Sperm Competition and the Function of Masturbation in Japanese Macaques *(Macaca fuscata)*

Dissertation

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Zusammenfassung

Daß Männer masturbieren ist Gegenstand einer Reihe von wissenschaftlichen Untersuchungen. Die vorliegende Studie handelt vom Masturbieren bei nicht-menschlichen Primaten im Allgemeinen. Im Detail beschäftigt sie sich mit wilden und gefangenen japanischen Rotgesichtsmakaken *(Macaca fuscata)* aus der Perspektive der Verhaltensökologie.

Aus einem interspezifischen Vergleich von 52 Primatenarten folgt, daß das Masturbieren von Männchen eine gängige Verhaltensweise auch bei nicht-menschlichen Primaten darstellt. Es kommt häufiger bei Arten vor, die ein Vielmännchen-Vielweibchen-Paarungssystem haben, als bei Arten, die monogam, in Haremsgruppen, solitär oder zerstreut leben. Das Ergebnis steht in direktem Gegensatz zu etablierten Theorien über Spermakonkurrenz. Die nämlich vermuten, daß in jenen Arten, in welchen eine hohe Wahrscheinlichkeit zu Spermakonkurrenz besteht, die Männchen möglichst voluminöse Ejakulate in diese Konkurrenz einbringen sollten. Männchen dürften demnach also nicht ihr Ejakulat produzieren, und es dann beim Masturbieren verschleudern.

Ich prüfte zwei miteinander zu verknüpfende Thesen, denen zufolge das Masturbieren für Männchen vorteilhaft sei. Rotgesichtsmakaken leben in Vielmännchen-Vielweibchengruppen, und die Weibchen sowohl als auch die Männchen sind hoch promisk. Zuerst untersuchte ich, ob das beim Masturbieren ejakulierte Sperma von niederer Qualität ist. Dann untersuchte ich, hierauf aufbauend, ob das daran anschließend produzierte Ejakulat „frischer“, ob es von höherer Qualität sein würde.

Den Mechanismus, wie einzelne Parameter des Ejakulats sich hinsichtlich der Zeitdauer, die das Ejakulat im männlichen Genitaltrakt gespeichert ist, verändern, bestimmte ich mit Testreihen an gefangenen Männchen, die in Einzelkäfigen gehalten wurden. Es ergab sich, daß die Ejakulate in ihrem Volumen umso größer waren und mehr Spermien hatten, je länger sie gespeichert waren. Zugleich aber schwammen die Spermien umso langsamer, und umso geringer war auch der Prozentsatz von lebenden Spermien in diesen lange gespeicherten Ejakulaten.

Auf diesen Ergebnissen aufbauend unterschied ich grob zwei Typen von Ejakulaten und ordnete sie den auf der Insel Yakushima auftretenden Paarungsstrategien von wildlebenden Männchen zu. Die sogenannten Guarders (die Bewacher-Männchen), in der Regel ältere, hochrangige Männchen, sind in der Lage, viele aufeinanderfolgende Kopulationen mit demselben Weibchen auszuführen. Das Ejakulat, das sie in die Spermakonkurrenz einbringen, bezeichnete ich als vom Typ A. Das sind jene länger

gespeicherten Ejakulate von großem Volumen und einer hohen Anzahl von Spermien, von denen jedoch viele bereits langsam schwimmen oder gar tot sind. Den Guarders gegenüber stehen die sogenannten Sneakers (die Betrüger-Männchen). Sie sind in der Regel jünger und von mittlerem oder niederem Rang. Sneakers können nur gelegentlich mit einem Weibchen kopulieren (eigentlich nie aufeinanderfolgend und immer versteckt vor den Guarders), und ihre Ejakulate stehen unausweichlich immer in Spermakonkurrenz zu denen der Guarders (oder auch anderer Sneakers). Es kommt vor, daß Sneakers auch noch kurz vor der Kopulation, masturbieren. Demnach bringen sie zumindest in diesen Fällen Ejakulate von geringer Menge in das Weibchen ein, die jedoch durch eine hohe Anzahl lebender und schnell schwimmender Spermien ausgezeichnet sind (Ejakulat vom Typ B).

Die höherrangigen Männchen wiederum masturbieren nur dann mit Ejakulat, wenn kein paarungsbereites Weibchen zur Verfügung steht und im Gegensatz zu den Sneakers vor allem nie vor einer Kopulation.

Erstens ist Masturbieren also ein Mechanismus bei jenen Primaten, die sich durch einen hohen Grad an Spermakonkurrenz auszeichnen. Zweitens können durch das Masturbieren niederrangige Männchen das Bestmögliche aus ihrer schlechten Ausgangsposition machen, indem sie eher in die Qualität des Ejakulats als in dessen Quantität investieren.

Unterstützung finden diese Ergebnisse zusätzlich noch durch eine DNA-Vaterschaftsanalyse. Diese ergab, daß sechs von neun Babies der Studiengruppe auf Yakushima von Sneakers gezeugt wurden. Masturbieren ist also eine physiologische Adaption an Spermakonkurrenz. Es wird, zwar in unterschiedlichen Zusammenhängen, jedoch von Guarders als auch von Sneakers betrieben. Es dient dazu, altes Ejakulat auszuwaschen und führt so zu einer qualitativen Verbesserung des nächsten Ejakulats. Demnach ist das Masturbieren eine Evolutionsstabile Strategie (ESS).

SUMMARY

Male masturbation or sexual-auto stimulation is well documented in humans. This study dealt with the occurrence of masturbation in non-human primates in general, and in Japanese macaques *(Macaca fuscata)* in particular, from the perspective of behavioural ecology.

In an interspecific comparison of 52 primate species, male masturbation was found to be a common behaviour that correlates more strongly to species that exhibit a multi-male multi-female breeding system than to species living in monogamous, one-male units, solitary or dispersed breeding groups. This result is in direct contrast to established theories of sperm competition, which predict that species with a higher risk of sperm competition (i.e. those with multi-male multi-female breeding systems) should invest in large ejaculate volumes so as to out-compete conspecifics. They should not, therefore, produce and seemingly waste ejaculate by masturbating.

In wild living (on Yakushima Island) and captive Japanese macaques, a multi-male multi-female seasonal breeding species with a high incidence of female and male promiscuity, two hypotheses concerning how masturbation may be beneficial to males in regard to sperm competition were tested. First, I tested whether or not males flush out low quality sperm from their genital tracts when they masturbate. Second, I tested for whether or not the subsequent ejaculate was "fresher".

The mechanism of how ejaculate parameters change in response to storage time in the male genital tract was determined by performing experiments with singly-caged Japanese macaque males. I found that the longer an ejaculate was stored, the larger its volume and total sperm number became. However, the longer an ejaculate was stored, the lower the sperm swimming velocity and percentage of vital sperm became.

Based on this result, roughly two types of ejaculates could be distinguished and correlated with commonly known male mating strategies. Guarders tend to be older, high ranking males who have the opportunity to perform many consecutive matings with the same female. They bring into sperm competition games Type A ejaculates, which have been stored for more or less lengthy periods and are characterised by a large volume and a large total sperm number comprised of slow swimming and many dead sperm. In contrast, sneakers, tend to be younger males of middle or low rank, who are usually able to mate only opportunistically (almost never consecutively and almost always covertly) and their ejaculates inevitably face sperm competition from guarders' (or from other sneakers')

ejaculates. Sneakers masturbate before mating and thus bring into the female only a small volume of ejaculate but one with fast moving sperm, all of which are a live (Type B). In contrast, guarders exclusively masturbate out long stored ejaculate only on days when there are no estrus females available in the troop. Guarders never were seen to masturbate before mating.

Masturbation, then, is one mechanism by which lower ranking males attempt to make the best of their limited mating opportunities in a species characterized by high levels of sperm competition by investing in ejaculate quality as opposed to quantity. This result is supported by a DNA-paternity-exclusion analysis in the study troop on Yakushima Island, in which six of nine babies were sired by sneakers. Masturbation is, thus, physiologically adaptive to sperm competition in primates. Both, guarders and sneakers use masturbation to improve ejaculate quality. Therefore, masturbation can be regarded as an evolutionarily stabilised strategy (ESS).

Figure 1: Masturbating Japanese macaque *(Macaca fuscata yakui)* on Yakushima Island. NINA-A study troop, mating period 1998 (photo made by Ruth Thomsen).

PREFACE

Why study male masturbation?

During the 3 years it took to complete this Ph.D. research, the question I was most frequently asked was not really of a scientific nature per say, but more of a general nature concerning the scientific value of such a study. The question was, of course, "why study masturbation?" Some of the "whys" looked completely amused, others were sympathetic or half-embarrassed, and yet a third category came close to anger. Most of these people were not working in science, but some of them did, so I feel I must set forth, in a few words, how my interest in male masturbation came about.

In 1993/94, while collecting data for my Master Thesis, I was following Japanese macaques in the beautiful forest of Yakushima Island. Every minute, I recorded in a little notebook, on which item a monkey was feeding. After some months, I was able to identify around 50 individuals, about 75 plant species and I was even able to make notes in Japanese. From then on, however, feeding records became routine - and routine is boring. I, therefore, started to write down anecdotal observations, identifying birds, butterflies, strange insects and the recording presence of deer and snakes. One day, it happened that my focal animal was masturbating. The male was sitting on a tree, concentrating on his penis, and with one hand, vigorously rubbing it back and forth for about the minute it took before ejaculate appeared. This white ejaculate not only looked like but also had the consistency of chewing gum. The male instantly tore it from where it had splattered on his hand and ate it without ceremony. I was halfway between feeling shocked and amused. The thing that shocked me was that, like humans, wild monkeys also masturbate. The amusing part was that they do it in almost the exact same manner.

Naturally, once a new behaviour is detected, a researcher wants to observe it again. Thus, my eyes focused on masturbating males and the white ejaculate so frequently found on the ground. My anecdotal pages in the notebook became full of data about masturbatory ejaculate, and I realised that masturbation is a common behaviour in wild living, unprovisioned Japanese macaques. At that time, however, I was completely at a loss as to why they perform such behaviour so often and under so many different social situations. The most striking observation for me during that time, was a male, who masturbated to ejaculation in the presence of an oestrus female. I could not fathom why he hadn't mated instead of

wasting his ejaculate or, if he hadn't wanted to mate, why he hadn't "saved" his ejaculate for later?

Upon my return to "civilisation", first to the excellent library of the Primate Research Institute of Kyoto-University, and later to Germany and the University of Munich, I realised from talking to other researchers with different interests, that male masturbation in wild monkeys may have something to do with questions concerning sperm competition in primates. From what I learned in these discussions, I knew I had found a problem that was worth examining in detail.

1. INTRODUCTION

1.1 Masturbation in humans

Masturbation or sexual-auto-stimulation in human males is well documented by a large body of scientific literature. The most famous reports on human male sexuality, including masturbation, are the Kinsey-Study (1948) and the Hite-Report (1981), in which men gave descriptions of their masturbatory habits.

Perhaps the most surprising aspect of male masturbation is the fact that ejaculates are produced and seemingly wasted. Andrologists have long considered the function of human male masturbation as a natural part of human sexuality. Zimmerman *et al.* (1965) argued that one possible function of male masturbation might be that old or low quality sperm is flushed out from the genital tract. Unfortunately, this study lacks, among other details, a definition of what constitutes sperm quality.

Baker & Bellis (1993, 1995) were the first to rigorously examine human male masturbation from an evolutionary perspective. They proposed that, even though ejaculate is lost through masturbation, what seems at first to be paradoxical might in fact be an adaptation for sperm competition. Male masturbation, they concluded, is a strategy whereby sperm fitness can be enhanced without having to increase the number of sperm in the female genital tract. The next ejaculate, produced some time after masturbation, should, they assert, be fresher and able to outcompete any old sperm from rivals during sperm competition. However, since modern humans live under a variety of influences caused by civilisation, which should deeply change natural pattern of reproduction, other study subjects than humans provide better opportunity to study mechanisms and ultimate function of masturbation.

In animals, reasons for male masturbation have yet to be studied in any depth. The occurrence of masturbation has either been ignored altogether or regarded merely as a compensatory act for a lack of mating opportunities during sexual arousal (e.g. Bielert & van der Walt 1982, Linnankoski *et al.* 1981). It has even been viewed as a pathological pattern of sexual behaviour, exacerbated by captivity (Dittrich 1968, Savage & Malick 1977, Beck & Power 1988, Mootnick & Baker 1994).

1.2 Sperm competition and the theory of sexual selection

Sperm competition is part of the theory of sexual selection, which was originally proposed by Darwin in 1859 to account for phenomena that he was unable to explain in terms of natural selection, as he described it at that time. Darwin argued that sexual selection "depends, not on a struggle for existence, but on a struggle between the males for possession of the females" such that "the result is not death to the unsuccessful competitor, but few or no offspring" (Darwin, p.136). Thus, the drive for reproductive success affects the behavioural, physiological and genomic aspects of male mating activity. Sexual selection operates in a variety of ways, which are not necessarily mutually exclusive. The most obvious aspect of sexual selection is the competition that occurs between members of the same sex to mate with the opposite sex. This kind of competition favours conspicuous secondary sexual ornaments and signals, mainly in males and, since Darwin catalogued these phenomena, such adaptations have been extensively investigated (Andersson 1994).

Sexual selection also occurs at the genomic level between competing ejaculates from different males to fertilise eggs. Much of the biologically meaningful sexual intrigue occurs after mating has occurred. Although many scientists may have inherently recognised this fact, sperm competition was defined relatively late by Parker (1970) as: "...competition within a single female between the sperm from two or more males for the fertilisation of the ova." Parker conducted pioneering work in the field of sperm competition with insects, in particular, flies. More recently, to incorporate the phenomenon of external fertilisation, he has modified his definition to "...competition between the sperm from two or more males for the fertilisation of a given set of ova" (Parker 1998).

Currently, sperm competition studies are rapidly spreading to include a vast array of groups including reptiles, fish, birds and mammals. Sperm competition is now widely recognised as a major and pervasive force in evolution (Choe & Crespi 1997, Birkhead & Møller 1998).

Mechanisms of sperm competition in insects have been tested empirically since the 1940's (e.g. Kaufman & Demerec 1942, Bateman 1948, Vandehay & Craig 1958, Schlager 1960, Parker 1970, Price *et al.* 1999). As such studies became more and more sophisticated, they revealed astonishing details and it became evident that there is no general rule as to how sperm competition works or to what extent males or females can influence it. Data on wild-living mammals, however, are rare (Gomendio *et al.* 1998) and data on the ejaculate of wild-living non-human primates are almost non existent.

My approach to the study of sperm competition in non-human primates is based upon three innovations. First, I focused directly on the ejaculate as opposed to indirect measures of potential ejaculate volumes, such as testicular size. Second, I combined data from both wild living and captive animals and third, I collected and analysed data specifically on masturbatory ejaculates, i.e. ejaculates that do not enter the female, but are nevertheless part of sperm competition strategies.

1.3 Sperm competition in animals

It has been suggested that sperm competition resemble a raffle, where the male with the largest amount of sperm is more likely to fertilise the ova (Parker 1984, Parker *et al.* 1990, Parker 1990 a). According to Parker's definition (1970), sperm competition operates only in species where female promiscuity occurs and the presence, at around the same time during the oestrus period, of at least two fertile males is necessary. Since many species fulfil these criteria, it is not surprising to find in a wide variety of taxa adaptations for sperm competition to occur.

Adaptations to enhance sperm competition often provoke sexual conflicts between males and females (Stockley & Purvis 1993). Sperm competition is not just a lottery giving each male and each ejaculate the same chance. Rather, each sex has developed strategies to influence fertilisation independently. Female strategies, however, have not been studied as extensively as those of males.

Female choice has been studied mainly in insects and arachnids (Eberhard 1996) but only a few studies based on empirical data have been conducted in primates (*Macaca fuscata:* Huffman 1987, Soltis *et al.* 1997 a & b; *M. mulatta:* Manson 1992). Competition within the female genital tract has been described as "cryptic female choice" in some insects (Eberhard 1997), because females may have developed mechanisms within their genital tract that allows them to choose between the sperm of different males. In other groups, this topic has also lead

to speculation concerning mechanisms of cryptic female choice but these studies still lack empirical data.

In contrast to females, various overt adaptations to sperm competition are evident in males. For example, one behavioural adaptation in males to reduce the risk of sperm competition is that of mate-guarding to prevent the female from mating with competitors (Berard *et al.* 1994). A well known physiological adaptation is the copulatory plug, formed by coagulating ejaculate, which acts, with varying degrees of success, to prevent subsequent males from achieving successful intromission into the female. Such plugs are found in many taxa and are very common in insects (Parker 1970), reptiles (Devine 1975), rodents (Martan & Shepherd 1976) and primates (Dixson 1998). Morphological adaptations include penises formed into sophisticated shapes to displace rival sperm from the females' genital tract. These penile adaptations are evident in several primate taxa where sperm competition is severe (Dixson 1987, Verell 1992). Large sized testes are commonly cited as an anatomical adaptation to sperm competition brought about by female promiscuity and have been documented in species as diverse as butterflies (Svärd & Wiklund 1989, Gage 1994), bats (Hosken 1998), ungulates (Ginsberg & Rubenstein 1990) and primates (Harcourt *et al.* 1981).

On the level of the individual, it is known in rhesus monkeys *(Macaca mulatta)*, that sperm production is related to the mass of testicular parenchyma. 23 million sperm are produced per gram of testicular parenchyma per day in this seasonal breeding species (Amann *et al.* 1976). Furthermore, a correlation of testis volume with body weight and with age is known from other primates (*M. fuscata:* Matsubayashi & Mochizuki 1982, *Papio cynocephalus anubis*: Bercovitch 1989). Thus, in wild living Japanese macaques, guarder males (see 1.7, H5) should potentially produce a greater volume of ejaculate than sneakers simply because, they tend to be older, larger animals.

Testis mass, however, is dependent not only on testicular size, but also on other factors. Starvation is known to reduce testis mass in mammals and thus ejaculate production becomes concomitantly smaller (Mann & Lutwak-Mann 1981). It is thinkable then, that males with small testes but who are in good nutritional condition can outcompete, in terms of ejaculate volume, males who normally would have larger testis mass, but suffer from starvation.

Ejaculate, in general, is not only costly to produce (Dewsbury 1982) but becomes limited for variable periods during mating activities because it takes time for the testes to reproduce new sperm before mating becomes possible again. The time of new sperm production may vary greatly with species and individual, what makes it impossible to predict next ejaculation in general. Nakatsuru & Kramer (1982) found empirical evidence that, due to

previous mating, sperm limitation occurs in the wild lemon tetra fish *(Pisces, Characidae)*. They also found that female choice comes forcefully into play the moment a male has depleted his sperm reserves. Females will then favour males who have not yet mated. Thus, males with large testes potentially run out of sperm at times of high mating success and so males with smaller testes may be afforded opportunities to out-compete them during sperm competition. Moreover, it does not necessarily follow that males with a large ejaculate volume succeed in mating so often that large volume would be such a great advantage. Bercovitch (1989), for example, failed to find any relationship between male mating success and testes size in baboons *(Papio cynocephalus anubis)*.

The feature common to all of these studies is that they relied upon indirect parameters to draw conclusions about the degree or severity of sperm competition. Even though these numerous studies give more or less consistent results, an analysis of the ejaculate itself was nevertheless lacking.

1.4 The non-human primate ejaculate

To date, only Møller (1988) has compared the number of sperm per ejaculate in different primate species and brought it in relation to their mating system. He demonstrated that the ejaculate of species living in a multi-male multi-female breeding system contains significantly more sperm than that of harem breeders. Møller's findings are consistent with common sperm competition theories in general (see 1.3) but data on intraspecific comparisons of ejaculate from different males in for example, one population of the same species were not included. Furthermore, many of Møller's data were derived from reports employing electro-ejaculation to collect primate ejaculates. The use of electro-ejaculation as a means to study "normal" ejaculate volumes and composition is problematic because monkeys are often anesthetized or, when not, they are generally extremely stressed (see 2.5.4.1) and this may well lead to aberrant ejaculate. Consequently, ejaculate derived under such circumstances must be viewed with caution.

Until now, data on the ejaculate of primates won from unanesthezised monkeys using methods other than electro-ejaculation have been reported in only two studies of chimpanzees (*Pan troglodytes:* Marson *et al.* 1989, Gould & Young 1996) and one study of squirrel monkeys (*Saimiri boliviensis:* Yeoman *et al.* 1997). Presently then, lack of data precludes the possibility of interspecific comparisons.

1.5 Paternity in different taxa

Different species compete using different methods, so mechanisms of how sperm competition works in one species cannot be extrapolated directly to another. Irrespective of which species are being investigated, the central question remains the same: the sperm of which male(s) succeed(s) in fertilisation? The main methodological problem in the study of sperm competition in internal fertilisers in general and, in particular, mammals, is that it occurs after the observable part of mating is completed, taking place entirely within the darkness of the female genital tract. Once DNA fingerprinting technologies became widespread in the 1980's, however, investigations of mating and reproductive success could be conducted and the study of sperm competition gained new perspectives.

The results of these many studies have not always been consistent and, in general, paternity cannot be related to any specific type of male or mating pattern. The general trend in insects is that either the first or the last male to mate sires the most offspring (Parker 1970). Other patterns, however, also exist. Bateman (1948), for example, found in *Drosophila melanogaster* that the 2nd male to mate with a female was most successful at fertilizing her eggs whereas Kaufmann & Demerec (1942) found a complete mixture of fertilisation in the same species of *Drosophila* after multiple matings.

Investigations of birds using DNA analysis have revealed that reproductive success within some avian populations could not have been accurately determined from behavioural observations alone, leading to major revisions in the mating classification of certain species (Poldmãa *et al.* 1995). A high proportion of extra-pair fertilisation success was found in some birds (Dixon *et al.* 1994) due to the maximization of a large amount of high quality sperm during extra-pair copulations (Birkhead *et al.* 1995). In the common shrew *(Sorex araneus)*, a significant positive correlation between sperm count and the number of offspring fathered has been found (Stockley *et al.* 1996) and in prairie dogs (*Cynomys* spp.), multiple paternity within litters is determined by the extent of female promiscuity (Travis *et al.* 1996). In yet other rodent species, the manner by which sperm competition works may depend on the mating order (first male advantage: Martan & Shepherd 1976, last male advantage: Dewsbury & Baumgardner 1981). Advantages independent of order, however, have also been detected in laboratory rats (Dewsbury & Hartung 1980).

In summary then, it appears that the probability of paternity is not universally correlated with any single one of the numerous factors studied to date. Rather, paternity may

depend on many factors, each influencing the other, or even on some as yet undetected mechanisms.

1.6 Sperm competition, paternity and masturbation in primates

The most interesting question concerning sperm competition in primates would be to detect the mechanism how it works inside the female`s genital tract and then to correlate this pattern with observable male behavioural strategies. However, since modelling sperm competition how it works inside the primate female has not been done yet, probably due to methodological difficulties, the most close method to detect at least its results, is to combine data on the inbrought ejaculates together with DNA paternity analysis. Since up to date, ejaculates were not studied, only information on indirect measuring of ejaculate input, i.e. male dominance rank or mating success, are available. In polygynandreous primates, in general, male rank is correlated with mating success (Berard *et al.* 1994) and thus, should be correlated also with the volume of inbrought ejaculate, even this assumption lacks of empirical proof, yet.

Furthermore, even though hypotheses concerning interspecific testes size, sperm number and sperm-producing tissue generally concur with commonly accepted ideas about sperm competition, intraspecific studies of sexual selection have revealed a lack of consistent correlation between male dominance rank and reproductive success. In groups having at least four adult males, only 27 % (N = 7 from 26) of studies have demonstrated a statistically significant correlation between male rank and reproductive success (Bercovitch 1992). Furthermore, only two of the seven reports of positive correlation between male rank and reproductive success came from wild populations of primates.

Of the only four macaque studies to date using DNA fingerprinting to identify sires, all have concluded that mating success is not a reliable indicator of reproductive success because males who were seen to copulate only rarely sired a disproportionately large number of offspring (*Macaca fuscata:* Inoue *et al.* 1992, Soltis *et al.* 1997 b, *M. fascicularis:* de Ruiter *et al.* 1992, *M. sylvanus:* Ménard *et al.* 1992). There is then, no guarantee that males of high rank endowed with large reproductive organs and probably large ejaculatory input will necessarily sire more offspring. Indeed, it appears that also criteria other than rank or the size of male reproductive organs are important for reproductive success (Figure 2). Observations on captive primates have revealed that, in contrast to males of other mammalian orders, primate males are unique in that they masturbate more or less regularly until ejaculation

occurs (*Macaca arctoides:* Linnankoski *et al.* 1981 & 1993, Nieuwenhuijsen *et al.* 1986, *Papio ursinus*: Bielert & van der Walt 1982, *Pan troglodytes:* Kollar *et al.* 1986). A novel prediction based on sperm competition theory concerning male behaviour, therefore, would be that it is masturbation that accounts for the lack of positive correlation between mating and reproductive success in polygynandreous primates such as those of the family *Cercopithecidae*, which includes macaques, baboons, guenons and colobines. Exactly how masturbation might contribute to this lack of correlation is outlined below.

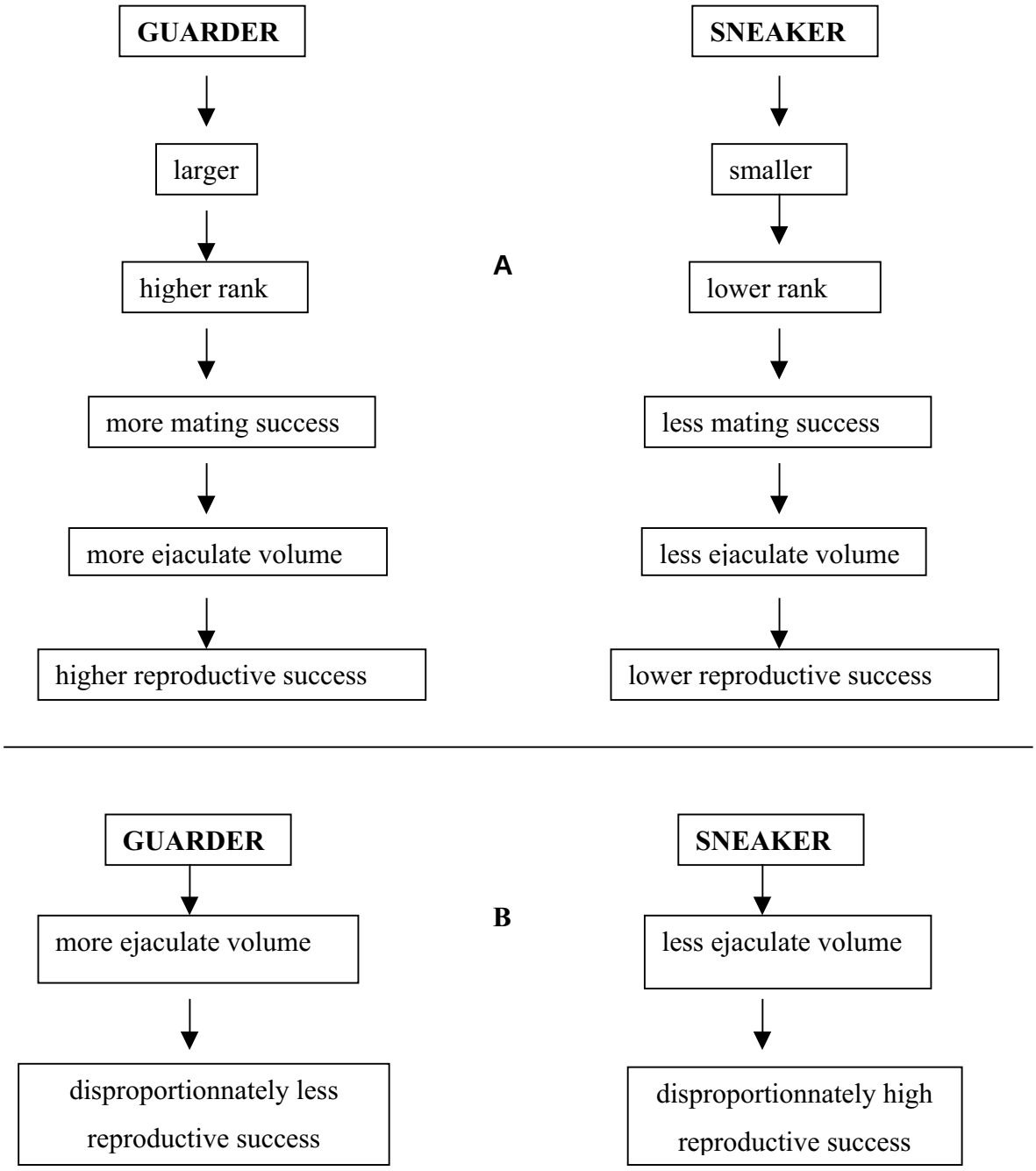


Figure 1: The problem of how male rank, mating success, ejaculate volume and reproductive success is connected in primates. (A) shows the actual hypothesis due to common theories taken from various literature. (B) shows the results taken from studies revealing paternity, which are in contrast to the assumptions from (A).

1.7 Aims and hypotheses of this study

I hypothesise that masturbation is an adaptation relating to sperm competition in non-human primates living (mainly) in a multi-male multi-female breeding system and that the result of masturbation is to wash out old ejaculate and thus increases ejaculate quality of the next produced ejaculate. High quality ejaculate should outcompete then, at least sometimes ejaculate of large quantity, but of low quality, during sperm competition. This would be of considerable evolutionary consequence were it demonstrated to hold true. I examine male masturbation in non-human primates empirically from different angles (see below). Furthermore, I discuss my results from the perspective of reproductive success and concentrate on the question of whether masturbation is an evolutionarily stabilised strategy (Maynard-Smith 1982) for males adopting a sneaker or a guarder mating strategy. More specifically, I tested the following hypothesis in this study.

(H1) Questionnaire

To assess the frequency of occurrence of masturbation in wild living non-human primates, I sent a questionnaire to a large number of field biologists.

a) If masturbation is observed often enough to be considered a common behaviour in wild living species, why, at least for species in which a high level of sperm competition exists (with multi-male multi-female breeding system), do males paradoxically waste costly ejaculate by masturbating instead of conserving it for when mating opportunities arise, as it is the case in some bat species (Racey 1979)? Were masturbation found not to be common in wild living primates but occurred mainly in captive primates, then this behaviour might simply be a byproduct of captivity not worthy of detailed study from an evolutionary viewpoint.

b) If masturbation is related to sperm competition in primates according to my hypothesis, it should be observed to occur more often in multi-male multi-female breeding species than in species living in one-male units, family groups, monogamous, solitary or in dispersed breeding systems.

(H2) Patterns of masturbation in wild and captive Japanese macaques

To evaluate details of male masturbatory behaviour, observations were conducted on wild living Japanese macaques *(M. f. yakui)* on Yakushima Island and on singly-caged Japanese macaques *(M. f. fuscata)*. Japanese macaques live in multi-male multi-female groups and are seasonal breeders that exhibit a high degree of both female and male promiscuity (Soltis *et al.* 1997 a, b). It was known from casual observation in unrelated studies, that male masturbation occurs frequently in the Yakushima population. Since spermatogenesis in Japanese macaques is restricted to the mating period (Matsubayashi & Enomoto 1983), the seasonal prevalence of masturbation until ejaculation should be restricted to it.

a) If masturbation is merely an act that compensates for a lack of mating opportunities as suggested by Bielert & van der Walt (1982) and Linnankoski *et al.* (1981), individual males should masturbate only during that distinct period of time when they have no mating success at all in the wild. This should happen independent of whether males adopt a guarder or sneaker mating strategy (see H5).

b) If, however, wild males also masturbate a short time prior to mating, this would lend support to the hypothesis that masturbation occurs to ensure a fresh supply of ejaculate (Zimmerman *et al.* 1965, Baker & Bellis 1993).

c) Singly-caged males are never allowed mating opportunities but ejaculate production during the mating season is nonetheless continuous (Matsubayashi & Enomoto 1983). Theoretically, there are two patterns thinkable. First, males stop sperm production at the moment the sperm storage organs are fulfilled and just keep holding this sperm until they might get a chance for mating. Second, males masturbate out these sperm regularly due to the amount of sperm production rate. Thus, masturbatory activity in these males would serve to empty the testes of their ever-increasing ejaculate volume. Assuming the rate of sperm production is steady and that males wait until a certain "threshold" volume of ejaculate is produced, masturbation would be expected to occur at fairly regular intervals, when storage capacity of the *cauda epididymis* is finished.

(H3) Subsequent ejaculations in singly-caged Japanese macaques

To find out what actually happens after ejaculation in terms of subsequent sperm production and ejaculate quality, I conducted experiments with captive males.

The five ejaculate parameters ejaculate volume, total sperm number, sperm velocity, motility and morphology were measured within 15 minutes of "human hand ejaculation" (HHE, see 2.5.4.1) to evaluate the ejaculate quality in response to storage time inside the male genital tract. The two most important parameters for fertilisation in humans (and thus should be important also during sperm competition) are the swimming velocity (Mortimer & Templeton 1982, Birkhead *et al.* 1995, Schirren 1995) and the percentage of vital sperm per ejaculate (Barrat & Cooke 1991, Schirren 1995). Even though reproductively active male Japanese macaques range in age from about 4-18 years (Matsubayashi & Mochizuki 1982), ejaculate parameters other than volume are not expected to differ with age, since the most important factor influencing the ejaculate should be storage time.

a) Thus, if masturbation functions to flush out old, low quality sperm, the longer ejaculate is stored, the lower its quality should become in terms of swimming velocity and the percentage of vital sperm.

b) However, since ejaculate production in Japanese macaques is continuous during the mating period, the longer ejaculate is stored, the greater the ejaculate volume and number of sperm per ejaculate should become.

(H4) Ejaculate types

If the above predictions are confirmed, the "ideal" ejaculate, that of a large volume with a large number of highly motile sperm, should not exist. Instead, there should be two broadly defined types of ejaculate:

a) Type A ejaculate should be characterised by a large volume and a high proportion of slow moving, senescent sperm due to a long storage time. This is an ejaculate of large quantity.

b) Type B ejaculate should be of a low volume with relatively fewer, but faster, fresher sperm due to masturbation having happened only a short time before. This is an ejaculate of high quality.

(H5) Guarders and sneakers: ejaculate quantity versus quality?

In many species, there are two types of males distinguished by their different mating strategies, namely, sneaker-males and guarder-males (Trivers 1972, Parker 1990 b). To align

ejaculate types with male type in Japanese macaques, I modified the sneaker-guarder model of Parker (1998) as follows.

Guarders are typically high ranking, older, larger males with larger testis, who attempt to avoid sperm competition by preventing females from mating with sneaker males (Berard *et al.* 1994). The guarder males' mating strategy is to perform multiple, consecutive matings with the same female. If their mate guarding succeeds, they will first, avoid sperm competition and second, bring in large amount of ejaculate into the female. Thus, they should not masturbate on days with mating success, but, if ever only on days without mating success.

In contrast, sneakers are younger, medium or small sized males with smaller testis of middle to low rank in the troop. Non-troop male sneakers, such as solitary males or males from neighbouring troops, are also known in the Yakushima population (Sprague 1989). Sneakers have a limited number of chances to mate and they do sopportunistically (Berard *et al.* 1994). They, therefore, inevitably face sperm competition. To make out the best of their worse situation, sneakers theoretically have two opportunities to outcompete guarders (or other sneakers) ejaculate.

a) They try to store large amount of ejaculate and then bring this large amount during one copulation into the female. In this case then, sneakers should not (or at least not regularly) masturbate. This would be type A ejaculate and thus, sneakers would invest more in ejaculate volume than in ejaculate quality.

b) They masturbate and flush out low quality ejaculate, thereby allowing for the rapid production of a new ejaculate and thus they would invest rather in ejaculate quality than in quanitity (type B ejaculate).

I argue that based on the fact, that sneakers may only very rarely have the chance to outcompete guarders ejaculate in quantity anyway, they rather should invest in type B ejaculate during sperm competition. Thus, sneakers should be observed masturbating regularly in the wild, if they have no mating success, but also on days with mating success (i.e. before mating).

2. MATERIALS AND METHODS

2.1 Definition of masturbation

In this study, I defined masturbation as a rhythmic rubbing of the erect penis with either a hand or the feet, which resulted in a complete ejaculation. Masturbation without ejaculation or that resulting in an undefined fluid secretion, such as a mixture including a urine-like coloured material, was excluded from behavioural data collection. In the case of the questionnaire, however, a looser "masturbation only" category was used.

2.2 Questionnaire: which primate species masturbate?

A questionnaire concerning only male masturbatory habits was sent to about 120 primatologists between 1996 and 2000. Since contributors were free to pass the questionnaire on to colleagues, the exact number of recipients is unknown. The questionnaire asked for information concerning the species (common and scientific names), study site (wild, semi-free ranging, captive), breeding system (multi-male multi-female, monogamous, dispersed, family group or one-male unit) and the approximate amount of observation time the researcher had spent observing that particular species. The scientific name was verified using Rowe, 1996. If a researcher did not mention the scientific name of the species, I used the common name to locate it in Rowe (1996). Where the scientific names used by contributors differed from those of Rowe, I used Rowe's classification to maintain consistency.

As it may be difficult to observe masturbatory ejaculation in the wild, especially in small sized species or in those occupying the higher strata of a tropical rainforest, the question concerning masturbation was broken down into three types of observations: a) rhythmic rubbing of the penis (to exclude the possibility of simply holding the penis), b) masturbation with ejaculation and c) no observation of masturbation. Observations had to be noted as either yes or no, excluding possibilities such as perhaps or probably. Questionnaires that contained ambiguous answers were excluded from the analysis.

In cases where a species was represented more than once in the questionnaire replies, information from the researcher with the longest period of observation was used in the analysis. If, however, two different answers concerning the same species were received, such as one questionnaire recording "yes" for masturbation with ejaculation and another "no", the

positive answer was used and the negative answer ignored, irrespective of the length of time spent studying the species.

The data collected from questionnaires were tested using phylogenetic regression analysis with the Glim 4 program (Royal Statistical Society, London). All tests used a 1-tailed p - value and the primate phylogeny adopted was that of Purvis (1995). The phylogenetic regression analysis contained two parameters concerning the breeding system, multi-male multi-female (MM-MF) versus other (O: dispersed, monogamous, family, one-male unit), and three parameters concerning masturbation, rhythmic masturbation (M), masturbation with ejaculation (ME) and no observation of masturbation (N). All five parameters (O: tested for M, ME and N versus MM-MF: tested for M, ME and N) were tested in three test rows as follows, to adjust for whether masturbation correlates with a higher probability of a species having a multi-male multi-female breeding system independent from phylogenetic relationships.

(A) M and ME versus N
(B) ME versus M and N
(C) ME versus N

2.3 Animals and study sites

Based on previous own observations on masturbation in different populations of Japanese macaques in Japan, I choosed Yakushima Island as study site. The most important criteria was that the macaques on Yakushima were continuously habituated without provisioning up from the beginning (Maruhashi 1980). Furthermore, I observed masturbation on nine different locations on this Island. This fact indicates that masturbation is common for these macaques. As main study site, I choosed Hanyama (Figure 3).

2.3.1 The study species: Japanese macaques

Japanese macaques belong to the superfamily *Cercopithecoidea*, the family *Cercopithecidae* and the subfamily *Cercopithecinae* (Rowe 1996). They are found only in Japan where two subspecies occur (see for an overview: Yamagiwa & Hill 1998). *Macaca fuscata fuscata* inhabits the Islands of Honshu and Kyushu and has the northernmost distribution of all primate species (except humans), extending to the Shimokita Peninsula

(41°N 131°E, Nakagawa *et al.* 1996). The other subspecies, *M. f. yakui*, is endemic to Yakushima Island (30°N, 131°E, Maruhashi 1980), which constitutes the limit of this species distribution to the south (Figure 3).

The two subspecies exhibit a few differences in phenotype and genotype (Shotake *et al.* 1975). Both, however, adopt a multi-male multi-female breeding system with a restricted mating season from mid September to mid February, the peak being from October to December (Yamagiwa 1985).

Field studies of Japanese macaques have been conducted for more than 40 years in many parts of the Japanese archipelago on a variety of populations from wild living to semi-free ranging populations, those in monkey parks and zoological gardens and laboratory-held individuals. Japanese macaques are relatively easily available, and compared with other primate species, about which little may be known, their morphology, anatomy and aspects of their reproductive behaviour have been extensively studied. This knowledge base makes them the perfect species in which to study a completely new topic, such as masturbation.

Because of agricultural development, especially forestry, totally wild living Japanese macaques are now a rarity (Yamagiwa & Hill 1998). Only two small Islands remain where the macaques have been habituated to human observers without provisioning and where habitat destruction has not yet become widespread. One is Kinkazan (38°N, 141°E; Nakagawa *et al.* 1996), a small Island off the Northeast of Japan, and the other is Yakushima.

2.3.2 The Study site in the wild: Yakushima Island

Yakushima (Figure 3) is 500 km^2 in size and its highest point is 1935 m in altitude. The mean annual temperature is 20° C and its vegetation ranges from broad-leaved evergreen forest near the coast to subalpine grassland at higher altitudes. Because of the steep topography of the Island, human settlement is restricted to the coastal region.

Research on the macaques of Yakushima started in 1976 (Maruhashi 1980) by habituating the first troop without provisioning at the Hanyama study site on the Northwestern coast. The vegetation inside Hanyama consists of subtropical coastal forest, warm temperate broadleaf evergreen forest and secondary road side forest (Maruhashi 1980). Since habituation, various topics on the ecology and social behaviour of this wild population of Japanese macaques have been investigated (for an overview see: Yamagiwa & Hill 1998).

Hanyama lies within the Yaku-Kirishima National Park and thus macaques are protected from hunting by law. During my study period, three troops were fully habituated

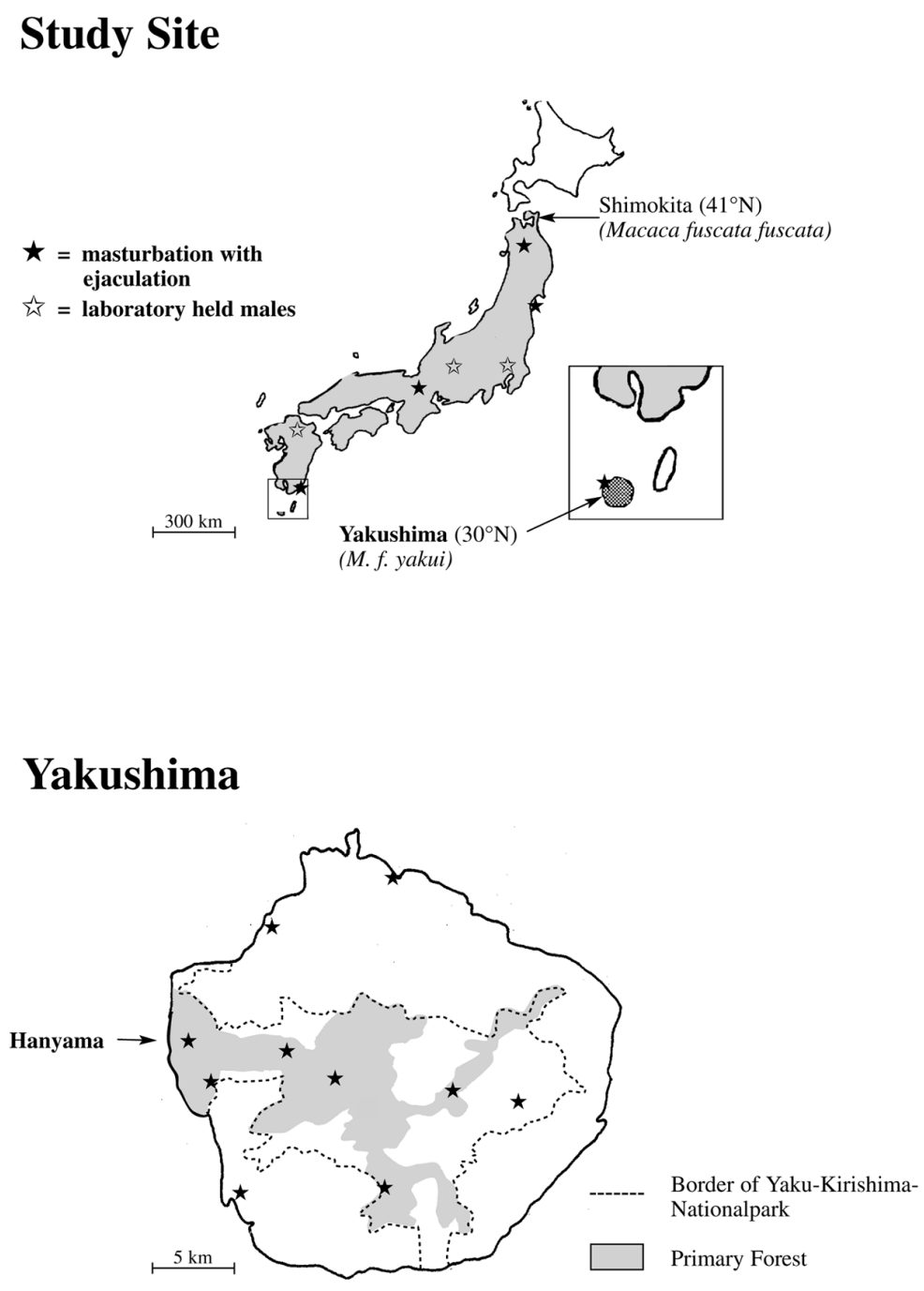


Figure 3: Japan and Yakushima Island. The stars indicate own obervations of masturbating males in different populations of Japan and Yakushima.

and habituation had just begun on two other troops. Long-term data on troop compositions, male immigration and emigration and feeding ecology of the macaques have been collected by the members of the Yakushima Research Group and information is housed at the research-station of the Primate Research Institute (PRI) of the Kyoto-University in the village of Nagata.

2.3.2.1 The study troop

The NINA-A troop occupied a 2 x 0.7 km range (0 - 230 m asl) inside Hanyama surrounded by at least four other troops with home-range overlap of approximately 60 %. NINA-A troop, which has never been provisioned, was habituated to human observers starting in 1994 (Toshiaki Tanaka, Juichi Yamagiwa & Ruth Thomsen). Complete habituation, identification and the naming of all individuals, including juveniles and infants, was done in the summer of 1997 (Joseph Soltis & Ruth Thomsen). Troop composition changed during the study period due to natural population dynamics, such as male immigration and emigration, births and deaths (Table I). The age of the 15 males was estimated using body size and other external features (Table II) based on 25 years of continuous research on this population.

Table I: NINA-A-troop composition during the 1997 and 1998 mating periods. Adult › 4 years, juvenile 1-3 years, infant 0 years.

	Males		**Females**		**Total**	
	1997	1998	1997	1998	1997	1998
Adult	15	12	15	13	30	25
Juvenile	10	10	7	4	17	14
Infant	3	3	2	4	5	7
Total	28	25	24	21	52	46

Table II: NINA-A troop males´ code, dominance rank and age in years in 1997 and 1998 mating periods.

Rank 1997	**Code**	**Age**	**Rank 1998**	**Code**	**Age**
1	SH	12	1	SH	13
2	MO	14	2	MO	15
3	KA	12	3	KA	13
4	GO	12	4	IN	10

5	KU	11	5	AR	10
6	TE	10	6	NM	9
7	IN	9	7	SK	8
8	AR	9	8	MM	8
9	NM	8	9	BA	6
10	SK	7	10	PA	6
11	MM	7	11	JK	5
12	BA	5	12	TO	5
13	PA	5	----------	----------	----------
14	JK	4	----------	----------	----------
15	TO	4	----------	----------	----------

2.3.2.2 Study period and observation time

During the two mating periods (September 1997 - January 1998 and September 1998 - December 1998) I conducted focal animal sampling on the males (15 in 1997 and 12 in 1998, Tab. 2) for 435 hours 30 min (mean ± SD = 30.1 ± 22.5 hours/male). The total study period ranged from October 1997 until November 1998. Total time of focal animal sampling (Altmann 1974) of males and females over the 13 months was 1204 hours. Data from this 13-month study were used to get an overview of male masturbatory activity throughout a whole year, to determine the male dominance rank (2.6.5) and to detect sneakers and guarders (2.6.6). Focal sampling data on females were used for another study (Thomsen & Soltis 2000).

2.4 Singly-caged Japanese macaques at the Primate Research Institute

Eight adult male Japanese macaques were selected from the breeding colony at the PRI, Kyoto-University, Inuyama, Japan, for ejaculation experiments. Two males were wild caught, one had come from a monkey park some months previously and the remaining five were bred at PRI and reared by their mothers. None had been used in invasive experiments, but some had previously taken part in behavioural experiments (PRI, veterinarian records).

The eight males were housed in individual cages within a breeding room in which a total of 22 single cages housing males and females were arranged. Olfactory, visual and auditory access to the other monkeys was not restricted, but poor due to the single cage

circumstances. During the entire experimental period, at least one estrus female was present in her own breeding cage and she was housed together from time to time with males to mate. The eight males chosen for this study, however, were not allowed to mate during the experimental period, but they did so before and after.

The light cycle was from 8:00 a.m. to 8:00 p.m. Room temperature was continuous at 20 ± 5 °C. The cages were raised 1m above the ground and as the bottom of the cage consisted of bars, fallen masturbatory ejaculates, so called spots (Bielert & van der Walt 1982) could be easily noted. The caretakers usually cleaned the cages and the space below the cages in the morning after 8:00 a.m. and thereafter fed the monkeys monkey chow, sweet potatoes and seasonal fresh fruit. They changed this routine, however, in accordance with the time of the experiments (2.6.4) to avoid interruptions.

2.5 Sexual behaviour and reproductive parameters

Japanese macaques perform a series of non-ejaculatory mounts culminating in ejaculation (Tokuda 1961), which can be verified by the white copulatory plug on the females red pudenda and/or remaining ejaculate on the male's penis. A female was judged to be in oestrus if a copulatory plug was observed at the opening of her vagina at least once on the day of observation. In males, sperm production starts at approximately 4-5 years of age (Nigi *et al.* 1980, Matsubayashi & Enomoto 1983). Males were judged to be adult from the time ejaculation was observed by masturbation or copulation.

Gestation length is 173 ± 8 days (Nigi 1976) and females on Yakushima give birth from mid March until mid July with a peak occurring in April and May (Thomsen & Soltis 2000). The inter-birth interval in wild-living Japanese macaques usually is 2 years (Takahata *et al.* 1998).

2.6 Data on the ejaculate

To compare the quality of auto-masturbatory ejaculates of wild and captive males, from both populations, 45 ejaculates (captive N = 24, wild N = 21) were collected immediately after auto-masturbation and analysed within 30 min after ejaculation.

Furthermore, this data set was also used to check, whether any of the 15 males from NINA-A troop were excluded from reproduction, due to bad ejaculate quality, even they were observed to mate.

2.6.1 The storage time of an ejaculate

In the wild, masturbation by individual males was recorded (see 2.7.2). Due to the difficulties of conducting continuous focal sampling in the dense forest of Yakushima, inevitably, some ejaculations went undetected. Thus, observations were also made on captive males. For each of the eight captive males, the occurrence of ejaculatory spots (Bielert & van der Walt 1982) on the floor under the cage was checked over 42 consecutive days during the mating period, from December 12th 1998 until February 17th 1999. Initially this check was made once each hour over a 24-hour period. However, once the preferred individual masturbatory time of each male was known (after about 1-5 days), the check was made only at these times, mainly at dusk and dawn.

2.6.2 Collection of masturbatory ejaculates in the wild

After a wild male was observed to ejaculate, the individual was displaced to prevent him from eating the ejaculate. Fresh masturbatory ejaculates were collected from rocks, the leaf litter, or from the road with a pipette (fluid part) and tweezers (plug part) and after measuring the total volume in ml with a scaled tube, put into a small plastic bag (squeezing bag). Only ejaculate samples from which a minimum of 70 % (by estimation) could be collected, attaining a volume of at least 1.0ml, were used for further analysis, since too small a volume may not have contained enough sperm (Kiyoaki Matsubayashi, personal communication). The ejaculate was transported in my pocket to be maintained at body temperature (since sperm swimming speed diminishes if it becomes too cold) and analysis was done in the forest within 30min of ejaculation, using a light microscope.

2.6.3 Analysis of masturbatory ejaculates in the wild

The common method for human ejaculate analysis without a computer-assisted-semen-analysis (CASA) system, used at the Technical University (TU) of Munich, Department of Dermatology and Andrology, was modified according to field conditions. Since this was done for the first time in wild living primates, I performed all possible kinds of analysis concerning sperm and only later excluded unimportant parameters.

For all analyses described below, only the fluid part of the ejaculate was used, since most of the sperm are in the fluid, whereas the plug contains only few (Mann & Lutwak-

Mann 1981). To separate the fluid from the plug without laboratory facilities (such as an incubator or chymotrypsin, Mann & Lutwak-Mann 1981), the ejaculate was pressed by hand inside the squeezing bag until sufficient fluid (about 0.5ml) was released. Following parameters were measured:

pH-value

The pH-value was measured with a special indicator paper for ejaculates (range from 6.4 - 8.0 pH) from Merck Corp. Germany, immediately upon collection of the ejaculate.

Sperm motility (percentage of each stage)

To determine sperm motility, the swimming speed of 200 sperm per ejaculate were estimated in the native ejaculate, characterised as "rapidly progressive", "progressive" or "non progressive" (dead) and the percentage of each stage present was calculated.

Agglutinations per ejaculate

Agglutinations were checked in the native ejaculate. Whenever more than twenty sperm were detected to be clumped together in one ejaculate, the ejaculate was defined as “with at least one agglutination“. The percentage of ejaculates with agglutination was calculated in relation to all observed ejaculates.

Percentage of vital sperm per ejaculate

If more than 40% of sperm were non-progressive, an Eosin-test was done to separate living (but not moving) from dead sperm (WHO-Instructions 1993). One drop of ejaculate was mixed with one drop of 1% Eosin-solution. After 5min, dead sperm became red, whereas live sperm remained uncoloured. The proportion of dead sperm per ejaculate was calculated by counting 100 sperm and thus the percentage of vital sperm (vitality) per ejaculate could be detected.

Morphology of sperm

The percentage of abnormal sperm was calculated per ejaculate. Sperm morphology was checked at 1000x magnification (using the oil-inversion technique) with special coloured glasses (Testsimplets, Boehringer Corp., Mannheim, Germany) for abnormal forms such as: double-heads, micro- or macro-heads, double-tails, side-tails, deformed heads, deformed midpieces, bent tails, cytoplasmic concentration around the midpiece, round cells or other forms known from human ejaculates (WHO-Instructions 1993, Schirren 1995).

Total sperm number in mio per ejaculate

Total sperm number was measured in million per total volume of ejaculate (mio/ejaculate) with a common Makler–chamber for cell counts. To stop sperm movement, one drop of ejaculate was mixed with 3 % NaCl-solution in the ratio of 1: 20 (TU-laboratory). After 5min, the mixture was put into the Makler–chamber and viewed at 400x magnification. Sperm number was counted and calculated for the whole ejaculate. Total volume of ejaculate was calculated by adding the measured volume of collected ejaculate to the estimated percentage of lost ejaculate.

2.6.4 Ejaculates from singly-caged males

Two types of ejaculates were collected from singly-caged males. First, auto-masturbatory ejaculates (spots) were collected from surveyed males from below the cages to compare this ejaculate type with them of wild living males (see above).

Second, ejaculates from trained singly-caged males were collected in relation to the time of their last masturbatory ejaculation to detect changes in ejaculate volume and quality in response to ejaculate storage time in the male's reproductive tract. Experiments were conducted between 1 and 32 hours after the last masturbatory ejaculation was detected (2.6.1).

2.6.4.1 Ejaculate collection technique: human hand ejaculation (HHE)

The four generally employed techniques for semen collection in non-human primates have been the artificial vagina, removal of the ejaculate from an impregnated female immediately following copulation, electro-ejaculation and semen collection after auto-masturbation (Martin & Gould 1981).

An artificial vagina can be used quite successfully in chimpanzees, but for as yet unknown reasons, macaques cannot be trained to learn this method. By removing semen from a female, only parts of the ejaculate can be won and furthermore, this is mixed with female's genital fluid so natural parameters would have changed. Auto-masturbation in general is a very good noninvasive method to collect semen from captive animals (Brown & Loskutoff 1998), and especially from wild monkeys. However, the time of ejaculation cannot be manipulated by the researcher, and thus I could not use the method to answer questions for which exact storage times were needed. The remaining technique of electro-ejaculation

(whether by the penile - or rectal probe methods), are well developed for mammals in general (Ball 1976) and for macaques (Mastroianni & Manson 1963, Weisbroth & Young 1965, Matsubayashi 1982, Sarason *et al.* 1991). With both types of electro-ejaculation techniques, however, there are problems.

First, if not anaesthesized (penile method), most monkeys are stressed during the procedure, as indicated by a non-erect penis (Bercovitch 1999), probably due to the use of electricity. Second, the negative effects of the anaesthesia (rectal probe method) or electricity on the semen itself range from a small volume of ejaculate to a disproportionately large amount of dead sperm (Yeoman *et al.* 1997). Such semen still may be usable for artificial insemination, but it cannot be used to draw conclusions concerning evolutionary biology. I, therefore, modified a method used successfully on chimpanzees and squirrel monkeys (Marson *et al.* 1989, Hiyaoka & Cho 1990), known as human hand ejaculation (HHE), in a manner appropriate for Japanese macaques. Using this method, the monkeys ceased to be stressed after several days of training (as indicated by penile erection during the experiments) and I could decide on the time of ejaculation without using electricity or anaesthesia.

Experiments were conducted inside the breeding room, to avoid transportation stress for the monkeys, but in a special cage designed originally for electro-ejaculation using the penile method (Matsubayashi 1982). Males were restrained on hands and feet. The penis and testes were then cleaned with warm water and the monkeys were fed peanuts and fruits throughout the procedure. Ejaculation was stimulated by massaging the genital region with the human hand. To begin, males were trained once a day at times other than when they normally performed masturbation. After 10 days of training, three of the eight males still refused to co-operate with HHE and were thus eliminated from further experiments. Five males (numbers: 974, 1134, 1209, 1316 and 1438, age: mean = 11, SD = 4.7 years, range: 6 - 18 years) responded to HHE with full ejaculations. The procedure was performed for not longer than 20min and if a male did not ejaculate during this time, the experiment was stopped without the final reward of fresh fruit. HHE was attempted again either later that same day or the following day.

After 10 days, HHE was performed on the remaining five males depending on the time elapsed since the males last auto-masturbatory episode in the cage to detect changes in ejaculate quantity and quality in reference to storage time. The male ejaculated directly into a graduated tube (0.1ml - 10ml) to measure the volume of the entire ejaculate. The ejaculate then was incubated immediately at 35°C for 30min to ensure liquefaction (fluid separates from coagulum).

2.6.4.2 Ejaculate analysis with the computer assisted semen analysis (CASA) software system

Ejaculates won using HHE were analysed with laboratory facilities for the same parameters as those of wild males in the forest (2.6.3). The CASA-system works faster than the analyses conducted in the field but both methods give equal results for Japanese macaque ejaculates (own data). One difference, however, is that with the CASA-system, velocity is measured exactly rather than relatively (wild: motility). Sperm morphology, total sperm number per ejaculate, sperm swimming velocity and vitality were measured immediately after incubation with CellSoftTM - Series 4000, Neuroscience Inc. Japan. For the CASA-analysis, only 1-4 drops of fluid ejaculate were used. The average swimming velocity of 200 sperm per ejaculate was measured in micrometers per second (μm/s). Dead sperm were separated from live ones automatically and vitality was calculated for each ejaculate.

2.7 Behavioural data collection on Yakushima

2.7.1 Animal sampling methods

All 15 males of NINA-A-troop were chosen as focal animals. The males were listed according to rank (Table II) and from the time the troop was detected in the morning, the chosen male was located and followed using focal animal sampling (Altmann 1974) over a 2-day period. During behavioural observation, if the focal male could not be detected within a 30-minute- period, the male listed next was followed for the rest of the day and the male lost was followed again the next day he could be located.

Data were used for analysis only when I succeeded in following a focal male for at least 6 hours per observation day without interruptions of more than 5 minutes. Since day length from dawn to dusk is approximately 12 hours on Yakushima during the mating period, I considered a minimum of a half-day of focal sampling to be adequate for analysis.

2.7.2 Male masturbation

Masturbation with ejaculation was noted whenever a male was observed performing it, independent of whether he was the focal animal or not. Thus, data on masturbatory

ejaculations were collected both by focal animal and the *ad libidum* sampling techniques (Altmann 1974).

2.7.3 Male mating success

Whenever a focal animal was seen copulating with a female, time of ejaculation and female identity was recorded.

2.7.4 Individual timing of masturbation and mating

The time and number of masturbatory ejaculations and mating ejaculations were calculated for each of the 15 males individually to detect ejaculatory patterns of sneakers and guarders (see 2.7.6).

2.7.5 Male dominance rank

To determine male to male dominance interactions, displacements (when one male approached within 1m and the other moved away) and submissions (facial grimaces, cowering, fleeing) were recorded. A total of 1576 dominance interactions between the 15 males over the 13 months were recorded (median per dyad = 8, range 1 - 143). A matrix was formed with winners on one axis and losers on the other. There were a few dyads that showed bi-directional displacements, and in these cases the highest ranking male was the one who won the majority of interactions (Table II).

2.7.6 Male mating strategies: guarders and sneakers

The aim of mate guarding by males is to prevent females from mating with other males and thus to avoid sperm competition and to maximise their own mating success (Berard *et al.* 1994). For the Yakushima population, I defined guarders as males who were observed to ejaculate into the same female during focal animal sampling more than three times per day over at least two days for at least once. Sneakers, on the other hand, were defined as males, who were observed to succeed only once per focal sampling day to ejaculate into the same female and thus never did consecutive ejaculations.

2.8 Statistical analysis

Non-parametric statistical tests were used (Conover 1980, Zöfel 1988, Lamprecht 1992) and calculations were performed using InStat Version 2.01 for Macintosh. With the exception of the phylogenetic regression analysis (2.2), all p-values were 2-tailed. Significance was set at alpha = 0.05.

3. RESULTS

3.1 Results of the questionnaire

I first tested, whether masturbation, which to date has mainly been documented for captive species (Savage & Malick 1977, Linnankoski *et al.* 1981, Bielert & van der Walt 1982, Beck & Power 1988, Mootnick & Baker 1994), is a regularly performed behaviour in wild living primate species as well. Second, using a phylogenetic regression analysis, I tested whether species exhibiting a multi-male multi-female breeding system are more likely to masturbate than species exhibiting other breeding systems.

3.1.1 Masturbation in non-human primates

Of the 131 returned questionnaires (see 2.2), 52 of them, each representing a separate species, were tested. The 79 questionnaires not included in the analysis were rejected based on lack of, overlap of or conflicting information, as outlined in the Methods section. Of the 52 species analysed, 33 exhibit a multi-male multi-female breeding system. The remaining 19 species exhibit one-male unit, family group, monogamous, solitary or dispersed breeding systems. 16 species lived in captivity, 5 under semi-free conditions, 30 in the wild and one under unknown conditions (Table III).

Rhythmic masturbation was observed in 13 of the 52 species (25%). Masturbation with ejaculation was observed in 21 species (40.4%) and 18 species (34.6%) were not observed to masturbate. There was no significant difference in the occurrence of masturbation between wild living and semi-free/captive species (Fisher's exact test, $N = 52$, $p < 0.05$). Thus, I conclude that masturbation is not forcible a byproduct of captivity or partial captivity but occurs independent of living conditions. It is further evident that male masturbation is a

natural behaviour performed by more than 65% (34 from 52) of the investigated non-human primate species.

Table III: Returned questionnaires refering to the observation of masturbation in 52 primate species in alphabetical order. BS = breeding system, MM-MF = multi-male multi-female, other = one-male-unit, family group, solitary, monogamous or dispersed breeding system. ME = masturbation with ejaculation, M = rhythmically masturbation, N = no observation of masturbation. German Primate Center (DPZ), Primate Research Institute, Japan (PRI), National Institutes of Health, USA (NIH).

No	Species	BS	Masturbation	State	Study site	Contributor
1	*? saki (Pithecia sp.)*	MM-MF	M	wild	Venezuela	MA Norconk
2	*Alouatta palliata*	MM-MF	M	wild	Hacienda La Pacifica/Costa Rica	JM Whitehead
3	*Brachyteles arachnoides*	MM-MF	ME	wild	Caratinga/Brazil	KB Strier
4	*Callicebus cupreus*	other	N	captive	DPZ/Germany	E Heymann
5	*Callithrix jacchus*	other	N	wild	Eflex Ibama/Brazil	L Digby
6	*Cebus apella*	MM-MF	M	captive	NIH/USA	GC Westergaard
7	*Cebus olivaceus*	other	N	wild	Hato Pinero/ Venezuela	L Miller
8	*Cercocebus albigena*	other	N	captive	Zoo Hannover/ Germany	M Böer
9	*Cercopithecus aethiops*	MM-MF	ME	wild	Amboseli/Kenya	M Hauser
10	*Cercopithecus campbelli*	other	M	captive	Zoo Leipzig/ Germany	E Loser
11	*Cercopithecus neglectus*	other	M	captive	Kenya	P Adoyo
12	*Cheirogaleus medius(?)*	other	N	captive	Univ.Bochum/ Germany	U Nieschalk
13	*Colobus badius badius*	MM-MF	M	wild	Tai-Forest/ Ivory Coast	A Korstjens
14	*Colobus verus*	MM-MF	ME	wild	Tai-Forest/ Ivory Coast	M Krebs
15	*Eulemur coronatus*	MM-MF	N	semifree	Duke/USA	P Kappeler
16	*Gorilla gorilla beringei*	other	N	wild	Karisoke/Rwanda	J Yamagiwa
17	*Gorilla gorilla gorilla*	other	ME	captive	Apenheul/ Netherlands	I Weiche
18	*Hylobates lar*	other	N	wild	Kao Yai/ Thailand	U Reichhard
19	*Lemur catta*	MM-MF	N	semifree	Duke/USA	P Kappeler
20	*Loris tardiagradus*	other	N	captive	Univ.Bochum/ Germany	U Nieschalk

21	*Macaca fascicularis*	MM-MF	ME	wild	Ketambe/Indonesia	M van Noordwijk
22	*Macaca fuscata fuscata*	MM-MF	ME	wild	Kinkazan/Japan	H Sugiura
23	*Macaca fuscata yakui*	MM-MF	ME	wild	Yakushima/Japan	J Soltis & M Matsubara
24	*Macaca maurus*	MM-MF	N	captive	Zoo Hannover/ Germany	O Petit
25	*Macaca mulatta*	MM-MF	ME	wild	Cayo Santiago/ Costa Rica	M Hauser
26	*Macaca nemestrina*	MM-MF	ME	captive	PRI/Japan	K Matsubayashi
27	*Macaca nigra*	MM-MF	ME	wild	Karanenta/ Indonesia	S Matsumura
28	*Macaca radiata*	MM-MF	ME	wild	India	G Hohmann
29	*Macaca silenus*	other	M	wild	India	A Kumar
30	*Macaca sylvanus*	MM-MF	ME	semifree	Salem/Germany	A Paul
31	*Macaca tonkeana*	MM-MF	ME	semifree	Univ. Strassburg, France	B Thierry
32	*Mandrillus sphinx*	MM-MF	M	wild	Lope/Gabon	L White
33	*Microcebus murinus*	other	N	wild	Madagascar	E Zimmermann
34	*Miopithecus talapoin*	MM-MF	ME	?	Stuttgart/Germany	E Zimmermann
35	*Nasalis larvatus*	other	M	wild	Borneo	CP Yeager
36	*Nycticebus coucang*	other	N	captive	Univ. Stuttgart/ Germany	E Zimmermann
37	*Otolemur crassicaudatus*	other	N	wild	South Africa	S Bearder
38	*Pan paniscus*	MM-MF	ME	semifree	Planckendaal/ Belgium	Bonobo Research Group
39	*Pan troglodytes*	MM-MF	ME	wild	Gombe/Tanzania	CB Stanford
40	*Papio cynocephalus*	MM-MF	ME	wild	Awash/Ethiopia	J Phillips-Conroy
41	*Papio anubis*	MM-MF	ME	wild	Amboseli/Kenya	B Noe-Slijter
42	*Papio hamadryas*	other	ME	wild	Ethiopia	W Angst
43	*Papio ursinus*	MM-MF	ME	wild	Natal/South Africa	RW Byrne
44	*Petterus fulvus*	MM-MF	M	captive	DPZ/Germany	J Ganzhorn
45	*Pongo pygmaeus*	other	M	wild	Suaq Balimbing/ Sumatra	C van Schaik
46	*Presbytis entellus*	MM-MF	ME	wild	Ramnagar/Nepal	C Borries

47	*Presbytis pileata*	other	N	wild	Madhpur/ Bangladesh	CB Stanford
48	*Saguinus fuscicollis*	MM-MF	N	captive	Philadelphia/USA	G Epple
49	*Saguinus labiatus*	MM-MF	N	captive	Philadelphia/USA	G Epple
50	*Saguinus mystax*	MM-MF	N	wild	Quebrada Blanco/ Peru	E Heymann
51	*Saguinus oedipus*	MM-MF	M	captive	DPZ/Germany	S Benning
52	*Saimiri boliviensis*	MM-MF	M	captive	Univ. Munich/ Germany	F Hübener

3.1.2 Masturbation is positively correlated with a multi-male multi-female breeding system

Figure 4 shows the occurrence of rhythmic masturbation (M), masturbation with ejaculation (ME) and no observation of masturbation (N) in relation to the breeding system (MM-MF or other) of the 52 primate species.

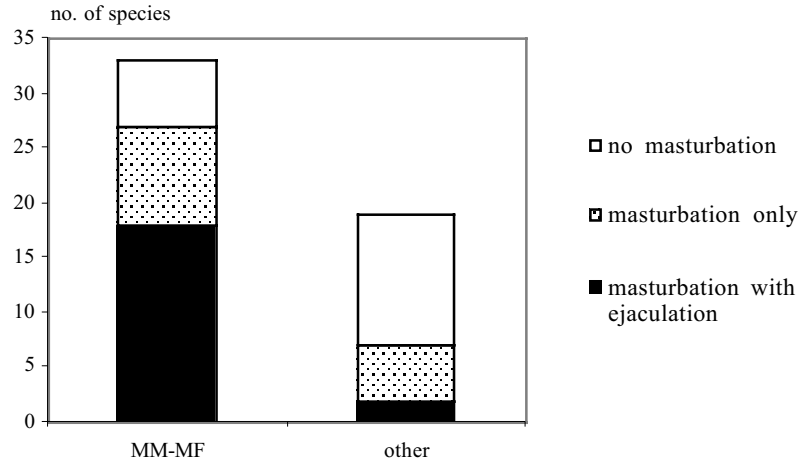


Figure 4: The occurence of masturbation in 52 primate species in relation to their breeding system. MM-MF = multi-male multi-female. Other = one-male-unit, family group, monogamous, solitary or dispersed breeding system.

Since cases in which only rhythmic masturbation had been observed remained a little unclear in terms of ejaculation, three test rows were examined (see 2.2). All three showed a positive correlation (Table IV). The hypothesis then that, independent of phylogenetic relationships, species exhibiting a multi-male multi-female breeding system have a higher

probability of masturbation with ejaculation than species exhibiting other breeding systems is upheld. Thus, the type of breeding system, i. e. the degree of sperm competition is the most important factor in determining whether or not masturbation to the point of ejaculation occurs.

Table IV: Phylogenetic regression analysis of the occurrence of masturbation in 52 non-human primate species. ME = masturbation with ejaculation, M = masturbation only, N = no masturbation was observed. For variables see methods.

	1. Wild	**2. Captive**	**3. All combined** (wild/captive/semifree/undefined)
A. Number of species: ME and M vs N	**30**	**16**	**52**
ß	0.44	0.22	0.43
F	9.86	0.3	9.62
df	1 and 9	1 and 8	1 and 28
p	< 0.025	NS	NS
B. Number of species: ME vs M and N	**30**	**16**	**52**
ß	0.57	0.05	0.49
F	12.86	0.02	16.36
df	1 and 16	1 and 4	1 and 23
p	< 0.005	NS	< 0.001
C. Number of species: ME vs N	**23**	**10**	**39**
ß	0.66	0.05	0.51
F	176.68	0.02	16.41
df	1 and 7	1 and 3	1 and 15
p	< 0.001	NS	< 0.005

3.2 Patterns of masturbation in wild and singly caged Japanese macaques

Written reports on masturbation in wild and/or captive primates are almost entirely anecdotal, often providing individual explanations for why a male masturbated. Examples of these supposed triggers of masturbation include: 1) that the caretaker arrived or food was offered (*Hylobates* spp. Mootnick & Baker 1994), 2) that no female was available for mating (*Macaca arctoides:* Nieuwenhuijsen *et al.* 1986, 1987) or 3) because of eye contact with a female in a neighbouring cage (Linnankoski *et al.* 1993). To redress this lack of data, one of my principal aims was to describe patterns of masturbation as they occurred in wild Japanese macaques over a period of 13 months, in relation to mating and non-mating periods.

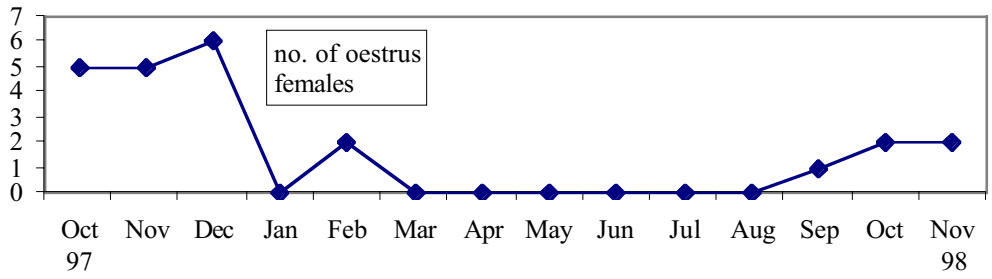
As masturbatory ejaculations may be difficult to detect in the wild, I incorporated data from systematic observations on singly caged males, on which 24 hour focal observations

could be made. Thus, exact time lapses between masturbation events could be determined for captive Japanese macaques and compared with the observations on wild males.

3.2.1 Seasonality of ejaculate production in wild males

Ejaculate production in captive Japanese macaques is restricted to only some months of the year (Matsubayashi & Enomoto 1983), so wild males should perform masturbation with ejaculation and mating only during their mating period. To determine if this were so and to discover the periods during which wild males start and finish masturbatory ejaculations, I collected focal and *ad libidum* sampling data on mating and masturbation for each of the 15 adult NINA-A troop males over 13 months (1204 hours of observation time). I also noted the maximum number of oestrus females per month in NINA-A troop to determine if ejaculate production correlated with oestrus (or vice versa).

On Yakushima, ejaculate production occurred in parallel with the number of oestrus females per month from September until February/March (Figure 5). This concurs with the findings of Matsubayashi and Enomoto (1983) concerning ejaculate production during the mating period. Although the last oestrus female during the study period was observed in February, the last masturbatory ejaculation was noted for March. The peak for both oestrus females and male ejaculation, however, was during October, November and December. Therefore, in wild Japanese macaques, seasonality of masturbatory ejaculations shows the same pattern as copulatory ejaculations. This demonstrates that males do not use masturbation to compensate a lack of mating opportunities at other times of the year, because they masturbate during the same period as when mating occurs. During the non-mating period, masturbation can be observed in the Yakushima population, but it occurs much less frequently and never with ejaculation.



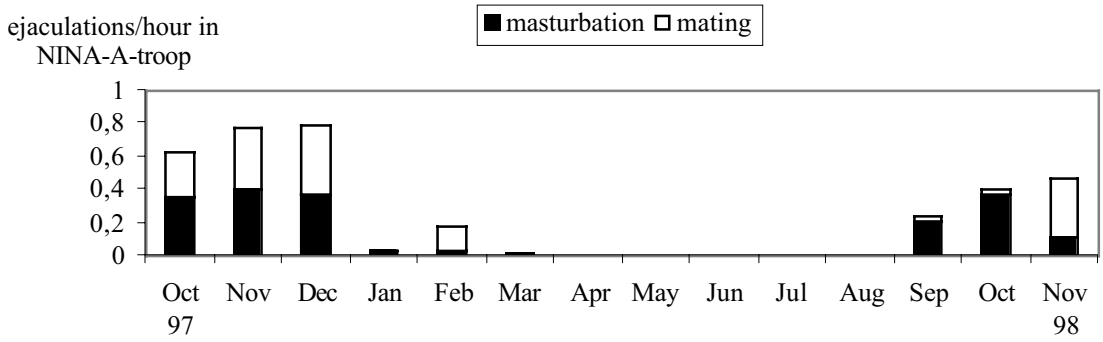


Figure 5: Sesonality and number of oestrus females and seasonality of masturbatory and copulatory ejaculations from the 15 males of NINA-A troop on Yakushima Island from October 1997 until November 1998. Observed ejaculations are expressed per hour of observation time.

3.2.2 Ejaculate dispersion: males' strategies

The 15 males of NINA-A troop could be divided into three guarders (rank 1 - 3, codes: SH, MO and KA) and 12 sneakers (rank 4 - 15, codes: GO, KU, TE, IN, AR, NM, SK, MM, BA, PA, JK, TO) according to their mating strategies (2.7.6). During the 1998 mating period, three males (GO, KU, TE) left the troop so, from IN (7th ranked in 1997), all males rose by three ranks in 1998. Total animal focal sampling time during the mating periods of 1997 and 1998 on the three guarders reached 206 hours (mean = 69.0 hours, SD = 19.3, range: 54.0 - 90.8) and on the 12 sneakers it was 247 hours (mean = 20.5 hours, SD = 9.5, range: 12.0 - 40.4).

3.2.2.1 Masturbation and mating of guarders and sneakers

In total, 171 ejaculations were observed during both mating periods. Guarders were observed to ejaculate on average 0.49 times per hour of focal sampling time (N = 101 ejaculations over 206 hours). Sneakers on average ejaculated 0.28 times per hour of focal sampling time (70 ejaculations over 247 hours).

The proportion of copulation to masturbation in guarders and sneakers showed a significant difference (x^2 - test, df = 1, $p < 0.01$). Guarders used a higher proportion of their ejaculate for copulation, whereas sneakers spread their ejaculates nearly equally between masturbation and copulation (Table V).

Table V: Ejaculate dispersion of guarders and sneakers in wild Japanese macaques *(Macaca fuscata yakui)* during two mating periods (1997 and 1998) on Yakushima Island.

	Guarders	**Sneakers**	**Total**
No. of males	3	12	15
Mating	71 (70,3%)	34 (48,6%)	105 (61,5%)
Masturbation	30 (29,7%)	36 (51,4%)	66 (38,5%)
Total	101 (100%)	70 (100%)	171 (100%)

3.2.2.2 Timing of masturbation in relation to mating success

Sneakers, if they manage to mate at all, succeeded in mating once a day with the same female and on days without mating success, they masturbated once until ejaculation. Notably, only sneakers were observed to masturbate even before they mated. In contrast, guarders exclusively masturbated on days when they had no mating success and they were never seen to masturbate before mating in this wild population. Neither sneakers nor guarders were observed to masturbate after mating (Table VI). The incidence of masturbation occurring only a short time before (approximately 1 - 5 hours) mating in sneaker males is small in absolute number (N = 8) but it represents 22.2% (8 from 36 cases) of all observed masturbation by sneakers (see 4.8). That guarders did not masturbate before mating, seems fairly certain because, in terms of both observation time and of mating success, I collected more data for guarders so the chances of detecting masturbation before mating was higher.

Table VI: Timing of masturbation in relation to the timing of mating success (ms) in wild Japanese macaques *(Macaca fuscata yakui)* on Yakushima Island.

	Without ms	**Before ms**	**After ms**	**Masturbation total**	**Ejaculations total**
Guarders	30	0	0	71	101
Sneakers	28	8	0	34	70
Total	58	8	0	105	171

The important difference in the masturbation patterns of guarders and sneakers, in terms of sperm competition, is that sneakers masturbated disproportionately more often on days when they later succeeded in mating than guarders did (Fisher's exact test, $p < 0.01$). The important mutuality is that both masturbated on days without mating success and did not store ejaculate over more days.

3.2.3 Natural storage time of a Japanese macaque ejaculate

In providing an overview of masturbatory patterns in wild and captive macaques in which the natural storage time of an ejaculate is described, I used only those days during which Yakushima males were not observed to mate. The captive males were restricted from mating throughout this study. Mean male age in NINA-A troop in 1997 (N = 15 males) was 8.6 years, SD = 3.2, range 4 - 14, and in 1998 (N = 12 males) it was 9.0 years, SD = 3.3, range 5 - 15. Mean age for the eight captive males was 11.6 years, SD = 4.4, range 6 - 18. The two populations did not differ significantly in age during either mating period (Mann-Whitney-U-test: 1997: U' = 36.5, U = 83.5, N1 = 15, N2 = 8, $p > 0.05$; 1998: U‘ = 30.5, U = 65.5, N1 = 12, N2 = 8, $p > 0.05$). This similarity in age should mean that the volume of ejaculate production was almost the same (Matsubayashi 1982).

In the wild, both guarders and sneakers were observed to masturbate once a day when they did not succeed in mating during that day (the exception being eight cases in which sneakers masturbated before mating (Table VI). Perhaps due to the difficulties of following a male from dawn to dusk and because of the dense forest, on only 64.5 % of all the days they did not succeed in mating (N = 72, 15 males over 72 days) was the focal male observed to masturbate.

The eight singly caged males, who were restricted from mating, could be checked continuously over 24 hours (N = 336, 8 males over 42 days). These singly caged males masturbated exactly once in a 24 hour period in 89.5 % of cases (Figure 6). This suggests that Japanese macaques masturbate about once more or less every 24 hours. This time elapse probably represents the end of storage capacity of the *cauda epididymis* in this particular species.

Captive males had clear individual preferences for the period in which they masturbated. For example, male No. 1134 preferred to masturbate in the early morning between 5:00 - 7:00 a.m, whereas male No. 1209 masturbated mostly in the late afternoon between 6:00 - 8:00 p.m. For laboratory held Japanese macaques at PRI, early morning and

late afternoon in general seem to be the most preferred times to masturbate, probably because during the day, the caretakers are working around them or the monkeys are used for experiments. From about 10:00 p.m until 5:00 a.m masturbation was not detected in the singly caged macaques, even though this would be the least disturbed time of the day. I conclude, therefore, that wild males also don't masturbate during night.

In the wild, there seems to be a similar tendency in individuals for preferred times to masturbate. However, as daily activity patterns in this population changed due to changing ecological conditions and since masturbation was restricted to resting periods (it never happens when feeding or moving), the individual timing of masturbation may be more variable than that observed in the captive monkeys. For example, the sneaker male MM (rank 11 in 1997, rank 8 in 1998) masturbated mostly between about 9:30 - 12:00 noon. On two days, however, he masturbated early in the morning around dawn and in another two cases he succeeded in mating after he had already ejaculated due to his normal masturbatory habits.

Guarders showed a tendency to more variability in their masturbatory patterns than sneakers. This might reflect the fact that they succeed in mating more than sneakers and therefore their ejaculate storage pattern was not as regular as that of sneakers. However, on days when guarders did not succeed in mating, they also tended to masturbate in the morning or around noon. For both male types in the wild, masturbation was rarely observed later than 4:00 p.m. (N = 3 cases).

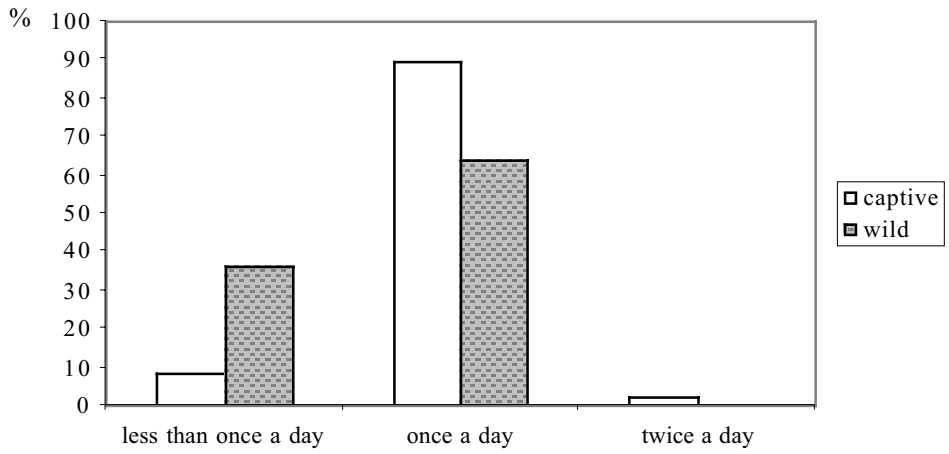


Figure 6: Frequency of masturbation in 15 wild and 8 singly-caged Japanese macaques. Percentage of the days during which auto-masturbatory ejaculation was observed for the focal individual (wild) or the ejaculatory spot below the cage was found (captive). captive: N = 336 checks, wild: N = 72 days of focal animal sampling.

3.3 Analysis of wild and singly-caged Japanese macaques' ejaculate

To exclude the possibility that ejaculates from captive and wild males differ in important parameters, I performed the same kind of ejaculate analysis on both populations. This was necessary because nothing is known from ejaculates of wild living primates (and other mammals) in general.

From the 15 wild males, 21 auto-masturbatory ejaculates could be collected (mean = 1.4, SD = 0.5, range: 1 - 2 ejaculates per male). From the eight singly caged males, I collected 24 fresh ejaculates from the bottom of the cage (3 ejaculates per male). This comparative analysis was necessary also to ensure that the results obtained from singly caged males by HHE experiments could be extrapolated directly to the wild population.

With the exception of ejaculate volume, none of the other measured ejaculate parameters differed significantly between the two populations (Table VII). The ejaculate volume collected was significantly higher in captive males, than in the wild population. Vitality did not differ significantly between populations but one of the ejaculates collected from the wild population contained only dead sperm (vitality 0 %). Since the second ejaculate collected from the same male exhibited normal vitality of around 60 %, the chances that sperm from the first ejaculate died during transportation or due to other handling errors is high. Thus, this male like the other was judged to be fertile in regard to sperm vitality.

It is especially notable that the percentage of morphologically normal sperm was high in both populations. Morphologically abnormal shaped sperm were found at less than 1% in both populations and are thus negligible in Japanese macaques. In other studies of primate ejaculates, high percentages of morphologically abnormal sperm have been found (e.g. *Macaca mulatta:* 29 %, *M. fascicularis:* 23 %, Sarason *et al.* 1991; *M. fascicularis:* 17.5 %, Gago *et al.* 1999, *Gorilla gorilla gorilla:* 92.5 %, Platz *et al.* 1980). The reason for this extreme difference probably is due to the fact that the ejaculates of this study were won by auto-masturbation and human-hand ejaculation, both of which are non-invasive methods of ejaculate collection. The results of this study confirm these as the best methods of ejaculate collection in primates. The importance of this result cannot be over emphasized, since only from natural-like ejaculates conclusions concerning evolution can be made.

Although the two methods used to measure sperm swimming velocity are not directly comparable, both populations fell within the range of normal levels for Japanese macaques according to the methods used (wild: estimation of the percentage of rapidly progressive,

progressive and non progressive sperm, captive: CASA-analysis of the sperm swimming velocity in µm/s).

The number of ejaculates that contained agglutinations did not differ significantly between the two populations. In 18 of the 24 ejaculates (75 %) from captive males and in 14 of the 21 ejaculates (67 %) from wild males agglutinations were found. It is difficult to interpret why sperm agglutinate occurs or how important agglutinations are for fertilisation (Schirren 1995) and during sperm competition (Baker & Bellis 1995). Nevertheless agglutinations did not reach high levels in which, for example, more than 200 sperm were involved (in average, Japanese macaque ejaculate contained around 535 mio/ml, thus the number of agglutinated sperm reached less than 0.04 % only), nor did they differ in amount between the two populations. Thus, I excluded agglutinations from further analysis and interpretation.

The reason why the only significant difference found between the two populations concerned the volume of ejaculate remains unclear. Possible explanations would include that: 1) due to the composition of the forest floor (leaf litter, earth, moisture and other material), ejaculate got lost during collection from the ground or stocked at the males' fur, or 2) since I estimated the total volume of ejaculate (2.6.2), it could be an error of estimation. Given that all other measured parameters of ejaculate were comparable, measured differences in the ejaculate volume between these two populations are likely to reflect an error on my part rather than any real difference.

In summary, auto-masturbatory ejaculates which were stored for approximately 24 hours in captive and wild Japanese macaques did not differ in important parameters and contained negligible amounts of agglutinations and abnormally shaped sperm. The following results won from captive macaques (3.4), therefore, are, in all probability, directly applicable to the wild population.

Table VII: Analysis of auto-masturbatory ejaculates from wild and captive Japanese macaques. * Wild: in 14 from 21 ejaculates agglutinations were found. Captive: agglutinations in 18 from 24 ejaculates. ** normal shaped sperm in %. Mean±SD (range).

	Wild *(M.f.yakui)*	**Captive *(M.f.fuscata)***	**U-test**
No. of males	15	8	----------
Ejaculates	21	24	----------
Volume in ml	2.7±1.0 (0.8-4.1)	4.2±1.4 (1.9-6.4)	U´= 358, U = 83 p ‹ 0.001
pH-value	7.2±0.3 (6.4-8.0)	7.2±0.4 (6.7-8.0)	NS

Agglutinations*	66,6%	75%	Fisher´s test p › 0.05
Morphology**	99.5±0.9 (97-100)	99.9±0.2 (99-100)	NS
Vitality in %	45.1±26.2 (0-100)	42.8±13.5 (3-70)	NS
Sperm number in mio/ml	572±3.7 (12.5±1600)	502±440 (2-2300)	NS
Velocity	**rapid (in %)** 42±28 (0-100) **progressive** 21±12 (0-50) **non-progressive** 36±28.4 (0-90)	**in µm/s** 42.3±19.4 (10.1-74)	not directly comparable

3.4 Change of ejaculate parameters in response to storage time in the male's genital tract

To detect changes in ejaculate parameters in response to the time ejaculate was stored in the genital tract, human hand ejaculation (HHE) experiments were performed with the captive population. Three of the eight captive males refused to HHE over more than 10 days of training and were thus excluded from further experiments. Their reasons for refusal were not apparent but possibilities include handling mistakes during earlier experiments or individual differences that makes them impossible to train in the HHE method. From the remaining 5 males (mean age = 11 years, SD = 4.7, minimum: 6, maximum: 18) 29 ejaculates could be collected using HHE.

Times of individual auto-masturbatory behaviour were known from checking for the presence of spots (Bielert *et al.* 1980). Thus, the previous ejaculation before human-hand-ejaculation was known and the time difference could be accurately recorded. Due to the regular auto-masturbatory behaviour of these macaques, the oldest ejaculate collected by HHE had been stored for no more than 32 hours. The freshest ejaculates had been stored only for less than 1 hour. Ejaculation using HHE was accomplished in 29 of the 98 trials (29,9%) on the 5 males (No. 1134: N = 11 ejaculates, No. 1316: N = 3, No. 1209: N = 6, No. 1438: N = 5, No. 974: N = 4).

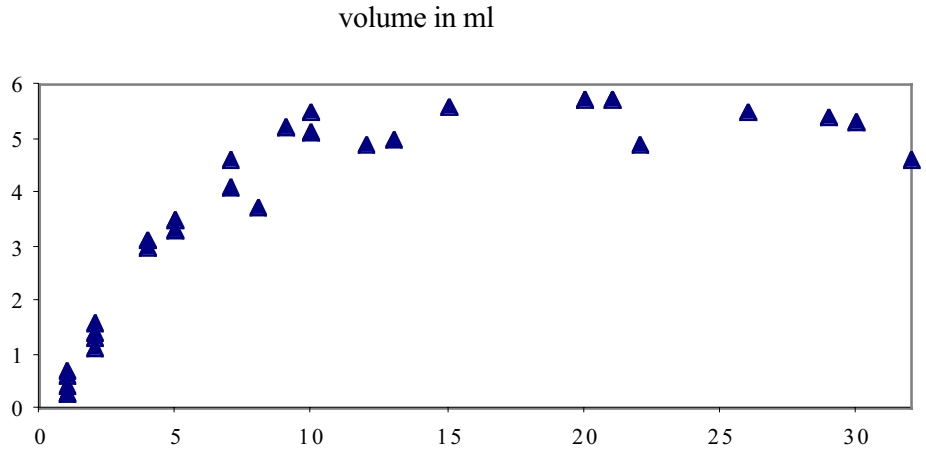
For ejaculate analysis, sperm morphology and agglutinations were ignored, since the impact of both parameters had already been judged as negligible (3.3). The other four

parameters, which should be important during sperm competition (volume, total sperm number per ejaculate, sperm swimming velocity and sperm vitality) were measured with the CASA-system.

Of these four measured parameters, no significant differences between individual males were evident (KW - Test: Volume: H = 5.4034, p › 0.05; Total sperm number: H = 2.6417, p › 0.05; vitality: H = 2.2026, p › 0.05, velocity: H = 3.0356, p ›0.05; N = 29 ejaculates).

Effects of storage time on the ejaculates were detected (Figure 7). The longer an ejaculate was stored, the larger ejaculate volume (r_s = 0.7008, N = 18, $p < 0.01$) and the greater the total number of sperm became (r_s = 0.8776, N = 18, $p < 0.001$). Within 1 hour until about 15 hours since last auto-masturbatory ejaculation, however, these two parameters increased and then plateaud (volume: $y = 5.535(1\text{-}e^{-0.1868x})$, R^2 = 0.959, F = 5.593; sperm concentration: $y = 1848(1\text{-}e^{-0.0526x})$, R^2 = 0.827, F = 1.475 x 106).

In contrast, sperm swimming velocity and the percentage of vital sperm per ejaculate showed clear negative correlations in response to storage time. Both reached high levels soon after the previous ejaculation, but then steadily decreased as storage time increased (velocity: r_s = - 0.8434, N = 18, $p < 0.01$; vitality: r_s = - 0.8012, N = 18, $p < 0.01$). Thus, all four measured parameters (volume, sperm concentration, velocity and vitality) changed in response to the time an ejaculate was stored in the *cauda epididymis*. The time an ejaculate is stored, therefore, is the most important factor in determining the quantity and the quality of an ejaculate (Figure 7).



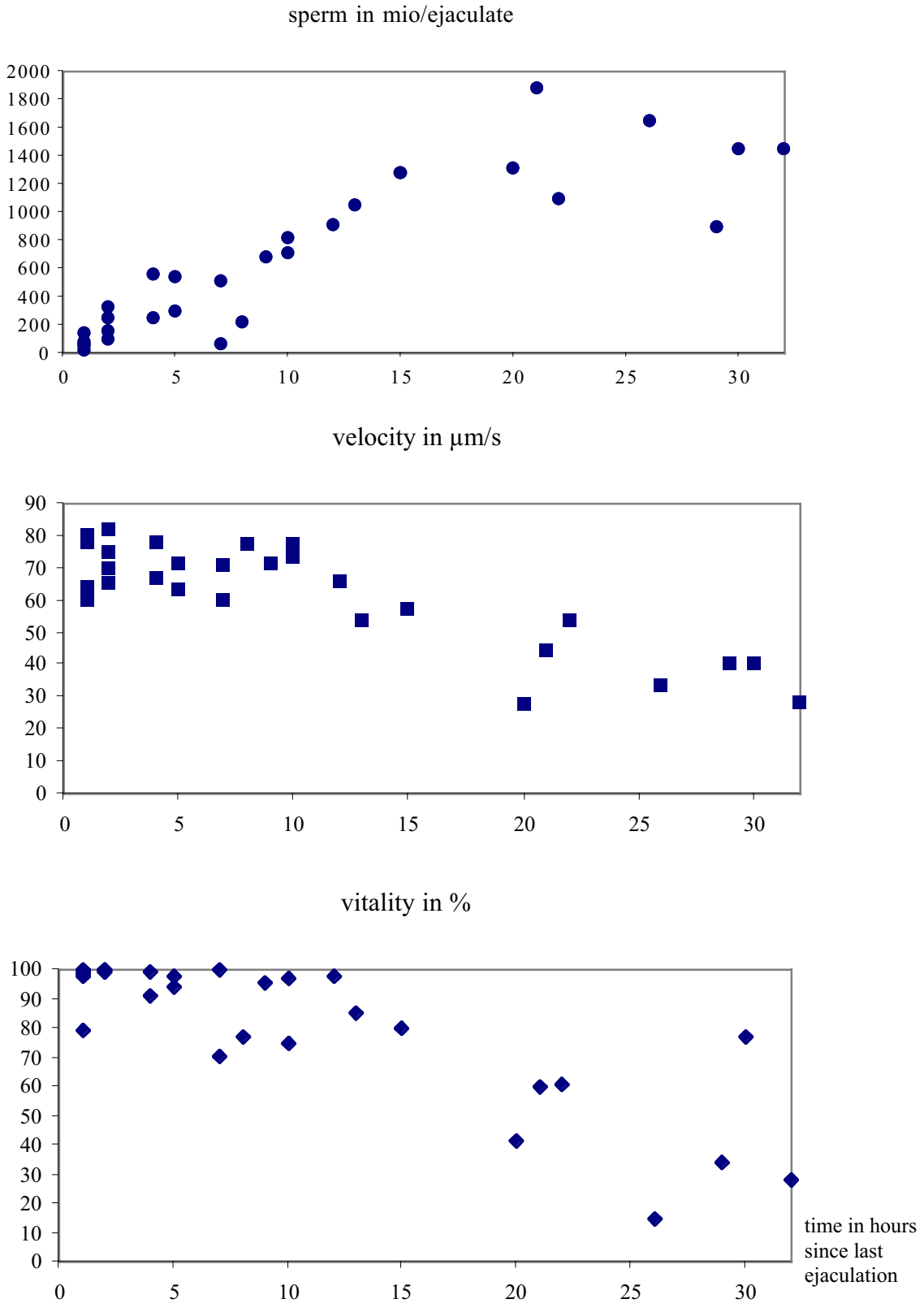


Figure 7: Change of the four ejaculate parameters volume, sperm number, sperm swimming velocity and percentage of vital sperm per ejaculate to the time an ejaculate was stored in the genital tract of male Japanese macaques *(Macaca fuscata fuscata)*. N = 29 ejaculates collected with HHE from N = 5 males.

4. DISCUSSION

4.1 Occurrence of masturbation and sperm competition

Masturbation occurs in non-human primates at an astonishingly high frequency of 65.4 %, in 34 of 52 investigated species. As this study is the first in which data concerning masturbation in wild living primates has been systematically collected, I wish to emphasise that the common perception of masturbation as a mainly pathological or abnormal behaviour in primates can now be considered defunct, even though pathological patterns of masturbation may nevertheless be exhibited by some captive species.

With regard to primate phylogeny, males of species exhibiting a multi-male multi-female breeding system, and thus facing a higher risk of sperm competition, masturbate with a higher probability than males of species in which almost no or considerably less risk of sperm competition occurs. This result is in direct contrast with pervious theories of sperm competition, which emphasise exclusively the importance of quantitative parameters, such as volume and sperm number (e.g. testis size: Harcourt *et al.* 1981, sperm number: Møller 1988), without considering the qualitative ejaculate parameters of velocity and motility, which are known to be the most important factors for fertilization (Mann & Lutwak-Mann 1981, Mortimer & Templeton 1982) and also during sperm competition in humans (Baker & Bellis 1995).

In considering the incidence of pathological forms of masturbation, harem-group living mountain gorillas *(Gorilla gorilla beringei)*, solitary living orang-utans *(Pongo* spp.) and monogamous gibbons *(Hylobates* spp.*)*, all of which exhibit breeding systems that should not, according to my results, masturbate, reports of masturbation for captive members of these species are available. For example, Mootnick & Baker (1994) describe masturbation with ejaculation in captive gibbons *(Hylobates pileatus, H. concolor, H. agilis)*. Since *Hylobates* spp. live monogamously, these observations are an example of the fact, that masturbation can be triggered by the abnormal circumstances of captivity: masturbation in these males was directed towards humans or food. In wild gibbons *(Hylobates lar)* and wild mountain gorillas, masturbation has never been observed (Ulrich Reichard, Juichi Yamagiwa, Table III). Thus, when describing masturbation in non-human primates in future studies, I strongly suggest description of all details concerning the behaviour so as to determine whether it can be identified as normal or pathological.

4.2 Stimuli of masturbation

The results of my study show fairly conclusively that if Japanese macaque males can mate regularly, they tend not to masturbate (or at least not regularly) but if they are restricted from mating, they have to masturbate about once within 24 hours (Figure 6). Even though the ultimate cause of masturbation may be related to physiological constraints (such as the end of the storage capacity of the *cauda epididymis*), at a proximate level of explanation masturbation results from the coincidence of sexual desire and lack of sexual opportunity. For example, sexual skin swellings on females, female soliciting behaviour, such as lip smacking, or the sight of other males mating (*Macaca nemestrina:* Linnankoski *et al.* 1993, *M. fuscata:* own observations) leads to sexual arousal in macaques and if mating is impossible then masturbation occurs.

This, on the first view, simple mechanism has, however, a beneficial consequence for the next ejaculate, which a male may then bring into sperm competition games because his sperm will presumably be fresher and more motile than that of his competitors, or at least as fresh and motile. Thus, I conclude, that the reaction to such proximate stimuli, whereby old ejaculate is discarded via masturbation, is adaptive on the ultimate level and that masturbation should not be regarded a behaviour without meaning or as maladaptive.

4.3 Adjustment of a fertile ejaculate in Japanese macaques

In this study, abnormal sperm morphology was virtually absent in both populations (Table VII). This finding is consistent with previous studies on Japanese macaques even when the electro-ejaculation method was used (Matsubayashi 1982). In contrast, abnormal morphology in non-human primate sperm is known for various other species (e.g. gorillas: Platz *et al.* 1980, vervet monkeys: Seier *et al.* 1996) and is common in humans (e.g. WHO-Instruction 1993, Baker & Bellis 1995, Schirren 1995). The reason why Japanese macaque ejaculates do not or only very rarely contain morphologically abnormal sperm remains inexplicable at present, but this seems to be the natural state in this species. If the ejaculates of other wild living species (be they primates or otherwise) were to be investigated the data set could be enlarged.

Despite the absence of a large body of comparative data, I feel confident in interpreting this result as a sign, that a) the Yakushima population does not seem to suffer unduly from any environmental pollution (presumed factors of which influence sperm

morphology) and b) that for Japanese macaques the methods of ejaculate collection used (HHE and electro-ejaculation with the penile method) do not influence sperm morphology. Therefore, the data derived from this study are appropriate for explanations concerning evolution.

With regard to sperm velocity, the faster the swimming velocity, the faster sperm will be transported through the hostile acidic environment of the female genital tract (Barrat & Cooke 1991). Thus, faster swimming sperm should not only enhance the chances of fertilisation (Mann & Lutwak-Mann 1981, Mortimer & Templeton 1982) but should also have a greater chance of outcompeting slower swimming sperm during sperm competition, especially after having entered the oviduct (Gomendio *et al.* 1998)

For macaques, exact values on the minimum volume of semen necessary for fertilisation are unavailable and must thus be estimated. In humans, 2-6ml of ejaculate is considered necessary for a high chance of fertilisation to occur (Montagna & Sadler 1974, Mann & Lutwak-Mann 1981, WHO-Instructions 1993, Schirren 1995). Nevertheless, smaller volumes can also result in fertilization. In Japanese macaques, volumes of less than 0.25ml are used successfully in artificial insemination. To be on the safe side, I estimated 0.5ml of ejaculate as necessary for successful fertilisation in Japanese macaques under natural circumstances.

The minimum sperm concentration necessary for fertilisation in humans to occur is about 20 mio/ml of ejaculate (WHO-Instructions 1993), which, with a 2ml minimum volume, equates to 40 million in total. Since Japanese macaques in general produce much more sperm than human (from twice to about twenty times the amount, Matsubayashi 1982), it is difficult to estimate the minimum sperm number necessary for natural fertilisation in this species. Nevertheless, I feel confident in estimating that an ejaculate of at least 0.5ml with at least 100 million of sperm should be sufficient to achieve fertilisation in Japanese macaques.

This minimum estimated volume and sperm number in Japanese macaques is accomplished approximately one hour after the previous ejaculation (Figure 7) and so, even if a male masturbated only a very short time before mating, his ejaculate may still succeed in fertilization.

4.4 Sperm production rate and storage time in macaques

This study found that for Japanese macaques, the natural storage time of an ejaculate, i.e. the time until the *caput, corpus, cauda epidydimis* and proximate parts of the *ductus*

deferens become filled to capacity with sperm is approximately 24 hours (Figure 6). Since Japanese macaques are seasonal breeders and face intense sperm competition, the efficiency of spermatozoal production should be high compared to non-seasonal breeders or species that don't face sperm competition (Amann *et al.* 1976, Møller 1988). Unfortunately, only very few data are available concerning how sperm production varies in primates that have been won without electro-ejaculation techniques. For rhesus macaques *(Macaca mulatta)*, a seasonal breeding species closely related to Japanese macaques, daily (24 hour) sperm production during the peak of the mating period reached 23 million sperm per gram of testicular parenchyma (Amann *et al.* 1976).

A 10 year old Japanese macaque male with a testicular weight of 70g (Matsubayashi & Mochizuki 1982) could, therefore, produce up to 1610 million sperm per day (23 million x 70g) during the breeding season. Taking in account mean sperm concentration for wild males of this study in million/ml (Table VII), with an average ejaculate volume of 2.7ml per day, sperm concentration would be 596 million/ml. This calculation demonstrates that even though I probably underestimated the ejaculate volume for these wild males (see 3.3) the results of this study overlap with those of Amann *et al.* (1976).

4.5 Proximate function of masturbation: ejaculate quantity versus quality

In inter-species comparisons of primates (Harcourt *et al.* 1981) and bats (Hosken 1998) testis weight can be considered to reflect variation in the intensity of sperm competition, assuming that for first, the larger the testis the larger the volume becomes and for second, the larger the volume the higher the chances of reproductive success becomes for a male during sperm competition. The relationship between testis size in a species and the number of males that a female copulates with has been demonstrated from insects to mammals as highly correlated making it one of biology's most general laws (Ridley 1999). This fact, however, gives the impression that only ejaculate quantity, as expressed by ejaculate volume and sperm number, is important in sperm competition games.

For first, unfortunately, the presumed importance of ejaculate volume in intraspecific sperm competition still lacks empirical evidence. In laboratory rats, however, mechanisms of sperm competition not dependent on the volume are known, such as an advantage for the first male (e.g. Foltz & Schwagmayer 1989) or for the last (e.g. Dewsbury & Baumgardner 1981). Nevertheless, distinct mechanisms of sperm competition in non-human primates have only been speculated upon to date.

For second, large ejaculate volume (and thus testis size), is of little value in sperm competition games if a large volume of ejaculate contains many dead or only slowly moving sperm due to a too long storage time (Figure 7). A high volume but low quality ejaculate may indeed lose in a sperm competition with a low volume but high quality ejaculate simply just because dead sperm can't fertilise the ova. Therefore, ejaculate quality (swimming velocity and the percentage of motile sperm) must be incorporated also into common theories related to sperm competition games (e.g. Parker *et al.* 1990, Parker 1990 a & b, Parker 1984, 1998). Not quantity of sperm alone, but quantity and quality jointly determine the competitiveness of an ejaculate.

The longer ejaculate is stored in a males' genital tract, the lower the parameters of swimming velocity and sperm vitality, i.e. the lower its quality. On the other hand, the longer an ejaculate is stored the larger its volume and the number of sperm. Furthermore, type A ejaculate (large volume, many slow or dead sperm) and type B ejaculate (small volume, few, fast moving, healthy sperm) are generated as a function of storage time. It is difficult to allocate a distinct period of storage time to each ejaculate type and there may exist some fluid stages of transition. Based on the trends evident in Figure 7, after about 15 hours ejaculate volume and total sperm number plateau, whereas sperm swimming velocity and the percentage of vital sperm are likely to start to decrease somewhat before 15 hours. Thus, 15 hours of storage may be the time frame that roughly divides "high quality" ejaculate from "high quantity" ejaculate in Japanese macaques. The question arises, however, of what is more important during sperm competition: ejaculate quality, quantity, or both?

It appears that the single most important factor in penetrating the *cervical mucus* (Barrat & Cooke 1991) and for sperm transport in the human female reproductive tract (Mortimer & Templeton 1982) is sperm motility. This would suggest that the velocity of sperm and the number of motile sperm per ejaculate are most important for both conception and during sperm competition. High velocity sperm are more likely to pass rapidly through the vaginal region, which is extremely hostile to sperm (Birkhead *et al.* 1995). Furthermore, in mammals, sperm velocity is positively associated with fertilisation success (Birkhead *et al.* 1995). Therefore, in the case of Japanese macaques, if males do not masturbate, after a time elapse of approximately 35 - 40 hours their sperm storage organs would contain only dead sperm. As, however, males masturbate more or less regularly once every 24 hours, the proximate function of masturbation is, as hypothesised, to wash out old sperm from the genital tract (Zimmerman *et al.* 1965).

The disadvantages of masturbation may be, that first, the energy required to produce ejaculate is wasted (Dewsbury 1982) and second, that masturbation results in a lower

ejaculate volume of the next ejaculate. All in all, however, betting on ejaculate quality probably outweighs the costs of masturbation, at least for males adopting the sneaker strategy (4.8).

However, there still remains a little discrepancy between the measured data and the behavioural observations in this study. Actually such males, who are betting on quality, should masturbate every 15 hours (and not every 24 hours) to keep up a high quality ejaculate. Two explanations for this lack of concurrence which both are based on the behavioural observations, are thinkable. As for the laboratory held males, they may have adopted the time elapse of masturbation to circumstances, such as feeding or cleaning or some other factors depending on laboratory routine which commonly occur regularly once a day. The wild males on the other hand, may be influenced from factors such as food shortage, less available feeding time as usual or injuries caused by male-male competition during the mating season. This kind of factors may prolonge the natural time elapse of sperm production in the wild and therefore influence the occurrence of masturbation.

4.6 Sperm storage mechanisms and the chemistry of ejaculates

Macaque sperm are produced in the testis, enter the *caput*, *corpus* and the *cauda* of the *epididymis* where they require less than five days for maturation (Amann *et al.* 1976). The testis and *epididymis* of macaques do not differ significantly from that of other mammals (Golarz de Bourne & Bourne 1975) but the seminal vesicle of the rhesus and Japanese macaques is relatively bigger than it is in humans (Golarz de Bourne & Bourne 1975) and the more a primate species faces sperm competition, the longer it is (Dixson 1998). The longer the seminal vesicle, the higher its sperm storage capacity should be. However, the longer sperms are stored, the older they become, and supplying the sperm with nutritional particles to maintain their cell metabolism becomes problematic. Male (and also female) bats can store live sperm over several months but mainly during hibernation (Racey 1979), so different mechanisms for storage must operate in different species. A comparison of these mechanisms would be of interest for future studies concerning sperm storage capacity and sperm competition.

4.7 Masturbation, female choice and cryptic female choice

The importance of female choice has only rarely been studied in primates. Theories abound on cryptic female choice and how it influences male behaviour and sperm competition or how females' pre-copulatory and post-copulatory behaviour may influence male competition in terms of genital morphology exist in theory but empirical data is rare (Eberhard 1985, 1990, 1996, 1997, 1998; Dixson 1998). Most of the literature concerning this topic seems to be based on conjecture regarding how female choice and cryptic female choice should work based on theories of sociobiology.

Japanese macaques are one of the few primate species in which behavioural elements of female choice had been studied in a wild population. Females are known to mate with multiple males to avoid infant harassment and infanticide from males (Soltis *et al.* 2000). They do not choose only the largest, for example, or those of higher rank, but try to mate with each male at least once during their oestrus cycle. To this end, they have even developed a second oestrus, despite the fact that most of them fall pregnant in the early stages of the first oestrus period (Mitsunaga *et al.* 1992). Sperm competition is, therefore, intense and the question arises as to how females may influence post-copulatory phenomena.

The genital tract of female mammals selects against morphologically abnormal sperm (Short 1979). Acidity in the genital tract may have evolved to inhibit the growth of microorganisms and creates a hostile environment for sperm (Birkhead *et al.* 1995). So, sperm that can penetrate quickly into *cervical mucus* should be favoured by cryptic female choice. That masturbation gives rise to fresh, highly motile sperm in the next ejaculate would not be in conflict with presumed elements of cryptic female choice in mammals. Overall, however, details of how the mammalian female genital tract might select certain sperm over others presently are highly speculative. Raising or lowering the pH-value to kill or sustain sperm, for example, is an idea that has yet to be demonstrated. This topic, however, will become available in future studies.

4.8 Guarder and sneaker mating strategies: modelling sperm competition games in Japanese macaques

Males at some point have to choose between large ejaculate volume and high ejaculate quality, because as demonstrated in this study, the production of the theoretically most ideal ejaculate, with large quantity and high quality, is impossible. In Japanese macaques, as in

other species (e.g. birds: Birkhead & Møller 1995), the feature of guarders is to perform consecutive mating (Parker 1990 b), so their strategy is to place into the female many ejaculates. Studies concerning sperm competition in animals in general assume, that during consecutive matings, guarders deposit linear or similar amounts of sperm into the female (Parker 1990 a & b, 1998). With the exception of one study in chimpanzees (Marson *et al.* 1989), data on the change of ejaculate parameters during consecutive ejaculations in primates do not exist. So, in what follows, I present a modelling of how sperm competition should work inside the female based on the results shown in Figure 7. In contrast to previous assumptions, guarder's ejaculate quantity and quality changes over consecutive mating. The first ejaculate of a guarder's mating series is from type A, while the following ejaculates are roughly from type B (Figure 8 and Table VIII).

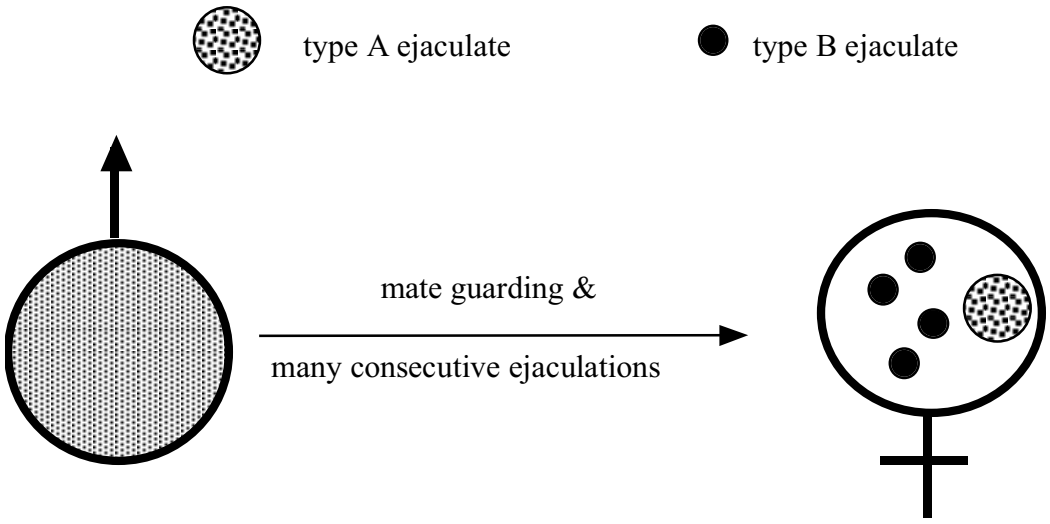


Figure 8: Mating strategy and ejaculate types of guarders (modification after Parker 1998).

Sneakers, who have few mating opportunities, must choose between using one large ejaculate that has been stored for a long time (Type A, Figure 9a) or to masturbate this out and use a small volume of high quality ejaculate (Type B, Figure 9b). The quality of an ejaculate stored for a long time and the quality of the first ejaculate after masturbation derived from the values of Figure 7 in conjunction with field observations of mating patterns in the Yakushima population.

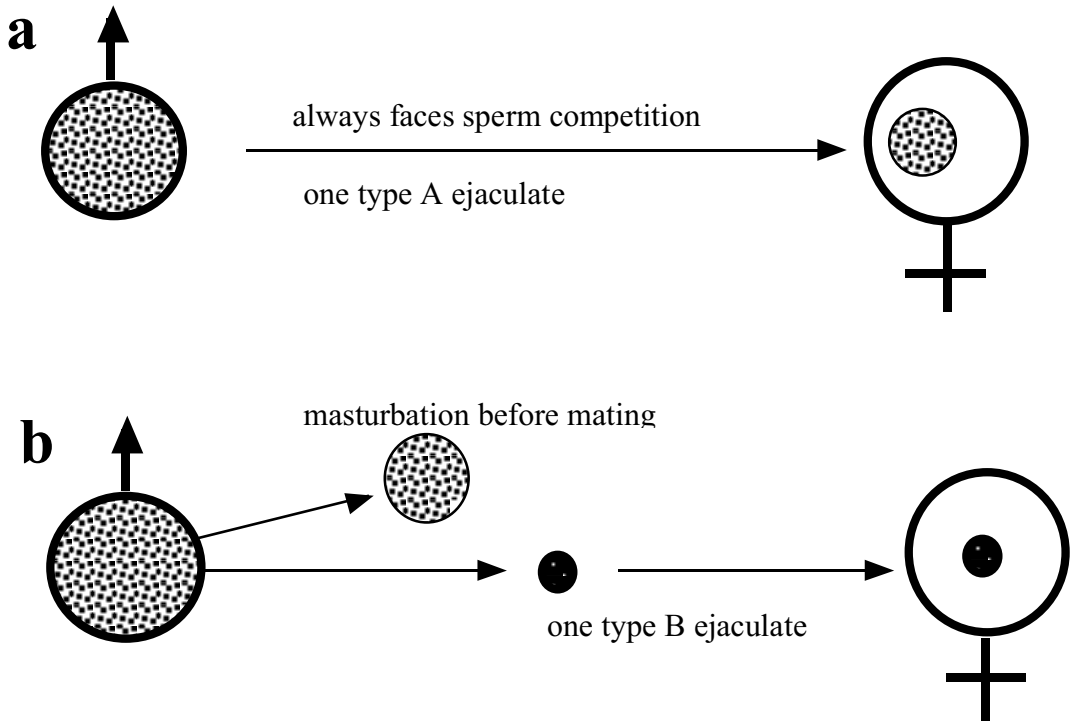


Figure 9a and b: Mating strategies and ejaculate types of sneakers (modification after Parker 1998).

It is highly unlikely, that males can predict future mating success and adjust masturbation times accurately enough to influence ejaculate type. Rather, masturbation seems to be stimulated more by proximate factors (see 4.2) together with physiological constraints. Nevertheless, whether ejaculate type A or type B is brought into sperm competition games during mating should influence the fertilisation chances of a male in response to his mating strategy.

Table VIII shows the observed timing of masturbation and mating of one guarder and three sneaker Yakushima males during one observation day, when they all managed to mate with the same female. Note first that only sneakers were observed to masturbate on the same day before they mated. Second, the guarder`s first ejaculate was estimated to have been stored for at least 24 hours based on observations of masturbation the previous day from around 10:00 a.m. Third, the storage time of the ejaculate that sneakers brought into sperm

competition games was known exactly due to observation of masturbation at a distinct time before they succeeded individually in mating with the female. Fourth, sperm parameters derived subsequently using the results of Figure 7 and the parameters of each individual ejaculate were calculated.

Table VIII: Modelling sperm competition in Japanese macaques. Behavioural data from Yakushima are combined with the results from HHE-experiments (Figure 7).

Male type	Time of masturba-tion	Time of mating	Volume in ml	Sperm number in mio/ml	Velocity in µm/s	Vitality in %
Guarder	24hrs before	10:00	5.5	1600	50	50
		10:30	0.25	100	60	100
		11:15	0.3	80	62	100
		12:15	1	150	70	100
		15:15	1.5	250	70	100
Total			8.5	2180		
Mean			1.71	436	62.4	90
Sneaker 1	2hrs10min (10:00)	13:10	1.5	250	70	100
Sneaker 2	4hrs (12:00)	16:00	3	350	70	100
Sneaker 3	9hrs (7:30)	16:30	4.5	600	70	90
Total			**9**	**1200**		
Mean			**3**	**400**	**70**	**96.6**

In summary, the one guarder male brought into sperm competiton games 1 type A and 4 type B ejaculates and the three sneakers managed it to bring in 3 type B ejaculates. Applying the data of Figure 7, the three ejaculates of the three sneakers combined (B) outcompetes the guarder's five ejaculates (A) in terms of sperm volume, velocity and the percentage of vital sperm, whereas guarder ejaculate outcompetes sneakers in terms of absolute sperm number and absolute number of vital sperm. Had the three sneakers not masturbated before mating (C), their combined input would have outcompeted the guarder in terms of sperm volume, absolute sperm number, and absolute number of vital sperm, but the

guarder would have outcompeted them in terms of sperm velocity and percentage of vital sperm. So why don't guarders also masturbate before mating? If the observed guarder male had masturbated around thirty minutes before his mating series began (D), he would have narrowly outcompeted the three sneakers only in terms of vitality, but he would have lost clearly in terms of all other parameters (Table IX).

Table IX: Calculated ejaculate parameters of 1 guarder and 3 sneaker male Japanese macaques from Yakushima Island based on data from Figure 7. (A) is the observed mating strategy of the guarder. (B) is the observed pattern, where sneakers mated after masturbation. (C) is the theoretical ejaculate input if sneakers not had masturbated before mating. (D) is the theoretical ejaculate input of the guarder if he had masturbated before his mating series started.

	(A) Guarder	(B) Sneakers	(C) Sneakers	(D) Guarder
No. of males	1	3	3	1
No. of ejacs	5	3	3	5
Volume in ml	8.5	9	16.5	3.3
Sperm in mio	2160	1200	4800	680
Velocity in µm/s	62.4	70	50	64.4
Vitality in %	90	96.6	50	100
No. of vital sperm in mio	1962	1159	2400	680

Another, however, speculative possibility for the disadvantage of a guarder to masturbate before mating may be based on the fact that ejaculates are of basic pH-value (Table VII) which might be helpful to dilute the acidity of the female genital tract and thus create a more comfortable environment for sperm. Therefore, if the first ejaculate in a mating series is from type A (as it is in reality) its intended purpose simply may be to create a reliable environment for the following type B ejaculates.

Even though such kind of observation is difficult to make on a wild population, it shows definitively, that masturbation offers the substantial benefit of improving ejaculate quality and that a higher quality ejaculate can outcompete guarder ejaculate in terms of the two important parameters, velocity and vitality.

Therefore, with masturbation, sneakers can make out the best of their bad situation while guarders exclusively should masturbate when they have no mating opportunities.

4.9 Masturbation and paternity

In captive Japanese macaques, Inoue *et al.* (1993) found a positive correlation between male dominance rank and mating success, but no correlation between mating success and reproductive success. In the three other macaque studies that used DNA fingerprinting to identify sires, all concluded that mating success is not a reliable indicator of reproductive success because males who were seen to copulate only rarely sired a disproportionately large number of offspring in relation to their ejaculate input (*Macaca fuscata:* Soltis *et al.* 1997 b, *M. fascicularis:* de Ruiter *et al.* 1992, *M. sylvanus:* Ménard *et al.* 1992). Thus, the question arises as to why males do not sire many offspring in spite of contributing a large ejaculate volume (Figure 2). There are several theoretical explanations to account for this phenomenon.

First, the ill-timed mating of highranking males during non ovulatory stages of the female could be one explanation for the discrepancy (Hausfater 1975). However, Soltis *et al.* (1999), found that high-ranking (captive) males mate especially frequently during the ovulatory period of females, thus negating this argument, at least for Japanese macaques.

A second possibility could relate to poor ejaculate quantity and/or quality in highranking males due to their age. In humans, ejaculate quantity decreases from the age of about 50 years on ward (Schirren 1995). In Japanese macaques, the same pattern occurs from the age of about 18 years (Matsubayashi & Mochizuki 1982). Regarding the ejaculate quality, data are unavailable, however, in this study, both wild and captive males showed no difference in ejaculate quality based on their age, males ranged between 4 - 18 years old, and thus the age-idea is irrelevant for this population.

Yet another explanation concerns the number of concurrently cycling females in a troop. In years with many concurrently cycling females, high ranking males cannot successfully perform mate guarding. Thus, with many mating opportunities for sneakers, paternity should be distributed among many males. In contrast, in years with only a few cycling females, highranking males can successfully guard mates. In these years, with sneakers afforded so few mating opportunities, paternity should be restricted to highranking males. In summary then, the relationship between dominance rank and mating success appears to be a (negative) function of the number of concurrently cycling females (Cowlishaw & Dunbar 1991). This “priority of access model” was tested on the NINA-A troop by relating, for each observation day, the number of females observed mating, the number of males observed mating, and the average rank of mating males. The number of males observed mating increased with the number of females observed mating and the average dominance rank of males observed mating decreased with an increase in the number

of mating females (Soltis *et al.* 2001). These results show that the greater the number of available females, the greater the mating opportunity for males of various dominance ranks.

A fourth possibility is that some males of a population could be infertile due to unknown factors. However, I established the fertility of each of the 15 troop males by analysing at least one auto-masturbatory ejaculate collected from the forest ground immediately after ejaculation (see 3.3). All males were judged to be fertile (Table VII). But this "infertile male hypothesis" could hold true in other troops or species and, therefore, ejaculate analysis should become a standard procedure in the study of reproductive patterns of wild and captive animals.

This study also dealt with the discrepancy between ejaculate input (mating success) and reproductive success. During the mating period, behavioural data on the mating success of the 15 troop males were collected. In the birth period of the next year, DNA from 9 babies was collected and a paternity exclusion analysis was performed (Joseph Soltis). The three guarders mated a total of 71 times, whereas the twelve sneakers were observed to succeed in mating only 34 times (own data). Based on established theories of sperm competition, guarders should sire approximately double the amount of offspring than sneakers do, since they contributed the double amount of ejaculate.

The three guarders, however, sired 3 babies and the 12 troop sneakers also sired 3 babies but the remaining 3 babies were sired by non-troop sneakers. In considering only troop males, sneakers enjoyed a disproportionately high reproductive success in relation to their ejaculate input into sperm competititon games, while guarders had a disproportionately low reproductive success. Furthermore, 33% of the offspring were sired by non-troop males, and, although mating with these males was not observed in the wild during this study period, it seems unlikely that non-troop sneakers achieved a high proportion of total ejaculatory input (Table X, Soltis *et al.* 2001).

Table X: Mating and reproductive success in NINA-A-troop in 1997. DNA-paternity exclusion-analysis was done by Joseph Soltis. NTM = non-troop-male.

	Guarders	**Sneakers**	**NTM-Sneakers**
No. of males	3	12	?
No. of copulations	71(67%)	34(33%)	?
No. of offspring	3 8(33,3%)	3(33,3%)	3(33,3%)

In fact, I propose that non-troop sneakers probably mated even less frequently than troop sneakers and pose the question as to why they also succeeded in siring offspring.

Similar patterns have been found in other species. In some birds for example, extra-pair copulations (corresponding to non-troop sneakers) result in a disproportionately high fertilisation success (Birkhead & Møller 1992, Lifjeld at al. 1993, Dixon at al. 1994), which is based on the maximisation of both, the number of sperm per ejaculate and sperm velocity (Birkhead *et al.* 1995). In summary then, I propose that some of those 6 babies sired by sneakers or non-troop sneakers conform to the "priority of access model", while the others conform to the "masturbation before mating model".

In conclusion, the function of masturbation is to wash out low quality sperm from the genital tract. The next produced ejaculate then contains more fresh sperm, which has higher chances during sperm competition. Masturbation, therefore, is an adaptation to sperm competition in non-human primates. In the case, the males of a population can be divided into sneakers and guarders it should be regarded as an ESS.

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A. Published papers

Thomsen R (1997): Observation of periparturitional behaviour in wild Yakushima macaques *(Macaca fuscata yakui)*. Folia primatol. 68: 338-341.

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Thomsen R, Soltis J & Teltscher C: Sperm competition and the function of male masturbation in non-human primates. In: J Cones (ed): Sexual Selection and Reproductive Competition in Primates - New Perspectives and Directions (*in press* 2002).

B. Submitted papers

Thomsen R, Soltis J, Matsubara M, Matsubayashi K & Takenaka O: Cost of ejaculates and male rejection of female presentations in wild Japanese macaques *(Macaca fuscata)*.

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