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Age Influences Male's Mating Preferences for Multiparous and Nulliparous Females in the Laboratory-bred *Macaca Fascicularis*

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Most studies of partner choice and preferences in nonhuman primates have been concerned with male social rank. Females select high-ranking males, and high-ranking females can more readily gain access to males. Although researchers have mentioned males' choices and their preferences in females, papers that focus on male preferences have been few. Past studies suggest that male primates prefer older females. This study was aimed at analyzing the male preference under minimized social factors. We analyzed data collected though the every-other-day mating system, in which a male alternately lived with each of two females. Multiparous and/or older females were more frequently pregnant. In the nulliparous group, females that became pregnant for the first time tended to be younger than the other non-pregnant females paired in the mating set. The results suggest that while males generally prefer females with higher gravidity, males prefer to mate with younger females among females that have never been pregnant. There is the possibility that females of high reproductive ability are chosen for mating. This strategy may be biologically advantageous for both males and females.

Male reproductive success is measured by successful competition with other males to have more offspring. On the other hand, female reproductive success in primates, including humans, is measured by the ability to acquire high-quality genes from a male and to fulfill her purpose to bear a child and bring it up safely (Penn, 2002). Although most studies conform to the notion that females take the initiative in mating partner choice, some studies have examined male mating preferences in nonhuman primates (Anderson, 1986; Muller, Thompson, and Wrangham, 2006; Parga, 2006).

Most studies of mating partner choice in nonhuman primates have been concerned with social rank and female choice. They have revealed that females prefer high-ranking males (Bercovitch & Strum, 1993; Bercovitch & Berard, 1993; Drickamer, 1974; Ellis, 1995; Gowaty, 1997; Huffman, 1991; Manson, 1992; Paul &Thommen, 1984; Small, 1989). Holding a higher rank in a dominance hierarchy seems to guarantee a male's genetic quality (Folstad & Karter, 1992; Havlicek, Roberts, & Flegr, 2005) and to show that he is in good health (Waynforth, Hurtado, & Hill, 1998). On the other hand, Nikitopoulos, Heistermann, de Vries, van Hooff, & Sterck (2005) suggested that female preference is independent of a partner's dominance rank. Their work was congruous with Bercovitch's (1995) finding regarding the mating of female savannah baboons with multiple males apart from their social rank. Thus, since there remain several controversies about female preference and male's social rank in partner choice, this research was excluded social rank factors.

Male preference studies are few. Bercovitch (1997) stated that offspring survivorship is a more important component of female reproductive strategy than choice of mating partner. Some other researchers also suggested that males choose fertile, old females with higher offspring survivorship (Muller et al., 2006). A high gravidity rate suggests that a female is fertile and also guarantees higher offspring survivorship.

This study was aimed at analyzing male preference in females. We focused on the female attributes that determined male preference and led to pregnancy. Which attributes of the female affect male preference? At first, we compared physical and/or biological factors, such as age and past gravidity between the subject females who became pregnant and those that did not within this mating system. Weight also is a good index of physical condition (Bercovitch, 1997); it shows the momentum of the individual and congenital individual differences. In this study, we compared the weight of pregnant and non-pregnant females. These female attributes may induce male preference and will be reflected in the difference in conception rate.

Furthermore, some studies have reported on the peak pregnancy rate (Gardin, Jerome, Jayo, & Weaver, 1989) and mating activity of primates (Kaufman, 1965). Because the number of viable oocytes in the ovaries decreases with age, age should be considered as much as weight or gravidity to be a factor that directly affects reproductive success.

In this study, we used individually housed cynomolgus monkeys (*Macaca fascicularis*) that were free from male–male competition and social rank factors, as much as possible. In order to compare a pair of females under the same condition, we adopted the every-other-day mating system, in which one male was set to copulate alternately with one of two females housed separately in adjacent cages. Although a male lived together with a distinct female for the same period, one female nevertheless became pregnant and the other did not after living together. The advantage of this system is that the condition of the male was controlled as much as possible for both of the females. This system enabled us to investigate male preferences in mating partners by analyzing the attributional or behavioral differences between pregnant and non-pregnant females after mating.

Method

Subjects

The subjects comprised 47 adult male and 94 adult female cynomolgus monkeys (*Macaca fascicularis*) that were born in the Tsukuba Primate Research Center (TPRC) breeding colony. Almost all of the males were confirmed their reproductive capability. Females were random-sampled from the individuals with regular menstruation. NIH safety standards were adhered to in the TPRC breeding and rearing environments.

All adult monkeys were housed in single cages (0.5 m wide × 0.8 m high × 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 a humidity of 50% to 60%. The room was lighted for 12 hours a day, from 0700 to 1900, and the monkeys were provided with fruit (100 g) and monkey chow (70 g) in the morning. All subjects were supplied the same amount of bait every day. Water was available ad libitum. Experienced animal technicians inspected the monkeys daily for any abnormalities and menstruation.

Mating Method

The TPRC uses several mating systems for effective breeding. In this study, we adopted the every-other-day mating system, in which one male is set to house a full day alternately with one of two females, each of which lives in an adjacent cage on either side of the male's cage. In this mating system, a male and a female stayed together without special consideration of the female's ovulation and menstrual cycle. It was randomly decided which female started living with a male at first. Combinations of female subjects' ages, weights and gravidity were not controlled to prevent unexpected deviation. In this experiment, females were housed with a male for up to 99 days. The menstrual cycle of cynomolgus monkey is 29 ±4.3 days on average. The typical gestational period of a cynomolgus monkey in the TPRC was 165 days.

A male's cohabitation with one of the females was started by removing the partition board on one or the other of the three consecutive cages, so there was no chance for the females to come in contact with each other. The female also did not come in contact with other males during the experiment period. Pregnancy was confirmed by ultrasonography, which was conducted on the 35th, 70th and 98th days after the start of cohabitation. This experimental condition assumed that the first ultrasonography included the female's one ovulation. Pregnancy testing and weight measurement were performed under anesthesia.

When pregnancy was confirmed in either of the females, that set of three monkeys was disbanded. If pregnancy was not confirmed in either female by the fourteenth week, we disbanded the mating combination and checked the females for pregnancy five weeks later. Mating ceased if one of the monkeys suffered a serious injury or became anorexic. We excluded several cases in which the females became pregnant simultaneously because the male's preference would not be discernible.

This study was approved by the Ethics Committee of the National Institute of Biomedical Innovation (NIBI), and the experiments were conducted according to NIBI guidelines.

Data Collection and Analysis

Data from 47 male-female-female mating sets were obtained from the records of every-other-day mating systems implemented between 2008 and 2011. The independent variable was a female's pregnancy in this experiment. The independent variable included two groups (pregnant females or non-pregnant females). We used three dependent variables; a female's past gravidity was defined as her number of pregnancies up to the experiment, and her age and weight were those at the start of mating. The females' ages ranged from 3 to 19 years, past gravidity from 0 to 11, and weight from 2410 g to 7770 g.

Since pregnant females and non-pregnant females were individually matched, logistic regression was applied for data analysis. With regard to the nulliparous females, we used one-way ANOVA between pregnant females and non-pregnant females, because of lack of past gravidity and small number of subjects. The statistical package IBM SPSS Statistics 21 was used for these analyses. The nulliparous females had no experience with pregnancy and became pregnant for the first time when mating during this experiment, while the multiparous females had experienced pregnancy before this study.

Results

First, we clarified the relationship between the variables using correlation. The pair-wise correlations between age, weight, and number of previous pregnancies (past gravidity) for the 94 females in the 47 mating sets are shown in Table 1. All three correlations were positive and significant, with the highest correlation seen between age and past gravidity. The lowest correlation was between age and weight; the heavier a female was, the older she was. The correlation between past gravidity and weight indicates that females with more previous pregnancies tend to be heavier.

Table 1. *Correlation between age, past gravidity and weight*

	Age	Past Gravidity	Weight
Age	⬜	0.85 **	0.60 **
Past Gravidity		⬜	0.66 **
Weight			⬜

Note. df = 1 & 92, *n* = 94.

** *p* < 0.01.

Table 2 illustrates the number of factors among pregnant females and non-pregnant females. In Table 2, it can be seen that the pregnant females were significantly older and had more past gravidity than the non-pregnant females.

We then distributed multiparous and nulliparous. First, we compared the multiparous pregnant females with the non-pregnant females that were in the same mating sets (*n* = 30) on three variables (age, past gravidity, and weight). Table 3 shows that the pregnant females were older and had more past gravidity than non-pregnant females.

Second, we compared the age and weight of the pregnant females with no previous gravidity versus the non-pregnant females in the same mating sets (*n* = 17). Table 4 shows that the pregnant females were younger than the non-pregnant females.

Table 2. *Factors of pregnancy: age, past gravidity and weight (logistic regression analysis)*

	Pregnant Females (n = 47)	Non-pregnant Females (n = 47)	
	Mean ± SD	Mean ± SD	Odds ratio (95% CI)
Age	8.3 ± 3.8	8.0 ± 2.9	0.61 (0.45-0.83) *
Past Gravidity	2.3 ± 2.6	1.0 ± 1.5	2.48 (1.50-4.10) **
Weight (g)	3491.6 ± 901.8	3196.8 ± 557.3	1.00 (1.00-1.00) *n.s*

Note. n = 94. *n.s.* = not significant, SD = Standard Deviation.
* *p* < 0.05. ** *p* < 0.001

Discussion

This study examined the relationship between the feasibility of becoming pregnant and the three variables of age, weight and gravidity in female cynomolgus monkeys. The weight of the females increased with age at the TPRC (even after maturation), and the number of previous pregnancies also rose with age. This means that the age of the female seemed to be most important for this study. As for the relationship between age and gravidity, previous studies reported that increasing age increases the chance of gravidity, and older females with greater gravidity have an increased chance of successful pregnancy (Anderson, 1986; Gardin, 1989; Muller et al., 2006).

Of the positive correlations between the chance to become pregnant and each of the three variables, past gravidity and age significantly affected the feasibility of becoming pregnant. Weight did not statistically influence pregnancy. Although the animals were kept indoors and were supplied with the same amount of food in the TPRC, weight did not influence the feasibility of becoming pregnant in this experiment.

To analyze the features of the females' gravidity in this study, we divided the pregnant females into groups of multiparous females with past gravidity and females with no history of pregnancy (instead of directly analyzing the influence of age). We found that the number of previous pregnancies was an important factor in the former group. The influence of age on pregnancy, however, should be considered in the group of females that became pregnant for the first time. That is to say, older females in the multiparous group became pregnant more readily, whereas the younger females in the group that became pregnant for the first time did so more readily. This means that the age factor appears to affect pregnancy differently, between the multiparous and nulliparous females.

In general, copulation is complete when a female accepts a male; it does not occur if a female refuses a male. In the every-other-day mating system used in this study, every female lived with a male through more than one ovulation cycle, and thus all should have had the opportunity to become pregnant. Nevertheless, we observed two groups of females: those that became pregnant and those that did not became pregnant. This suggests the possibility that males rather than females choose mating partners.

The every-other-day mating system allowed males to choose one of the females living in the adjacent cages. Even though we might suppose the females chose the males based on their reproductive ability, this seems less likely for the following reason: in a given mating set, the female with more previous pregnancies was more likely to become pregnant, whereas the younger female of a pair without a history of previous pregnancies was more apt to become pregnant. Our results suggest that the idea that a male chooses a mating partner is more reasonable than the converse. This is congruous with the male's preference for females (Muller et al., 2006).

It is worthwhile to notice that the male chose a younger female in mating with nulliparous females. If the young female became pregnant in the early stages of maturation, she would give more births to offspring than a female that became pregnant for the first time at an older age. The male who chose a younger, nulliparous female would thus have the possibility of producing more offspring. A male's choice of a younger female would raise the reproductive success for both the male and female.

It is important for males to choose females that more easily and regularly become pregnant and give birth to offspring with higher survival rates, because the male's choice is biologically significant with respect to leaving more offspring (Trivers, 1972). Apart from social rank, there is the possibility that females of high reproductive ability are chosen for mating. This strategy may be biologically advantageous for both males and females.

This study suggests that males may select females as part of their reproductive strategy. There is no evidence yet regarding the characteristics that males use to distinguish females of greater reproductive ability from other females. Who took the initiative in mating, the female or the male? That question will probably be considered in the future. We need further basic data on macaques' mating behaviors to determine whether males choose their mating partners. We could observe such behaviors as which individual initiates mating, the male or female, and whether his ejaculation was successful by checking for sperm in the female's vagina.

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