dieser Studie belegen, dass sowohl dominante als auch subordinierte Javaneraffen unter natürlichen Bedingungen dazu fähig sind, die fertile Phase eines ovariellen Zyklus zu erkennen. Die Anzeiger der fertilen Phase scheinen dabei im Zusammenhang mit weiblichen Östrogenwerten zu stehen. Die weiblichen Sexualschwellungen erwiesen sich in statistischen Berechnungen hingegen nicht als verlässliche Indikatoren der fertilen Phase, obwohl die Ovulation nachweislich immer zur Zeit der Maximalschwellung erfolgte. Im Gegensatz dazu erwiesen sich spezifische weibliche Verhaltensweisen – das „Beinfassen" während der Kopulation und sexuelle Aufforderungen – als verlässliche Hinweise auf die fertile Phase, da sie signifikant häufiger innerhalb der fertilen Phase auftraten, als außerhalb. Die genetische Vaterschaftsanalyse bestätigte die Erkenntnis von de Ruiter et al. (1994), wonach hochrangige Javaneraffenmännchen unter natürlichen Bedingungen einen höheren Reproduktionserfolg haben als niedrigrangigere Männchen. Während der fertilen Phase wurden die Weibchen entweder von dominanten Männchen bewacht oder die unbewachten Weibchen paarten sich mit verschiedenen Männchen. Daraus läßt sich schließen, dass unter natürlichen Gegebenheiten Monopolisierung von Weibchen einen zentralen Einfluss auf den Reproduktionserfolg von Javaneraffenmännchen hat. Eine Monopolisierung wird dadurch erleichtert, dass die fertilen

Phasen der weiblichen Zyklen bei Javaneraffen nicht synchron auftreten, und dass Javaneraffenmännchen die jeweiligen fertilen Phasen der unterschiedlichen Zyklen erkennen können. Die Monopolisierung von Weibchen ist jedoch nicht der einzige den Reproduktionserfolg von Männchen bestimmende Faktor. Ein entscheidender Teil von Vaterschaften wurde auf der postkopulatorischer Ebene determiniert. Damit scheinen postkopulatorische Mechanismen der Vaterschaftsfestlegung einen stärkeren Einfluss auf den Reproduktionserfolg von Javaneraffenmännchen zu haben, als bisher angenommen wurde. Außerdem scheinen hochrangige Männchen überproportional von diesen Mechanismen zu profitieren. Ob der Reproduktionserfolg der Männchen auf der pre- oder postkopulatorischen Ebene determiniert wurde, hängt dabei nach meiner Erkenntnis von zwei Faktoren ab, von der Stabilität der Dominanz des höchstrangigen Männchens und dem Interesse der Männchen an Weibchen, wobei hochrangige Weibchen bevorzugt werden. Weibliche direkte Partnerwahl hingegen hatte keinen direkten Einfluss darauf, welches Männchen Vater wurde. Die Partnerwahl wurde entweder durch dominante Männchen unterbunden oder die Weibchen kopulierten mit mehr als einem Männchen, so dass erst nach der Kopulation festgelegt wurde, welches Männchen das Weibchen befruchtete. Rangniedrige Männchen hatten ebenfalls keinen Einfluss auf ihren Reproduktionserfolg. Sie konnten die Monopolisierung von Weibchen in der Regel nicht verhindern, und selbst wenn sie es schafften, sich mit einem bewachten Weibchen zu paaren, führte dies nicht zur Befruchtung. Neben den genannten Ergebnissen zeigte sich in dieser Studie ebenfalls, dass es notwendig ist, für die Untersuchung von reproduktiven Prozessen bei Primaten objektive und valide Methoden 1. für die Bestimmung des weiblichen Reproduktionsstatus und 2. zur Bestimmung von Vaterschaften zu benutzen, da sich die fertile Phase nicht durch einfaches Rückrechnen vom Tag der Geburt des Kindes an bestimmen lässt, und weil der Paarungserfolg eines Männchens nicht unbedingt seinen Reproduktionserfolg widerspiegelt.

Ringkasan

with Selly Sita

Dalam penelitian ini sebuah kombinasi modern, teknik noninvasive analisis genetika dan hormon dari sampel kotoran digunakan sebagai penambahan untuk melengkapi pengamatan perilaku, yaitu dengan menggunakan teknik perkiraan hubungan kebapakan pada kelompok primata multimale dibawah kondisi alami. Untuk ini, monyet ekor panjang digunakan sebagai sebuah model. Sejak teknik determinasi hubungan kebapakan selama fase fertil siklus ovarium digunakan, sebagian besar analisis difokuskan pada bagian ini dari siklus betina. Keterangan yang diperoleh, antara lain : 1. Arti monopoli pada betina fertil oleh jantan dominan dan pilihan pasangan langsung betina pada sukses reproduksi jantan, serta 2. Potensi mekanisme postcopulatory yang menjelaskan determinasi hubungan kebapakan. Poin awal dari pertanyaan yang diajukan adalah apakah seekor jantan mampu menandai seekor betina fertil dan jantan mana yang sanggup menandai seekor betina pada fase fertil. Hasil penelitian menunjukan bahwa jantan monyet ekor panjang mampu mengetahui fase fertil pada sebuah siklus ovarium dibawah kondisi alami, dan bahwa kemampuan tersebut terdapat baik pada jantan dominan maupun subordinat. Lebih lanjut, hasil menunjukan bahwa pengamatan fase fertil oleh jantan tergantung pada keberadaan hormon estrogen pada betina. Hal tersebut dikarenakan pembengkakan alat kelamin betina bergantung pada siklus estrogen dan perilaku spesifik betina mengindikasikan betina dalan fase fertil, pembengkakan alat kelamin betina hampir tidak dapat dipercaya sebagai sebuah indikator pada saat fase fertil walaupun selalu terdapat pada saat pembengkakan maksimal. Disisi lain, perilaku spesifik betina, yaitu sosialisasi betina pada interaksi seksual dan perilaku betina dalam mendekati kembali jantan selama kopulasi, terjadi lebih sering selama fase fertil dibanding diluar masa tersebut. Hal ini melengkapi informasi bahwa jantan dapat berguna untuk mengamati status reproduksi betina. Data hubungan kebapakan telah diungkapkan oleh de Ruiter et al. (1994), bahwa hubungan kebapakan pada monyet ekor panjang liar lebih condong pada jantan peringkat tinggi. Selama fase fertil, betina tampak dijaga oleh pasangannya yaitu seekor jantan dominan, atau ketika tidak sedang memonopoli, mereka dapat berpasangan dengan beberapa jantan. Oleh sebab itu, dapat disimpulkan bahwa pada monyet ekor panjang liar, monopoli jantan sangat penting untuk sukses reproduksi jantan. Hal tersebut merupakan fasilitas reproduksi pada jenis ini sebab siklus betina terjadi secara tidak bersamaan dan karena jantan mampu melihat fase fertil betina. Monopoli jantan bagaimanapun juga bukan satu-satunya faktor yang dapat menunjukan hubungan kebapakan pada monyet ekor panjang. Adanya sebuah hubungan kebapakan lebih ditunjukan pada tingkat postcopulatory. Mekanisme postcopulatory merupakan peran yang lebih penting untuk sukses reproduksi jantan pada jenis ini dibanding anggapan sebelumnya. Lebih lanjut, mereka melihat terjadinya ini pada jantan peringkat tinggi. Derajat mekanisme pemilihan pada postcopulatory bergantung pada dua faktor, yaitu : ketidak stabilan posisi rangking alfa dan tidak adanya minat jantan dominan pada betina peringkat rendah. Disamping itu, pilihan pasangan langsung betina dan alternatif strategi reproduksi jantan tidak langsung mempengaruhi hubungan kebapakan. Pilihan pasangan betina lebih dibatasi oleh jantan dominan atau pada saat betina tidak sedang memonopoli atau saat betina sedang berhubungan dengan beberapa jantan yang lain. Pada penelitian ini, strategi reproduksi pada jantan subordinate, seperti formasi kelompok yang melawan jantan peringkat tinggi dan kopulasi secara diam-diam, bukan salah satu atau tidak mempengaruhi hubungan kebapakan. Akhirnya, fakta pada penelitian kami menunjukan bahwa jarak antara hari akhir fase fertil dan pada masa kelahiran bayi, dimana sering digunakan untuk melihat fase fertil, berbeda-beda untuk sebuah periode bersamaan sepanjang fase fertil itu sendiri. Namun, hal tersebut masih menunjukan bahwa metode ini tidak cukup diandalkan untuk mengetahui fase fertil. Sebagai penambahan, bukti statistik menunjukan bahwa sukses pasangan jantan, dimana sering digunakan sebagai sebuah ukuran pada sukses reproduksi jantan pada penelitian lapangan ini, bukan kebutuhan yang berhubungan dengan hasil hubungan kebapakan pada monyet ekor panjang, penekanan lebih lanjut diutamakan pada pentingnya penggunaan analisis genetika untuk melihat hubungan kebapakan pada saat mempelajari proses reproduksi primata dalam kondisi liar.

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Curriculum Vitae

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1998	Summer School at the Max-Planck-Institute of Behavioural Physiology: „Causes and Consequences of Sociality“
1999 – 2004	PhD project at the Institute of Human Biology and Anthropology, FU Berlin in cooperation with the Department of Reproductive Biology, German Primate Centre and the Institute of Medical Genetics, Charité, Humboldt-University Berlin

Occupation

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Publications

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