ARTICLE

Effects of Multiple Mating on Female Reproductive Output in the Cat Flea (Siphonaptera: Pulicidae)

M. H. HSU AND W. J. WU

Department of Entomology, National Taiwan University, Taipei, 1, Roosevelt Road, Section 4, Taipei, Taiwan 106, Republic of China

J. Med. Entomol. 37(6): 828–834 (2000)

ABSTRACT Multiple mating behavior of female cat fleas, *Ctenocephalides felis* (Bouché), was confirmed in this study, and its effects on fecundity and fertility were investigated as well. The number of fertile eggs produced by mated females was close to nil within 7 d after removal of males, but it was resumed when females were exposed to males again on day 7. Multiple-mated females displayed significantly higher fecundity (400.3 eggs per female) and fertility (182.8 viable eggs per female) than single-mated females (61.7 and 19.0, respectively) in the 24-d period, suggesting that multiple mating by females is an advantageous strategy for cat fleas. The duration of first mating averaged 63.1 min. The high ratio (55.56%) and short duration (34.0 min) of impotent mating suggested that cryptic female choice may be involved during copulation.

KEY WORDS *Ctenocephalides felis*, cat flea, fecundity, fertility, multiple mating, reproduction

MULTIPLE MATING BY female insects is widespread even in species where one copulation results in a sufficient sperm supply for the female (Reinhart and Köhler 1999). The topics of single versus multiple mating have been extensively studied because of its implications in understanding not only population dynamics but also its importance to control strategies (Romoser and Stoffolano 1998). Females that mate more than once may greatly reduce the efficiency of sterile male release techniques, given that multiple matings may provide excess sperm (Metcalf and Luckmann 1994). Despite the apparent medical and economic importance of the cat flea, *Ctenocephalides felis* (Bouché), the mating behavior of this pest has seldom been investigated in detail (Akin 1984). Other fleas such as *Ceratophyllus gallinae* (Schrank) and *Nosopsyllus fasciatus* (Bosc.) mate without a blood meal (Humphries 1967; Iqbal and Humphries 1970, 1974); nevertheless, Dean and Meola (1997) reported that unfed male cat fleas are unable to inseminate females. Furthermore, our unpublished data show that temperature is a critical factor in the mating behavior of the cat flea. Therefore, the copulation of this permanent ectoparasite may always occur on warm-blooded animals such as cats and dogs. Searching for mates in adult emergence areas should be adaptive if females mate just once or are receptive shortly after emergence. Instead, like other insects for which females mate more than once, the foraging and oviposition site of the cat flea is the most probable male and female encounter site on the host body (Thornhill and Alcock 1983). In addition, Shyu et al. (1993) reported that male cat fleas emerged 2.7 d later than females. This phenomenon is referred to as protogyny and suggests that last male sperm precedence may be exhibited in this species. When sperm precedence occurs, males should emerge to secure at least as many preoviposition copulations as any other males, rather than timing emergence in relation to the availability of fresh virgins. Because there is often a lag between female emergence and egg laying, males may begin to appear well after the peak of female emergence has passed (Thornhill and Alcock 1983). Despite the lack of direct evidence to date, the female cat flea was believed to mate multiply (Dryden 1993). Although repeated matings were recorded in females of *Nosopsyllus fasiatus* (Bosc) and *Echidnophaga gallinacea* (Westwood), the effect of mating frequency on reproductive output (fecundity and fertility) in Siphonaptera has not been thoroughly examined (Suter 1964, Iqbal and Humphries 1976). In some polyandrous species, females may gain nutrients, oviposition stimulants, and fresh viable sperm during each additional copulation, which eventually enhances their reproductive success, or increases genetic diversity and viability of the offspring (Cordero 1995, Reinhart and Köhler 1999, Wilson et al. 1999). Zakson-Aiken et al. (1996) found that virgin female cat fleas were able to lay nonviable eggs after blood feeding; however, they began ovipositing viable eggs and quadrupled their output within 24 h after male fleas were added.

The protocol for this study was conducted according to the "Guide for the Care and Use of Laboratory Animals" promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of the Laboratory Animals Resources, National Research Council, 1996. Protocol for the use of cats in this research was performed according to Animal Protection Law set forth by Council of Agriculture, Taipei, Taiwan, and approved by the Laboratory Animal Care Committee, Department of Entomology, National Taiwan University. The protocol is on file.

0022-2585/00/0828–0834$02.00/0

According to Ridley (1988), there are two main types of evidence that multiple mating occurs in females: (1) adequacy of sperm supply and (2) comparisons of the reproductive output between single and multiple-mated females. The study attempts to determine if multiple mating occurs in cat fleas and, if so, is it advantageous.

Materials and Methods

Cats. Cats used in these studies were mixed sex and housed individually in stainless steel wire cages (61 by 70 by 60 cm). Caged cats were maintained in a room where temperature and relative humidity fluctuated between 25 ± 4°C and 60–90% RH. For easy cleaning, newspaper, rather than commercial cat sand, was placed in the plastic pan (23 by 15 by 12 cm) as litter for cats. To maintain sanitation of the cage the litter was cleaned more than once daily. This routine was used to determine cat health by observing the condition of their feces and urine. Cats were given food and water daily.

Flea Rearing. The Taipei flea colony used in this study originated from five stray cats collected on streets in Taipei 1990 (Shyu et al. 1993). According to the 1-yr survey conducted by Shyu et al. (1993), all of the fleas collected on cats and dogs in Taipei are cat fleas. Eggs were collected daily from the pan beneath each cat's cage and placed in white sand that contained powdered porcine blood curd as the larval rearing medium. More than 75% of larvae become adult fleas when maintained at 27 ± 1°C and 75 ± 3% RH. The blood curd was purchased from a market in Kungkuan in Taipei City and it was dried in a 50°C oven for 48 h before it was ground. Porcine blood curd is a traditional Chinese food. According to Wang (1992), blood curd is made from porcine blood to which 0.9% sodium chloride, 0.5% sodium citrate, and 50% water is added during the early stages of the production process. The blood is cured with ascorbic acid and nitrite at 4°C for 24 h, heated to 90°C for 10 min, and cooled in ice water for 30 min.

Microcells. A microcell was constructed from a plastic cylinder (3 cm diameter by 1 cm tall) with a disc of 35-mesh/cm nylon screen that was glued to the bottom to effectively avoid egg loss. The shape of microcell was similar to, but smaller than, the flea chamber in the artificial membrane system purchased from FleaData (Freeville, NY) that was described by Pullen and Meola (1995). Our microcell contained no cat hairs, because hairs hindered the precision needed to count the eggs as well as inhibited the smooth operation required in the transfer of fleas. In addition, for humidity balance and air exchange, the microcell was sealed with a plastic cap that had the same nylon screen as the bottom of the cylinder. The application of microcells on cats followed the chambered flea technique described by Thomas et al. (1996). However, to prevent over-straining the cat's neck or skin trauma by rubbing, the bandaging tapes on the additional collar were tightened rather than extending them forward around the animal's neck. Two cats were used alternatively for application of microcells, and six to eight microcells could be placed under the bandaging tape on each cat simultaneously.

Test Fleas. Our unpubished data indicated that unfed males make no attempt to copulate with females. Therefore, individually maintaining flea larvae or pupae to obtain virgin adults was deemed unnecessary. Approximately 25–30 newly emerged (1-d-old) virgin fleas, previously separated by sex, were loaded into each microcell, which was then placed on a cat for a 5-d blood-feeding period. Thus, the fed virgin fleas were 6 d of age. They were then prepared for sperm depletion and female multiple mating experiments.

Sperm Depletion Experiments. Five pairs of virgin male and female fed fleas were transferred into each microcell. In group A (once-paired females) and group B (twice-paired females), only the males were removed from the microcell after 24 h. After the daily collection of flea eggs, the microcell was returned to the original cat. These experiments were conducted for a 24-d period. However, the same numbers of fed virgin males, as the remaining females, were added to each microcell in group B on day 7. After 24 h, the newly introduced males were removed. In group C (multiple-paired female), the introduced males stayed with females from the beginning to the end of experiments. There were six replications of each group.

Female Multiple Mating Experiments. The fed virgin fleas were paired at random and each pair was loaded into a microcell. The microcell was placed on a digital hot plate at 38.5°C (body temperature of cats). Thus, some of these fleas mated, and their mating duration was determined. The mated male in group 1, once-mated female ($n = 20$), and group 2, twice-mated female ($n = 15$), were discarded immediately after copulation had ceased. The female was confined individually in the microcell, which was returned to the cat where it was examine daily for egg production during the 24-d study period. However, on day 7 after the first mating, each female in group 2 mated again with a fed virgin male. In group 3, multiple-mated female ($n = 10$), the male in the first mating was not removed from the microcell, and each pair of the confined fleas was placed back on the cat for further investigation of egg laying.

Fecundity and Fertility. The eggs laid in each microcell were counted every day to determine the daily fecundity of the female flea and were put into a petri dish with blood feces of their parent female at 27 ± 1°C and 75 ± 3% RH. Four days later, the number of larvae in each petri dish was recorded for daily fertility. If the mated female did not deposit any viable eggs in 10 d after mating, it was defined as impotent, and the data of impotent mating was excluded from calculations of the reproductive output of groups 1 and 2.

Results

Sperm Depletion Experiments. *Fecundity.* Egg production for the 24-d period indicated that the females in group C, consistently exposed to males from day 0

Table 1. Effects of pairing frequency on fecundity (number of eggs laid), fertility (number of eggs hatched), duration of egg laying, and duration of viable egg laying per female cat flea for 24 d

Group	*n*	Fecundity	Fertility	Duration egg lying, d	Duration viable egg laying, d
A	6	114.2 ± 3.6^a	41.4 ± 3.1^a	10.3 ± 0.7^a	6.5 ± 0.4^a
B	6	189.5 ± 8.5^b	60.9 ± 6.5^b	18.6 ± 0.5^b	11.9 ± 0.4^b
C	6	335.9 ± 40.3^c	100.0 ± 8.5^c	22.6 ± 0.6^c	22.4 ± 0.7^c

Mean ± SE, means with different letter are significantly different ($P < 0.05$) using the Fisher least significant difference (LSD) multiple range tests. Group A, paired with males 24 h once; group B, paired with males 24 h twice; group C, paired with males for 24 d. *n*, number of replicates, each contains 5 females and 5 males from the beginning.

to 24, laid significantly more eggs than the females in group B that were exposed to males only on days 0 and 7. The females of group A, which were exposed to males only on day 0, laid the least number of eggs. In comparison with group A, the total fecundity of the females in group C increased nearly threefold, yet those of group B had only a 1.7-fold increase. The females in group A laid eggs for ≈10 d then stopped egg deposition after day 12. Because of the addition of males on day 7 in group B, the daily fecundity of females increased to a second peak on day 9, and oviposition was also extended (Table 1; Fig. 1a). The number of eggs deposited daily by females in group C peaked on day 5, and remained high as females aged (Fig. 1a).

Fertility. The females in group C laid ≈100 viable eggs over the 24-d period, and several females that stayed with long-lived males continued to produce viable eggs until the last few days of the experiment. The females of group A stopped laying viable eggs within 7 d after removal of the males. However, in group B, female fertility was restored when males were reintroduced on day 7. Sperm depletion occurred when the males were removed from the microcells of group B, and the deposition of viable eggs ended on day 15 (Table 1; Fig. 1b). Thus, multiple mating by the females of this species was confirmed.

Female Multiple Mating Experiments. *Ratio of Impotent Mating.* For the first mating of virgin fleas, 25/45 of matings were impotent. That is, 55.6% of once-mated females produced no viable eggs in the following 10 d. In contrast, only 6/21 (28.6%) of second matings were impotent when the previously mated females had produced viable eggs before day 7.

Duration of Copulation. Females of 20 pairs deposited viable eggs after the first mating and the duration of these matings, ranging from 2 to 157 min, were significantly longer than those that resulted in no viable eggs (Fig. 2). The relationship between mating duration and the number of eggs produced per female in group 1 was not significant ($r = -0.27$, df = 18, $P = 0.25$). No significant relationship was found between mating duration and the number of viable eggs deposited per female ($r = -0.16$, df = 18, $P = 0.50$).

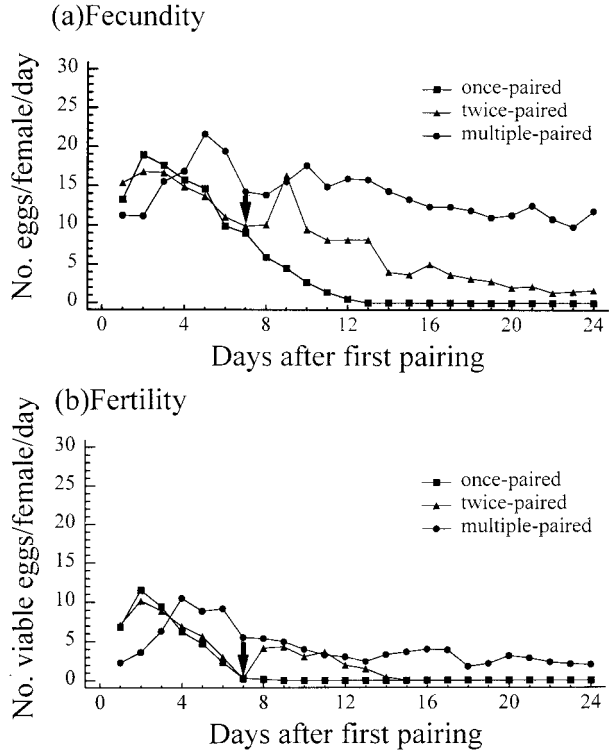

Fig. 1. Changes in (a) daily fecundity and (b) daily fertility for females paired singly ($n = 30$), doubly ($n = 30$), or multiply ($n = 30$). Filled points represent averages. Arrow indicates time at which males were added into the microcells of doubly paired females for 24 h.

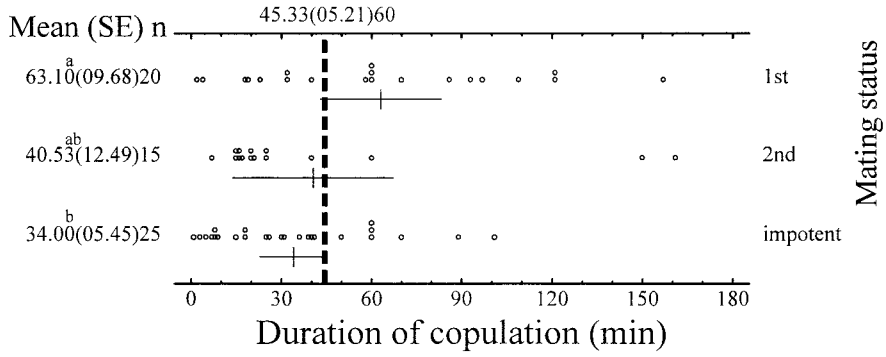

Fig. 2. Variations of the duration of copulation in different mating status including first mating of group 1, second mating of group 2, and impotent mating of group 1 female. The vertical line is drawn at the grand mean of the total observations. The plot displays the grand mean, standard error, and number of observations at the top of the vertical line. The plot also shows the location for each group mean, as well as the 95% confidence intervals around the means. The values of each group mean followed by the different letter are significantly different ($P < 0.05$) using the Fisher least significant difference (LSD) multiple range tests. SE, standard error; n, sample size.

Fecundity. Table 2 summarizes the effects of multiple mating on female reproductive performance. Repeated matings significantly increased the number of eggs laid and the number of offspring hatched (fertility) during the 24-d period. The multiple-mated females in group 3 laid significantly more eggs than the twice-mated females in group 2 and the once-mated females in group 1 in the 24-d period. Compared with group 1, the total number of eggs produced per female by group 2 increased 2.7-fold during the 24-d period, whereas those of group 3 had a 6.5-fold increase. The duration of egg laying per female in group 1 was only ≈1 wk, however the oviposition duration of group 2 lasted for 20 d, which was nearly equal to that of group 3 (Table 2). Seventy percent (14/20) of females in group 1 ceased oviposition within 1 wk. However, only 6.7% (1/15) of females in group 2 stopped laying eggs before day 14. Although the males died on day 17.7, most (7/10) of females in group 3 continued egg production until the experiment ended on day 24.

During the first week, the daily number of eggs deposited per female in both group 1 ($r = -0.97$, df = 5, $P = 0.0004$, $y = 12.39 - 1.31$ x) and group 2 ($r = -0.95$, df = 5, $P = 0.001$, $y = 13.97 - 1.37$ x) declined significantly with increasing female age. In contrast, the number of eggs deposited per female in group 3 ($r = 0.78$, df = 5, $P = 0.038$, $y = 14.07 + 1.99$ x) increased significantly with increasing female age. Without the second mating, the females of group 1 nearly stopped laying eggs by day 9. However, if remated on day 7, the daily deposition of eggs by group 2 peaked by day 10, and these isolated females continued to lay small numbers of eggs until the last few days of the experiment (Fig. 3a).

Table 2. Effects of mating frequency on fecundity (number of eggs laid), fertility (number of eggs hatched), duration of egg laying, and duration of viable egg laying per female cat flea for 24 d

Group	*n*	Fecundity	Fertility	Duration egg laying, d	Duration viable egg laying, d
1	20	61.7 ± 11.9^a	19.0 ± 4.3^a	7.5 ± 1.3^a	3.7 ± 0.6^a
2	15	166.8 ± 32.7^b	44.1 ± 6.4^b	19.9 ± 1.0^b	12.4 ± 0.6^b
3	10	400.3 ± 51.3^c	182.8 ± 20.1^c	22.3 ± 1.2^c	16.6 ± 1.2^c

Mean ± SE, means with different letter are significantly different ($P < 0.05$) using the Fisher least significant difference (LSD) multiple range tests. Group 1, once-mated female; group 2, twice-mated female; group 3, multiple-mated female. *n*, sample size, each contained a couple of cat fleas from the beginning.

Fertility. A trend similar to egg numbers was found in fertility. The females in group 3 laid significantly more viable eggs than those in group 2 and the females in group 1 deposited the smallest number of viable eggs in the 24-d period (Table 2). The number of viable eggs produced per female (fertility) in group 2 had only a 2.3-fold increase, as compared with the number produced by group 1, whereas group 3 fertility had a 9.6-fold increase. Ninety percent (18/20) of the females in group 1 stopped laying viable eggs within 1 wk, whereas 100% (15/15) of the females in group 2 stopped before day 14. The duration of viable egg deposition by the females in group 1 was 3.7 ± 0.6 d (mean ± SE), whereas that in group 2 after the second mating on day 7 was not affected by the first mating. Therefore, the estimate of the duration of viable egg production after the second mating was 4.7 ± 0.4 d, and the differences in the duration between the two groups was significant (*F*-test; $F = 3.03$; df = 1, 33; $P = 0.04$). Except for one female in group 3, which suspended egg production on day 12, the duration of viable egg laying was related significantly to the longevity of their mates ($r = 0.74$, df = 7, $P = 0.022$). Seven of nine females ceased producing viable eggs on the day of their mate's death.

The daily number of viable eggs deposited per female (fertility) in both group 1 ($r = -0.996$, df = 5, $P < 0.001$, $y = 5.64 - 0.74$ x) and group 2 ($r = -0.95$, df = 5, $P = 0.001$, $y = 7.90 - 1.07$ x) declined significantly with the increasing age of the females during the first week. The number of viable eggs increased significantly with increasing female age, except on day 6 in group 3 ($r = 0.80$, df = 4, $P = 0.05$, $y = 7.44 + 1.88$ x). Unlike group 1, which terminated the production

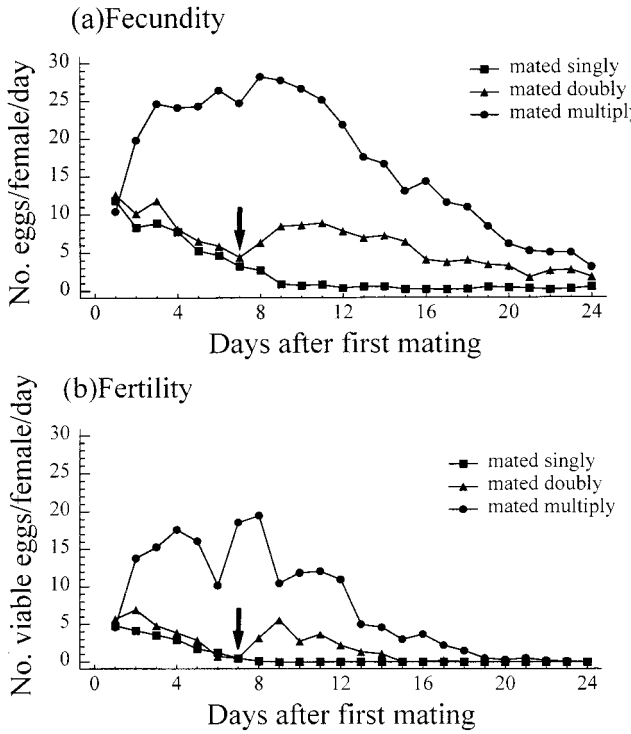

Fig. 3. Changes in (a) daily fecundity and (b) daily fertility for females mated singly ($n = 20$), doubly ($n = 15$), or multiply ($n = 10$). Filled points represent averages. Arrow indicates time at which doubly mated females were remated.

of viable eggs before day 8, the daily fertility of group 2 reached the second peak on day 9 because of female–male remating on day 7. However, the fertility of group 2 was 0 on day 15, the same day that the group B females ceased production of viable eggs in the sperm depletion experiment (Figs. 3b and 1b).

Discussion

Oviposition. Before the start of the current study, we observed that after blood feeding the virgin female cat fleas began laying a number of nonviable eggs. This had been reported by Zakson-Aiken et al. (1996), wherein they pointed out that the nonviable eggs were normal in appearance. However, we found that most of the nonviable eggs looked flat and transparent, not oval and milky white as do fertile eggs. When the proportions of flat eggs exceeded 50%, low or even zero fertility of flea eggs occurred. We therefore conclude that oviposition in the early stage of the cat flea was controlled by blood feeding, with increased mating frequency promoting the number, quality, and viability of eggs. Thus, the number of eggs decreased when there were fewer males. Potentially, the ejaculate substances of male cat fleas may provide oviposition stimulants, as reported in beetles (Wilson et al. 1999). Alternatively, the female may reabsorb ova when sperm supply is insufficient. Ridley (1988) postulated that long-lived species might be more likely to hold back unfertilized eggs, because they could be metabolized and reused later, although he suggested that females were not perfectly efficient in holding back eggs because their sperm supply ran out. We did not determine why even the female cat fleas in group C that were consistently exposed to more than one male and the females in group 3 that were consistently exposed with the same male laid only 29.8 and 45.7% viable eggs, respectively (see Tables 1 and 2). Zakson-Aiken et al. (1996) also reported a low hatchability (14.3%) of eggs in the 7 d after introducing the male fleas into the flea cages. Previous reports showed that the flea eggs collected from the pan beneath the cat cage had 87–100% hatchability at 27°C and 70% RH (Silverman et al. 1981, Shyu et al. 1993). Because of their flat shape, the newly deposited nonviable eggs may have little chance of falling into the pan beneath the cat cage. Thus, it is possible that these eggs stick onto the cat fur and are subsequently licked-up by the cat. This could explain why hatchability of flea eggs was so high in previous reports. Thus, the large number of nonviable eggs deposited by the multiple-mated female cat fleas suggests that nonviable eggs may have some functions. According to Hsu (2000), the highest emergence (81.82%) of adult cat fleas was displayed in the group in which the larvae were fed with a mixture of blood feces and some nonviable eggs, which was higher than the group fed with blood feces alone (46.67%). However, feeding the flea larvae with only the nonviable eggs led to the lowest adult emergence (13.51%). She also revealed that feeding the larvae

with nonviable eggs significantly shortens the duration of larval development of female fleas.

Suter (1964) indicated that the mating of female sticktight fleas, *Echidnophaga gallinacea* (Westwood), was multiple because of sperm replenishment and fecundity restoration in females after their second copulation. In cat fleas, fecundity enhanced by mating frequency may be limited by the females' ability to produce ova. Despite the 9.6-fold increase in the mean number of fertile eggs produced by the multiple-mated females when compared with the once-mated females, the total number of eggs produced by each multiple-mated female only had a 6.5-fold increase. The viable eggs produced by isolated female cat fleas decreased to zero by 7 d and the once-mated female only laid ≈20 eggs on average. This suggests that either the number of sperm transferred was small or that the longevity of sperm was short within the spermatheca. The female Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), and bean bug *Reptortus clavatus* (Thunberg) both underwent multiple matings and 3,212 sperm were in the spermathecae of a once-mated female fly; however, at each mating of bean bug, ≈550 sperm were transferred (Sakurai 1996, Yuval et al. 1996). In addition, the longevity of sperm of the male melon fly, *Bactrocera cucurbitae* (Coquillett), was only 12.6 d (Tsubaki and Yamagishi 1991).

Mating. Humphries (1967) indicated that the sequence of mating behavior begins when the male *Ceratophyllus gallinae* (Schrank) accidentally collides with a female and his maxillary palps receive a contact-chemical stimulus. This sexually stimulating substance is present on the abdominal cuticle of both males and females. To determine if a pheromone might be involved in the mating behavior of cat fleas, Akin (1984) used the glass probe, which rubbed against the body of a female flea, to touch the male fleas. However, none of the cat fleas mated. Our unpublished observations showed that male fleas would inseminate several females within 24 h after a blood meal. Marshall (1981a, 1981b) pointed out that the sex ratio is unequal in most of the ectoparasites in wild populations, females usually predominating. According to Shyu et al. (1993), the sex ratio of the newly emerged cat fleas is nearly 1:1. However, because of the shorter life span of male fleas, the sex ratio of the flea population on the cat or dog host was ≈1:3–1:5 (M:F) (Amin 1966, Osbrink and Rust 1985, Shyu et al. 1993). In Taipei, there are two flea seasons annually. Shyu et al. (1993) indicated that 1 mo before each flea season, the proportion of male fleas on cats or dogs peaked. Thus, the occurrence of multiple matings by females during that time may be higher than in the other months. One month later, most of the offspring, which were produced at the time of highest proportion of males, would become adult fleas.

Potentially, the female fleas mated with more than one male not only for enhanced fertility but also for increased genetic diversity of offspring. The production of blood feces by males is significantly less than that produced by females (unpublished data), indicating that males make smaller parental investments than females. The number of viable eggs produced by the females (fertility) that paired with males for 24 h was significantly higher than the fertility in the once-mated female group. This suggests that female cat fleas mated more than once within 24 h. When several fleas were confined in a microcell, we found that some males frequently disturbed mating pairs. Once the mating was interrupted, the female may choose another mate or remate with the same male. Moreover, harassment of courting males could decrease the reproductive success of the female. Our results showed that the females in group C confined with several males in the 24-d period and laid significantly less viable eggs than females that had multiple matings with one male. The duration of impotent mating was significantly shorter than potent mating, indicating that during copulation a sort of cryptic female control might exist. Despite the few observations of impotent matings reported in other insect species, when measurements were made the proportions of impotent matings were high (Ridley 1988). Eberhard (1985) suggested that this happened when the female blocked sperm transfer, after entering into copula because of the female finding fault with the male's genitalia. He also stated that in many animal groups that have internal fertilization copulation does not always result in insemination, nor does insemination always result in fertilization of eggs (Eberhard 1991). Humphries (1967) reported that the mating of *Ceratophyllus gallinae* (Schrank) lasted from a few seconds to nearly 9 h with an average of 2.8 h. This period was longer than the duration of copulation in northern rat fleas, *Nosopsyllus fasciatus* (Bosc.), recorded by Iqbal and Humphries (1974), and it was also longer than that of cat fleas in this experiment. The ratio of impotent mating was not given in their results for either species. However, 25% of the *C. gallinae* finished copulation within 30 min. The cryptic female choice may result in extremely short mating times. Thornhill (1983) pointed out that the female scorpionfly *Harpobittacus nigriceps* (Selys) controlled mating duration, and the number of sperm transferred during mating depended on the duration of copulation.

Conclusion. The sperm depletion experiment revealed the existence of multiple mating by female cat fleas. The female mating experiment indicated that those multiple-mated females had significantly higher fecundity and fertility than singly mated females, suggesting that multiple mating is an advantageous strategy that increases reproductive success. According to Thornhill and Alcock (1983), high risks of predation and the extra energy devoted to additional copulation are two possible disadvantages of remating by other insects. The mating pattern and receptivity of the female cat flea may be the next step for further understanding of its multiple mating behavior. The longevity of sperm within the spermatheca and the amount of sperm in each ejaculate may be essential in understanding a female's receptivity.

Acknowledgments

We express our gratitude to Ya-Chun Hsu and Yueh-Lin Yang for their patient and expert assistance to tame the cats. We really appreciate the suggestions and comments provided by Wei-June Chen, Shwu-Bin Horng, How-Jing Lee, and Shiuh-Feng Shiao, which greatly improved an earlier version of the manuscript.

References Cited

Akin, D. E. 1984. Relationship between feeding and reproduction in the cat flea, *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae). M.S. thesis, University of Florida, Gainesville.

Amin, O. M. 1966. The fleas of Egypt: distribution and seasonal dynamics of fleas infesting dogs in the Nile Valley and Delta. J. Med. Entomol. 3: 293–298.

Cordero, C. 1995. Ejaculate substances that affect female insect reproductive physiology and behavior: honest or arbitrary traits? J. Theor. Biol 174: 453–461.

Dean, S. R., and R. W. Meola. 1997. Effect of juvenile hormone and juvenile hormone mimics on sperm transfer from the testes of the male cat flea (Siphonaptera: Pulicidae). J. Med. Entomol. 34: 485–488.

Dryden, M. W. 1993. Biology of fleas of dogs and cats. Comp. Cont. Ed. Pract. Vet. 15: 569–579.

Eberhard, W. G. 1985. Sexual selection and the evolution of animal genitalia. Harvard University Press, Cambridge, MA.

Eberhard, W. G. 1991. Copulatory courtship and cryptic female choice in insects. Biol. Rev. 66: 1–31.

Hsu, Y. C. 2000. Effects of diet factors on larval development and survival of cat fleas, *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae). M.S. thesis, National Taiwan University, Taipei (in Chinese, English [abstr.]).

Humphries, D. A. 1967. The mating behaviour of the hen flea *Ceratophyllus gallinae* (Schrank) (Siphonaptera: Pulicidae). Anim. Behav. 15: 82–90.

Iqbal, Q. J., and D. A. Humphries. 1970. Temperature as a critical factor in the mating behaviour of the rat flea, *Nosopsyllus faciatus* (Bosc). Pak. J. Zool. 6: 163–174.

Iqbal, Q. J., and D. A. Humphries. 1974. The mating behaviour of the rat flea *Nosopsyllus faciatus* Bosc. Pak. J. Zool. 6: 163–174.

Iqbal, Q. J., and D. A. Humphries. 1976. Remating in the rat flea *Nosopsyllus faciatus* (Bosc.). Pak. J. Zool. 8: 39–41.

Marshall, A. G. 1981a. The ecology of ectoparasitic insects. Academic, London.

Marshall, A. G. 1981b. The sex ratio in ectoparasitic insects. Ecol. Entomol. 6: 155–174.

Metcalf, R. L., and W. H. Luckmann. 1994. Introduction to insect pest management, 3rd ed. Wiley, New York.

Osbrink, W.L.A., and M. K. Rust. 1985. Seasonal abundance of the adult cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae), on domestic cats in southern California. Bull. Soc. Vector Ecol. 10: 30–35.

Pullen, S. R., and R. W. Meola. 1995. Survival and reproduction of the cat flea (Siphonaptera: Pulicidae) fed human blood on an artificial membrane system. J. Med. Entomol. 32: 467–470.

Reinhart, K., and G. Köhler. 1999. Costs and benefits of mating in the grasshopper *Chorthippus parallelus* (Caelifera: Acrididae). J. Insect Behav. 12: 283–293.

Ridley, M. 1988. Mating frequency and fecundity in insects. Biol. Rev. 63: 509–549.

Romoser, W. S., and J. G. Stoffolano. 1998. The science of entomology, 4th ed. McGraw-Hill, Boston, MA.

Sakurai, T. 1996. Multiple mating and its effect on female reproductive output in the bean bug *Reptortus clavatus* (Heteroptera: Alydidae). Ann. Entomol. Soc. Am. 89: 481–485.

Shyu, M. H., T. C. Hsu, and W. J. Wu. 1993. Seasonal abundance of cat flea, *Ctenocephalides felis* (Bouché) (Siphonaptera: Pulicidae), in Taipei city. Chin. J. Entomol. 13: 59–67 (in Chinese, English [abstr.]).

Silverman, J., M. K. Rust, and D. A. Reierson. 1981. Influence of temperature and humidity on survival and development of the cat flea, *Ctenocephalides felis* (Siphonaptera: Pulicidae). J. Med. Entomol. 18: 78–83.

Suter, P. R. 1964. Biologie von *Echidnophaga gallinacea* (Westw.) und Vergleich mit andern Verhaltenstypen bei Flöhen. Acta Trop. 21: 193–238.

Thomas, R. E., L. Wallenfaels, and I. Popiel. 1996. On-host viability and fecundity of *Ctenocephalides felis* (Siphonaptera: Pulicidae), using a novel chambered flea technique. J. Med. Entomol. 33: 250–256.

Thornhill, R. 1983. Cryptic female choice and its implications in the scorpionfly *Harpobittacus nigriceps.* Am. Nat. 122: 765–788.

Thornhill, R., and J. Alcock. 1983. The evolution of insect mating systems. Harvard University Press, Cambridge, MA.

Tsubaki, Y., and M. Yamagishi. 1991. "Longevity" of sperm within the female of melon fly, *Dacus cucurbitae* (Diptera: Tephritidae), and its relevance to sperm competition. J. Insect Behav. 4: 243–250.

Wang, F. S. 1992. Studies of physicochemical properties of heat-induced porcine blood curd. M.S. thesis, National Taiwan University, Taipei. (in Chinese, English [abstr.]).

Wilson, N., T. J. Tufton, and P. E. Eady. 1999. The effect of single, double, and triple matings on the lifetime fecundity of *Callosobruchus analis* and *Callosobruchus maculatus* (Coleoptera: Bruchidae). J. Insect Behav. 12: 295–306.

Yuval, B., S. Blay, and R. Kaspi. 1996. Sperm transfer and storage in the Mediterranean fruit fly (Diptera: Tephritidae). Ann. Entomol. Soc. Am. 89: 486–492.

Zakson-Aiken, M., L. M. Gregory, and W. L. Shoop. 1996. Reproductive strategies of the cat flea (Siphonaptera: Pulicidae): parthenogenesis and autogeny? J. Med. Entomol. 33: 395–397.

Received for publication 17 December 1999; accepted 27 June 2000.