FEMALE CONTROL OF PATERNITY DURING COPULATION: INBREEDING AVOIDANCE IN FERAL CATS

by

YASUKO ISHIDA\textsuperscript{1,2)}, TETSUKAZU YAHARA\textsuperscript{1)}, EIITI KASUYA\textsuperscript{1)}
and AKIHIRO YAMANE\textsuperscript{3,4)}

\textsuperscript{(1} Department of Biology, Faculty of Sciences, Kyushu University, Hakozaki, Fukuoka 812-8581, Japan)

\textsuperscript{(Acc. 2-XI-2000)}

\begin{center}
\begin{small}
\begin{tabular}{l}
\textbf{Summary}\\
Although among feral cats, \textit{Felis catus}, females copulate with multiple males, they do not accept all mounting or copulation attempts by males during their oestrous period. We observed eight female cats over their oestrous periods to examine whether or not female cats control paternity of their offspring in the field. The females were courted by between nine and 19 males, but copulated with only three to nine of them. Firstly, we compared female receptivity to male attempts among the eight females and tested how female traits affect their receptivity. Female receptivity to male attempts varied among the females both at mounting and at copulation. Females were more choosy at the time of copulation than at mounting. Females with a shorter oestrous duration and a lighter body weight tended to accept mounts more frequently than the females with a longer oestrous duration and a higher body weight. Older and lighter females tended to accept copulation more frequently than younger and heavier females. Females courted by fewer males per day also tended to accept
\end{tabular}
\end{small}
\end{center}

\textsuperscript{2)} Corresponding author; e-mail address: yishiscb@mbox.nc.kyushu-u.ac.jp
\textsuperscript{3)} Address: Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan
\textsuperscript{4)} We thank M. Hiraiwa-Hasegawa, M. Kawata, T. Koga, E. Natoli, and T. Saitoh for their fruitful comments on the manuscript, K. Ooi for his technical support in molecular analysis, and S. Katada, K. Tsuchida, and C. Wedekind for their advice in data analyses. We also thank M. Kinoshita, T. Kogawa, H. Koyanagi, Y. Nakashima, Y. Sakisaka, M. Shimizu, T. Shimizu, H. Tajiri, T. Yokogawa and N. Yoshihara for their assistance in trapping cats, colleagues of the laboratory of ecology, Kyushu University for their encouragement during the study and the people of Ainoshima Island for their kind cooperation to our project. This work was in part supported by grants #107572 to YI and #10874117 to TY from the Ministry of Education, Culture and Science. The English used in this manuscript was revised by Miss K. Miller (Royal English Language Centre, Fukuoka, Japan).

copulations more frequently. Secondly, combining behavioural observations and determined kinship, we assessed whether females avoided copulating with their kin. The results showed that female cats avoid inbreeding with their close kin during copulation but not with distant relatives. Copulation attempts by kin males were less frequently accepted than those by non-kin males. Thirdly, we tested whether male age and body weight affect their mating success, but we failed to find any such correlation. These results support the hypothesis that female cats control paternity of their offspring during copulation.

Introduction

Increased attention has recently been paid to female control of paternity (Birkhead & Møller, 1993; Gowaty, 1994; Zeh & Zeh, 1997). Birkhead & Møller (1993) and Gowaty (1994) have claimed that females can control paternity at the stages (1) before copulation, (2) during copulation, (3) after copulation and before fertilization, and (4) after fertilization. Based on previously known information regarding reproductive anatomy and physiology, Birkhead & Møller (1993) considered female control more likely to occur after copulation, particularly in sperm selection. Female animals with internal fertilization perform crucial postintromission activities which can control the chances of males to sire (Eberhard, 1996). Zeh & Zeh (1997) have also claimed that it is easier for females to choose genetically compatible sperm than to choose males before copulation. On the other hand, there have been several studies supporting female control of paternity before copulation (e.g. East et al., 1993; reviewed in Gowaty, 1994). Though behavioural mechanisms of pre-copulatory paternity control would include avoidance of mate-guarding by males, multiple mating by females, and female incitation of male-male behavioural contests (Gowaty, 1994), mate choice may be the most effective control over paternity before copulation. Female house mice, Mus musculus, are known to choose MHC dissimilar males before copulation (Potts et al., 1994), and it has been suggested that this preference functions as a mechanism through which inbreeding is avoided (Potts & Wakeland, 1993; Potts et al., 1994; Svensson & Skarstein, 1997).

Many studies on inbreeding avoidance in mammals and birds investigated the role of sex-biased dispersal, avoidance of familiar individuals who have grown up together, and delayed maturation (reviewed in Blouin & Blouin, 1988; Pusey & Wolf, 1996). Furthermore, several experiments under laboratory conditions suggested that animals also avoid copulation with unfamiliar kin that they encounter during their oestrus (Barnard &
Fitzsimons, 1988; Keane, 1990; Simmons, 1991). However, it remains uncertain how important this is within field populations.

Feral cats are suitable for studying female control of paternity in the field. First, copulations can easily be observed in a feral cat population. Observing copulations is the most direct way to characterize mating systems, although this may be difficult (Waser, 1993). Secondly, cats are promiscuous and several male cats surround and court with an oestrous female. Heavier males can occupy a closer position to a given female and can thus copulate more frequently (Liberg, 1983; Yamane et al., 1996). Thirdly, females show visible rejective behaviour such as pawing, hissing and rushing against mating attempts by males, and as such, a male is unable to achieve intromission if a female does not pose to accept it. Moreover, recent molecular analyses have shown that males dominant in male-male fighting do not always sire offspring (Yamane, 1998). All these facts suggest the possibility of female control of paternity in feral cats.

The purpose of this study is to demonstrate female control of paternity prior to copulation in feral cats. The questions addressed are: (1) Is there a difference among females regarding the degree of acceptance of copulations? (2) Is there a difference in copulatory acceptance between close relatives and non-relatives? (3) Do male body weight and male age influence the frequency of copulations? To answer these questions, we continuously observed the behaviour of certain oestrous females and analyzed their choice of mate with regard to kinship.

Methods

Study area and background information

The observation of feral cats was made within a southern area (about seven ha) of Ainoshima Island (125 ha), Fukuoka Prefecture in Japan (Fig. 1). In this study, all cats above eight years of age were regarded as being nine years old. In total, 48 mature males, 26 mature females, and 25 kittens (less than one year old) were found in the study area. Through continuous investigations since 1989, the ages of more than 80%, and the mother-offspring relationships of more than 60% of the cats had been identified. In addition, the paternity of 11 cats was determined by microsatellite DNA analysis (Yamane, 1998).

Individual cats are easily identifiable by their coat colour patterns and their eye colour. Some all black or all white cats wore collars for identification. Cats were weighed in December, 1996 before the breeding season, following the method of Yamane et al. (1996).
Fig. 1. The study area (shaded area).

Quantification of pedigree relationships and relatedness

In addition to pedigree information of Yamane (1998), we newly quantified kinship of 50 mature cats using 9 microsatellite DNA loci and DNA samples of 64 individuals including 14 cats that had died already but were possibly mothers or fathers of cats we observed in this study. We trapped cats using a box trap made of wire net to collect blood samples for DNA analyses. Before drawing blood, cats were injected with atropine sulfate (0.03 ml/kg) and xylazine (0.1 ml/kg), and then anaesthetized with ketamine hydrochloride (0.3 ml/kg). Obtained blood was stored at −80°C with 1x SSC. After drawing blood, we injected cats with ringer solution (25-35 ml) and Mycillin Injection NZ (Nihon Zenyaku, 0.1 ml/kg) hypodermically. The cats were released at the trapped point after they had fully recovered.

Genomic DNA samples were isolated by the phenol/chloroform extraction method from whole blood samples. Kinship of cats was determined using nine microsatellite loci; Fca 8, Fca 23, Fca 43, Fca 45, Fca 77, Fca 78, Fca 90, Fca 96, and Fca 126 (Menotti-Raymond & O'Brien, 1995). Polymerase chain reaction (PCR) amplification using fluorescent-labeled primers designed for those loci was carried out using the same methods as Yamane (1998). PCR products were resolved on a 5% Long Ranger gel on ABI PRISM™ 377 DNA Sequencer and genotyped with GeneScan Analysis Software version 3.1 using GENESCAN 400HD [ROX] Size Standard (PE Applied Biosystems, see the industrial protocol for the detailed method). We quantified their sibship and estimated relatedness using likelihood calculations of Kinship 1.2 (programmed by Goodnight, see details in Goodnight & Queller, 1999). Paternity of 18 mature cats and matrinity of 45 mature cats were determined.

Using our pedigree for the past two generations, we defined kin and non-kin male and female pairs in the following manner. From the known parent-offspring relationship, we
determined which male and female pairs had 1/4 or higher degrees of relatedness. For cats whose parents were not determined due to lack of DNA samples of their parents, genotypes were compared between pairs of individuals and pairwise relatedness was estimated using relatedness calculations (see details in Queller & Goodnight, 1989) of Kinship 1.2. We defined pairs that had 1/4 or higher degrees of relatedness as being kin and other pairs as being non-kin. Cats whose DNA samples were not obtained were omitted from the analyses except some tame cats whose pedigree information was supplied by their keepers.

Field survey and observed behaviour

From 14 January to 30 March, 1997, we observed the reproductive conditions and mating behaviour of the cats every day. When a female cat came into pre-oestrus, male cats gathered around her and performed courting behaviour. Female cats were understood to be in oestrus when they began to accept copulation attempts by males (Natoli & De Vito, 1988, 1991; Yamane et al., 1996). In the pre-oestrous period, females did not copulate even when they were courted. We observed each female cat throughout her oestrous period, although we could only observe cats in the daytime since using a torch at night would have greatly disturbed their behaviour (Yamane et al., 1996). While following particular females, we recorded the identity of the males courting the female and which of them the female rejected or accepted. Female cats rejected males by pawing, hissing, and rushing at two distinct stages; before mounting and after mounting before copulation. At mounting, a male grasps a female firmly at the nape of the neck, and he can achieve intromission only if she raises her hindquarters to present her genitals and poses to accept copulation. On the other hand, male cats sometimes appeared to mount coercively. However, it was difficult to determine whether a mount was done coercively or not. Thus, accepted mounts we counted include all mounting events we observed. Mounting behaviours were classified into the following categories.

(1) rejected mount (RM): mounting attempt (MA) was rejected by the female.
(2) accepted mount (AM): mounting attempt was accepted by the female; this category includes mounting once accepted by the female but then abandoned by the male himself or intercepted by other males, and the following cases.
(3) rejected copulation (RC): mounting was curtailed by the female after the male had already mounted her.
(4) accepted copulation (AC): mounting with intromission.

When intromission occurred, the female cried sharply, showing aggression against the male, before usually rolling on the ground and then grooming herself, as described by Natoli & De Vito (1991). Thus, accepted copulation could be easily discerned from rejected copulation. Episodes of such behaviour were counted for each male and a female pair.

Data analyses

Definition of variables
The following two variables were calculated for each male and female pair in courtship.

(1) Mount success proportion: the number of accepted mounts divided by the number of all mounting attempts (AM/MA).
(2) Copulation success proportion: the number of accepted copulations divided by the number of all copulation attempts (AC/(RC+AC)).
The following two variables were calculated for each female.

1. Per-female proportion of mounts: total number of accepted mounts during the 
oestrus period divided by total number of mounting attempts (total AM/total MA).
2. Per-female proportion of copulation: total number of accepted copulations during the 
oestrus period divided by total number of copulation attempts (total AC/(total RC 
+ total AC)).

Statistical methods
Logistic regression was used to test any difference in proportion of mountings or copulation 
among females, by treating females and males as nominal variables. This is an appropriate 
way to examine relationships between a proportion and continuous variables (Hardy & Field, 
1998). Repeating tests (e.g. Fisher's exact probability test) for each pair of females would 
lead to inflation of the type I error rate by multiple testing (Hochberg & Tamhane, 1987). To 
examine which factor influenced the differences in per-female proportion of copulation or in 
per-female proportion of mounts, multiple logistic regression was used, designating oestrus 
duration, female age, female body weight, and the mean number of courting males per day as 
explanatory variables. To remove the effects of autocorrelation between explanatory variables 
and per-female proportion of copulation or in per-female proportion of mounts, we excluded 
the explanatory variables that were correlated with total number of copulation attempts or 
total number of mounting attempts from the analyses. LogXact-Turbo (Mehta & Patel, 1993) 
and JMP Ver. 3 (SAS Institute INC., 1998) were used for the logistic regression.

In the observational design of the present study, we treated individual females as blocks 
('strata' in logistic regression) when comparing among males. If we pool the data from 
different females together, the differences in observational conditions among females may 
confound the result.

We examined the effect of male traits on mating performance of males by testing 
the relationships between the traits and two variables; mount success proportion, and 
copulation success proportion. When analyzing relations between the variables of mating 
performance and male body weight and age, the body weight or age was the same value 
across blocks of females for a given male. To avoid inflating the apparent sample size, we 
tested these correlations as follows. The variables of mating performance (e.g. copulation 
success proportion) were standardized among males who courted the same female, and 
the standardized values for each male were averaged. Then, the correlation between this 
standardized mating performance and male body weight or age is tested. Thus, one trait value 
of a male was used only once in each of the tests.

To test whether females avoid their kin, the data of females courted by both their kin 
and non-kin were analyzed as follows. Males that courted a given female were ranked in 
the descending order of mount success proportion (defined as ranking of mount success of 
males) or copulation success proportion (defined as ranking of copulation success of males). 
Males that made no attempts at mounting, and thereby no attempts at copulation were ranked 
above those males whose attempts were all rejected, but below those males that achieved any 
osuccessful mount or copulation. By treating females as strata, the differences in ranks between 
kin and non-kin were examined using stratified Wilcoxon rank sum test with StatXact-Turbo 
(Mehta & Patel, 1992). We also tested the difference in the number of accepted mounts and 
the number of accepted copulations between kin and non-kin.
To examine whether acceptance of females varies with the degrees of relatedness, relatedness was calculated for each male and a female pair using Kinship 1.2. The correlations between the relatedness and ranking of mount success of males or ranking of copulation success of males were analyzed with Kendall's tau with a blocking variable (Korn, 1984). Only cats that had been subjected to DNA analyses were used in these analyses.

We examined whether females had a tendency to accept those males that persisted in mounting in the following way. In each pair in which mounting attempts of a male to a female and acceptance/rejection of the female to the male were observed, all mounting attempts were consecutively numbered. For example, in the case that a male attempted to mount a female five times in a bout and were rejected, accepted, rejected, rejected and accepted, two accepted mounts were numbered as 2, 5 and three rejected mounts were 1, 3, 4. These consecutive numbers indicate the order of acceptance and rejection in a bout of a male's mount attempts. If a female had no tendency to accept a male that persisted in mounting, it is expected that there is not difference between consecutive numbers of attempted mount and consecutive numbers of rejected numbers. We tested this null hypothesis using stratified Wilcoxon rank sum test. Because some males attempted to mount a female, each male was treated as stratum. By performing this test independently for eight females, we obtained eight probabilities that the observations occur under the null hypothesis. Then, we combined these probabilities following Sokal & Rohlf (1995, p. 794-797) to examine a general tendency. A tendency in copulation attempts was examined in the same way.

Results

Traits and mating behaviour of females

We observed eight females over their whole oestrous periods, and 39 males courted these females. We confirmed that all of them were pregnant after their oestrus. The average body weight of females was 3.2 ± 0.2 kg (mean ± SE, N = 7), while that of males was 4.5 ± 0.1 kg (N = 31). We were unable to measure the body weight of one female and eight males. The average oestrous duration of these females was 4.0 ± 0.5 days (N = 8), and their average age was 4.8 ± 1.1 years (N = 6). The number of males that courted a female varied from nine to 19, with an average of 12.5 ± 1.1 (N = 8). The average age of males was 4.6 ± 0.8 years (N = 25). We observed a total of 106 episodes of copulation, with each female copulating 13.3 ± 2.5 times on average (N = 8), ranging from six to 27.

Oestrous duration was correlated neither with female age nor with female body weight (Kendall's rank correlation coefficient: age; τ = 0.00, p > 0.99, N = 6, body weight; τ = 0.45, p = 0.16, N = 7). In addition, oestrous duration was not correlated with the total number of courting males (Kendall’s rank correlation coefficient: τ = 0.39, p = 0.18, N = 8).
The number of courting males per female was correlated neither with female age nor with female body weight (age; \( \tau = -0.14, p = 0.70, N = 6 \), body weight; \( \tau = 0.14, p = 0.65, N = 7 \)). There was no significant correlation between female age and female body weight (\( \tau = -0.6, p = 0.14, N = 5 \)). There were no indications that a female accepted persistent males for both mounting (combining probabilities from independent test of significant after stratified Wilcoxon rank sum test: \( p > 0.05 \)) and copulation (combining probabilities from independent test of significant after stratified Wilcoxon rank sum test: \( p > 0.05 \)).

Mount success proportion was significantly different among individual females (nominal logistic regression: \( p = 0.004, \chi^2 = 20.63, df = 7 \)). Per-female proportion of mounts was positively correlated with oestrous duration but negatively with female body weight, to a significant degree (Table 1). The average per-female proportion of mounts was 0.76 ± 0.04.

Copulation success proportion also varied significantly among females (nominal logistic regression: \( p < 0.0001, \chi^2 = 38.7, df = 7 \)). Per-female proportion of copulation was positively correlated with female age, but negatively with both female body weight, and the mean number of courted

**Table 1. Correlations between per-female proportion of mounts and female traits (unstratified multiple logistic regression)**

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>( \beta^* )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oestrous period</td>
<td>1.09</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Age</td>
<td>0.01</td>
<td>0.89</td>
</tr>
<tr>
<td>Body weight</td>
<td>-2.93</td>
<td>0.03</td>
</tr>
<tr>
<td>Mean number of courting males per day</td>
<td>-0.21</td>
<td>0.36</td>
</tr>
</tbody>
</table>

* partial regression coefficient.

**Table 2. Correlations between per-female proportion of copulation and female traits (unstratified multiple logistic regression)**

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>( \beta^* )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.28</td>
<td>0.02</td>
</tr>
<tr>
<td>Body weight</td>
<td>-2.33</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean number of courting males per day</td>
<td>-0.67</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>

* partial regression coefficient.
males per day (Table 2). The average per-female proportion of copulation was 0.44 ± 0.10.

Per-female proportion of copulation was significantly lower than per-female proportion of mounts (Wilcoxon's signed rank test: $z = -2.24$, $p = 0.03$, $N = 8$). There was no significant correlation between per-female proportion of copulation and per-female proportion of mounts (Kendall's rank correlation coefficient: $\tau = 0.21$, $p = 0.46$, $N = 8$).

Performance of kin or non-kin males

Among eight females, seven females were courted by both kin and non-kin males, while the eighth was courted by only non-kin males. Figures 2 and 3 show the ranking of mount success and copulation success of those males. Females rejected significantly higher proportions of mounting attempts

![Diagram showing ranking of mount success between non-kin males and kin males. Open circles, maternal kin males; closed circles, paternal kin males; shaded diamonds, males thought to be kin; crosses, non-kin males. Males were ranked in descending order of success. Figures indicate individual males which are kin of the seven females observed. Maternal half sibling, 1, 4, 5, 12 (litter), 13 (litter); paternal half sibling, 2, 3 for PG; mother/son, 9, 10, 11. Familial males, 1, 3 for TAM and for PG, 4, 5, 9, 10, 11, 12, 13; unfamiliar males, 2, 6, 7, 8. Male 3 used the same feeding site with TAM and PG by coincidence and it seems to be familiar.]

Fig. 2. Difference in ranking of mount success between non-kin males and kin males. Open circles, maternal kin males; closed circles, paternal kin males; shaded diamonds, males thought to be kin; crosses, non-kin males. Males were ranked in descending order of success. Figures indicate individual males which are kin of the seven females observed. Maternal half sibling, 1, 4, 5, 12 (litter), 13 (litter); paternal half sibling, 2, 3 for PG; mother/son, 9, 10, 11. Familial males, 1, 3 for TAM and for PG, 4, 5, 9, 10, 11, 12, 13; unfamiliar males, 2, 6, 7, 8. Male 3 used the same feeding site with TAM and PG by coincidence and it seems to be familiar.
Fig. 3. Difference in ranking of copulation success between non-kin males and kin males. Open circles, maternal kin males; closed circles, paternal kin males; shaded diamonds, males thought to be kin; crosses, non-kin males. Males were ranked in descending order of success. Figures indicating individual males correspond to those in Fig. 2.

(stratified Wilcoxon rank sum test: $p = 0.01$) and copulation attempts (stratified Wilcoxon rank sum test: $p = 0.0005$) by kin males than by non-kin males. Females also significantly mounted and copulated with non-kin males more frequently than kin males (stratified Wilcoxon rank sum test: the number of accepted mounts; $p = 0.04$, the number of accepted copulations; $p = 0.003$). That is, females mostly avoided mounting and copulating with their own kin.

Relatedness was neither correlated with ranking of mount success (Kendall’s tau with a blocking variable: $T = 0.04$, $p = 0.67$) nor with ranking of copulation success of males (Kendall’s tau with a blocking variable: $T = 0.06$, $p = 0.49$).

**Effects of male traits and on mating performance of males**

To establish whether male traits affect their mating performance, correlations between male body weight or age, and mount or copulation success proportion were analyzed (Table 3). There were no significant correlations between
**TABLE 3. The relationships between male traits and male behaviours**

<table>
<thead>
<tr>
<th></th>
<th>( \tau )</th>
<th>( p )</th>
<th>( N )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized average of copulation success proportion</td>
<td>0.10</td>
<td>0.46</td>
<td>26</td>
</tr>
<tr>
<td>Standardized average of mount success proportion</td>
<td>0.08</td>
<td>0.58</td>
<td>27</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Standardized average of copulation success proportion</td>
<td>-0.17</td>
<td>0.26</td>
<td>23</td>
</tr>
<tr>
<td>Standardized average of mount success proportion</td>
<td>-0.10</td>
<td>0.50</td>
<td>23</td>
</tr>
</tbody>
</table>

* Kendall’s rank correlation coefficient.

the standardized average of copulation success proportion or the standardized average of mount success proportion and body weight of males or age of males.

**Discussion**

While it has been widely accepted that males compete for females, the idea that females control the paternity of their offspring is rather controversial (Birkhead & Möller, 1993). In many mammals, male-male competition is overwhelmingly prevalent and female control in mating behaviour is either unimportant or very hard to detect (e.g. Clutton-Brock et al., 1982). However, increasing attention has been paid to the female role in shaping animal mating systems, and this study clearly demonstrated that female cats have some control over which males they allow to mount and which males they copulate with. During courtship, females frequently rejected the male’s attempts to mount and copulate by pawing, hissing, or rushing, and females responded differently to courting males. Receptivity of females depended on female condition and whether or not the given male was kin. Male-male competition and different physiological and/or environmental conditions may well affect female behaviour, but the preference by females for non-kin males over kin males can not be readily explained by these factors.

In mammals, male-biased dispersal and female philopatry have been considered to be the major mechanisms through which inbreeding is avoided (e.g. Hoogland, 1982; reviewed in Clutton-Brock, 1989). Several laboratory studies have documented how females selectively avoid mating with genetic
relatives, even if they were unfamiliar to them (mice: Barnard & Fitzsimons, 1988; white-footed mice (*Peromyscus leucopus*): Keane, 1990; field crickets (*Gryllus bimaculatus*): Simmons, 1991). Nevertheless, it still remains uncertain whether inbreeding avoidance based on kin recognition by genetic similarity occurs in the field. To demonstrate inbreeding avoidance by genetic similarity in the field, (1) it must be possible to identify a mate, and (2) kin relationships between females and males must be known. The population of cats studied here meets these conditions and in fact females mostly did avoid copulation with kin males. In this study, however, courtship by paternal kin males was observed only in two cases (shown by solid circles in Figs 2 & 3). Further, one of these (male 3 for PG) was observed to share a feeding site with the female (PG) and they possibly grew together or close during development and became familiar. Among the four males that had 1/4 or higher degrees of relatedness (shown by diamonds in Figs 2 & 3) three (males 6, 7, and 8) were unfamiliar males, because their maternity was different and they did not use the same feeding site. Their rankings were also low both at mounting and at copulations (see Figs 2 & 3). Further surveys are necessary to determine whether female cats avoid mating with kin males in the field based on familiarity and/or genetic similarity.

How important is inbreeding avoidance in the wild? Inbreeding often brings about an increased frequency of homozygosity and causes a reduction in survival and reproduction. Inbred offspring of zoo species have greater juvenile mortality than non-inbred ones, with the exception of reindeer (*Rangifer tarandus*), dik-dik (*Madoqua sp.*), and cheetah, *Acinonyx jubatus* (O’Brien et al., 1985). The extreme genetic monomorphism at the major histocompatibility complex loci in the cheetah may have resulted from inbreeding, and it could lead to hypersensitivity of cheetahs to viral pathogens (O’Brien et al., 1985), although the validity of this view has been questioned recently (e.g. Caughley, 1994). In lions, *Panthera leo*, it is suggested that an increased level of inbreeding has had an adverse effect on reproductive performance (Packer & Pusey, 1993). Males in the Ngorongoro Crater who had been genetically isolated for five generations have a higher proportion of abnormal sperm than the lions in Serengeti, while the more highly inbred Asiatic lions of the Sakkarbaug Zoo have even higher levels of sperm abnormality (Packer & Pusey, 1993). Thus it is advantageous for animals to avoid copulating with their relatives. However, animals do not always avoid inbreeding, for it is better to breed incestuously than to risk not breeding at
all in situations where only relatives are available as possible mates (Emlen, 1995). Thus, whether animals avoid inbreeding or not is likely to depend upon ecological conditions.

Do female cats in the population studied need to have the ability to discriminate between kin and non-kin males? The population is composed of several individual feeding groups (Izawa, 1984; Yamane et al., 1994). Males tend to disperse from their natal place after becoming mature (Izawa, 1984), although sometimes they remain within their natal group. Males expand their home territory during the breeding season (Yamane et al., 1994) and some of them court females that belong to other groups (Yamane et al., 1996). Thus, there is a possibility for male cats to court related females with whom they are not familiar. A kin recognition system would therefore seem to be an effective means through which cats could avoid inbreeding.

How do cats recognize kin? Familiarity seems to be the simplest mechanism. Communal nurses are observed between a mother and her daughters or between maternal sisters within feral cat populations (Izawa & Ono, 1986; Macdonald et al., 1987). Then, how do cats recognize unfamiliar kin? According to Grafen (1990), the kin recognition system depends on 3 kinds of loci, namely, matching, detection, and using, and matching loci affects the traits used to detect kin. Brown & Eklund (1994) proposed major histocompatibility complex (MHC) genes as matching loci that are potentially important. Potts et al. (1994) suggested that MHC dissimilar mating functions as an inbreeding avoidance mechanism, based on evidence that was obtained from house mice in an enclosure. It is possible that also cats recognize kin on the basis of MHC genes.

Female cats did avoid copulating with their close kin that had 1/4 or higher degrees of relatedness, but their acceptance did not vary with the degree of relatedness in this study. That is, females consistently avoided close kin, but their responses to other males were variable. Prairie dogs (Cynomys ludovicianus) also avoid extreme inbreeding with close relatives but not with distant relatives, though it was discussed that the main reason was the spacial distribution (Hoogland, 1992).

In our study, females rejected males at two stages, when males attempted to mount and when males attempted to copulate. It would be advantageous for females to reduce assessment errors when choosing mates. For example, the high ranking of mount success for one of the kin who courted 'NIN' may have been an assessment error (Fig. 2). Its ranking lowered at the copulation
stage (Fig. 3). Assessing at two stages may work to avoid copulation with kin males more effectively.

Females were more rejective of males' attempts at copulation than of their attempts at mounting. We observed some coercive mounts but no coercive copulation. Furthermore, our observations showed that females whose receptivity was high at mounting did not necessarily show high receptivity at copulation. This would suggest that different factors affect the receptivity of females at mounting and at copulation, and that various female traits influence the receptivity of females. The females whose oestrous durations were shorter accepted mounting less frequently. Since there were many male cats, it is most unlikely that females would fail to find their mates within this study area. Thus females whose oestrous durations were shorter could be choosy. Younger and heavier females may be good mates for males because of their high activity and good health for breeding. The low receptivity of heavier and younger females may suggest the possibility of females controlling mounting and copulation. It is also plausible that females are more choosy when they are in good condition. However, we can not exclude the possibility that this correlation is a consequence of indirect sexual coercion of lighter or older females.

Evidence supporting female control of paternity does not exclude the fact that males are active participants in this process (Birkhead & Möller, 1993). Aggressive encounters of male cats are frequently observed during the breeding season. Heavier males successfully compete with lighter males, occupy better courtship positions, and copulate with females more frequently (Liberg, 1983; Yamane et al., 1996). Further, Liberg (1983) reported that dominant males monopolized copulations, though such monopolization was observed neither by us nor by Yamane et al. (1996) and Natoli & De Vito (1991). In this study, neither male body weight nor male age was correlated with mounting or copulation success. Natoli & De Vito (1991) had found no such dominance hierarchy, either. These results suggest that females do not always copulate with those males who rank highly in male-male competition.

References


