A Male Cynomolgus Monkeys (Macaca fascicularis) Differentially Mated with a Female

Permalink
https://escholarship.org/uc/item/37v283bn

Journal
International Journal of Comparative Psychology, 30(0)

ISSN
0889-3667

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Publication Date
2017

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Peer reviewed
Male Mate Choice Among Captive Long-tailed Macaques (*Macaca Fascicularis*)

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The purpose of this study was to investigate male mate choice and to define whether a male’s behaviors were affected by a female’s reproductive status (multiparous/nulliparous). Our experimental conditions enabled us to measure successful mating by observing sperm in female vaginal washings and their sexual behavior. Our study subjects were long-tailed macaques that were bred in our primate institute. All the males had fathered young, thus, their reproductive capabilities were confirmed. All of the females were individuals with regular menstruation. During the study, one male would be grouped with two females, each of whom lived in a cage adjacent to the male’s cage. This enabled each of the females to be housed with the male in turn; 12 males and 24 females were in the study. Cohabitation continued until both females were observed over two menstrual periods. After a male cohabited with a female, we checked for sperm via microscope in the female’s vaginal washing, thus confirming copulation success. For some of the groups, behavioral observation was conducted on both male and female subjects. According to our findings, among multiparous females, successful mating was observed on 29% of cohabitation days. Among nulliparous females, the presence of sperm was observed during only 6% of cohabitation periods. Some 67% of the nulliparous females never mated with a male. Our observations also revealed sexual behaviors happened more frequently when a male lived with a multiparous female. Males groomed multiparous females more often than nulliparous ones. Our study suggests that male long-tailed macaques prefer multiparous females, as it is important that a male choose a female who more easily and regularly becomes pregnant and gives birth to offspring with a higher survival rate. Thus, male choice may provide him with some reproductive benefits.

A wide variety of studies have focused on describing the roles and importance of male–male competition and female mate choice in driving evolutionary change (Anderson, 1994; Hill, 1990; Möller, 1988; Ryan, 1980; Sullivan, 1987). Researchers have emphasized that in primates, a male having been chosen by a female appears to guarantee his genetic quality (Folstad & Karter, 1992; Havlicek, Roberts, & Flegr, 2005), demonstrate his good health (Waynforth, Hurtado, & Hill, 1998), and signify his high rank (Ellis, 1995; Gowaty, 1997; Huffman, 1991; Manson, 1992; Small, 1989). In addition, genetics may influence sexual selection. Genetic disassortative mating preferences offer fitness and benefits for animals as well as humans (Penn, 2002). Though male mate preference has been recognized since the time of Darwin, the reasons for a male’s choice of mate remains unresolved (Bonduriansky, 2001). There are few studies on male preference and mate choice in primates; however, some researchers have suggested that males choose fertile, high-ranking, older females with higher offspring survivorship as a means of ensuring male reproductive success (Berenstain & Wade, 1983; de Ruiter, van Hooff, & Scheffrahn, 1994; Fitzpatrick, Altmann, & Alberts, 2015; Girard-Buttoz et al., 2014; Keddy-Hector, 1992; Kuester & Paul, 1996). In our study, we also explore male mate choice under certain experimental conditions.

In our previous study, we conducted a statistical analysis of breeding data; the results showed that a female’s pregnancy history predicted future pregnancy. Our findings suggested that male choice affects breeding data, because male long-tailed macaques (*Macaca fascicularis*) prefer to mate with a multiparous female (one who has been pregnant multiple times) rather than one who is nulliparous (one who has never...
been pregnant) for reproductive success (Kobayashi, Koyama, Yasutomi, & Sankai, 2015). Furthermore, these phenomena have been observed in male chimpanzees (*Pan troglodytes*) and rhesus monkeys (*Macaca mulatta*), which have also been shown to prefer multiparous females (Anderson, 1986; Muller, Emery Thompson, & Wrangham, 2006; Paul, 2002). Multiparous females are considered to have had higher reproductive success and are thus more easily selected by a male. This agrees with Trivers’ parental investment theory (Trivers, 1972), which posits that a male shall have more advantage and increase his reproductive success (i.e., realize greater offspring survivorship) if he mates with a multiparous female rather than one who is nulliparous.

The female ovulation cycle also has a strong influence on sexual behavior. Males of several species have been observed to concentrate their mating efforts on females during their conception cycles (Emery Thompson & Wrangham, 2008; Weingrill, Lycett, Barrett, Hill, & Henzi, 2003). The exaggerated estrous swelling displayed by many primates is commonly cited as a trait upon which males base mate choice. Swelling at its maximum coincides with the period of ovulation. The swelling consists of a marked distension of tissues with fluid; the skin becomes brightly colored, shiny and convoluted (Napier & Napier, 1994). Only Old World primates show this phenomenon. These swellings are most prominent in baboon (*Papio* sp.), the pig-tailed macaque (*Macaca nemestrina*), mangabeys (*Cercocebus* sp.), chimpanzees (*Pan troglodytes*), talapoins (*Miopithecus talapoin*), and bonobos (*P. paniscus*). In some species, females with more extensive swellings have been shown to be preferred by males (Domb & Pagel, 2001; Higham, Semple, MacLarnon, Heistermann, & Ross, 2009; Huchard et al., 2009; Napier & Napier, 1994). In the long-tailed macaque, the menstrual period has been found to be 29 ± 4 days (Kobayashi et al., 2015). Changes in the extent of sexually related swelling showed a similar pattern in all cycles; however, males were better able to identify fertility during the females’ conception cycles than when they were not ovulating (Engelhardt, Hodges, Niemitz, & Heistermann, 2005; Engelhardt et al., 2004). In other words, female ovulation was hard to identify by changes in the sexual organs. Yoshida, Hanari, Fujimoto, and Sankai (2010) suggested that the serum follicle stimulation hormone (FSH) in the long-tailed macaque increased at 10 to 11 days after menstruation and ovulation was observed 1 or 2 days later. So, the optimal cohabitation time was judged to be about 12 days after menstruation. To summarize, a female’s ovulation period was not visible to the animal technician and their menstrual period was for about one month, which included one ovulation period.

However, there is no evidence on the characteristics that males use to distinguish females of greater reproductive ability from other females. Thus, the objective of our study was to examine whether female pregnancy history affected male preference and whether males distinguished past female gravidity as a consideration for selection of a mate, using an experimental model that involved the behavioral observation of laboratory-bred long-tailed macaques in one-on-one living conditions. Our previous results could not make a reference as to whether or not a nulliparous female mated with or socially interacted with a male, because we did not observe their behavior. For this study, we established cohabitation conditions that included females who had been pregnant and those who had not. We checked for the presence of sperm and observed the monkeys’ social behavior. This experimental model enabled us to investigate as to whether or not the male long-tailed macaque preferred a multiparous female as a mating partner.

**Method**

**Subjects**

Our study subjects comprised 12 adult male (age 7 to 25 years; weight range of 3.57 to 6.97 kg) and 24 adult female (age 5 to 16 years; weight range of 2.60 to 4.20 kg) long-tailed macaques (*Macaca fascicularis*) that were born in the Tsukuba Primate Research Center (TPRC), National Institutes of Biomedical Innovation, Health and Nutrition breeding colony.
All of the males had fathered young, thus, their reproductive capabilities were confirmed. All of the females were individuals with regular menstruation (12 multiparous females [age 11 to 16 years, \( M = 12.67 \) years] and 12 nulliparous females [age 5 to 12 years, \( M = 8.17 \) years]). Our study methods were approved by the Ethics Committee of the TPRC. National Institutes of Health (NIH) safety standards were adhered to in the TPRC’s breeding and rearing environments. All of the adult monkeys were housed in single cages (0.5 m wide \( \times \) 0.8 m high \( \times \) 0.9 m deep; stainless steel mesh). The air of the breeding room was replaced 12 times hourly while maintaining a temperature of about 25 °C and humidity of 50 to 60%, and was lighted 12 hrs a day from 0700 to 1900. All of the subjects were supplied the same amount of food (100 g of fruit and 70 g of monkey chow) every day, and water was available ad libitum. Experienced animal technicians inspected the monkeys daily for any abnormalities and for menstruation (Tsuchida, Yoshida, Sankai, & Yasutomi, 2008).

Cohabitation Methods

For this study, we adopted an every-other-day mating (cohabitation) system in which one male was set to house a full day alternately with one of two females, each of whom lived in a cage adjacent to each side of the male’s cage. We established three types of cohabitation groups, consisting of one male and two females for observation: Multiparous-Male-Multiparous, Nulliparous-Male-Multiparous and Nulliparous-Male-Nulliparous. The difference in age of the females in the groups was within 2 years, on average. Four males and eight females were set for each group and a total of 12 groups were investigated. There were two conditions for which a group was disbanded: when both of the females in the group were observed as having menstruated twice or when pregnancy was confirmed in either of the females. Both of the females should have had at least one ovulation period. Pregnancy was confirmed by ultrasonography conducted on the 35th and 70th days after the start of cohabitation.

Determination of Successful Mating

The determination of successful mating was done using a saline-filled syringe along with a sonde to obtain a vaginal washing from each female on the days the female cohabited with a male. We checked for sperm in the vaginal washing using a microscope. When sperm were observed, we judged mating to be successful that day.

Behavioral Observation

Behavioral observation was conducted on the Nulliparous-Male-Multiparous groupings to compare the male’s preference for type of female: multiparous or nulliparous. Their behaviors were videotaped for one hour per week immediately after their cohabitation period started. Gathered video data was for 40 hrs (20 hrs of multiparous-male cohabitation data and 20 hrs of nulliparous-male cohabitation data). An analysis was performed using the focal animal sampling method (both male and female) and one-zero sampling method every 10 s (Lehner, 1998). Eight behaviors in three categories were observed, as shown in Table 1.

Analysis

For analyzing the frequency of successful mating days, a statistical analysis was used to compare whether mating was successful between the males and the multiparous or nulliparous females. Successful mating ratios (successful mating days/female cohabitation days) were performed using one-way ANOVA on arcsine (\( \sqrt{x} \) )-transformed data by ANOVA4 (Forsgren, Amundsen, Borg, & Bjelvenmark, 2004).

In this study, we also ran a comparison between the multiparous and nulliparous groups to determine the ratio of females who never mated with a male during any of their cohabitation periods (never-mating animals). We calculated the data as a percentage (never mating animals/multiparous females or nulliparous females). For the statistical analysis, we performed a 2-sample test for equality of proportions without continuity correction. We used the prop.test function on R Version 3.3.0 for Windows, with a significance level of 5% (Yamamoto, 2013). We analyzed 20-hr behavioral records for both the multiparous and nulliparous females using Welch’s \( t \) test.
Table 1

**Behavioral Definitions**

<table>
<thead>
<tr>
<th>Class of Behavior</th>
<th>Behaviors</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual Behavior</td>
<td>Mounting</td>
<td>Male climbs onto the back of a female in order to copulate.</td>
</tr>
<tr>
<td></td>
<td>Observation of female genitals</td>
<td>Male brings his face close to the female’s genitals occasionally to sniff or touch them.</td>
</tr>
<tr>
<td></td>
<td>Female presenting</td>
<td>Female raises her tail and shows her genitals to a male.</td>
</tr>
<tr>
<td>Social Affinity</td>
<td>Female grooming of male</td>
<td>Female cleans the fur or skin of the male.</td>
</tr>
<tr>
<td></td>
<td>Male grooming of female</td>
<td>Male cleans the fur or skin of the female.</td>
</tr>
<tr>
<td></td>
<td>Social contact</td>
<td>Two individuals have physical contact with any part of the body.</td>
</tr>
<tr>
<td>Individual Behavior</td>
<td>Stereotypy by male</td>
<td>Male repeats the same behavior or moves over three times (e.g., repetitive swaying, pacing, circling, bouncing, and/or rocking).</td>
</tr>
<tr>
<td></td>
<td>Stereotypy by female</td>
<td>Female repeats the same behavior or moves over three times (e.g., repetitive swaying, pacing, circling, bouncing, and/or rocking).</td>
</tr>
</tbody>
</table>

**Results**

First, we compared the successful mating data between the multiparous and nulliparous groups. Using an ANOVA with arcsine (\(\sqrt{x}\))-transformed data, a significant difference was found, \(F(1, 22) = 7.80, p = 0.01\). Among multiparous females, we observed that successful mating took place on 29% of the days they cohabited. Among nulliparous females, however, we observed the presence of sperm (i.e., confirmation of successful mating) on only 6% of the days they cohabited.

We analyzed the animals who never mated in both the multiparous and nulliparous groups. For statistical analysis, the 2-sample test for equality of proportions without continuity correction was used with a significance level of 5%. According to the results, 66.7% of the nulliparous females never mated with a male during a cohabitation period. In other words, more multiparous females took the opportunity to mate with a male than did the nulliparous females (9:4) under this every-other-day cohabitation model, \(\chi^2(1, 24) = 4.20, p = 0.04\).

A behavioral comparison between multiparous and nulliparous groups was tested by Welch’s \(t\) test, in the next step. We used eight dependent variables: mounting, observation of female genitals, female presenting, female grooming of male, male grooming of female, social contact, stereotypy by male, and stereotypy by female.

Table 2 shows the average frequency of sexual behaviors during each hour of observation for both the multiparous and nulliparous females. The difference between the two groups of females was statistically significant in terms of *mounting*, *observing female genitals*, and *female presenting* (\(p = 0.03, p = 0.01, p = 0.05\) for each) and marginally significant in *male-grooming-of-female* behaviors (\(p = 0.06\)). That is, these behaviors were more frequently observed in the pairs consisting of a male and a multiparous female than in those consisting of a male and nulliparous female.
Table 2

Welch’s t-test Comparing Multiparous Female and Nulliparous Female Behavior

<table>
<thead>
<tr>
<th>Behaviors</th>
<th>Multiparous Females M ± SD</th>
<th>Nulliparous Females M ± SD</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mounting</td>
<td>2.2 ± 3.8</td>
<td>0.1 ± 0.3</td>
<td>5.80*</td>
</tr>
<tr>
<td>Observation of Female Genitals</td>
<td>2.2 ± 2.5</td>
<td>0.4 ± 0.9</td>
<td>8.60**</td>
</tr>
<tr>
<td>Female Presenting</td>
<td>3.3 ± 5.3</td>
<td>0.7 ± 1.3</td>
<td>4.33*</td>
</tr>
<tr>
<td>Female Grooming of Male</td>
<td>25.8 ± 43.3</td>
<td>18.6 ± 29.0</td>
<td>0.36 ns</td>
</tr>
<tr>
<td>Male Grooming of Female</td>
<td>18.5 ± 33.3</td>
<td>2.4 ± 4.4</td>
<td>3.93+</td>
</tr>
<tr>
<td>Social Contact</td>
<td>83.2 ± 147.3</td>
<td>51.8 ± 100.1</td>
<td>0.59 ns</td>
</tr>
<tr>
<td>Stereotypy by Male</td>
<td>26.0 ± 63.7</td>
<td>6.0 ± 8.8</td>
<td>1.84 ns</td>
</tr>
<tr>
<td>Stereotypy by Female</td>
<td>0.8 ± 2.9</td>
<td>4.9 ± 10.3</td>
<td>2.27 ns</td>
</tr>
</tbody>
</table>

Note. Mean shows times the behavior occurred per hour.
+p < 0.10. *p < 0.05. **p < 0.01. ns = not significant.

Discussion

The intent of this study was to investigate male preference in choosing a female mate rather than female preference, in terms of sexual behavior. We established experimental conditions that enabled us to measure successful mating by gathering sperm from the females’ vaginas, as well as by observing sexual behavior through at least one ovulation period.
In comparing the frequency of successful mating days between the multiparous and nulliparous groups of females, on 29% of the total number of days of cohabitation the multiparous females mated with a male. Some of the multiparous females were observed successfully mating for most of their cohabitation period, that is, mating with a male almost every day. However, the nulliparous females mated with a male on only 6% of the days they cohabited with a male. In short, the mating activity of the nulliparous females was extremely low; however, regular menstruation had been observed in these subjects.

In investigating whether mating was successful during the females’ cohabitation periods, what we found fascinating was that 66.7% of the nulliparous females never mated at all with a male, despite the fact they lived with a male through more than one ovulation cycle. Conversely, only 25% of the multiparous females did not mate successfully with a male during the course of the study. Put another way, 75% of the multiparous females successfully mated during their cohabitation periods.

The results of our behavioral observation also showed that all three sexual behaviors (mounting, observing female genitals, female presenting) were more frequently seen when a male was living with a multiparous female. Male observation of female genitals and female presenting behavior are associated, and it is not an exaggeration to say that this is a series of sexual behaviors. The observation of female genitals often followed female presenting. In addition, male-grooming-of-female behaviors were more frequently observed between a male and a multiparous female; in short, the males groomed these females more often. Intriguingly, with respect to female-grooming-of-male behaviors, a female would provide regular grooming to a male regardless of whether she was multiparous or nulliparous. These results suggest that both types of females (multiparous and nulliparous) endeavor to establish social relationships with males. Some researchers have postulated that grooming behaviors generally increase the chances of mating (Clarke, Halliday, Barrett, & Henzi, 2010; Gumert, 2007; Koyama, Caws, & Aureli, 2012). Taking these studies into account, the nulliparous females in our experiment did indeed show social affinity for the males, and without having engaged in sexual behavior. Conversely, the males tended to use grooming of a multiparous female as an approach to copulating with her.

Since the males may express their preference for multiparous over nulliparous females with respect to prior mating performance and sexual behavior, it is necessary that we explore the reasons a male would show this preference. First, it could be said that males are concerned with offspring survivorship, even though they are not as directly involved in raising offspring as the females are. Some researchers have suggested that males choose fertile, older females with higher offspring survivorship (Muller et al., 2006). Also, that older females are likely to have higher gravidity (Anderson, 1986; Kobayashi et al., 2015). In addition, a high gravidity rate means that a female is fertile and also guarantees higher offspring survivorship (Domb & Pagel, 2001). It is important for males to choose females who can more easily and regularly become pregnant and give birth to offspring with higher survival rates, since male choice is biologically significant with respect to leaving more offspring, according to Trivers’ parental investment theory (Trivers, 1972).

Secondly, although our experimental conditions eliminated the social rank factor (Kobayashi et al., 2015), we could not help but refer to it, as in primates this factor does affect both male and female preference in a mating partner. Male nonhuman primates have been shown to prefer older, experienced females as well as high-ranking females (Anderson, 1986; Keddy-Hector, 1992; Paul, 2002). These females’ offspring are more likely to survive; consequently, a preference for these females is beneficial. Some researchers have suggested that the daughters of these females inherit their mothers’ higher status and the sons of higher-ranking females achieve greater reproductive success than those of lower-ranking females (Gerloff, Hartung, Fruth, Hohmann, & Tautz, 1999; Kuesterl & Arnemann, 1992; Smith & Smith, 1988; van Noordwijk & van Schaik, 2015).
1999). Therefore, it could be said that males gain indirect yet long-term benefits from choosing dominant females (Paul, 2002). In future studies, we plan to take into account the social dominance factor.

In conclusion, our study suggests that male long-tailed macaques distinguish prior female gravidity and prefer a multiparous female as a choice of mate, which may ultimately provide him with some reproductive benefits (Berenstain & Wade, 1983; de Ruiter et al., 1994; Girard-Buttoz et al., 2014; Keddy-Hector, 1992; Kuester & Paul, 1996). The males and the females were not familiar with each other. However, we are not yet able to clarify exactly how a male distinguishes a multiparous female from one who is nulliparous. In long-tailed macaques, sexual organ swelling does not change in all cycles; however, the males seem better able to recognize when females are most fertile during their conceptive cycles than when they were not ovulating (Engelhardt et al., 2004, 2005). It is also not clear as to whether a female may have specific chemical traits (e.g., vaginal scent) during their ovulation cycle that are attractive to a male. In addition, the dominance factor was excluded in our experimental model, but we will include it in future studies. These topics are definitely worthy of further study, along with the pursuit of even better investigational methods for this type of primate research.

References


Financial conflict of interest: This study was supported the grant by A MED (15ak0101020h0003) of Japan.
Conflict of interest: No stated conflicts.

Submitted: July 5th, 2016
Resubmitted: October 4th, 2016
Accepted: December 16th 2016