The adaptive significance of multiple mating in female mink (*Mustela vison*) and its effects on the mating system.

by

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Abstract

The adaptive significance of multiple mating in female mink (*Mustela vison*) and its effects on the mating system.

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Chairperson of the Supervisory Committee: Professor David P. Barash
Department of Psychology

Female multiple mating is common in mammals, but studies of its advantages are often constrained by our limited understanding of the reproductive physiology underlying it. The reproductive physiology underlying female multiple mating in the American mink (*Mustela vison*) is well-known, but this is the first study to focus on the functions of female multiple mating and its possible effects on the mating system.

Multiple mating by female mink has different effects depending on the interval between matings. Matings at eight day intervals (8DI) during delayed implantation induce additional ovulations while matings at one day intervals (1DI) around ovulation promote sperm competition. To determine female mating preferences and males' response, I gave fur farm females opportunities to remate at 1DI and/or 8DI with the same male (familiar) and/or unfamiliar males.

Females mated multiply in the absence of male coercion. Females remated after eight days regardless of male familiarity, possibly because females that experience only matings early breeding season are more often barren. For familiar pairs at the 1DI, males showed less interest in

remating than females did, which led to females mating different males on consecutive days and to their fecundity not being reduced when one male was infertile. Allozyme electrophoresis confirmed the paternity advantage of males mating around later ovulations and showed a first male advantage in consecutive day matings.

Copulation duration increases over the course of the breeding season beyond what is necessary to induce ovulation and achieve fertilization. Copulation duration increased for unfamiliar pairs and pairs that had last mated more than a week before, but not for pairs that had mated the previous day—further illustrating the decreased interest of familiar males at the 1DI and suggesting that prolonging copulation is a male strategy in sperm competition.

Wild-caught *Mustela vison evagor* mated in May rather than March, but otherwise showed multiple mating behavior similar to fur farm mink. Female mink may multiply mate during delayed implantation to insure maximum fecundity and to increase offspring quality by increasing opportunities for male-male competition and mate choice.

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"We need another and a wiser and perhaps a more mystical concept of animals. Remote from universal nature, and living by complicated artifice, man in civilization surveys the creature through the glass of his knowledge and sees thereby a feather magnified and the whole image in distortion. We patronize them for their incompleteness, for their tragic fate of having taken form so far below ourselves. And therein we err, and greatly err. For the animal shall not be measured by man. In a world older and more complete than ours they move finished and complete, fitted with extensions of the senses we have lost or never attained, living by voices we shall never hear. They are not brethren, they are not underlings; they are other nations, caught with ourselves in the net of life and time, fellow prisoners of the splendour and travail of the earth."

-- Henry Beston, 1928, 'The Outermost House'

CHAPTER 1:

Multiple mating preferences in female mink: matings that induce additional ovulations versus contribute additional sperm.

INTRODUCTION

Although female multiple mating is common in animals (Smith 1984, Birkhead & Moller 1992, Eberhard 1996), there is considerable controversy about the extent to which mating with more than one male per reproductive cycle may be adaptive for females (Halliday & Arnold 1987, Arnold & Halliday 1988, Sherman & Westneat 1988, Keller & Reeve 1995). Mating multiple females is considered adaptive for males because of the higher number of offspring they can potentially sire. Even when mating females that have already mated, males may increase their reproductive success through post-copulatory competition for fertilizations, such as sperm competition (Parker 1970, 1984). On the other hand, females that mate multiple males usually do not have more offspring than females with a single mate, leaving the benefits, if any, to females of these 'extra' matings more difficult to detect and quantify.

Whether multiple mating is a female strategy or something they are compelled to do by males is not always easy to determine by simply observing female behavior (Parker 1974, Birkhead & Moller 1992). In birds, a number of studies have shown females seeking out and soliciting copulations from multiple males (Birkhead et al. 1985, Alatalo et al. 1987, Moller 1990, Burley et al. 1994), clearly implying that their behavior is adaptive. In other cases, there is good evidence that females resist and are

forced to copulate with additional males (McKinney et al. 1983, 1984; Clutton-Brock & Parker 1995), implying that these additional matings are not adaptive for them. In most species, however, female behavior is harder to interpret; females may appear to passively accept additional mates or to put up limited 'resistance' (or 'surrender selectively', Eberhard 1996), perhaps as a test of male quality rather than an absolute indicator of female unwillingness to mate (Cox & LeBoeuf 1977, Westneat et al. 1990).

Such species' differences in female multiple mating behavior have less to do with differences in the potential advantages or disadvantages of multiple mating than with differences in their mating systems. That female birds engage in overt mate-seeking behaviors as part of their multiple mating strategy is not surprising because these females often search for and approach their initial mates as well. In contrast, many mammals and insects have mating systems that involve males searching for females. In these cases, females may not need overt mate-seeking behaviors to acquire multiple mates any more than they needed them to acquire initial mates.

In these behaviorally ambiguous cases, looking at female reproductive physiology may provide clues as to the nature of female multiple mating (Gomendio & Roldan 1993). If multiple mating were disadvantageous to females, then natural selection should favor the evolution of mechanisms that not only decrease female receptivity after mating but quickly decrease their fertilizability as well, to prevent males from getting any benefit from mating an already mated female. On the other hand, if multiple mating were advantageous to females, then natural

selection should favor mechanisms that increase its likelihood of occurring, such as prolonging physiological receptivity and its advertisement after mating. In this latter case, prolonging the fertile period as well would imply that females derive genetic benefits (via heterogeneous offspring, sperm competition, or cryptic female choice) from multiple mating. If the fertile period is not prolonged concomitant with receptivity, then females may be deriving primarily non-genetic benefits from multiple mating, e.g., protection from infanticide (Packer & Pusey 1983, Jeppson 1986).

The American mink (*Mustela vison*) is a mammal whose physiology provides females extensive opportunities to mate multiply and secure additional fertilizations. First, mink are induced ovulators that remain receptive to additional matings during the 28-72 hour interval between a mating that induces ovulation and ovulation itself (Sunqvist et al. 1988). This interval between mating and ovulation is long compared to that in other induced ovulators (e.g., vole, 9-12 hours; rabbit, 9-10 hours; ferret, 24-35 hours; cat, 26-58 hours, Ramirez & Beyer 1988) and could allow additional males time to locate a mated female and compete for fertilizations. Indeed, males mating the same female on consecutive days are usually both represented in her litter and no simple mating order advantage has been established (Johansson & Venge 1951; Jorgensen 1985; Fleming chapter 3).

Second, a female mink can remate and ovulate again at approximately weekly intervals throughout the 3-5 week breeding season and produce a litter that contains kits sired by males that mated weeks apart ('split litters'; Shackelford 1952). Like many other mustelids, mink have

delayed implantation (Wright 1963, Mead & Wright 1983, Mead 1989)—an extended period of time in which the embryos (blastocysts) are quiescent and free-floating in the uterus. Unlike most other mammals, new follicles continue to develop in the ovaries after ovulation, and are mature 6 days later (Hansson 1947, Shackelford 1952). At this point, mated females become sexually receptive again and mating will induce ovulation as before (Hansson 1947). This cycle continues approximately every 8-10 days until changing photoperiod around the vernal equinox stimulates implantation (Allais & Martinet 1978) and a single litter of kits is born about 30 days later. Thus, split litters result from different males mating a female a week or more apart and fertilizing two different sets of ova that implant at approximately the same time (Shackelford 1952). However, matings during subsequent receptive periods typically result in the loss of unimplanted embryos from previous weeks' matings (Adams 1981), so that 86-90% of kits born are conceived at the female's last ovulation (Johansson & Venge 1951, Shackelford 1952). Thus, females can encourage multiple paternity and sperm competition both by mating repeatedly prior to or shortly after a single ovulation, and by replacing embryos from previous mates with those of new mates at weekly intervals.

In the wild, mink are solitary animals and typical carnivores in having relatively large home ranges for their body size (Gittleman & Harvey 1982). Most mink populations appear to conform to the pattern of intrasexual territoriality common in mustelids (Powell 1979), in which larger male ranges may encompass a number of smaller female ranges (Mitchell 1961, Gerell 1970, Whitman 1981). Males and females are sexually mature at 10

months of age (Hansson 1948, Dunstone 1993). During the breeding season, both juvenile and adult males are reported to travel widely in search of receptive females (Gerell 1970, Mitchell 1961). Males have been reported to remain with females for up to 2 days after mating (Ireland 1990 in Dunstone 1993), but there are no reports of longer male-female associations, of males providing resources to females, or of paternal care in mink.

Several hypotheses that have been proposed to explain multiple mating in female mammals (Schwagmeyer 1984, Moller & Birkhead 1989) do not apply to mink because they are solitary and lack paternal care. Given female minks' unusual reproductive physiology and their mating system, the three hypotheses that I considered to explain the function of female multiple mating in mink involve female strategies to 1) reduce male harassment, 2) assure fertilization, and 3) manipulate paternity.

Female mink may multiply mate simply because it would be too costly in terms of time, energy, and risk of injury to avoid ardent males (Alcock et al. 1977, Daly 1978, Parker 1979, McKinney et al. 1983). Male mink are 2-3 times larger than females so 'harassment reduction' could certainly apply to a fur farm situation in which females are placed directly in a cage with a male. It could also apply in the wild, where Hatler (1976) observed female condition deteriorating during the breeding season and speculated that their foraging was restricted by their remaining in their dens to avoid male harassment.

Mating with several males during a single fertile period could guard against male infertility (Dewsbury & Sawrey 1984, Huck et al. 1986, Huck et

al. 1989), and could simultaneously increase the genetic diversity within a litter (Williams 1975, Walker 1980), or simply promote sperm competition and/or cryptic female choice in which the best male sires most of the kits (Cohen 1971, Birkhead et al 1993, Birkhead & Moller 1993, Keller & Reeve 1995, Eberhard 1996). The physiology of domestic mink, and presumably of wild mink (Linscombe et al 1982), also provides females with greater options for influencing the paternity of their litters than they would have if they only ovulated once. If a female cannot be sure to encounter a number of males over the course of the breeding season, she may opt to mate indiscriminately with the first male encountered to insure fertilization and then be more discriminating at any subsequent mating opportunities. This strategy, termed 'fertilization assurance/exertion of preference' by Schwagmeyer (1984), could increase the mate choice options considerably for mink. A female that encounters a preferred male more than a week after previous matings can remate and replace 86-90% of the blastocysts sired by earlier mates (Johansson & Venge 1951, Shackelford 1952).

To test these hypotheses, I presented female mink with the opportunity to remate at intervals that would either provide additional sperm (1 day interval or 1DI) or induce additional ovulations (8 day interval or 8DI). The males were either ones they had already mated (familiar males) or ones they had not encountered before (unfamiliar males). Females were placed in cages connected to a male's cage by tunnels that were wide enough for the females to pass easily from one cage to the other but that restricted the males, which are 2-3 times larger, to one side. Females were either presented with a single male—familiar or unfamiliar—and given the

opportunity to mate or not (sequential choice experiment) or presented with a choice of two males—one familiar, one unfamiliar—and the option of not mating (simultaneous choice). The former simulated a situation in nature in which females would be approached by one male at a time (i.e., low population densities, low competition) while the latter simulated a situation in which females were located by more than one male at a time (i.e., high population densities, high competition).

If females were simply multiply mating to avoid resistance costs, they should avoid 'extra' matings by not entering males' cages after their first mating. If females were multiply mating so as to expel blastocysts from previous matings, they should be more selective about mates that would induce later ovulations than about their initial mates and less likely to remate a previous mate than to mate an unfamiliar male. If females were multiply mating during a single fertile period to reduce the risk of mating an infertile male, to increase the heterogeneity of their litter, or to promote sperm competition and/or cryptic female choice, then they would be more likely to mate different males on consecutive days than the same male. These last two hypotheses are not mutually exclusive, but refer to the two different types of multiple mating in mink: matings that will induce additional ovulations versus those that will contribute additional sperm to that already present in the female tract.

METHODS

I conducted my research on a private fur farm in western Washington in March 1989 and 1990 between dawn and dusk (700-1800 hours). Mink

were maintained under standard fur farm conditions as approved by the Mink Farmers' Animal Welfare Board. Testing cages were built of polyvinyl-coated wire with 2.5 x 5 cm squares. Fifteen pairs of cages (77.5 cm x 50 cm x 35 cm each) were connected by two 25 cm tunnels spaced 35 cm apart on the long side of the cage. Each male was housed in one of these cages throughout the study. The tunnels were ~7 cm in diameter to restrict males to their home cage while the females had access to both. I shut males in their nest boxes before females were placed in the opposite cage. Females were allowed to explore both cages and return to their own before the male was released from his nest box. Thus, a female could remain in or return to her cage at any point to avoid mating without having to fight a persistent male.

I used adult ($\geq$2 years old) and juvenile (10 month old) mink of both sexes in the study. Adults are a selected subset of successful breeders from the previous year, meaning that the variance in adult characteristics should be less than in juveniles'. Because adult females come into breeding condition earlier than juveniles (Hansson 1947), I did not start my experiments until both age classes were likely to accept males, which I determined by the mating success of females in the rest of the fur farm population.

Sequential choice experiment

Twenty-five adult females and 35 juvenile females were paired with 14 adult old males and a juvenile male between March 4 and March 19. The juvenile male proved to be a competent mater on his first exposure to a

female. Trials began when males were released and ended either after copulation, if the pair began to fight, or after two hours if mating had not commenced. Males mated no more than twice per day at least 2 hours apart ($\bar{X} \pm$ SE: 248.29 $\pm$ 13.981 minutes, n=21). This is typical of fur farm practices and does not reduce male sexual interest or fertility (J. Adair, Oregon State University Experimental Fur Farm, personal communication).

I gave females the opportunity to mate a second time after either a ~1 day (16-24 hours) or 8-10 day interval (1DI or 8DI, respectively). Females were exposed to either the same male they had mated previously (familiar) or a different male (unfamiliar). In addition, I gave females that mated a second time after 8-10 days an opportunity to mate a third time the following day. These third matings were also categorized as 1DI matings and I again presented females with either the previous day's mate (familiar) or another male (unfamiliar). Matings fell into one of seven categories from the perspective of the female: 1) first matings (males unfamiliar by definition), second 1DI matings the following day with 2) familiar or 3) unfamiliar males, second 8DI matings the following week with 4) familiar or 5) unfamiliar males, and third 1DI matings with 6) familiar and 7) unfamiliar males (Table 1.1).

Simultaneous choice experiment

The following year, I tested 18 juvenile females with 10 juvenile males in a similar manner, except that I gave females a simultaneous choice between two males at each mating opportunity. I joined third cages to each testing unit by an additional pair of tunnels, so that tests began with

the female in the cage between two males' cages. As in the sequential choice experiment, I gave females opportunities to mate a second time after an 8 day interval and a third time on the following day (1 day interval). At each mating opportunity, I presented females with both their previous mate (familiar) and a male that they hadn't mated (unfamiliar).

Behavioral descriptions

I recorded each female's latency to enter the male's cage as the interval between the male's release from his nest box and the female's last entrance into the male's cage prior to mating. I recorded the female's latency to mate as the interval between her last entrance and penetration leading to a successful mating. To achieve penetration, the male mink must grasp the back of the female's neck with his jaws (grab) and grasp her torso just behind her forelegs with his forelegs (mount). From this position, the male engages in repeated series of rapid, shallow thrusts (for 3-6 seconds, ~2-3 thrusts per second) until he achieves penetration (intromission). Intromission is characterized by an extreme arch of the male's back while mounted, such that his pelvis is almost under the female's, and the cessation of rapid, shallow thrusting. During copulation, pairs alternate between standing and lying on their sides. During the latter, sperm is thought to be transferred during a series of deep thrusts (Enders 1952; J. Adair, personal communication). Four to 10 of these deep thrusts typically occur within one or two minutes at 8-20 minute intervals during the mating. The end of a mating (withdrawal) is recognized by the straightening

of the male's back, moving his pelvis away from the female's. Pairs typically separate within 10-15 seconds after this.

I recorded females as having mated if the interval between intromission and withdrawal was longer than 6 minutes and included at least one series of deep thrusts. Sperm are easily detectable in a sample of female vaginal fluids after even a 2 minute mating if a series of deep thrusts have occurred (M. Fleming, unpublished data).

Some females (see below) that did not mate but had entered a male's cage and were grabbed and mounted by him left as soon as they could break away or were released by the male. Other females fought with the male first. Because struggling, screaming, and hissing are common responses of females to males' initially grasping and mounting them, I defined actual fights as interactions in which a female pursued a male and bit at his face. Males would typically respond by trapping females against the side of the cage with their bodies (faces averted), and by vigorously biting at the female's torso if she persisted.

Analyses

I performed the analyses using Systat 5.2.1 for Macintosh (1992). Unless otherwise noted, means are presented with standard errors and tests were two-tailed. I compared proportions of females mating in various categories using chi-square tests when sample size permitted and Fisher's exact tests when sample sizes were smaller (Siegel 1956). I compared individual females' mating tendencies across mating numbers using McNemar's test of changes when sample sizes were sufficient and binomial

tests when sample sizes were smaller (Siegel 1956). For other behavioral measures, when data were normally distributed I used parametric tests, otherwise I used nonparametric tests.

RESULTS

Sequential choice experiment

Effects of mating number and mating interval on female mating preferences

Of the 60 females paired, 35 (58.3%) mated at their first opportunity, and another ten mated the second male they were exposed to (45 of 59 that were paired twice or 76.3%). Twelve females (20%) refused to mate within three opportunities, and another three did not mate within one (1) or two (2) opportunities and did not get subsequent opportunities to choose. Twelve of these females ultimately mated: one at her fifth opportunity, six with the first male they were placed directly into a cage with, and five after being placed directly in the cages of 2-4 males. One female was never paired more than once. The two remaining females did not mate even after being placed in the cages of 6-7 males.

Forty-two of the mated females were given the opportunity to mate a second time eight days later (8DI). These females were no more or less likely to remate the first male they encountered at the 8DI, when mating would induce an additional ovulation, than they had been to mate the first time (McNemar test of changes $X^2 = 0.286$, $p = 0.593$; Fig. 1.1). Sixteen females (38.1%) did not remate the first male they were exposed to but 14 of these were given up to three opportunities to mate so as to induce a

second ovulation. Eight of these 14 females remated at their second or third opportunity, for a total of 34 females (81%) remating.

Thirty-three females that mated at the 8DI were given the opportunity to mate a third time the following day (third mating after a 1DI). Eleven females that mated for the first time during the second week of the breeding season were also given the opportunity to mate again one day after their initial mating (second mating after a 1DI).. Because females' likelihood of remating did not differ significantly with mating number (Fisher's exact test $p = 0.738$; Fig. 1.1), I combined data from second and third 1DI opportunities for the remaining analyses. I also included three females that had intromissions considered too short to be fertile matings during the first week of breeding, but which I did not expose to males again until the following week. All three ultimately mated during the second week, but because the brief intromissions during the first week may or may not have induced ovulations in these females, I could not categorize them unambiguously as either singly-mated females or females that ovulated a second time. I did, however, expose them to males one day after their mating and thus, included them in the 1DI analyses.

More females refused to remate at their first opportunity at the 1DI than had the previous day (20 of 47 vs. 13 of 47), but the difference was not significant (McNemar's test of changes $X^2 = 2.33$, $p = 0.127$). Because these 1DI mating opportunities were offered toward the end of the breeding season, there was no time to offer all the non-maters second and third mating opportunities. Of the six females offered second opportunities, four mated and two did not.

Females that did not mate at a particular opportunity avoided doing so in various ways (Table 1.2). At their first mating opportunities, four females (16.0%) did not enter the males' cages, while 10 females (40%) entered once and 11 (44%) entered 3-21 times (median = 7). Of the females that entered, five (23.8%) left again before the male could grab or mount them, and 16 broke away from or were released by the male: 12 (57.1%) before intromission and 3 (14.3%) after, but before ejaculation. At multiple mating opportunities, females that did not mate were more likely to not enter a male's cage than they had been initially (McNemar test of changes; 8DI: X^2= 7.36, p = 0.007; 1DI: X^2= 11.27, p = 0.001). Females were also less likely to enter a male's cage at the 1DI than they had been the previous day (McNemar test of changes X^2= 5.40, p = 0.02).

Effects of familiarity on female mating preferences

At the 8DI, 20 females were paired with their mates from the previous week (familiar males) while 22 were paired with unfamiliar males. There was no effect of familiarity of the potential mate on females' likelihood to remate the first male encountered (X^2 = 0.115, p = 0.694; Fig. 1.2), or to remate within three opportunities (Fisher's exact test p = 1.0). Overall, 80% of females (16 of 20) remated the familiar male and 86.4% (19 of 22) remated an unfamiliar male.

At the 1DI, 22 females were paired with males they had mated the previous day (familiar males), while 22 females were paired with different (unfamiliar) males. Females were significantly more likely to remate the same male they had mated the previous day than to mate unfamiliar males

(X^2 = 9.464, p = 0.002; Fig. 1.2). Females that had mated the first male they were exposed to on the previous day were no more or less likely to remate a familiar male the next day (binomial test p = 0.454), but were significantly less likely to mate an unfamiliar male (McNemar's test of changes X^2 = 7.143, p = 0.008).

Of the females that chose not to remate after an 8DI, 68.75% did so by not entering the male's cage (Table 1.2) and this did not differ with the familiarity of the male (Fisher's exact test p = 1.0). Eighty-four percent of females that did not remate after a 1DI did not enter the male's cage, and this tended to occur more often with females exposed to unfamiliar males (93.3%) than familiar ones (50%; Fisher's exact test p = 0.097).

Effects of female age on their mating preferences

Juvenile and adult females did not differ significantly in their likelihood of mating the first time that they encountered a male (X^2 = 0.127, p = 0.722; Fig. 1.3). When paired a second time after an 8DI, adult females were less likely than juveniles to remate at their first opportunity (X^2 = 9.24, p = 0.002), particularly when paired with unfamiliar males (Fisher's exact test p = 0.007; Fig. 1.3). This difference did not persist after I took females' second and third opportunities into account (Fisher's exact test p = 0.30). Overall, 82.4% (14 of 17) adults and 95.5% of juveniles (21 of 22) remated so as to induce a second ovulation.

Juvenile and adult females showed similar tendencies in remating after a 1DI, whether they were paired with familiar (Fisher's exact test p = 1.0) or unfamiliar males (Fisher's exact test p=0.667). Both juveniles and

adults paired with unfamiliar males tended to avoid them, while those paired with familiar males tended to mate (Fisher's exact tests; juveniles: $p = 0.05$; adults: $p = 0.07$; Fig. 1.3).

Effects of age, mating interval, and familiarity on other behaviors

Females are rarely passive when males attempt to copulate with them. They vocalize (hisses, screams and 'tch' sounds), writhe their bodies, arch their backs, and crawl around with the male on top of them. Females that are not active in these ways are released and not pursued by males, suggesting that "struggling" may be correlated with sexual receptivity in females. However, males cannot achieve intromission until a female stops moving temporarily, straightens her back, and lifts her tail. If the male does not succeed, the female starts moving again.

I recorded how long it took females to enter a male's cage ('latency to enter') and how long it took for a male to achieve intromission ('latency to mate') once she did so. Female sexual 'willingness', then, was measured not simply by whether a female mated or not, but her latency to do so. If adults are more selective about 8DI matings than juveniles, and both age classes are more selective about matings that add sperm to that already present in the female tract as suggested by the previous results, then females' latency to enter and remain in a male's cage and their latency to mate may vary in a similar pattern. Alternatively, the predicted pattern of female interest in multiple mating (e.g., favoring unfamiliar males at the 8DI or 1DI) may be supported by these aspects of female behavior.

Twenty-one percent of females that ultimately mated a male initially entered his cage and left again either before the male could grab them, after breaking free, or after being released by the male. These females returned 1-16 times prior to mating with a male, taking from 0 -109 minutes to return to mate (median = 6 minutes). Females that mated did not enter males' cages more quickly than females that entered but did not mate (Mann-Whitney $U = 414$, n = 21,41, p = 0.794). Because the latency to enter and stay was not normally distributed, I used Wilcoxin signed-ranks test to determine whether females' behavior changed across multiple matings and Mann-Whitney U-tests to determine whether female behavior differed depending on their age and the familiarity of the male.

The females that took longer to enter male cages were most often adults, particularly for first and 1DI matings, but latencies varied widely and these differences were not significant (Fig. 1.4a). Adults' decreased likelihood of mating at their first opportunity at the 8DI relative to juveniles was not reflected in longer latencies for adult females to enter at the 8DI as well.

Females also did not differ in latencies to enter the cages of familiar or unfamiliar males at the 8DI (Mann-Whitney $U = 78$, n=16,12, p = 0.399; Fig. 1.4b) and took as long to enter at the 8DI as they had previously (Wilcoxin signed-ranks test $W = 91.5$, n = 22, p > 0.10). Consistent with their being more likely to remate familiar males at the 1DI, females tended to enter the cages of males they'd mated the previous day more quickly than those of unfamiliar males (Mann-Whitney $U = 76$, n=19,13, p = 0.062; Fig. 1.4b), but overall, latencies to enter at the 1DI did not differ from that at

the previous day's mating (Wilcoxin signed-ranks test $W = 171.5$, n = 29, $p > 0.10$).

Once a male had grabbed a female, he took 3-44 minutes to achieve intromission ('latency to mate'). Because latencies to mate were normally distributed, I used paired t-tests to look for changes across mating number and 2-way ANOVAs to determine the effects of familiarity and female age. Females took no longer to mate when mating would induce a second ovulation than they had when mating induced the first ovulation (paired t-test $t = 0.123$, df = 27, $p = 0.903$). Females' latency to mate when mating would induce an additional ovulation did not differ with female age ($F = 0.506$, $p = 0.483$; Fig. 1.5a) or familiarity of the male ($F = 0.227$, $p = 0.638$; Fig. 1.5b). When matings were only one day apart, females' latencies were significantly shorter for matings on the second day (paired t-test $t = 4.2$, df = 28, $p < 0.001$), but did not vary with familiarity ($F = 0.419$, $p = 0.523$; Fig. 1.5b). Although adult latencies tended to decrease when remating would add sperm to that already present in the female tract (paired t-test, $t = 1.655$, df =9, $p = 0.132$), juvenile females' latencies to mate decreased significantly (paired t-test $t = 3.992$, df = 18, $p = 0.001$) such that they tended to mate even more quickly than the adults' ($F = 2.728$, $p = 0.111$; Fig. 1.5a).

Effects of males on female mating preferences

Individual males' mating opportunities were distributed evenly across mating categories and female ages whenever possible (Table 1.3). Males averaged 9.73 ($\pm$ 2.12 SD) opportunities to mate at females' first opportunity at each mating number. Deviations in the numbers of times I

used individual males in multiple mating categories occurred primarily due to the need to use males of particular genotypes in certain mating categories for paternity analysis (details in Fleming, chapter 3), and because males that did not mate a given female were generally given another opportunity sooner than males that mated.

Six males had more than the typical 0-2 opportunities to mate with familiar and/or unfamiliar females at the 8DI and 1DI, and 10 males had more than 0-2 opportunities to mate with either juvenile or adult females in these multiple mating categories (Table 1.3). Over representation of these males in these categories cannot account for the observed differences in female preference, however. When I used only the males' first 2 opportunities in each category in the analyses, the results remained the same: juveniles were more likely to mate than adults at the 8DI ($X^2 = 6.655$, $p = 0.01$), but not the 1DI ($X^2 = 1.072$, $p = 0.301$); and females were just as likely to mate familiar and unfamiliar males at the 8DI ($X^2 = 0.056$, $p = 0.813$), but more likely to mate familiar males at the 1DI ($X^2 = 9.227$, $p = 0.002$).

On average, 60.88 (±18.26 SD) % of males' pairings resulted in matings, but males varied in their success rates (Table 1.3). All but the juvenile male (#14) had been successful maters the previous year, and the juvenile's success rate was comparable to the adult males'. Although one male mated every female he was presented with and another male mated as few as one in three, there was no correlation between males' success rates at females' first matings and subsequent matings (Spearman rank correlation, $r = 0.233$, $n = 15$, $p = 0.294$).

Could the observed variation in female preferences be accounted for by the distribution of mating opportunities of particular males — those more or less successful than average? To address this question, I looked at the first mating opportunities for 1) the nine males that mated both juvenile and adult females at the 8DI and 2) the 12 males that mated both familiar and unfamiliar females at the 1DI. At the 8DI, males tended to mate the first juvenile female they were exposed to more often than the first adult female (binomial test $p = 0.062$, 1-tailed; Table 1.4). At the 1DI, males were significantly more likely to mate the first familiar female they were exposed to than the first unfamiliar female (binomial test $p = 0.004$, 1-tailed; Table 1.4). Furthermore, the mean overall success rates of males paired with juvenile and adult females at the 8DI did not differ (independent t-test $t = 0.171$, $df = 40$, $p = 0.865$; Table 1.4), nor did the mean overall success rates of males paired with familiar and unfamiliar males at the 1DI (independent t-test $t = 0.17$, $df = 45$, $p = 0.866$; Table 1.4). This suggests that adult females at the 8DI and unfamiliar females at the 1DI were not less likely to mate because they were more often exposed to less sexually competent males.

Simultaneous choice experiment

First matings

Eight of 17 females mated the first competent males they were exposed to and two mated at their second opportunity. Because earlier mountings by incompetent males could have induced the first ovulations of five females(Enders 1952), these females were placed directly into the cages of competent males with no more than one opportunity to choose

among them to ensure that any ova already ovulated would be fertilized. Two females (11.8%) never mated even after 4 or 5 encounters or direct placements with males. Thus, eight of 15 females (53.3%) that ultimately proved capable of mating did so at their first opportunity with competent males.

Females' latency to enter and remain in a male's cage ranged from 0-55 minutes (median = 9.5). The three longest latencies (46,49,55 minutes) occurred when females visited the other male before mating. In two of these three cases, females first visited the male they eventually returned to mate.

Females' latency to mate after having entered a male's cage ranged from 7-51 minutes (median = 12). The five females that had been placed directly into a male's cage did not prove more reluctant to mate by this measure: their latencies ranged from 9-41 minutes (median = 13; Mann-Whitney $U = 19$, $n = 5,10$, $p = 0.462$).

Mating preferences at an opportunity to induce a second ovulation

Of the 10 females that chose to mate initially, 6 (60%) remated at their first opportunity at the 8DI, one remated at her second. More females mated familiar males (5) than unfamiliar males (2), but this difference was not significant (binomial test $p = 0.454$). Two of the remaining three females subsequently mated unfamiliar males when placed directly with them. Of the five females that were placed directly into the male's cage for their first mating, two mated an unfamiliar male at their first opportunity and two mated the familiar male. The fifth female refused three mating

opportunities. Including the four females that chose their second mate only, females' preferences for familiar versus unfamiliar males were still not significantly different (binomial test $p = 0.454$).

Latencies to enter a male's cage and stay did not vary consistently between females' first and second matings (Wilcoxin signed-rank test $W = 7$, $n = 7$, $p > 0.10$). Latencies between familiar pairs ranged from 0-38 (median = 10) and one female visited the unfamiliar male prior to returning to remate her previous mate. The two females that mated unfamiliar males had latencies of 0 and 42 and neither visited their previous mate.

Latencies to mate (having entered a male's cage) ranged from 6-26 (median = 8) and did not differ between first and second matings (Wilcoxin signed-rank test $W = 11$, $n = 7$, $p > 0.10$), nor between familiar and unfamiliar males at the 8DI (Mann-Whitney $U = 15$, $n = 7,4$, $p = 0.848$).

Matings that promote multiple paternity

Of the 11 females that chose to mate at the 8DI, nine (81.8%) remated at their first opportunity on the following day, one remated at her second, and the last was placed directly with a male after two opportunities. There was a strong tendency for females to mate unfamiliar males (8) rather than remate their previous day's mate (2; binomial test, $p = 0.055$, one-tailed).

Latencies to enter a male's cage and stay did not vary consistently between females' matings on consecutive days (Wilcoxin signed-rank test $W = 17.5$, $n = 9$, $p > 0.10$). Latencies between the familiar pairs were 0 and 36, while latencies between unfamiliar pairs ranged between 2-82 (median

= 19.5). The three of the longest latencies (21, 32, and 82) were again due to females visiting the other male—in these cases their previous mates—prior to entering the cage of the unfamiliar male.

Five females visited familiar males first and five visited unfamiliar males first, however, only two females stayed to remate their previous mate whereas all five females visiting unfamiliar males mated them. The three females that visited the familiar males but did not mate them spent 6, 32, and 52 minutes playing and fighting with these males before leaving and entering the unfamiliar males' cages where they copulated within 9-13 minutes. Overall, the latencies to mate at the 1DI tended to be shorter (median = 7.5, range 4-57) than those for the female's previous mating (Wilcoxin signed-rank test $W = 8$, $n = 10$, $p > 0.05$).

DISCUSSION

Are female mink coerced into multiple matings?

Female mink behavior was not consist with the harassment reduction hypothesis of multiple mating. Approximately 60% of females mated upon first exposure to a male at both 1DI and 8DI and at their initial mating opportunity. Furthermore, 60% of those given second and third exposures also remated. Seventy-six percent of females that avoided mating at multiple mating opportunities did so by not entering male cages, but females that entered male cages prior to first or subsequent matings were also able to avoid mating by fleeing or fighting. Females that fled or fought particular males often entered these males' cages repeatedly and were ultimately ignored by them, showing that males do not persist in mating

attempts indiscriminately. Some of these females may not have been physiologically receptive at the time, but others probably were, because they mated the following day (Table 1.2). This suggests that females are capable of rejecting the advances of particular males in favor of others and is consistent with the finding that physiological indicators of estrus (i.e., vaginal smears, estrogen levels) are better predictors of when females will absolutely not mate, than when they will (Travis et al. 1978).

Both female multiple mating and multiple ovulations ('superfetation', Shackelford 1952) are made physiologically possible by delayed implantation. In most mammals, new follicles could not mature and additional ovulations could not be induced because progesterone from the corpora lutea of the ovary would inhibit pituitary production of the hormones that stimulate both follicle development and ovulation—FSH and LH, respectively (Knobil & Neill 1988). During delayed implantation, the mink ovary does not produce sufficient progesterone (Canivenc & Bonnin-Laffargue 1967 in Sundqvist et al. 1988) so the pituitary-gonadal axis continues to function as though the female were not pregnant.

But even if the physiology underlying multiple mating in mink originated simply as a side effect of delayed implantation (present for other reasons), it would not necessarily be maintained if it were costly for females and provided no benefits. Copulation in mink may last as long as 2-3 hours (Hansson 1947, Enders 1952) and, while on the female's territory, males would be competitors for food. Thus, weeks of cyclical receptivity in female mink could be quite costly if numerous males were likely to locate her

during the breeding season. Under such circumstances, multiple mating would be unlikely to persist purely as a harassment reduction strategy.

Are female mink multiply mating at 8DI to improve offspring quality?

Because matings more than a week after a previous mating can induce another ovulation and expel most of the blastocysts already in the uterus, I hypothesized that females may use this as a way to exercise mate choice when they are likely to encounter only one (or few) male(s) at a time. I predicted that if this were the case, then females would be less likely to remate a previous mate than an unfamiliar male when remating will replace previous blastocysts with new ones. They should also be more selective about mates (i.e., less likely to mate) that would induce later ovulations than those that induced earlier ones.

In contrast to my predictions, females were, if anything, more likely to remate the same male than to mate an unfamiliar one at their first opportunity to mate at the 8DI, suggesting that 'trading-up' was not the primary goal of mating so as to induce an additional ovulation. In the simultaneous choice experiment, females (all juveniles) mated more familiar than unfamiliar males (sevem versus four), but the difference was not significant. In the sequential choice experiment, juveniles did not show this tendency but adults did (Fig. 1.3). This preference for familiar males did not persist when adult females were exposed to other unfamiliar males on subsequent days, however. Thus, females may not mate familiar males to

the exclusion of unfamiliar ones, but rather may be more selective about mates that would induce later ovulations, favoring familiar males.

Why should females remate previous mates so readily if doing so will simply replace offspring conceived the week before with others from the same male? One possibility is that there is an advantage to ovulating again regardless of the identity of the mate. Inducing later ovulations with any male may be important to females because females that mate and ovulate only once early in the breeding season often have fewer or smaller litters than females that mate and ovulate once later in the season (Adair et al. 1988, Elofson et al. 1989) and females that ovulate more than once (Park et al 1988, Elofson et al. 1989). This is not due to fewer ova being ovulated at early matings (Hansson 1947, Shackelford 1952, Elofson et al. 1989), but may be due to failure in sperm transport (Adams & Reitveld 1981) or failure of blastocysts to survive delay or implant (Elofson et al 1989).

Why mate early if such matings are more likely to be nonproductive? The answer may have to do with a conflict of interest between the sexes over timing and number of matings. Having cyclical receptive periods coupled with a later male advantage in paternity may benefit solitary females by prolonging their opportunities for sequential mate choice (trading up) and/or for male-male competition. Males, on the other hand, would benefit most from mating females only around their last ovulations. Determining the timing of a female's last ovulation may not be possible without mating her whenever she is receptive, however. Female mink remain receptive until mating induces ovulation and are then nonreceptive until a new set of ova mature 6 days after ovulation (Hansson 1947). If a

male encounters a receptive female and mates her, he would have six days to seek additional mates before further guarding or remating of his previous mate might be necessary. On the other hand, if he does not mate a receptive female when he first finds her, then he will not know when she mates and, more importantly, when she may become receptive again. Thus, males are likely to maximize their reproductive success if , early in the breeding season, they mate receptive females just long enough to induce ovulation, and, later in the breeding season, return to guard their investment. Females may encourage the regular attendance by previous mates to facilitate pre- or post-copulation comparisons and competition with other males that may locate her during subsequent receptive periods.

While studies of mink in the wild are necessary to test these ideas, certain aspects of mink reproductive biology provide some support. Females' first ovulations are induced with less stimulation than later ones (less than 5 minutes versus greater than 12; Venge 1956, Adams 1981), maximizing females' chances of conceiving at their first mating. This may be necessary because copulation durations are considerably shorter early in the breeding season than they are later (15-30 minutes versus 2 hours or more), which is appropriate for a male strategy of simply stimulating ovulation versus maximizing their likelihood of paternity (see Fleming, chapter 2). That neither males nor females prevail in this conflict is apparent from the incidence of barren females and males that do not succeed in mating females around their last ovulation.

Females could also be nonselective about whom they mate at the 8DI because intrasexual selection results in males mating later in the

breeding season being of better quality on average than males that mated earlier. Because the paternity advantage to males that mate around a female's last ovulation should promote greater male-male competition then compared to earlier in the breeding season, female mink may not have to be very selective about later mates in a direct sense to get a superior male. Observations of mink mating in the wild or of direct competition between males for access to females are rare. Hatler (1976) observed males near a mating pair that did not attempt to interfere with the mating. Furthermore, when the mating male was finished, he went to sleep on a nearby rock and did not interfere with other males pursuing his mate. Ireland (1990 in Dunstone 1991) observed a juvenile male in four brief fights with other males upon his leaving a female's den. Both researchers reported wounds on the face and neck of males during the breeding season, but these could result from fights with reluctant females as easily as from fights with other males.

In solitary mammals, male-male competition for females often involves scramble rather than contest competition in the sense that the males compete primarily to be the first male to find an estrus female (Schwagmeyer & Wootner 1986). The extent of females' adaptive behavior in this situation may be to mate the first male that finds them late in the breeding season, even if it is their previous mate returning. Because the onset of female receptivity prior to mating is less fixed in time than the interval between an ovulation and the next receptive period (Hansson 1947), the advantage to males of mating early in the season may be that it makes it easier for them to locate and remate those females again when

remating will induce a later and a last ovulation. From the perspective of the female, the ability of a previous mate to return may provide more information about his quality than she would have about an unfamiliar male that located her—perhaps accounting for females' slight preference in this study for familiar males. Similarly, adult females' one day hesitation to remate unfamiliar males could also be a female tactic to delay remating until previous mates can also find her.

Do females prefer mating with different males around a single ovulation?

I hypothesized that females would be more likely to mate different males when remating would contribute additional sperm in order to receive the potential benefits of heterogeneous litters, sperm competition, cryptic mate choice, or fertility assurance. The results of the simultaneous and sequential choice experiments did not reveal consistent preferences of females for familiar or unfamiliar males at the 1DI. In the sequential choice experiment, females were more likely to mate with familiar males at their first opportunity and took longer to enter the cages of the unfamiliar males that they did mate. The opposite was true when females had a simultaneous choice between an unfamiliar male and the previous day's mate. To further complicate matters, having entered the male's cage, females in both experiments began to copulate more quickly at the 1DI than when mating would induce an ovulation, regardless of familiarity.

Because most females did not get second or third opportunities to mate at the 1DI, I could not determine whether the females that did not mate

right away were being selective about who they mated with (as may have been the case at the 8DI) or whether they were no longer receptive to matings. But because four of the six females that got second opportunities mated, the latter is clearly not the only explanation.

The discrepancy between the results of the two experiments and between the two measures of female 'willingness' to mate may be accounted for in large part by the behavior of males. In the simultaneous choice experiment, females showed no preference for entering the cages of familiar or unfamiliar males at the 8DI and all mated the male whose cage they entered first. Similarly at the 1DI, females were as likely to enter the cages of familiar as unfamiliar males first (five versus five) and the females who entered the cages of unfamiliar males first all remained to mate with them. In contrast, of the five females that entered the cages of males they had mated the day before, three ultimately left and entered the cages of unfamiliar males whom they mated. In each of these cases, the familiar males had grabbed and mounted the females repeatedly but released them without achieving intromission. Subsequently, while the females remained in the cage, they were either ignored by the males or the pairs engaged in virtually soundless, exaggerated bounding around that looked like play. When these females subsequently entered the cages of unfamiliar males, they began to copulate within a few minutes. The familiar males who had been left mated other, unfamiliar females successfully; thus the males' apparent lack of interest was restricted to females they had already mated the previous day.

Males' relative lack of interest in females they'd just mated the previous day compared to unfamiliar females was also apparent in the sequential choice experiment. First, females that mated familiar males at the 1DI entered the males' cages more quickly than females that mated unfamiliar males. Ad libitum observations of male behavior prior to a female's entrance show that males could slow and perhaps prevent the entrance of females by blocking the tunnels in efforts to grab them before they could get through. It is possible that this was less likely to occur with males that had already mated a female on the previous day because they were less interested in remating than an unfamiliar male would be. Second, matings between familiar pairs at the 1DI were considerably shorter than matings between unfamiliar pairs and also shorter than they had been the previous day (details in Fleming, chapter 2). The two matings between familiar pairs at the 1DI in the simultaneous choice experiment were also relatively short. Sperm is transferred at intervals throughout copulation, thus prolonging copulation may be a male strategy in sperm competition (see Fleming, chapter 2). If so, then a male may increase his reproductive success by avoiding repeated matings with the same female during a single fertile period (as in the simultaneous choice experiment), or at least not mating with her as long the second time (as in the sequential choice experiment), thus saving time, energy and sperm for matings with females whom he had not yet mated during a given fertile period.

Because multiple paternity is common when two or more males mate with a female during her last fertile period (Johansson & Venge 1951, Fleming chapter 3), it would be to a male mink's advantage to prevent his

previous day's mate from mating with another male. The females' behavior suggests that it is to their advantage to mate as much as possible. When females were not removed from the test cages immediately after mating in the sequential choice experiment, some returned to the male's cage within the hour and began to copulate again. Similarly, when females had access to two males in the simultaneous choice experiment, they sometimes finished copulating with one male and were found, minutes later, visiting or copulating with the other. Regardless of whether females mate different males during a single fertile period because they prefer to or because such males are more persistent, it seems likely that females will mate other males if their previous mates do not copulate with them continuously while they are receptive or drive off other males that comes near them. Female mink may not have explicit mating preferences because having a high libido is sufficient to enable them to secure at least one or two high quality mates through the pre- or post-copulatory male-male competition it promotes.

An accurate interpretation of the role of female mating preferences in the mink's mating system requires information that can only be obtained from studies of these small, solitary, largely nocturnal animals in the wild. In particular, observations of male movement patterns are necessary to determine whether males return to the territories of previous mates at weekly intervals and whether they mate guard during their mate's fertile period. Paternity analyses of litters would also determine the extent to which multiple mating occurs whether males mate guard or not. Given female physiology and behavior, if densities are such that several males are likely to find females during their last fertile period, then sperm

competition could be a considerable problem for males. Further captive studies are also necessary to identify the role of sperm competition and cryptic female choice in determining the paternity of offspring when females mate multiply.

SUMMARY

Female mink will mate multiply during the 2-3 days between a mating that induces ovulation and ovulation itself, and also at 8-10 day intervals during 1-5 weeks of delayed implantation. The former matings add to sperm already present in the female tract, while the latter actually induce the ovulation of additional sets of ova and expel blastocysts from previous matings.

Females were not prompted to multiply mate simply to avoid male harassment. Given the opportunity to avoid mating, females were as like to multiply mate as they were to mate the first time. When remating would induce another ovulation and expel blastocyst from previous matings, females did not behave as though they were 'trading up'—that is, mating a later male they deemed better by some criterion than their previous mate(s) to improve the quality of their litters. Overall, females paired with previous mates and unfamiliar males were equally likely to mate, to mate as quickly, and were no less likely to mate than they had been initially. Females may not discriminate among males for these matings because they must ovulate late in the breeding season to insure that they are not barren. Short matings typical of the early breeding season may not be sufficient to stimulate sperm transport or the maintenance of embryos during delay.

Also, females may not have to be more selective later in the breeding season to get a higher quality mate than they encountered earlier. The regular interval between one ovulation and the maturation of a new set of follicles could enable males to predict when their mates will be fertile again, promoting competition between returning mates and new males that locate females by the time mating would induce their last ovulations.

The identity of females' mates during a single fertile period also does not have to be determined by females' exercising direct, precopulatory choice. I found that females retained a strong interest in mating multiply throughout their fertile period, but did not prefer to associate with unfamiliar males despite the potential benefits of promoting heterogeneous litters, sperm competition, cryptic female choice, or fertility assurance. Nonetheless, because males were less interested in remating females they had mated the previous day (as shown by shorter or no copulations), females were likely to mate with unfamiliar males when available. Presumably, in a similar situation in nature, males would try to prevent their mates from copulating with another male. Thus, females' prolonged receptivity and continued high libido after mating may function to increase male-male competition (pre- and postcopulatory) and insure that females have access to one or more high quality mates.

Table 1.1: Numbers of females exposed to males in seven mating number/interval/familiarity categories. Numbers in parentheses are females that were given the opportunity to remate after a 1DI, but could not be unambiguously categorized as having mated once or twice previously. Four juvenile females that did not mate under the conditions of the study are excluded here.

MATING:	**First** (other)		**Second**		**Third** (other)
INTERVAL:			**1DI**	**8DI**	**1DI**
Juveniles	28 (3)	Familiar:	3	10	11 (2)
		Unfamiliar:	2	13	9 (1)
Adult	25	Familiar:	2	10	6
		Unfamiliar:	4	9	7

Table 1.2: Behavior of females that did not mate at their first opportunity at a given mating interval/familiarity (F, U) combination, subdivided by whether females never entered the male's cage, entered only once, or entered repeatedly. Females that fought with males and those that mated within three opportunities are also noted.

	# of females	% of non-mating	Fought	Chose to mate later
First				
Never entered	4	16%		1 juvenile
Entered once		40%		
not grabbed	1			
not mounted	3		1 adult	1 adult
no intromission	4		1 adult	1 juv., 2 ad.
failed mating	2			1 juvenile
Entered >once		44%		
not grabbed	1			
not mounted	0			
no intromission	9		1 juv.	2 juv., 1 ad.
failed mating	1			1 adult
8DI				
Never entered	11 (5F, 6U)	68.8%		1 juv., 4 ad.
Entered once		18.8%		
not grabbed	1 (F)			1 adult
not mounted	1 (U)			
no intromission	1 (U)			1 adult
Entered >once		12.5%		
not grabbed	0			
not mounted	1 (F)		1 adult	
no intromission	1 (U)			1 juvenile
1DI				
Never entered	15 (2F, 13U)	83.3%		
Entered once		5.6%		
failed mating	1 (U)			
Entered >once		11.1%		
not mounted	1 (F)			
no intromission	1 (F)			

Table 1.3: Number of matings per pairings (m/p) for 15 males with juvenile (Juv) and adult (Ad.) females at various mating interval/familiarity combinations. Individual male success is shown as the % of pairings in which a male mated at females' first matings (First), multiple matings (Mult), and all mating types combined (Tot.). Success rates are shown for each mating type as m/p and %.

	First		8 Day Interval				1 Day Interval				% success (mating/pairing)		
			Familiar		Unfamiliar		Familiar		Unfamiliar				
	Juv	Ad.	Juv	Ad.	Juv	Ad.	Juv	Ad.	Juv	Ad.	First	Mult	Tot.
1	1/1	2/2		1/1			1/1	1/1	2/2		100	100	100
2	1/3	0/1	1/1			0/1	1/1		0/1		25	50	37.5
3	1/1	2/2		0/1			1/1		0/1	1/1	100	50	71.4
4	2/3	1/1	1/1	0/1	1/1		2/2		0/1		75	66.7	70
5	2/3	1/1	1/1		1/2	0/1	1/1		1/1		75	66.7	70
6	2/2	2/2		1/1				1/2	2/2		100	80	88.9
7	2/2	1/2	1/1						0/2	1/2	75	40	55.6
8	2/2	1/2	1/1	0/1				1/2		0/1	75	40	55.6
9	1/1	1/1			2/2	0/1	2/3			0/1	100	57.1	66.7
10	3/5	0/1	1/2		0/1	1/2	3/4				50	55.6	53.3
11	0/2	1/1	1/1		3/3		2/2		C/1	0/2	33.3	66.7	50
12	1/1	1/3		1/1		1/1		1/1	0/1		50	75	62.5
13	1/3	0/2	1/1		1/1	0/3		1/1		1/2	20	50	38.5
14	0/2	2/3	0/1	1/1	2/2			1/1			40	80	60
15	0/1	1/1		1/3	1/1		0/1			0/2	50	28.6	33.3
m/p	19/32	16/25	8/10	5/10	11/13	2/9	13/16	6/8	5/12	3/11	X = 64.6	X = 60.4	X = 60.9
%	59	64	80	50	84.6	22.2	82	75	41.7	27.3			

Table 1.4: Number of males mating at their first exposure to a) juvenile (J) and adult (A) females that last mated eight days before (8DI) and b) familiar (F) and unfamiliar (U) females that last mated the previous day (1DI). Mean (± SE) total success rates (%) of males paired in each category are also shown.

					Mean total success %	
Mate:	J, not A	A, not J	Both	Neither	J	A
a.) 8DI	6	1	2	0	55.32 ± 2.58	56.16 ± 4.40
Mate:	F, not U	U, not F	Both	Neither	F	U
b.) 1DI	8	1	2	1	61.67 ± 3.66	60.82 ± 4.13

Figure 1.1: Percentages of females mating at their first opportunities at four mating number/interval combinations. Sample sizes are shown in the bars.

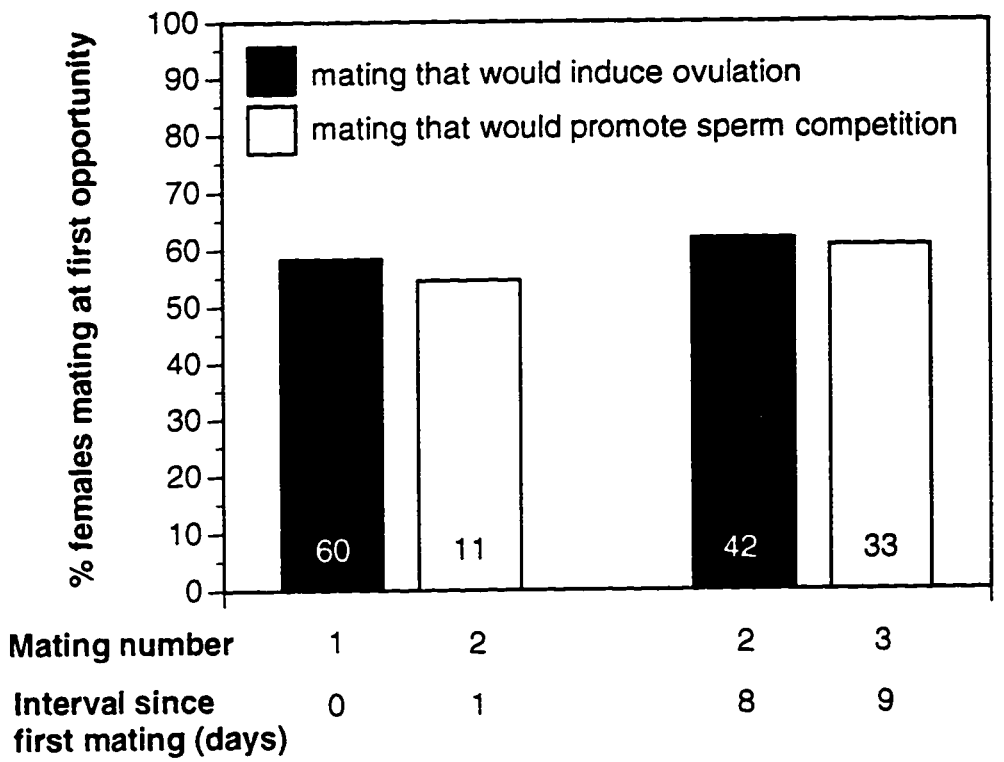

% females mating at first opportunity
100
90
80
70
60
50
40
30
20
10
0
mating that would induce ovulation
mating that would promote sperm competition
60
11
42
33
Mating number
1
2
2
3
Interval since first mating (days)
0
1
8
9

Figure 1.2: Percentages of females mating at their first opportunities for their first mating and when paired with familiar or unfamiliar males for multiple matings that would either induce additional ovulations (8DI) or add sperm (1DI). Sample sizes are shown in the bars.

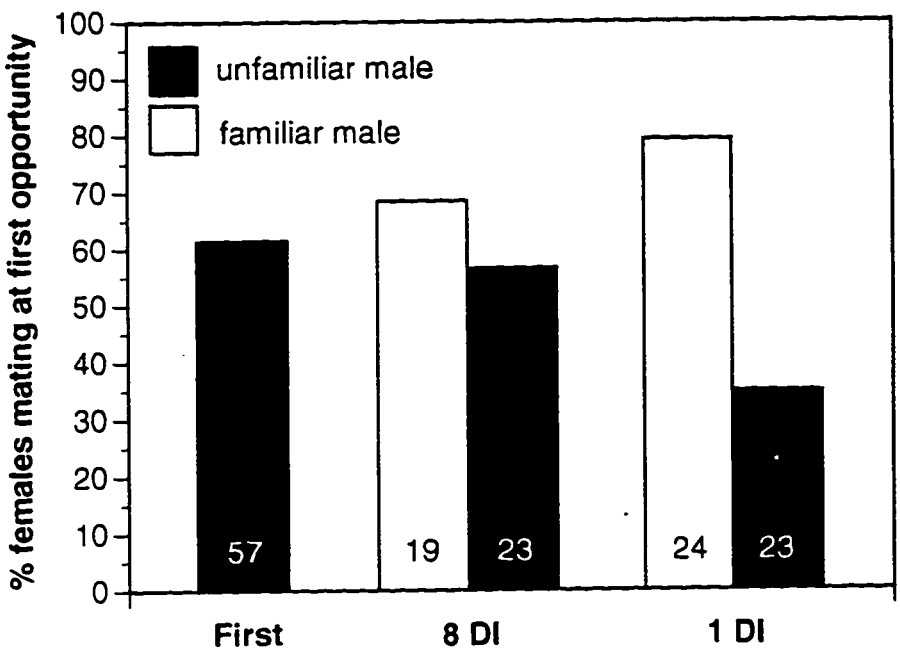

% females mating at first opportunity
100
90
80
70
60
50
40
30
20
10
0
unfamiliar male
familiar male
57
19
23
24
23
First
8 DI
1 DI

Figure 1.3: Percentages of adult or juvenile females mating the first familiar or unfamiliar males they were exposed to for their first matings and multiple matings that would induce additional ovulations (8DI) or add sperm (1DI). Sample sizes are shown in the bars.

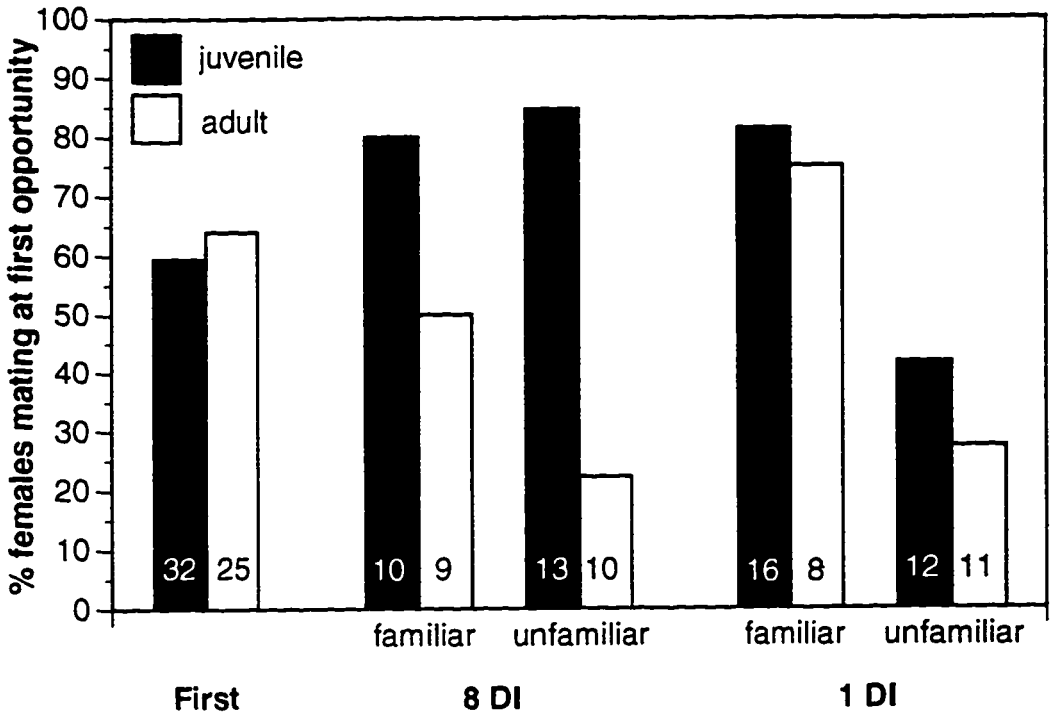

% females mating at first opportunity
100
90
80
70
60
50
40
30
20
10
0
juvenile
adult
32
25
10
9
13
10
16
8
12
11
familiar
unfamiliar
familiar
unfamiliar
First
8 DI
1 DI

Figure 1.4: Latencies (medians, 25th and 75th percentiles) to enter males' cages and remain to mate for a) adult and juvenile females with b) familiar and unfamiliar males.

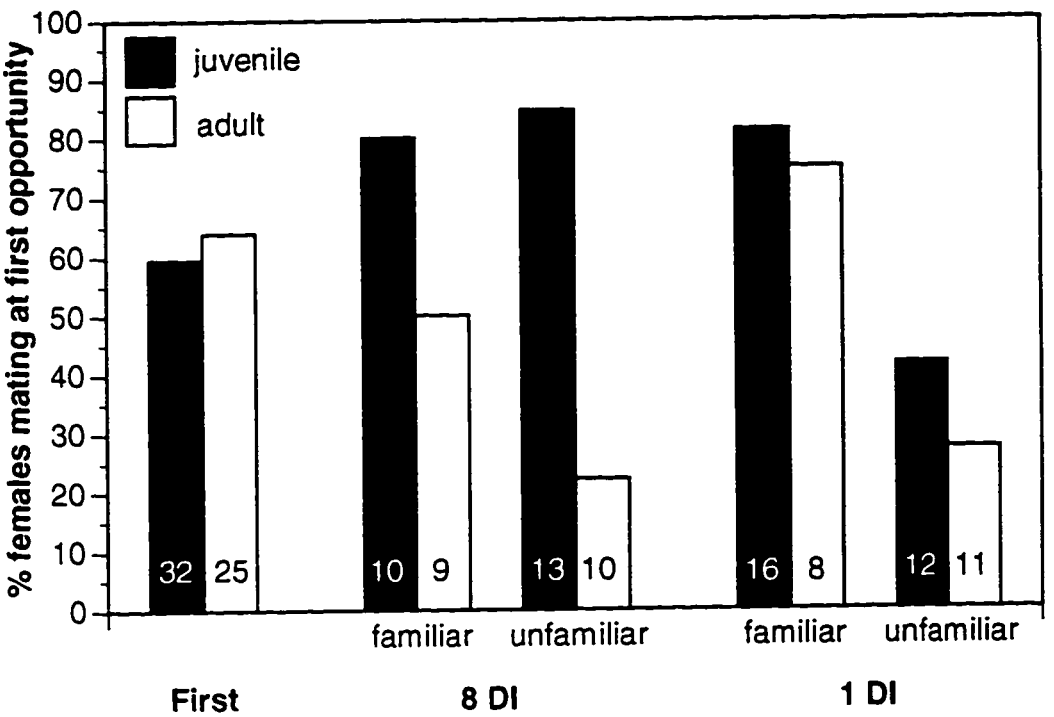
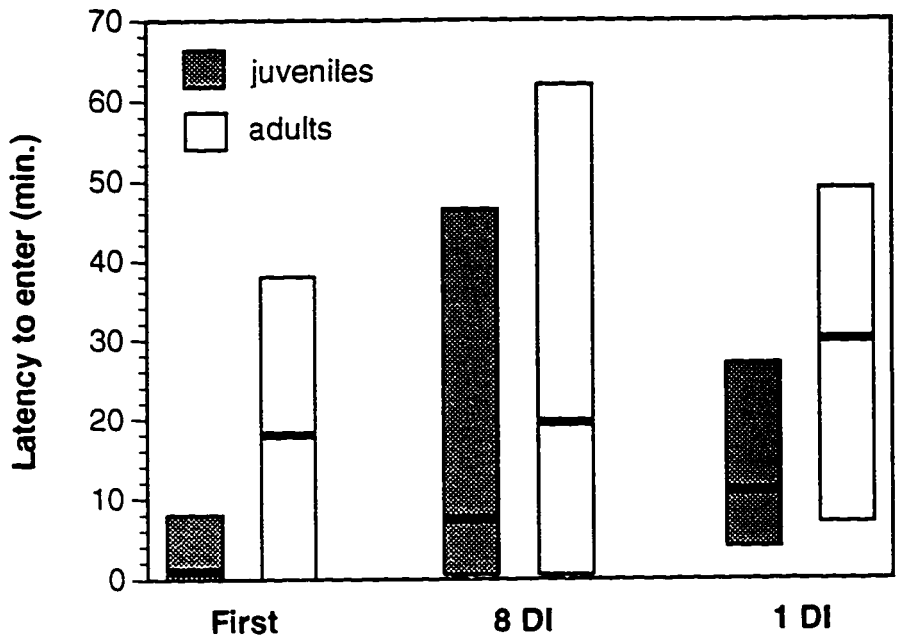
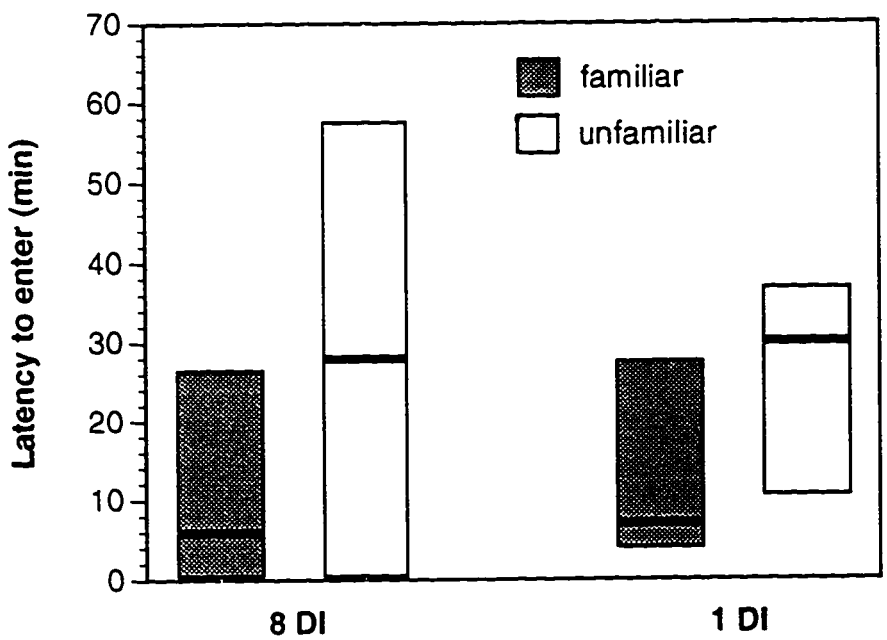

% females mating at first opportunity
100
90
80
70
60
50
40
30
20
10
0
juvenile
adult
32
25
10
9
13
10
16
8
12
11
familiar
unfamiliar
familiar
unfamiliar
First
8 DI
1 DI

Figure 1.5: Latencies (mean ± SE) to mate after having entered a male's cage for a) adult and juvenile females with b) familiar and unfamiliar males. Sample sizes are shown in the bars.

a.


70
60
50
40
30
20
10
0
juveniles
adults
Latency to enter (min.)
First
8 DI
1 DI

b.


70
60
50
40
30
20
10
0
familiar
unfamiliar
Latency to enter (min)
8 DI
1 DI

CHAPTER 2:

Prolonged copulation in mink as a male strategy in sperm competition

INTRODUCTION

Sexual selection theory predicts that polygynous males should produce more sperm than monogamous males so as to successfully fertilize numerous females (Trivers 1972). The quantity of sperm transmitted to females may also depend on whether the female is likely to mate monogamously or polyandrously (Harcourt et al 1981, Kenagy & Trombulak 1986, Moller 1989, Ginsberg & Rubenstein 1990); in other words, whether females behave so as to promote sperm competition.

Male mammals have evolved two basic ways of coping with sperm competition: restricting the access of other males to a female and increasing the quantity and quality of sperm deposited in her (Ginsberg & Huck 1989). Restricting access not only includes behavioral mate guarding but other 'sperm competition avoidance mechanisms' (Knowlton & Greenwell 1984; SCAMs) such as prolonged postejaculatory intromissions (e.g., copulatory locks in canids, Parker 1974) and copulatory plugs (Martan & Shepherd 1976, Hartung & Dewsbury 1978, Brock Fenton 1984). Inseminating females beyond what is necessary for fertilization is another way for males to increase their likelihood of paternity. This may involve increased ejaculate sizes (Baker & Bellis 1989) and ejaculate numbers, the latter either via multiple intromissions during a single copulatory bout (Lanier et al. 1979, Dewsbury & Baumgardner 1981, Oglesby et al. 1981) or through repeated matings (Eaton 1978, Lott 1981). Of course, males are in

competition with other males to evolve better SCAMs and ways of getting around the SCAMs of others, e.g., penile scoops or spines to remove previous males' sperm or copulation plugs (Eberhard 1985), bigger ejaculates to swamp others' bigger ejaculates (Parker 1982). This spermatic arms race is not restricted to intrasexual competition however; conflict between the sexes also contributes (Birkhead et al 1993, Keller & Reeve 1995, Eberhard 1996).

When acquiring multiple ejaculates is advantageous to females, then they should also be expected to evolve mechanisms for getting around male SCAMs. Female can resist repeated matings by a single male, avoid being guarded or forming consortships (Tutin 1979, Goodall 1986), and remove sperm plugs themselves (Voss 1979). The possible advantages to females' of resisting SCAMs are two-fold: females either receive the benefits of multiple mating in the first place, or produce offspring from only the male(s) with the best SCAMs (Knowlton & Greenwell 1984). If male ability to avoid sperm competition also has implications for 'good genes', i.e., older, larger, healthier males are best able to guard, produce sperm, or remain locked with a female, then females benefit all the more from their attempts to resist SCAMs.

SCAMs are difficult to study in animals with internal fertilization. Many of the most intricate studies of SCAMs are in insects (Walker 1980), the physiology and anatomy of which can be studied easily in large numbers. Studies of mammals have identified mating order advantages (Dzuik 1965, Dewsbury 1984), copulation or ejaculate number advantages (Lanier et al. 1979, Oglesby et al. 1981), and differential fertilization

capacities of sperm (Martin & Dzuik 1977, Lanier et al. 1979, Huck in Ginsberg & Huck 1989). However, characteristics of the female tract that influence SCAMs have been less well studied, particularly as female adaptations to enhance sperm competition or cryptic mate choice. Furthermore, the mammals whose physiology is well known tend to be domesticated; thus the adaptive significance of various aspects of female reproductive physiology has scarcely been addressed.

The American mink (*Mustela vison*) is a small, semi-aquatic, solitary carnivore in the family Mustelidae (Linscombe et al. 1982, Eagle & Whitman 1987). Since early in this century, mink have been raised on fur farms and as a result, their reproductive physiology has been extensively studied. Little is known about their reproductive behavior and mating system in nature, but they are thought to be like other small mustelids in being intrasexually territorial, with females in particular being territorial all year around while males appear to roam widely during the breeding season in search of receptive females (Gerell 1970, Powell 1979, Erlinge & Sandell 1985). Males are not known to remain with females after mating or to take part in parental care. Some unusual aspects of female mink reproductive behavior and physiology appear to extend the opportunity for sperm competition over several weeks and pose a considerable challenge to the ability of any one male to secure exclusive paternity in a females' litter.

As in many mustelids (Wright 1963, Mead & Wright 1983, Mead 1989a), mink have delayed implantation and the timing of both mating season and implantation of embryos is influenced by photoperiod (Duby & Travis 1972, Allais & Martinet 1978; Fig. 2.1). Unlike most other mustelids,

however, the delay of implantation in mink is short and highly variable—1-5 weeks—depending on how early in the breeding season a female mates. During delay, ovarian follicles continue to develop such that females become receptive and mating again can induce additional ovulations (and fertilizations) every 8-10 days (Fig. 2.2; Shackelford 1952). Female mink will also mate multiply during the 28-72 hours between the mating that induces each new ovulation and ovulation itself. No relationship has been established between mating order and reproductive advantage for males competing to fertilize ova from a single ovulation (Johansson & Venge 1951; Shackelford 1952). However, matings during subsequent receptive periods result in the loss of unimplanted embryos from previous weeks' matings (Adams 1981) and 86-90% of kits are conceived at the female's last ovulation (Johansson & Venge 1951; Shackelford 1952). Thus, a male must mate with a female within a day or so of her last ovulation in order to achieve paternity in her litter and, as a result, sperm competition should be most intense late in the breeding season.

In domestic mink, copulation duration has been reported to increase from less than 30 minutes at the beginning of the 3-5 week breeding season to longer than 2 hours by the end (Hansson 1947; Elofson et al. 1989). Although mink are induced ovulators, this dramatic increase is not proportional to changes in female responsiveness to stimulation (Venge 1956, Adams 1981, Elofson et al. 1989). Longer copulations may correlate with more sperm transferred (Venge 1956). Because female mink multiply mate and have multiply-sired litters, if longer matings result in the transfer of

more sperm, then prolonging copulation may be a male adaptation to sperm competition.

To investigate the proposed relationship between prolonged copulation and sperm competition, I paired males with females that they had mated previously and new females, both at intervals that would induce additional ovulations (8-10 days) and intervals that would contribute sperm to that already present in the female tract (~1 day). I hypothesized that males would adjust copulation duration in response to the familiarity of a female and the interval since her last mating so as to maximize success in sperm competition. When the interval since a female's last mating is greater than 8 days, males should copulate just as long with a previous mate (familiar) as with an unfamiliar female to increase their probability of paternity by fertilizing ova from the female's latest ovulation. In contrast, when the interval since a female's last mating is only 1 day, males should be more likely to prolong copulation with unfamiliar females than females that they had just mated the day before because of the lower return for repeatedly inseminating the same female during a single periovulatory period versus inseminating a number of different females. In addition, the number of times a male or female has mated should not influence copulation duration independent of mating date, familiarity, and female mating interval. This is because the important criterion for male reproductive success is mating at the female's last ovulation, which is ultimately determined by photoperiod regardless of how many times a female has mated.

I also sought to confirm that sperm is transferred throughout long copulations rather than simply being deposited early with the male

constituting a mating plug for the remainder of the copulation. If sperm is being deposited throughout copulation, then the numbers of motile sperm in a sample of vaginal fluid taken at the end of a 1.5-2 hour mating should be greater than that in sample taken 1.5-2 hours after a mating that was interrupted (ended) after only 15-20 minutes.

METHODS

Copulation duration

I conducted my research on a private fur farm in western Washington in March 1989 and 1991 between dawn and dusk (700-1800 hours). Mink were maintained under standard fur farm conditions as approved by the Mink Farmers' Animal Welfare Board. Fifteen pairs of testing cages (77.5 x 50 x 35 cm each) were built of polyvinyl-coated wire (with 2.5 x 5 cm squares) and connected by two 25 cm tunnels spaced 35 cm apart on the long side of the cage. Tunnels were ~7 cm in diameter to restrict males to one cage while females—which are 33-67% smaller—had access to both. Thus, females could avoid mating without having to fight a persistent male.

Experiment 1: 1989

I paired 25 2-year old ('adult') females and 35 1-year old ('juvenile') females with 14 adult males and a juvenile male between March 4 and March 18. Trials began after I placed females and males in the separate cages and ended either after copulation ended, if the pair began to fight, or after two hours if mating had not commenced. Males mated no more than twice per day at least 2 hours apart ($\bar{X} \pm$ SE: 248.29 $\pm$ 13.98 minutes, n =

21 male with two matings per day). This is typical of fur farm practices and does not reduce male fertility or sexual interest (John Adair, Oregon State University Experimental Fur Farm, personal communication). Fourteen females did not mate with any of the males they were exposed to for their initial mating. Eleven of these females mated subsequently when placed directly into the cage of a male and the copulation durations of these pairs did not differ from those involving the same males with females that had entered voluntarily ($\bar{X}_{voluntary}$ = 63.91 ± 4.47, $\bar{X}_{placed}$ = 63.78 ± 8.17; paired t-test, t = 0.022, df = 8, p = 0.983).

After their first mating, I gave 54 females the opportunity to remate after either a 1 day (16-24 hours) or 8-10 day interval (1DI or 8DI, respectively). Females were exposed to either the same male they had already mated (familiar) or a different male (unfamiliar). In addition, I gave females that mated a second time after 8-10 days an opportunity to mate a third time the following day. These third matings were also categorized as 1DI matings. Matings fell into one of 7 categories from the perspective of the female: 1) first matings (males unfamiliar by definition), 2) second 1DI matings the following day with familiar or 3) unfamiliar males, 4) second 8DI matings the following week with familiar or 5) unfamiliar males, and 6) third 1DI matings with familiar and 7) unfamiliar males.

Experiment 2 : 1991

This experiment was similar to that in 1989 except in the following particulars. I paired 6 juvenile males with 30 juvenile females between March 7 and March 20. The males were all experienced breeders, having

mated 3-5 times on March 3-6 ($\bar{X}$ = 4.0 ± 0.26, n = 6). I gave all females opportunities to mate a second time after 9-10 days rather than 8-10, and to mate a third time on the following day. Because experiments started 3 days later and I waited an extra day before pairing females for their second matings, the study was more time-constrained in 1991 than 1989. To insure that all females had the opportunity to mate three times, I separated pairs if they had not begun to copulate after approximately 1 hour ($\bar{X}$ = 72.85 ± 3.27, n = 34) and males had at least 1 hour between matings on the same day ($\bar{X}$ = 146.32 ± 17.77, n = 19). To insure that each of the six males mated females in each familiarity/mating interval category, I introduced 11 of the 21 females that remated after 9 days and seven of the 17 females that remated 1 day later directly into the cages of the six males.

Behavioral descriptions:

Copulation duration was measured from intromission to withdrawal. Prior to intromission, male mink grasp the back of the female's neck with their jaws and grasp her torso just behind her forelegs with their forelegs. Intromission is characterized by an extreme arch of the male's back while in this position, such that his pelvis is almost under the female's, and the cessation of rapid, shallow thrusting seen prior to penetration. During copulation, pairs alternate between standing and lying on their sides. During the latter, sperm is thought to be transferred during a series of 4-10 deep thrusts (Enders 1952; J. Adair, OSU Experimental Fur Farm, personal communication) which typically occur over one or two minutes at 8-20 minute intervals. Withdrawal is recognized as a straightening of the male's

back, moving his pelvis back away from the female's. Pairs typically separate within 10-15 seconds after this. If the male retained his hold and achieved intromission again, total time spent in intromission was recorded as the pair's copulation duration.

Because female struggling, screaming, and hissing are common responses of females to males' initially grasping and mounting them, fights were defined as interactions in which a female actually pursued a male and bit at his face. Males would typically respond to this aggression by trapping females against the side of the cage with their bodies (faces averted), and by vigorously biting at the female's torso if she persisted.

Analyses

I performed the analyses using Systat 5.2.1 for Macintosh (1992). Means are presented with standard errors and, unless otherwise noted, tests were two-tailed. Because copulation durations were normally distributed, I used parametric tests. Because the same males were mating repeatedly in all familiarity/ female mating interval categories and some males tended to copulate longer or shorter than average (One-way ANOVA, $F = 4.551$, df = 98, $p < 0.001$; Table 2.1), I used males' mean copulation duration for given mating categories and paired t-tests in all comparisons unless otherwise noted. All males were not represented in all categories, because females sometimes refused mating opportunities.

Sperm deposition during mating

Females reliably produce litters when copulations are disrupted after

only 15 minutes late in the breeding season (Venge 1956, Adams 1981), meaning that sufficient sperm must be transferred early in long copulations to achieve fertilization. To confirm that sperm is also transferred later in these long copulations, I compared samples of vaginal fluid collected from females in 3 conditions: females whose 1.5-2 hour copulations I did not disrupt but whom I sampled 1) within 1-10 minutes after the mating ended (n = 8), or 2) after a 1.5-2 hour delay (n = 7) and 3) females whose copulations I disrupted after 15-20 minutes but whom I sampled 1.5-2 hours later (n = 8). Samples were collected by inserting a ~2 cm long, 1 mm diameter glass tube with a rubber squeeze bulb full-length into the vagina of a female. Samples contaminated with urine were discarded. I immediately placed samples on a microscope slide, and viewed them under the 43X power of a standard light microscope. Due to the difficulty in making precise counts, I estimated sperm quantities and categorized them as 1 = 0-10 sperms, 2 = 11-100 sperms, 3 = 101-500 sperms, 4 = 501-1000 and 5 = >1000 sperms in the field of view. Similarly, I estimated motility to the nearest 5% as the percentage of motile sperm versus immotile sperm or sperm heads in the field of view. I estimated sperm numbers and motility blind with regard to which conditions the samples came from.

RESULTS

Effects of male remating interval on copulation duration.

Of 113 copulations observed in 1989, 21 of them took place on the same day as another mating by the same male. Of 59 copulations observed in 1991, 19 were males' second matings of the day. I performed

multiple regressions to determine whether males' second matings were influenced by the duration of the first mating and the interval between the matings. I first reduced the variance of the measures by removing the effect of mating date on copulation duration. In 1989, I adjusted copulation duration by the linear regression of females' first copulation durations on mating date, using only males' first matings each day (3.843 ± 0.446; $R^2 = 0.445$, $n = 92$, $p < 0.001$). Because all first matings in 1991 took place over 4 days rather than over two weeks (in 1989), I used the linear regression of copulation durations for unfamiliar pairs on mating date, again only using males' first matings each day (3.443 ± 1.083 minutes per day; $R^2 = 0.265$, $n = 30$, $p < 0.01$). In both years, I calculated the difference between the mean adjusted and unadjusted copulation duration for males' first matings each day ($\bar{X}_{1989} = 30.39$, $\bar{X}_{1991} = 29.34$). Adding this constant to each copulation duration made the mean unadjusted and adjusted copulation durations equivalent for easier comparison.

In both years, copulation durations were similar when males mated twice per day (paired t-tests; 1989: $t = 0.963$, df = 15, $p = 0.351$; 1990: $t = 0.497$, df = 10, $p = 0.630$). Neither duration of the first mating (1989: $p = 0.669$; 1991: $p = 0.917$) nor the interval between them (1989: $p = 0.096$; 1991: $p = 0.694$) had a significant effect on the duration of the second mating (multiple regressions; 1989: $R^2 = 0.201$, $n = 16$, $p = 0.233$; 1990: $R^2 = 0.034$, $n = 11$, $p = 0.872$). Thus, when a male had copulated more than once in a day in a given mating category, I used the male's averaged copulation duration for that day and recalculated the effects of date on copulation duration in both years.

Effects of date on copulation duration.

Copulation duration for the females' first matings in 1989 increased 4.424 ± 0.654 minutes per day over 15 days in 1989 (R^2 = 0.510, n = 46, p < 0.001; Fig. 2.3). I used this regression coefficient to remove the effect of mating date from all copulation durations prior to further analyses. The relationship between copulation duration and mating date was similar when the 58 multiple matings were included (3.721 ± 0.419 minutes per day; R^2 = 0.436, n = 104, p < 0.001). Individually, eight of 15 males in 1989 showed significant increases in copulation duration with date, while five other males showed similar tendencies (p < 0.10; Table 2.1). In 1991, copulation duration increased 2.3 ± 0.807 minutes per day (R^2 = 0.160, n = 37, p < 0.05). Two of these six males showed significant increases in copulation duration with date.

Effects of familiarity and female mating interval on copulation duration.

Experiment 1:1989

Both the interval between female rematings and the familiarity of pairs affected copulation duration. When the females remated after an 8 day interval—inducing a second ovulation—copulation duration did not vary with the familiarity of the pairs (paired t-test t = 0.989, df = 8, p = 0.351; Fig. 2.4). Males also did not copulate longer with either familiar or unfamiliar females when inducing second ovulations than they had when inducing females' first (paired t-tests; familiar: t = 0.662, df =12, p = 0.521;

unfamiliar: $t = 0.723$, df = 9, $p < 0.488$; Fig. 2.4). Copulations were significantly shorter when familiar pairs remated a day after mating one another than when unfamiliar pairs mated one day after matings with other individuals (paired t-test $t = 2.405$, df = 6, $p < 0.05$, one-tailed; Fig. 2.5). These 1DI matings between familiar pairs were also shorter than matings between familiar pairs that would induce ovulation (paired t-test $t = 3.536$, df = 9, $p < 0.01$, one-tailed; Fig. 2.5). By comparison, there was no difference between copulation durations at unfamiliar 1DI pairings and unfamiliar pairings that would induce ovulation (paired t-test $t = 0.532$, df = 7, $p = 0.611$; Fig. 2.5).

Experiment 2:1991

As in 1989, familiarity of the pairs in 1991 did not affect copulation durations when mating would induce an additional ovulation (paired t-test $t = 1.382$, df = 4, $p = 0.239$; Fig. 2.6). Nor did pairs copulate longer when mating would induce a second ovulation than they had when inducing the first (paired t-tests; familiar: $t = 1.574$, df = 4, $p = 0.191$; unfamiliar: $t = 0.324$, df = 5, $p = 0.759$; Fig. 2.6). Once again, second copulations during a single periovulatory period were significantly shorter when familiar pairs had already mated the previous day than when unfamiliar pairs were mating (paired t-test $t = 2.830$, df = 5, $p = 0.018$, one-tailed; Fig. 2.6). These 1DI matings between familiar pairs were also shorter than matings between familiar pairs that could induce another ovulation (paired t-test $t = 2.176$, df = 4, $p < 0.043$, one-tailed; Fig. 2.6). There was no difference between

copulation durations of unfamiliar 1DI pairings and unfamiliar 8DI pairings (paired t-test $t = 0.832$, d f= 5, p = 0.443; Fig. 2.6).

Effects of mating number and age on copulation duration

The number of times males or females mated did not affect their copulation durations. In 1989, it appeared that copulation duration decreased as male mating number increased ($R^2 = 0.044$, n = 117, $p < 0.05$), but this relationship disappeared when 1DI matings between familiar pairs, which were later matings, were removed ($R^2 = 0.000$, n = 100, p = 0.984; Fig. 2.7). There was no relationship between copulation duration and the number of times that males had mated in 1990 either ($R^2 = 0.006$, n = 59, p = 0.544). In 1989, this could be shown to be true for females as well; after a 1 day interval, the durations of matings did not differ whether they were a female's second mating or her third (independent t-tests; familiar: $t = 0.504$, df = 13, $p > 0.10$; unfamiliar: $t = 0.515$, df = 10, $p > 0.10$; Fig. 2.8). Copulation durations of 14 males that mated both 1 year and 2 year old females did not differ with female age ($\bar{X}_{1\text{-year old}} = 54.32 \pm 4.02$, $\bar{X}_{2\text{-year old}} = 57.41 \pm 3.60$; paired t-test $t = 1.372$, df = 13, p = 0.193).

Sperm deposition during mating

The proportion of motile sperm in the vagina was significantly higher immediately after a long mating than 1.5-2 hours after a mating that had been interrupted after only 20 minutes (Mann-Whitney $U = 60$, p = 0.002; Fig. 2.9a) The proportion of motile sperm in the sample taken immediately after a long mating was also higher that in the sample taken 1.5-2 hours

after long matings (Mann-Whitney $U = 2.5$, $p = 0.002$; Fig. 2.9a), which showed percent motility similar to samples from matings that had been interrupted 1.5-2 hours before (Mann-Whitney $U = 29$, $p = 0.905$; Fig. 2.9a).

Sperm numbers did not differ in samples taken immediately after or 1.5-2 hours after long matings (Mann-Whitney $U = 21.5$, $p = 0.425$; Fig. 2.9b). However, samples taken immediately after a long mating had more sperm than samples taken 1.5- 2 hours after matings that were interrupted after 20 minutes (Mann-Whitney $U = 50.5$, $p = 0.045$; Fig. 2.9b). Sperm numbers taken from long and interrupted matings did not differ significantly when the samples were taken after 1.5-2 hours (Mann-Whitney $U = 41.5$, $p = 0.108$; Fig. 2.9b).

DISCUSSION

The observed increases in copulation duration in both years of the study are comparable to those reported in two other studies. In 1989 and 1991, copulation duration increased 4.424 and 2.3 minutes per day, respectively. Hansson (1947) reported a mean length of copulation after March 26 of 114 minutes compared to 49 minutes prior to March 10 (a 4.33 minute per day increase) and Elofson et al. (1989) reported a 4.2 ± 0.2 minute per day increase between March 8-22. Furthermore, wild mink in captivity increased copulation duration by 1.96 ± 0.54 minutes per day over the course of the breeding season (Fleming, unpublished data). As expected, the increase in copulation duration in this study was independent of the number of times either males or females mated. If males prolong copulation late in the breeding season to increase the probability of

fertilizing ova released at a female's last ovulation, then copulation duration should be determined by whether the female is likely to ovulate again the following week (i.e., mating date), not the mating number of the female involved. That males did not differentiate between 1 year old and 2 year old females in terms of copulation duration also makes sense as minks' life expectancy in the wild is short (3-5 years; Mitchell 1961, Gerell 1971, Hatler 1976, Askins & Chapman 1984) and differences in the productivity, if any, of the two age classes is small (Elofson et al. 1989).

Copulation duration increased with date only for pairs that had not previously mated during the female's latest periovulatory receptive period. Pairs that had mated one another the previous day had significantly shorter copulation durations than either 1) pairs that had mated other individuals the day before or 2) familiar and unfamiliar pairs that had mated when mating would induce an additional ovulation. These results are also consistent with the view that long copulations towards the end of the breeding season may be a male strategy for coping with sperm competition. Because 85-90% of a female's litter is conceived during her last periovulatory period (Johansson & Venge 1951, Shackelford 1952), sperm competition is as much of a problem for males when mating with females they mated last in a previous week as when mating females they've never mated. In contrast, having mated a female during her 2-3 day receptive period around her last ovulation, males would not benefit as much from prolonging additional copulations with them as from conserving time, energy and sperm for matings with other females.

Do males actually control copulation duration, however? Copulation is obviously a pair behavior—could the female in fact be determining the duration of mating? Females appear to 'struggle' at intervals throughout copulation: squealing and hissing, twisting their heads and bodies, and sometimes bending around to bite a males' feet. Matings typically end during one of these struggles, suggesting a female role in terminating the mating. The fact that some males consistently copulate significantly longer or shorter than average suggests that male characteristics contribute to copulation duration as well. Copulation duration may be determined both by both how strongly a female struggles and how long a male can hold on to her, with some males being better at holding females than others, making female 'resistance' a potential test of male vigor. Regardless of the extent to which the durations of copulations are attributable to how quickly males release females (i.e., male interest in the female) versus how much females struggle (i.e., female interest in the male), copulation duration can have important implications for sperm competition.

How prolonged copulation may influence sperm competition.

This study also provides some empirical support for the claim that male mink transfer sperm at intervals throughout copulation. Sperm numbers and motility were high in samples taken from the vagina at the end of matings longer than two hours. Motility was significantly lower in samples taken after a 2 hour delay from both the end of short (~20 minutes) and long (>2 hour) matings. Sperm numbers were also lower 2 hours after short, interrupted matings than immediately after long matings. To be so

motile at the end of a 2 hour mating, sperm must be deposited not only early in a copulation, but at least once more toward the end of long copulations. The presence of white blood cells (indicating that the semen in the sample was in the female tract long enough to elicit an immune response) in the samples taken 2 hours after a mating ended, but not in the samples taken at the end of 2 hours matings also supports the idea that fresh sperm is deposited late in long matings. Venge (1956) found more sperm in females that had mated for 24 minutes than 12 minutes. Fur farmers also typically find large quantities of motile sperm whenever they "sperm check" males by breaking up matings anytime after 30-60 minutes, which implies that sperm may be transferred at more regular intervals later in copulation as well.

Male mammals often deposit sperm in a single female over prolonged periods of time. Commonly, this involves multiple intromissions (Eaton 1978, Lott 1981, Dewsbury 1984) rather than sperm being transferred at intervals during a single prolonged intromission. Larger quantities of sperm transferred during multiple or prolonged copulations probably dilute the sperm of other males, making sperm competition primarily a matter of relative numbers (Parker 1982).

In some species, prolonged copulatory stimulation is necessary to induce ovulation (Gray et al. 1974, Eaton 1978) or reduces female receptivity to further matings (Carter 1973). Neither appears to be the case in mink. Matings 5 minutes in length are sufficient to induce a female's first ovulation (Venge 1956, Adams 1981) and 12-15 minute matings are sufficient to induce subsequent ovulations (Adams 1981, Elofson et al. 1989). Females remain as likely to remate the day after a previous mating

as they were the day before (details in Fleming, chapter 1) and there is no evidence that copulation duration affects females' likelihood of remating (Fleming, unpublished data). Nonetheless, there are other ways in which prolonging copulation could affect male success in sperm competition in mink.

Copulations may be prolonged beyond the time necessary for sperm transfer in order to remove sperm or plugs from previous matings (Adler & Zoloth 1970, Martan & Shepherd 1976, Dewsbury 1984) or disrupt sperm transport (Adler & Zoloth 1970, Matthews & Adler 1977, Dewsbury 1985). Adams (1981) suggested that the observed paternity advantage for male mink that mated around a female's last ovulation was because mating either induced uterine contractions that mechanically expelled blastocysts or introduced semen components that destroyed them. Whether longer copulations in mink may also expel recently deposited sperm or slow its transport to the site of fertilization is unknown. Experiments using vasectomized males and varying the duration of 1DI matings could help determine whether short copulations, like those observed between familiar pairs, simply add more sperm to that already present and whether longer copulations also decrease the representation of previous mates in a litter.

Another possible mechanism by which prolonged copulation may be advantageous in sperm competition is by inducing ovulation more quickly than shorter copulations. In spontaneous ovulators, mating can cause earlier ovulation (Zarrow & Clark 1968, Jochle 1975). Estimates of the interval between induction of ovulation and ovulation itself in mink range from 28-72 hours (Sundqvist et al. 1988) and this considerable variation

has not been explained. If prolonging copulation induces ovulation more quickly in mink, then males that prolong copulation would be reducing the amount of time that other males would have to find and mate the female as well. Males that find females that have mated within the last day or two could still be prolonging copulation for reasons already discussed: to interfere with sperm from previous males and to introduce large quantities of their own sperm.

Females may also benefit from males' attempts to prolong copulation. Females struggle and vocalize during copulation, particularly at the beginning and during the periodic series of deep thrusts thought to be associated with sperm transfer. This may not only help females to determine a male's health and experience (through his ability to hold her) but it may also attract other males to the area to promote male competition (Cox & LeBoeuf 1977). If prolonging copulation increases a male's chances of prevailing in sperm competition with a female's previous or future mates, a female would benefit from the likelihood that her sons would inherit genes for their sire's success (Keller & Reeve 1995)

How might males determine how long to copulate with a given female?

Because it is to a male's advantage to copulate as long with females last mated more than a week before as with unfamiliar females, there is no reason for males to remember previous mates for 8 days. Thus, the mechanism for prolonging copulation with females that have not recently mated could be tied to photoperiod. But how might males distinguish

between females that they mated the day before and those they didn't? Do they recognize individual females and remember them over the short term or do they distinguish between females that recently mated a different male and those that they recently mated?

Both mechanisms could be at work in mink given the variety of information mustelids can gather from one another's scents. Male mink can determine sex and estrous status of females from scent marks (Robinson 1987). Chemical 'signatures' of anal glands secretions mink used in scent marking are complex enough to allow for individual identification (Brinck et al. 1978). Polecats (*Mustela furo*) have been shown to distinguish between their own odors, stranger odors, and odors of individuals dominant to them (Clapperton et al. 1988). Prior to intromission, male mink paid particular attention to sniffing two areas of the female's body - the anus/vulva and the back of her neck. The anal secretions could provide information about female identity and vulval secretions could provide clues as to whether she had mated recently (if changes in vaginal chemistry occur or there are traces of semen) and possibly with whom (if males can tell their semen odors from others). Neck sniffing may detect small puncture wounds indicating that a female has been grabbed recently and perhaps saliva traces that could be recognizable to the male as 'self' or 'stranger.' Females lick their vulvas and the surrounding area vigorously after copulations, however, and as mink are semi-aquatic, saliva traces may be washed off if a female has been in the water recently. Nonetheless, the females in this study had not been in water and any of these residual odors could be useful to males in the wild at least over the short term.

SUMMARY

Mink engage in increasingly long copulations as the breeding season progresses: males and females that engaged in 15-30 minute copulations during the first week of the study copulated for up to 2 hours during the second week. These copulations are considerably longer than necessary to induce ovulation or achieve fertilization late in the breeding season. Because matings more than a week after a female's previous mating induce another ovulation and expel embryos from previous matings, males must mate with a female within a day or so of her last ovulation to have a reasonable chance of paternity in her litter. Thus, male competition should be most intense towards the end of the breeding season and I hypothesized that prolonged copulation is a male strategy in sperm competition.

The increase in copulation duration was not attributable to the numbers of times either males or females had mated. As predicted, males prolonged copulation with females they had not yet mated during the females' current fertile period (whether they had ever mated them before or not) but did not copulate as long with females they had mated the previous day. Furthermore, semen samples from the vaginas of females mated for two hours contained large quantities of motile sperm while samples from females that had mated for 15-20 minutes 2 hours previously did not. This indicates that additional sperm is being transferred well into prolonged copulations, and sperm competition by dilution is liable to be one function of

prolonged copulation. Other possible functions could include facilitating sperm transport and hastening ovulation.

Table 2.1: Mean copulation durations and rates of increase per day (regression coefficient) for all matings by the 15 males in the 1989 experiment.

Male	Number of Matings	Mean Cop. Duration	Regression Coefficient	R^2	P-value
1	**11**	**53.47**	**2.493**	**0.368**	**0.033**
2	**5**	**52.80**	**4.322**	**0.976**	**0.002**
3	**8**	**71.84**	**5.972**	**0.837**	**0.006**
4	9	55.15	3.396	0.439	0.052
5	**7**	**43.82**	**3.578**	**0.770**	**0.009**
6	**8**	**43.66**	**4.372**	**0.771**	**0.004**
7	**7**	**58.72**	**4.806**	**0.770**	**0.009**
8	8	46.34	3.408	0.432	0.077
9	7	65.95	2.336	0.152	0.388
10	9	45.63	3.066	0.228	0.194
11	**10**	**54.11**	**4.886**	**0.638**	**0.003**
12	7	40.01	4.318	0.523	0.066
13	6	53.18	5.174	0.651	0.052
14	**6**	**67.95**	**4.409**	**0.709**	**0.035**
15	5	88.22	3.888	0.745	0.059

Figure 2.1: Seasonal timing of reproductive events in female mink.

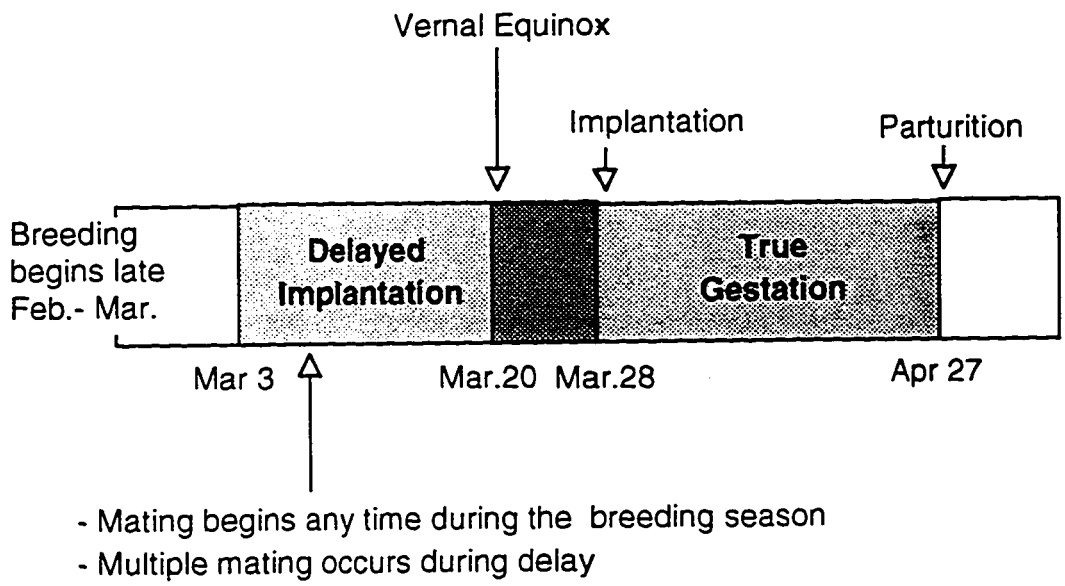

Vernal Equinox
Implantation
Parturition
Breeding
begins late
Feb.- Mar.
Delayed
Implantation
True
Gestation
Mar 3
Mar.20
Mar.28
Apr 27
- Mating begins any time during the breeding season
- Multiple mating occurs during delay

Figure 2.2: Pattern of sexual receptivity in female mink during delayed implantation and some of its physiological correlates.

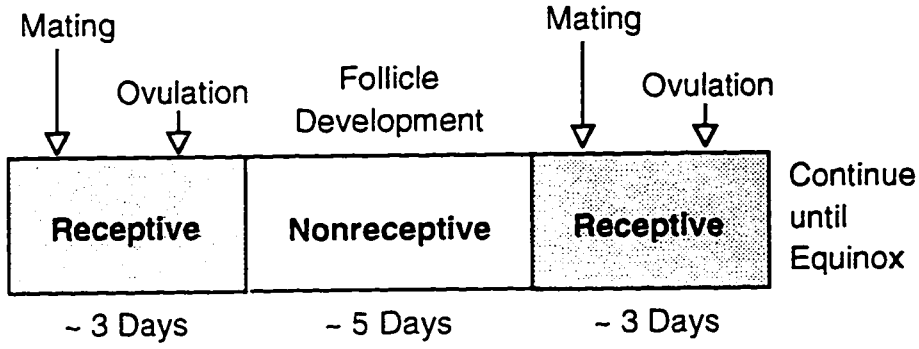

Mating
Ovulation
Follicle
Development
Mating
Ovulation
Receptive
Nonreceptive
Receptive
Continue
until
Equinox
~ 3 Days
~ 5 Days
~ 3 Days

Figure 2.3: Linear regression of copulation duration on mating date for 46 females' first matings in 1989.

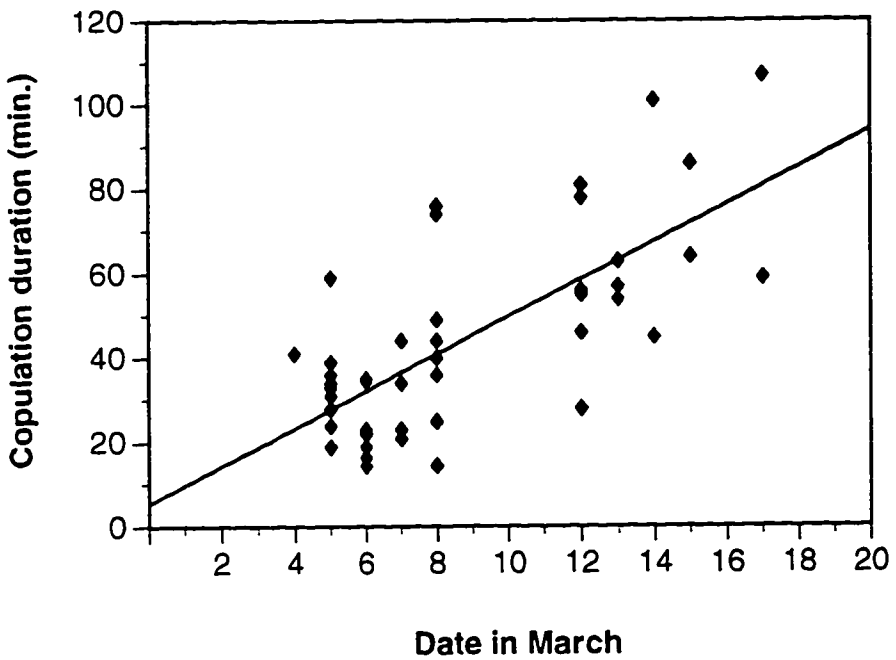

Copulation duration (min.)
120
100
80
60
40
20
0
2
4
6
8
10
12
14
16
18
20
Date in March

Figure 2.4: Mean (± SE) copulation durations (adjusted for mating date) of males that mated both familiar and unfamiliar females when remating would induce additional ovulations (8DI) in 1989. "First matings" refers to the males' copulation durations when mating females that had not yet mated. Sample sizes are shown in bars.

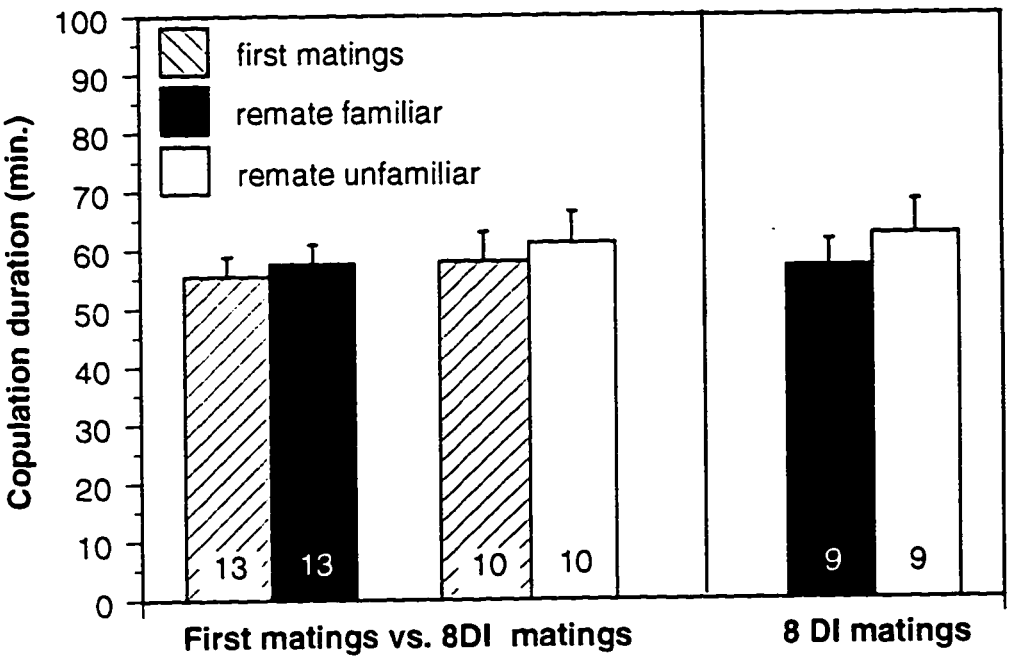

100
90
80
70
60
50
40
30
20
10
0
Copulation duration (min.)
first matings
remate familiar
remate unfamiliar
13
13
10
10
9
9
First matings vs. 8DI matings
8 DI matings

Figure 2.5: Mean (± SE) copulation durations (adjusted for mating date) of males that mated both familiar and unfamiliar females when mating would add to sperm already present in the female tract (1DI) in 1989. "Previous days' matings" refers to the males' copulation durations when mating with females who were mating for the first time in that receptive period. Sample sizes are shown in bars. * $p < 0.05$.

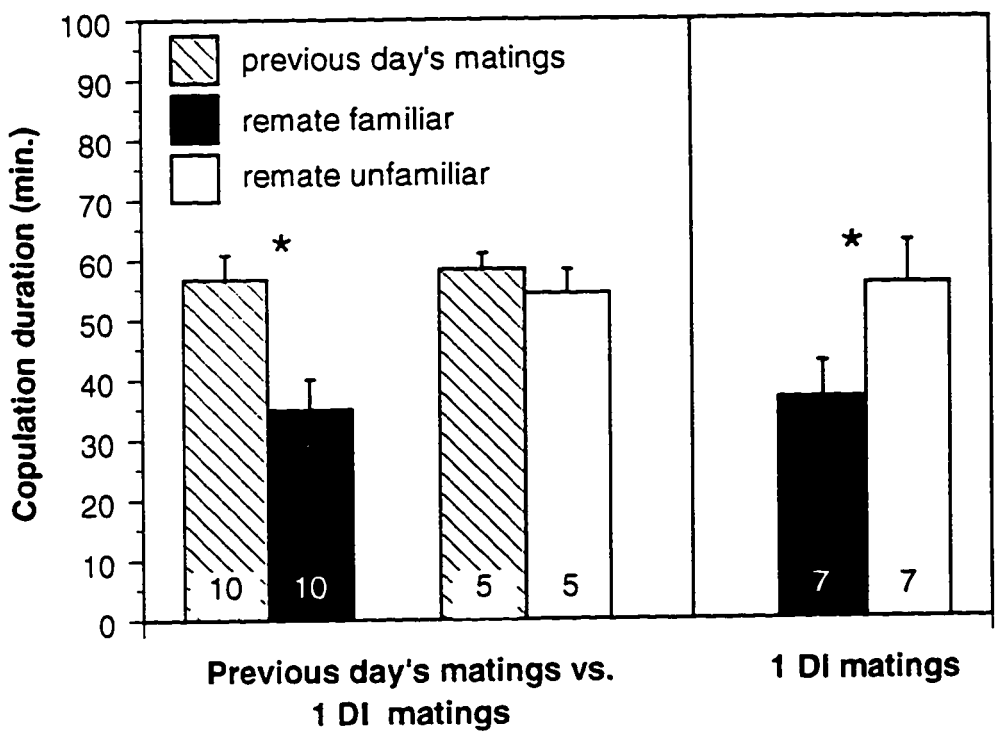

100
90
80
70
60
50
40
30
20
10
0
Copulation duration (min.)
previous day's matings
remate familiar
remate unfamiliar
*
*
10
10
5
5
7
7
Previous day's matings vs.
1 DI matings
1 DI matings

Figure 2.6: Mean (± SE) copulation durations (adjusted for mating date) of six males (unless otherwise noted in the bars) that mated females in each mating number/interval/familiarity category in 1990.
* $p < 0.05$.

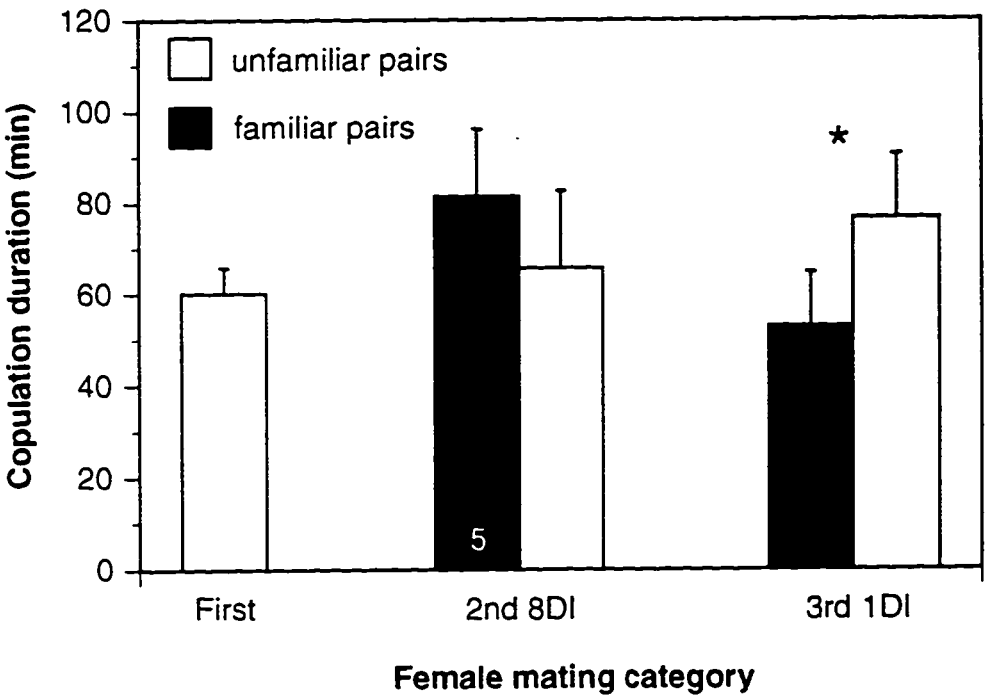

120
100
80
60
40
20
0
unfamiliar pairs
familiar pairs
*
5
Copulation duration (min)
First
2nd 8DI
3rd 1DI
Female mating category

Figure 2.7: Linear regression of copulation duration, adjusted for the effect of mating date, on male mating number in 1989.

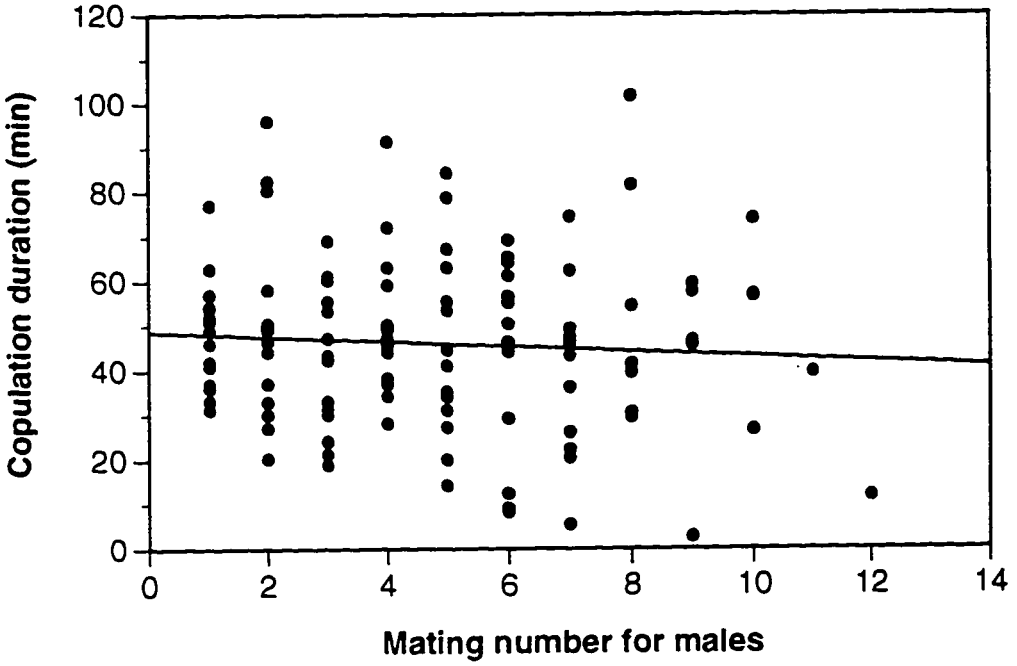

Copulation duration (min)
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Mating number for males

Figure 2.8: Mean (± SE) copulation durations (adjusted for mating date) for females remating for a second or third time with either familiar or unfamiliar males after a one day interval. Sample sizes are shown in the bars.

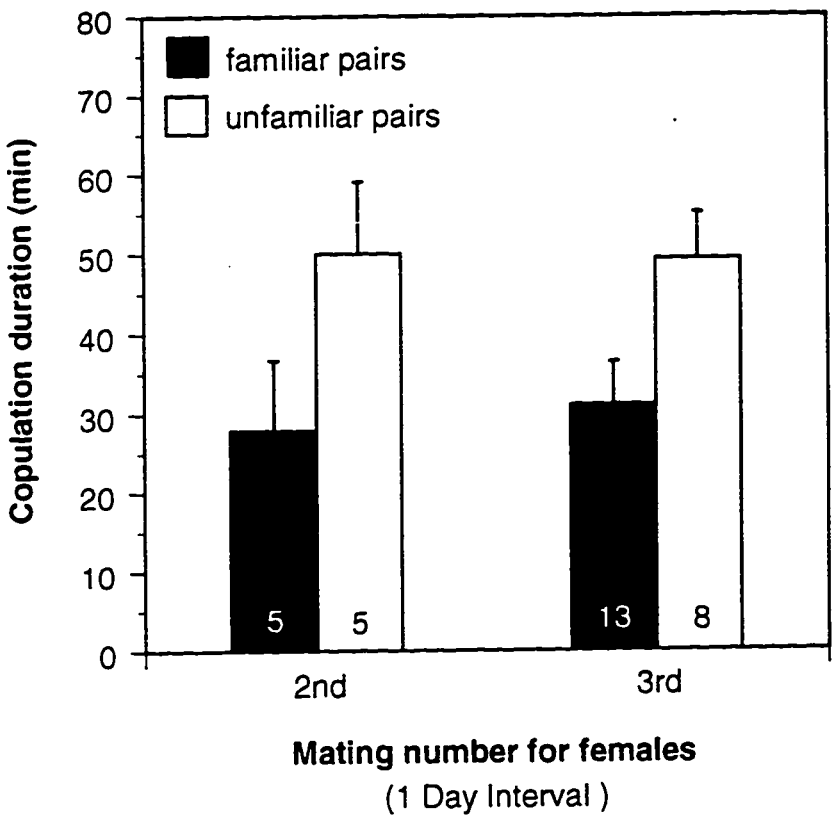
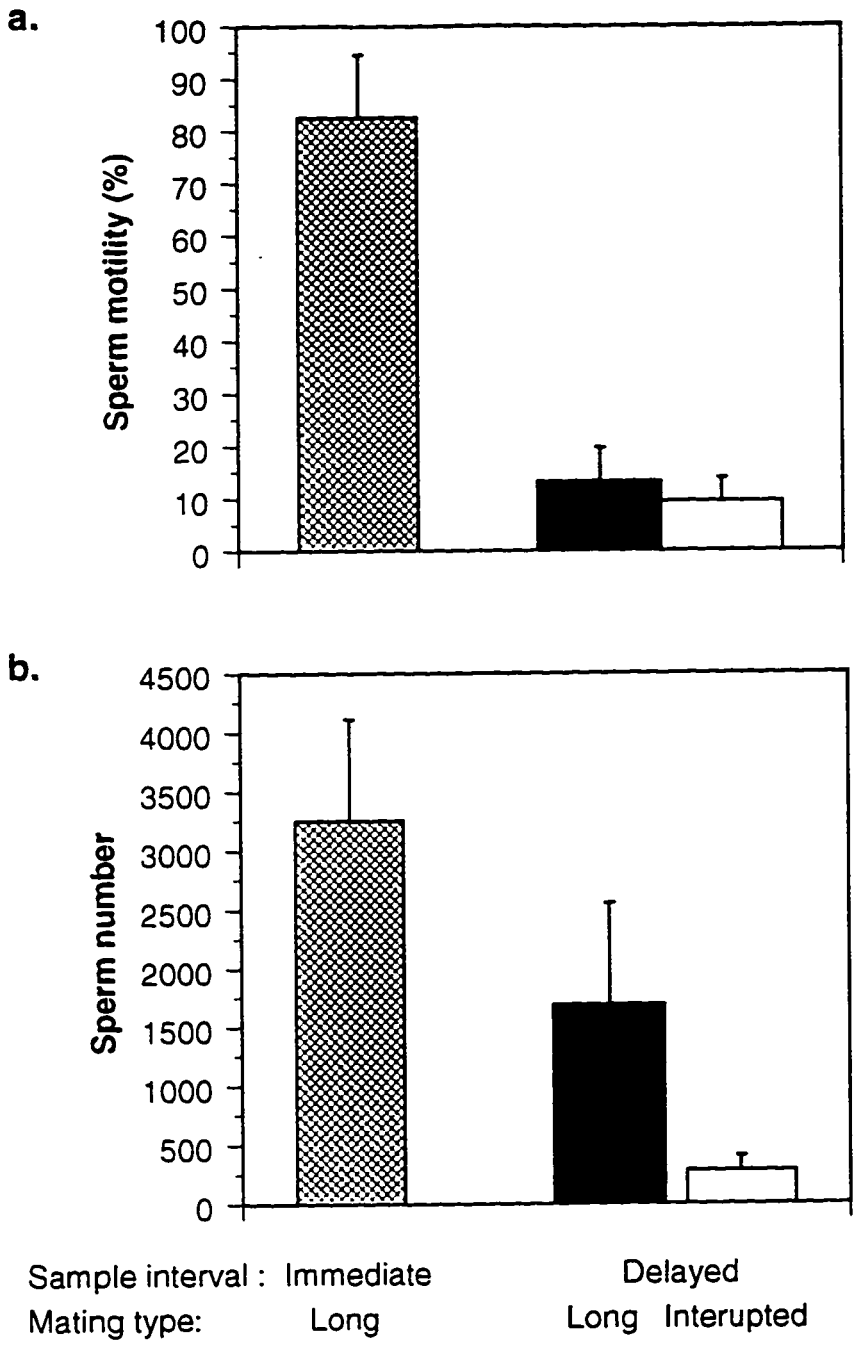

80
70
60
50
40
30
20
10
0
familiar pairs
unfamiliar pairs
Copulation duration (min)
5
5
13
8
2nd
3rd
Mating number for females
(1 Day Interval)

Figure 2.9: Mean (± SE) percentages of a) motile sperm and b) numbers of sperm in samples of vaginal fluid taked from females either 1) immediately after a 1.5-2 hour copulation ended, 2) 1.5-2 hours after, or 3) 1.5-2 hours after a copulation that was interrupted after only 15-20 minutes.


a.
Sperm motility (%)
100
90
80
70
60
50
40
30
20
10
0
b.
Sperm number
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Sample interval : Immediate
Delayed
Mating type:
Long
Long
Interupted

CHAPTER 3:

Effects of multiple mating on female fecundity and paternity of litters in mink

Multiple mating by females can potentially affect the intensity of sexual selection and shape the mating system of a species. The evolutionary consequences of female promiscuity depend foremost on whether multiple mating leads to multiple paternity, which in turn depends on patterns of sperm precedence, the timing of mating relative to ovulation, and the outcome of sperm competition (Ginsberg & Huck 1989, Birkhead & Hunter 1990). Thereafter, the extent to which females exercise mating preferences or cryptic female choice also plays a role (Birkhead et al 1993, Eberhard 1996). Schwagmeyer (1984) pointed out that if females express the same preferences for males when mating multiply as when mating initially, then female multiple mating should have little influence on the intensity of sexual selection. On the other hand, if females have no preferences or evaluate additional mating partners on different criteria than their initial partners, then female promiscuity should result in a decrease in the intensity of sexual selection by reducing the variance in male mating success (Schwagmeyer 1984). If males vary in their ability to prevent females' ova from being fertilized by sperm from other males, then whether females mate indiscriminately or have preferences, intrasexual competition among males should also influence which males fertilize a female (Parker 1970, 1972, 1984).

The American mink (*Mustela vison*) is a small, solitary, seasonally breeding carnivore native to waterways throughout North America

(Linscombe et al. 1982, Eagle & Whitman 1987). Like many other solitary mammals (Powell 1979, Erlinge & Sandell 1985), male mink roam widely during the breeding season in search of sexually receptive females, which remain on the home ranges year around (Mitchell 1961, Gerell 1970). Little is known about mink mating behavior in the wild except that several males can be found in the vicinity of a sexually receptive female (Hatler 1976, Ireland 1990 in Dunstone 1993), suggesting that females may multiply mate if their mates do not prevent it.

Studies of the reproductive biology of mink on fur farms suggests that some unusual aspects of female physiology could increase the opportunity for female multiple mating and for sperm competition. Female mink will mate repeatedly for 2-3 days after a mating that induced ovulation and produce multiply sired litters as a result (Johansson & Venge 1951, Shackelford 1952). Ovulation of 8-9 ova (from two ovaries combined, Hansson 1947) occurs some 28-72 hours after the mating that induced it (Hansson,1947; Enders 1952). This interval is long compared to other induced ovulators (e.g., vole, 9-12 hours; rabbit, 9-10 hours; ferret, 24-35 hours; cat, 26-58 hours, Ramirez & Beyer 1988) and in the wild, it may be sufficient to allow additional males to find a mated female and potentially contribute to her litter. Johansson & Venge (1951) found that when female mink mated different males on consecutive days, the second male sired more of the young (63%), however when there were 2 days between matings, the first male sired more of the young (73.2%). This discrepancy may be accounted for by differences in the activity of sperm at ovulation and the arrival times of sperm relative to ovulation. Sperm deposited on the

second day may be more active at the time of ovulation than sperm from the previous day, and sperm from the third day may arrive at the site of fertilization too late to capacitate and compete successfully for fertilizations (Johansson & Venge, 1951).

Female mink will also remate at 8-10 day intervals, at which time mating will induce the ovulation of additional sets of ova (Hansson 1947, Enders 1952). These additional sets of ova can also be fertilized and will implant, allowing females to produce 'split litters' containing kits that were sired by males that mated weeks apart ('superfetation'; Shackelford 1952). Like many other mustelids (Wright 1963, Mead & Wright 1983, Mead 1989a), mink have delayed implantation—an extended period of time in which embryos are quiescent and float free in the uterus—which ends in response to changing photoperiod. In mink, implantation is stimulated by increasing photoperiod around the vernal equinox after 1-5 weeks of delay (Allais & Martinet 1978). Shackelford (1952) argued that superfetation in mink is made possible by delayed implantation in that the inactivity of the corpora lutea during delay allows for the continued development of ova in technically pregnant females. Furthermore, delay allows for kits conceived weeks apart to be born at the same stage of development because blastocysts resulting from successive ovulations implant in response to the same photoperiod cue.

These additional ovulations by themselves do not appear to increase a female's ultimate litter size, however. Despite the ovulation of 8-9 ova each time, only 83.7% of ova implant and only 50% result in kits (Hansson 1947). Thus, the litter sizes of singly-mated and multiply-mated

females are similar, as are the litter sizes of females that mate multiply in various mating number/mating interval combinations (Hansson 1947, Elofson et al. 1989). Johansson & Venge (1951) showed that 85.6% of kits were sired by males that mated around a female's last ovulation and Adams (1981) showed that the loss of previous blastocysts was stimulated by copulation during later receptive periods.

Female mink appear as willing to remate when mating will induce an additional ovulation—after an 8 day interval—as when it will add sperm to that already present in their reproductive tract— after a 1 day interval (details in Fleming, chapter 1). They also appear as willing to remate a previous mate (familiar) as a new one (unfamiliar), although when both are present the day after females' previous matings, females more often mate the unfamiliar males. This is due, in part, to males being 'less interested' in remating females they mated the day before than unfamiliar females (Fleming, chapter 1). In this part of my study, I was interested in whether females that mate multiply with different males show an increase in fecundity relative to females that mate the same male repeatedly. In particular, I was interested in whether remating so as to induce a second ovulation and then mating again the next day with a unfamiliar male would increase a female's litter size or likelihood of having a litter relative to other combinations of mating number, mating interval, and familiarity.

I also replicated Johansson & Venge's (1951) experiments regarding mating order advantages when females mate with different males to fertilize the same set of ova. However, instead of using different strains of males with different coat colors, I used all standard dark mink screened and

chosen for allozyme polymorphisms prior to breeding. My reasons for this were two-fold. First, although Johansson & Venge (1951) reported that "there was no evidence of selective fertilization by spermatozoa from males of a certain color phase (p. 256)," they presented no results pertaining to this point and subsequent studies have shown fertility or 'semen quality' differences among various coat color varieties (Sundqvist & Sundqvist 1986). Second, color phases of mink differ in a number of characteristics other than fur color, including timing of breeding and mating behavior. For example, 'blue' varieties breed 1-2 weeks later (Jorgensen 1985) than standard dark mink which usually breed at approximately the same time as wild populations in the same geographic region (Dunstone 1993). Blue mink are also larger and the males are more aggressive when mating (personal observation). Thus, using all one coat color variety of mink removed the potential confounding effects of strain specific physiological and behavioral differences between males.

METHODS

I conducted my research on a private fur farm in western Washington in March 1989 and 1990 between dawn and dusk (700-1800 hours). Mink were maintained under standard fur farm conditions as approved by the Mink Farmers' Animal Welfare Board. Testing cages were built from polyvinyl-coated wire with 2.5 x 5 cm squares. Thirty cages (77.5 cm x 50 cm x 35 cm each) were connected in series by two 25 cm tunnels spaced 35 cm apart on the long side of the cage. Individual males were housed in every other cage in the series throughout the study and individual females

were introduced to the empty cages in between for each trial. The tunnels between cages were ~7 cm in diameter to restrict males to their home cage while the females, which were 33-50% the size of the males, had access to both. Thus, a female could choose to mate or not by remaining in or returning to her cage without having to fight persistent males. I opened and closed doors on the tunnels as needed to restrict female movement to only one or two males. I shut males in their nest boxes before females were placed in the next cage and allowed females to explore male cages and return to their own before releasing the males from their nest boxes.

Twenty-five 2 year old ('adult') females and 48 10 month old ('juvenile') females mated with 22 males (14 adults and eight juveniles) between March 4 and March 20. Trials began when males were released and ended either after copulation, if the pair began to fight, or after two hours if mating had not commenced. Males mated no more than twice per day. This was typical of fur farm practices and does not result in reduced male fertility or sexual interest (J. Adair, Oregon State University Experimental Fur Farm, personal communication).

After their first mating, I gave females the opportunity to remate after either a ~1 day (16-24 hours) or 8-10 day interval (1DI or 8DI, respectively). They were exposed to either the same male they had mated previously (familiar) or a different male (unfamiliar) or two males: one familiar, the other unfamiliar. In addition, I gave females that mated a second time after 8-10 days opportunities to mate a third time the following day. These third matings were also categorized as 1DI matings and female were presented with either the previous day's mate (familiar) or another male (unfamiliar) or

one of each. Matings fell into one of seven categories from the perspective of the female: 1) first matings (males unfamiliar by definition), 2) second 1DI matings the following day with familiar or 3) unfamiliar males, 4) second 8DI matings the following week with familiar or 5) unfamiliar males, and 6) third 1DI matings with familiar and 7) unfamiliar males.

Behavioral descriptions

Copulation duration was measured from intromission to withdrawal. Prior to intromission, male mink grasp the back of the female's neck with their jaws and grasp her torso just behind her forelegs with their forelegs. Intromission is characterized by an extreme arch of the male's back while in this position, such that his pelvis is almost under the female's, and the cessation of rapid, shallow thrusting seen prior to penetration. During copulation, pairs alternate between standing and lying on their sides. During the latter, sperm is thought to be transferred during a series of 4-10 deep thrusts (Enders 1952; J. Adair, personal communication). Withdrawal is recognized as a straightening of the male's back, moving his pelvis back away from the female's. Pairs typically separate within 10-15 seconds after this. If the male retained his hold and achieved intromission again, I recorded the total time spent in intromission as the pair's copulation duration.

I considered females as having mated if the interval between intromission and withdrawal was longer than 6 minutes and included a series of deep thrusts. Sperm are easily detectable in a sample of female

vaginal fluids after a 2 minute mating if 3-4 deep thrusts have occurred (M. Fleming, unpublished data).

Paternity determination

I collected blood samples from 80 females and 40 males in February 1989 to look for allozyme polymorphisms that could be used as genetic markers for paternity analyses. Mink were restrained by hand and a hind toenail was clipped to the quick. Blood was collected in 4-6 heparinized micro-hematocrit capillary tubes per animal, which were subsequently sealed at one end with Critoseal. Within 1-2 hours of being collected, tubes were spun at 3000G for 10 minutes in a portable electric micro-hematocrit centrifuge to separate red blood cells (RBCs) and plasma. I then scored capillary tubes with a file and broke them at the interface between RBCs and plasma. I transferred RBCs and plasma for each mink into separate Eppendorf tubes and kept them frozen on dry ice for up to 8-16 hours until they could be placed in a -70° C freezer.

Frozen samples were homogenized in extraction buffer and screened for enzyme polymorphisms using starch gel electrophoresis by Paul Aebersold, at the National Marine Fisheries Service in Seattle WA, using methods described in Aebersold et al. (1987). Samples were screened for 52 enzyme systems of which 35 were present in RBCs, plasma, or both. All but four loci, PDPEP, TAPEP, NP, and CK were monomorphic. The resolution of both NP and CK were poor and may have been improved by using a different buffer, but each locus appeared to have only two alleles and only three mink (2.5%) were heterozygous for NP, 4

(5%) were heterozygous for CK, and no mink were homozygous for the rarer allele. TAPEP had two alleles which resolved clearly, but only 10 mink (8.3%) were heterozygous and none were homozygous for the rare allele.

PDPEP, a leucine-proline peptidase, was the only locus that was sufficiently polymorphic to be used as a genetic marker for paternity. PDPEP had three alleles with mobilities of 100, 110, and 122. Forty-five of the mink (37.5%) were homozygous for the common allele, and four (5%) were homozygous for one of the rarer alleles. However, 12 (10%) were heterozygous for two of the rare alleles, leaving 59 (49.2%) heterozygous for the more common and a rarer allele.

I chose 60 females and 15 males for the study in 1989, including 31 females that were homozygous for the common allele and five males and 11 females that were homozygous or heterozygous for the rarer alleles. For thirty-one females (15 juveniles, 16 adult), I determined possible pairings of individual females with two or more males that would allow contribution of at least one of males to the litter to be established in advance. Because females were allowed to choose whether to mate a particular male or not and males were allowed to mate no more than twice per day, only 25 of these females mated as planned.

In 1990, I selected 17 females and six males from among the offspring from the previous year's matings because their allozymes had already been screened as part of the paternity analysis. The females were all homozygous for the common allele as were three of the males. The remaining three males were heterozygous for the two rare alleles.

Unfortunately, two of the heterozygous males proved to be sexually incompetent, mating none of the 6-9 females they were introduced to during the first week of the breeding season. Furthermore, one of the homozygous males only mated one female. These three males were replaced with three sexually competent juvenile males of unknown genotype. By chance, one of these males was heterozygous for the two rarer alleles and I could determine paternity in four litters.

The number of kits in a litter was counted within a day of birth. After kits had been separated from one another and placed in individual cages at about 15 weeks of age, blood samples were collected and screened as described above.

Analyses

I performed the analyses using Systat 5.2.1 for Macintosh (1992). Unless otherwise noted, means are presented with standard errors and tests were two-tailed.

RESULTS

Effect of females' mating pattern on litter size

Multiply-mated females had larger litters (independent t-test t = 5.648, df = 64, $p > 0.001$), both because singly-mated females were less likely to produce a litter and because the litters they produced were smaller (Table 3.1). When the number of kits per litter for females that mated once during the first week of the breeding season (March 4-8) were distinguished from those of females that mated once later (March 12-17) and multiply-

mated females, the difference between singly- and multiply-mated females (ANOVA $F = 6.813$, df = 56, p = 0.002) was attributable to the smaller litter sizes of females that mated once early compared to both multiply-mated females (Fisher's least significant difference test p = 0.001) and females that mated once later in the breeding season (Fisher's LSD test p = 0.036; Table 3.1). Females that mated once late had litter sizes similar to multiply-mated females (Fisher's LSD p = 0.484; Table 3.1). When barren females were included in the analysis (ANOVA $F = 17.894$, df = 62 , p = 0.001), the difference in the number of kits per mated female between females that mated once early versus late was no longer significant (Fisher's LSD p = 0.092) and multiply-mated females had significantly larger litters than singly-mated females of both types (Fisher's LSD; early: $p < 0.001$; late: p = 0.006).

The litter sizes of multiply-mating juvenile (n=37) and adult (n=18) females did not differ ($\bar{X}_{juveniles} = 6.03 \pm 0.51$, $\bar{X}_{adults} = 6.0 \pm 0.45$; independent t-test $t = 0.045$, df = 53, p = 0.964). Multiply-mating females tended to have smaller litters when they mated the same male twice consecutively, particularly when the two matings induced separate ovulations (Fig. 3.1). When females mated three times—a second time after an eight day interval and a third time the following day—litter sizes were similar regardless of the familiarity of the 8DI and 1DI males (Table 3.2).

The previous analyses do not include seven females that mated an apparently infertile male. The male was the only one of 18 involved in the paternity testing who never sired any kits. Because this male was heterozygous for the two rare alleles used as genetic markers of paternity,

another possible advantage of female multiple mating was tested unintentionally: whether female multiple mating could reduce the impact of mating with infertile males. Not surprisingly, the influence of matings with this male on litter size depended on the females' multiple mating pattern (Fig. 3.2). When one female mated the infertile male first and a fertile male induced the second ovulation, the female had a normal-sized litter. When the infertile male induced a second ovulation however, that female did not have a litter. When two females mated multiply around the second ovulation, mating the same infertile male twice resulted in only one female having a litter comprised of a single kit. When only one of the consecutive day matings was with the infertile male, one female did not have a litter and two others did, but both litters were small.

Effect of females' mating pattern on paternity

Over the two years, 29 females mated such that the paternity of their litters could be established unambiguously. Of these, the infertile male mated seven, another female was barren, another lost her single kit, and two other litters could not be located for blood draw. This left 18 litters for paternity testing; five from females that had mated once at two separate ovulations and 13 that had mated on consecutive days. Of the latter 13, only four litters were from females that had mated different males around a single ovulation. The remaining nine litters were from females that had mated different males around their second (and last) ovulation and thus, some of the kits could have been sired at their dams' first ovulation. The data from these litters were included in the analysis only after adjusting the

number of kits sired by each male by subtracting those that could have been sired by one of the males (or a male of the same genotype) mating at a previous ovulation (Table 3.3). In eleven of 18 litters (61.1%), not all kits born were available to have blood samples drawn either due to natural mortality or to their histories being lost during cross-fostering of kits from larger litters to smaller litters or transfers post-weaning to cages of 3-4 siblings and then, a month later, to individual cages. Ninety of the 122 kits (73.8%) born to these litters were ultimately located for blood draws.

When females only mated once per fertile period, significantly more of their kits were sired by the male that induced the last ovulation (paired t-test $t = 4.781$, df = 4, p = 0.009; Fig. 3.3). One litter (20%) was multiply-sired and the other four were sired entirely by the last male. In contrast, the first male sired more kits when females mated on consecutive days during their last fertile period, although the difference was not significant (paired t-test $t = 1.218$, df =12, p = 0.247; Fig. 3.3). Six litters (46.2%) were multiply-sired, five (38.5%) were sired entirely by the first male, and two (15.3%) were sired entirely by the second male. The proportion of multiply-sired litters sired by the first male (60.9%) was similar to the proportion of kits sired by the first male overall (Table 3.3).

Copulations around a female's last ovulation lasted between 30 -158 minutes. Litter sizes were not affected by either the duration of the mating that presumably induced ovulation (Linear regression $R^2 = 0.047$, n = 39, p = 0.19) nor the total amount of time females copulated over two days (Linear regression $R^2 = 0.001$, n = 26, p = 0.876). The proportion of kits sired by the second (and last) male to mate a female during her last fertile

period was not affected by the interval between the two matings or the length of the second copulation relative to the first (multiple regression; R^2 = 0.244, p = 0.247; Table 3.4).

DISCUSSION

Fecundity of singly- versus multiply-mated females

Multiple mating appears to increase fecundity of female mink over that of singly-mated females. Singly-mated females were less likely to have a litter regardless of when they mated during the breeding season, and when females that only mated once early had litters, they were smaller. These results are consistent with those of other studies of female fecundity in mink but are also difficult to draw conclusions from for the same reasons.

The singly-mated females in this, and most other studies (Hansson 1947, Friend & Crampton 1960, Maciejowski et al. 1973, Backus 1982, Park et al. 1988) were self-selected; they were females that were given the opportunity to mate multiply but refused. Thus, it is impossible to determine whether these particular females were simply less fecund than those that were willing to mate repeatedly or whether their potential fecundity was reduced because they only mated once. The phenomenon of reduced fecundity in singly-mated females, particularly due to increased numbers of barren females, is well-known on fur farms and is largely the reason that farmers pair females repeatedly. But precisely because farmers pair females repeatedly in attempts to have them remate, so-called 'mating system' effects cannot be distinguished from effects due to female differences in fecundity correlated with willingness to mate (Hansson 1947,

Enders 1952). Researchers have often added to the confusion by not distinguishing between conclusions drawn from retrospective studies (done by comparing records of female productivity after the fact) versus studies in which females were randomly assigned to particular mating exposure categories ahead of time.

One set of studies that not only randomly assigned females to single vs. multiply mating categories, but also took into account the timing of single matings (Elofson et al. 1989) suggests that mating early, not mating once, may be responsible for the high proportion of barren singly-mated females. Females that mated once or twice on consecutive days ('singly-ovulating' females) early in the breeding season were more often barren than females that mated later. This was particularly true of females in their first breeding season ('juveniles'), although the significance of this observation is unclear. Both Hansson (1947) and Enders (1952) pointed out that comparisons between age classes of fur farm mink are suspect due to the ubiquitous practice of pelting females that do not mate or that produce a small or no litter during their first breeding season. As a result, adult females are a highly select group of the most productive females. Elofson et al.'s (1989) findings concerning the litter sizes of singly-ovulating females that had litters are difficult to interpret for similar reasons. They found no effects of mating date on litter sizes of juvenile females mated once or twice on consecutive days, but a slight increase in litter sizes of adult females, such that adult females mating at the end of the breeding season had more kits than juvenile females.

Discounting possible age differences, Elofson et al.'s (1989) results suggest that matings early in the breeding season are less likely to result in litters than later matings, but the litters sizes of females that have litters do not differ. There are three possible explanations for the failure of early mating females to produce litters: failure to ovulate, failure of fertilization, or failure to implant.

There is no evidence that directly implicates failure to ovulate during early matings in females' failure to have litters. Enders (1952) and Adams (1981) agreed that females' first ovulations required surprisingly little stimulus to induce regardless of when matings were attempted: copulations or mountings as brief as 2-5 minutes are sufficient. Venge (1956) also observed no failure to ovulate among females mated for only 2-6 minutes late in the breeding season. Elofson et al. (1989) found that all females ovulated when mated twice on consecutive days early or late in the breeding season.

Copulation durations are relatively short (less than 30 minutes) early in the breeding season compared to later (1-3 hours; Fleming, chapter 2; Hansson 1947, Elofson et al. 1989). Venge (1956) found that copulations of 12 minutes duration deposited sufficient sperm to achieve fertilization throughout the breeding season, although he found more sperm present when copulations were 24 minutes long. Copulations of only 6 minutes duration could transfer as much sperm as 12 minute matings, but more often transferred much smaller amounts. Because the females with even the shortest matings ovulated and only small proportions of the sperm deposited actually reach the site of fertilization in most mammals, Venge

(1956) argued that insufficient sperm ejaculated during short matings, or possibly failure to stimulate sperm transport, could be responsible for barren females. Adams & Reitveld (1981) extended Venge's work by showing that females that mated a fertile male for 5 minutes followed or preceded by 15-20 minutes of copulation with an infertile male produced more litters than females that mated for only 5 minutes with a fertile male. Apparently, the quantity of sperm transferred in 5 minutes was sufficient to fertilize the females' ova, and Adams & Reitveld (1981) concluded that the added stimulation of a 20-30 minute copulation facilitated sperm transport.

Adams & Reitveld (1981) did not actually demonstrate that sperm transport did not occur after 5 minute copulations. Venge (1956) documented the rate of sperm transport (just past the cervix within 5 minutes, and throughout the oviducts within 30 minutes), but he did not determine whether longer copulation durations speeded the process. Prolonged copulation has been found to facilitate the initiation of pregnancy by stimulating progesterone production in rodents (Adler 1969; Matthews & Adler 1977). While progesterone production is stimulated by photoperiod in mink, it is possible that prolonged copulation could stimulate other physiological processes involved in the maintenance of blastocysts during delay. Elofson et al. (1989) reported fewer normally developing embryos prior to implantation in juvenile females who mated on consecutive days early in the breeding season (the group also most likely to be barren). After implantation, no fetuses were found in six of 15 females (four juveniles and two adults) that mated twice early compared to two of 17 females (one juvenile and one adult) that mated late (Elofson et al. 1989). These results

suggest that embryos from early ovulations may not always develop or implant properly, but whether this is due to problems with early ova, sperm, or the reproductive tract supporting these embryos early in delayed implantation is not known.

The most common explanation for the poor productivity of early mated females is the loss of blastocysts during delayed implantation (Enders 1952; Elofson et al. 1989). Litter size is negatively correlated with gestation length, and because the post-implantation gestation period of mink is relatively stable relative to the length of delay, the small litter sizes have been attributed to long delays. But because implantation is stimulated by increasing photoperiod around the vernal equinox, long delays are also correlated with early matings. When mating date was held constant, Elofson et al. (1989) reported a tendency for increasing litter sizes with shorter gestations. Embryo loss during delay is not the only possible explanation for this however; studies from other litter bearing species have shown a tendency for larger litters to be born after shorter gestation periods (van Tienhoven 1968). On the other hand, in a typical fur farm situation, loss of blastocysts may explain the perceived relationship between mating date and litter size (as opposed to the percentage of barren females). The increased handling stress involved in repeatedly pairing singly-mated females in attempts to remate them could induce blastocyst loss during delay (Daniel 1971) as could mounting and failed intromissions by several males.

The present study suggests another factor to be considered in evaluating the reasons for female failure to produce a litter: the quality of a

copulation (Dewsbury 1988, Eberhard 1996). Although not particularly short, three of the single matings by barren females (two adults early and one juvenile late) were notable in having only one or two deep thrusts associated with ejaculation with 10-20 minutes and two intromissions between. Because typical matings involve several thrusts per minute at intervals of 8-10 minutes during a single intromission, matings with few irregularly spaced thrusts may either introduce insufficient sperm to the female tract or fail to stimulate sperm transport or processes related to the support of blastocysts. The other two barren females (an early and a late mating juvenile) had seemingly normal copulations, as did the three early-mating females that had litters of only 1-3 kits (two adults, one juvenile).

Why do females mate early if earlier matings are likely to be non-productive? There is no compelling evidence that ovulating twice is advantageous in terms of female productivity over ovulating once later in the breeding season (Adair et al. 1988, Elofson et al. 1989). In the wild, female mink are solitary, intrasexually territorial, and possibly intersexually territorial as well (Gerell 1970), so if there is any question of more than one male being able to locate a particular sexually active female during the breeding season, it may be advantageous for her to mate with the first male that comes along rather than hold out and risk going unmated (Schwagmeyer 1984). If females are liable to get multiple mating opportunities, particularly late in the breeding season, mating a male early in the season may allow a female to evaluate his qualities as a mate for later, when matings are more productive ('copulation as courtship', Dewsbury 1988).

If female mink have multiple mating opportunities in the wild, the question of why male mink bother to mate females early in the breeding season is also valid, given that earlier matings are not only less productive but also quite likely to be negated if females remate more than a week later, expelling 86% of blastocysts in the uterus (Shackelford 1952; Adams 1981). The fact that copulations are shorter and probably transfer less sperm early in the breeding season suggests that males are not investing as much in early as later matings, so why mate at all? Males' investment in actually copulating with females early in the breeding season could be explained if returning mates are more likely to be accepted later in the breeding season than strange males due to prior courtship. Female fur farm mink were somewhat more likely to mate familiar males over unfamiliar males when given a choice between them eight days after their previous mating (seven mated familiar, four mated unfamiliar, but the difference was not significant (Fleming, chapter 2). However, this did not take into account the quality of the male's initial copulation as a factor in the female's decision.

Male mink could also gain some advantage from early copulations because by inducing females to ovulate, males can predict when she will be sexually receptive again. Unmated females remain receptive and have similar numbers of follicles ready to ovulate throughout the breeding season, meaning they can mate at any time (Elofson et al. 1989). Females that have mated ovulate their mature follicles and do not become receptive and capable of ovulating again for at least 6-8 days. Thus, males that locate an unmated female and mate her can induce ovulation quite easily and have the advantage over other males of 'knowing' when she will be

capable of ovulating again later in the breeding season when matings will be more productive. Males would not even have to waste any sperm on early matings, since the purpose would be to be the first male to return to a female when it is possible to supplant any of one's own blastocysts with new ones. The easily induced ovulations and short, sperm-poor copulations early in the breeding season could then be interpreted as results of conflict between the sexes over the number of mates a female should have and the timing of matings.

Fecundity of females that mated multiply in different patterns

Females did not differ significantly in fecundity regardless of the interval between matings or how many times they mated. These results are similar to those of other studies that have compared fecundity when female mink mated twice on consecutive days late in the breeding season versus at eight day intervals (Friend & Crampton 1960, Backus 1982, Elofson et al. 1989) ; and when consecutive day matings were females' second and third matings versus their first and second matings (Maciejowski et al. 1973, Park et al. 1988).

The familiarity of mates also did not affect fecundity in any of the above mating patterns. In a study that I became aware of only after the first year of my own, Park et al. (1988) recorded litter sizes and the incidence of barren females in a retrospective comparison of the effects of various combinations of mating number, interval, and number of different mates per female, including the combinations that I used. The number of barren females was higher when females mated the same male repeatedly than

when multiple sires were used, but Park et al. (1988) found no differences in the litter sizes of females that had litters. They attributed their results to the risk of secondary infertility in male dark mink. Removing all females that mated the apparently infertile male from the analysis in advance precluded my finding similar results: only one of the 55 remaining multiply-mating females was barren.

The infertile male in my study had sired kits in several litters the year before, meaning that he was suffering from 'secondary infertility' as described by Tung et al. (1984). Pelletier (1986) described the seasonal breakdown of the blood-testis barrier after the breeding season in fur farm mink which may be associated with increased autoimmune testicular disturbances that often accounts for secondary infertility. The purpose of the blood-testis barrier breakdown and whether it occurs in wild mink with the same potential effects on fertility are unknown. The degree to which permanent infertility occurs in wild animals has not been widely studied, but some authors have suggested that temporary male infertility via sperm depletion could be common (Dewsbury 1982; Birkhead 1991). Because mink engage in 1-3 hour copulations late in the breeding season and copulation duration seems to correlate with the quantity of sperm transferred (Venge 1956, Fleming, chapter 2), temporary male infertility could occur and multiple matings either with the same male (Lott 1981) or, more likely, different males could be a female strategy to ensure that at least one mating during her last fertile period is fertile.

Effects of mating order and interval on multiple paternity

As in Johansson & Venge's (1951) study, when different males mated the same female more than a week apart, the second male sired the majority of the litter. My results disagreed with theirs when different males mated the same female on consecutive days. They found that 37% of kits were sired by the first male, while I found that 62.8% of kits were sired by the first male. The critical difference between the studies may involve the definition of '1 day'. Two of the 13 litters in this study actually mated 42.5 and 47.5 hours apart, or 2 days—an interval at which Johansson & Venge (1951) found 73.2 % of kits sired by the first male. When these two litters are removed, however, 58.8% of the remaining kits were still sired by the first male. Johansson & Venge (1951) did not specify when females were mated on the following day in their study but implied that the interval between matings was close to 24 hours, whereas the females in this study remated 20-32 hours after their previous mating. Nonetheless, neither the five females that mated after a 20-25 hour interval nor the six females that mated after a 26-32 hour interval had more kits by the second male (kits by first male: 20-25 hour interval = 65.8% ; 26-32 hour interval =60.8%.

Johansson & Venge (1951) attributed their observed switch of a second to first male advantage when the interval between matings was longer than a day to Hansson's (1947) results suggesting that ovulation occurred about 36 hours after the first mating. They suggested that if ovulation occurred 12 hours after the second mating, the sperm from the second male would have time to reach the ova and would perhaps be more vigorous than the 36 hour old sperm from the first mating. But, if the second

mating occurred after ovulation, then the sperm from the second male would arrive too late to fertilize many of the ova. This explanation relies on a fairly regular interval between the mating that induces ovulation and the ovulation itself, while estimates of this interval are quite wide ranging (28-72 hours; Sunqvist et al. 1988). Ovulation can be advanced by copulation in a number of spontaneously ovulating species (Jochle 1975). So, it is possible that some of the variation in the timing of ovulation in mink is due to greater stimulation from long copulations hastening ovulations.

Copulation duration could also influence paternity by varying the amount of sperm deposited by a particular male. In this study, unlike others (Schwagmeyer & Foltz 1990, Huck in Ginsberg & Huck 1989), neither copulation duration or the interval between matings were helpful in predicting the percentage of a litter sired by a male. The varying interval between mating and ovulation could have obscured any relationship, but more importantly, the ranges of both variables observed may have been too restricted. Copulations were not interrupted, but allowed to progress until the pair broke up naturally, thus the copulation durations of the two males that mated each female were, for the most part, quite similar (Table 3.4). Similarly, because my intent was simply to give females the opportunity to remate on consecutive days, the intervals between matings were mostly between 20-32 hours (Table 3.4). Testing mink over a wider range of both of these variables would provide better information about their influences on paternity.

SUMMARY

Multiple mating in female mink has implications for the reproductive success of both females and males. Females that mate so as to induce a second ovulation or mate twice around a single ovulation late in the breeding season have higher reproductive success than females that mate only once. This effect is largely attributable to the larger number of singly-mated females that do not have litters, and the smaller litter sizes of those females that mate once during the first week of the breeding season. Comparing these results to those of other studies of fecundity in mink, I suggest that short, 'poor quality' matings that do not transfer sufficient sperm or stimulate the female sufficiently to transport sperm or maintain blastocysts are more likely explanations for the high incidence of barren females than failure to ovulate.

The litter sizes of multiply-mated females that mated fertile males did not differ significantly depending on the number of matings, interval between matings, or the number of different mates a female had. Most incidences of barren females or females with small litters occurred when females mated only once or mated the same male twice during their last fertile period. These incidences were attributable to the females' last mate being a single infertile male and demonstrates that mating different males during the last fertile period is effective in counteracting the negative effects of mating with an infertile male.

As in a previous study, when different males mated a female a week apart, there was a clear second male advantage attributable to the expulsion of blastocysts sired by the first male during the mating that

induces the second ovulation. Unlike previous studies which reported a second male advantage for consecutive day matings, I found a first male advantage when two males mated during a single fertile period. The first male advantage persisted whether the interval between matings was 20-25 hours, 26-32 hours or 42-48 hours. The explanation for the first males' greater success at the shorter intervals is unclear, but one factor that must introduce considerable variability between litters is the variability in how quickly ovulation is induced. I did not find a relationship between paternity and the copulation duration of the two males that mated a female or the interval between their matings. The ranges of natural copulation duration differences and of intervals allowed between the two males' matings may well have been too small to detect a relationship, however.

Table 3.1: Reproductive performance of singly- and multiply-mated females. Singly-mated females were subcategorized as mating early (March 4-8) or late (March 12-17) in the breeding season. Multiply-mated females were subcategorized by the number of times they mated (2 or 3) and the intervals between matings (1 or 8 days, or both).

		# female	# w/	% w/out litter	kits / female (mean±SE)	kits / litter (mean±SE)
singly-mated		11	5	45.5	2.0 ± 0.76	3.67 ± 0.95
(time in season)	early	6	3	50	1.0 ± 0.52	2.0 ± 0.58
	late	5	2	40	3.2 ± 1.46	5.33 ± 1.2
multiply-mated		55	1	1.8	6.02 ± 0.28	6.13 ± 0.26
(interval between matings)	1	10	0	0	6.3 ± 0.73	6.3 ± 0.73
	8	13	0	0	6.08 ± 0.6	6.08 ± 0.6
	8, 1	32	1	3.1	5.9 ± 0.36	6.1 ± 0.31

Table 3.2: Litter sizes of females that mate a second time after an eight day interval and a third time the following day. Familiarity/mating interval categories are designated with three letters; the first two letters are separated by a dash to indicate the 8DI, the last two letters are adjacent to indicate the 1DI, and each male is represented by a letter (e.g., A-BB refers to females that mated an unfamiliar male after the 8DI and remated him the next day). Two-way ANOVA, 28 df ; familiarity of 8DI male, $F = 1.496$, $p = 0.23$; familiarity of 1DI male, $F = 0.661$, $p = 0.42$; interaction, $F = 2.718$, $p = 0.11$.

	Familiarity / mating interval categories			
	A-AA	A-BB	A-AB	A-BC
kits / female (mean ± SE)	6.5 ± 0.71	4.4 ± 0.89	5.89 ± 0.67	6.2 ± 0.62
# of females	8	5	9	10

Table 3.3: Ratios of kits sired by the first vs. second male to mate 18 females either eight days apart, on consecutive days around a single ovulation, or on consecutive days around a second ovulation (females' second and third matings).

	2 matings, 2 ovulations	2 matings, 1 ovulation	3 matings, 1 ovulation	3 matings, 1 ovulation (adjusted*)
	0 : 2	4 : 1	4 : 0	4 : 0
	0 : 6	2 : 0	3 : 0	3 : 0
	0 : 5	0 : 5	2 : 1	2 : 0.75
	0 : 5	6 : 0	4 : 0	3.67 : 0
	2 : 4		0 : 6	0 : 6
			3 : 4	2.42 : 4
			6 : 1	5.42 : 1
			2 : 2	1.67 : 2
			5 : 3	4.34 : 3
Totals	2 : 22	12 : 6	29 : 17	26.5 : 16.75
% from first male	8.3%	66.7%	63%	61.2%

* The number of kits attributable to the male with the same genotype as the female's first mate adjusted by 8.3%, the percentage of kits likely to be sired at the female's first ovulation.

Table 3.4: Percentage of 13 females' litters sired by the second male to mate during each female's last fertile period, as well as her litter size, the percentage of her total copulation duration over two days accounted for by the second male, and the interval between in the two matings.

Litter size (# of kits)	% of litter sired by 2nd male *	% of total copulation duration by 2nd male	Interval (hours) between matings
3	0	39.5	20.5
4	0	48	21
6	100	58	21
4	0	68	24.5
3	27	42	25
7	16	51	26
5	100	42	29
7	62	43	29
4	54	60	29
2	0	48	30
5	20	40	32
6	0	54	42.5
8	41	65	47.5
4.92 ± 0.50 kits	32.3 ± 10.2 %	50.65 ± 2.68 %	29 ± 2.36 hrs

* From numbers of kits adjusted for the 8.3% that could have been sired by a mating at a previous ovulation.

Figure 3.1: Litter sizes (mean ± SE) of females mated two or three times with the same male each time or different males at intervals that resulted in either one or two sets of ova being ovulated. Sample sizes are shown in the bars.

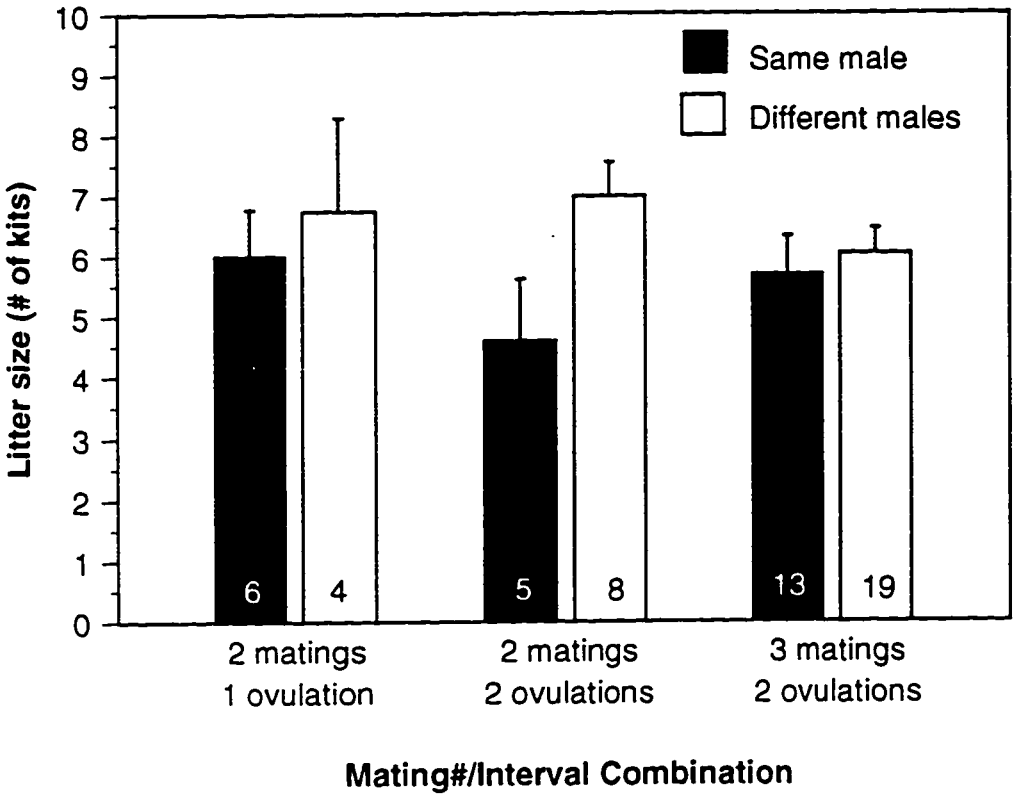

Same male
Different males
Litter size (# of kits)
10
9
8
7
6
5
4
3
2
1
0
6
4
5
8
13
19
2 matings
1 ovulation
2 matings
2 ovulations
3 matings
2 ovulations
Mating#/Interval Combination

Figure 3.2: Litter sizes (mean ± SE) of females mated with fertile males and/or an infertile male twice eight days apart ('1 Mating at 2nd ovulation') or on consecutive days ('2 Matings at 1 ovulation'). Dark bars also indicate whether the infertile male induced the 'First' or 'Last' ovulation in the former situation, or whether the female mated the infertile male twice ('Same') or only once ('Different') in the latter. Sample sizes are shown in the bars.

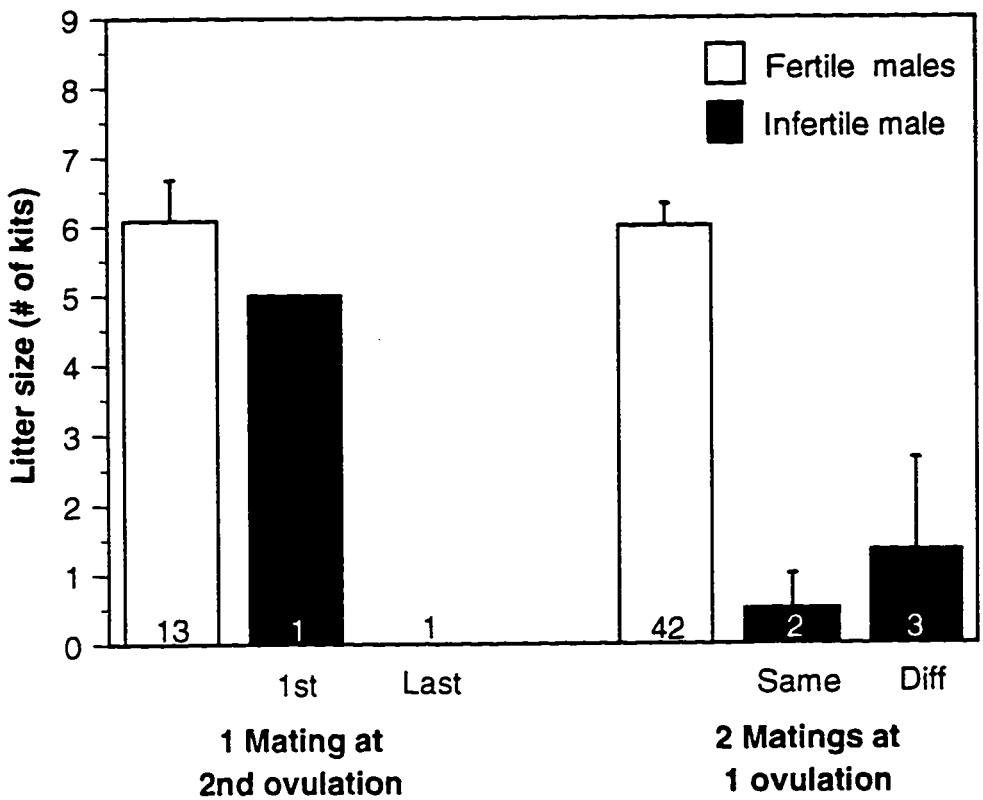
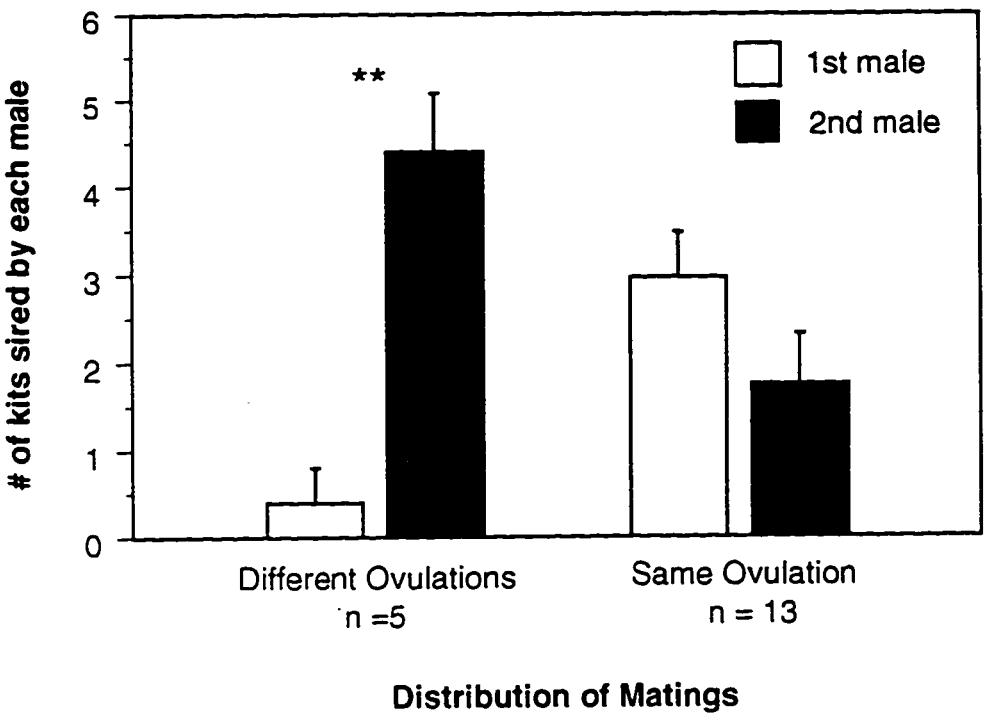

Litter size (# of kits)
9
8
7
6
5
4
3
2
1
0
Fertile males
Infertile male
13
1
1
42
2
3
1st
Last
Same
Diff
1 Mating at
2nd ovulation
2 Matings at
1 ovulation

Figure 3.3: Number of kits (mean ± SE) sired by the first and second male to mate when there were either eight or one day intervals between the females' matings. Sample sizes are below the bars.
** $p < 0.01$.


6
5
4
3
2
1
0
**
1st male
2nd male
# of kits sired by each male
Different Ovulations
n =5
Same Ovulation
n = 13
Distribution of Matings

CHAPTER 4:

Multiple mating and timing of breeding in *Mustela vison evagor*

INTRODUCTION

Reproductive physiology and behavior of the American mink (*Mustela vison*) have been studied extensively in fur farm populations established in the 1920-50s (Sundqvist et al. 1988). Seasonal changes in photoperiod are known to stimulate the onset of breeding in late February and March (Hammond 1951, Duby & Travis 1972, Boissin-Agasse & Boissin 1985) and embryo implantation shortly after the vernal equinox (Allais & Martinet 1978). Females will accept matings repeatedly during a 1-5 week period of delayed implantation and there is some evidence that multiply-mated females are more likely to produce a litter (Hansson, 1947, Enders 1952, Park et al 1988, Elofson et al. 1989). The extent to which reproduction in fur farm mink is similar to that in wild mink is unclear.

Little is known about reproduction in wild mink because these solitary and largely nocturnal carnivores are not easily observed in the wild. No studies have documented female multiple mating or delayed implantation in native, wild mink, but others suggests that there may be greater flexibility in the timing of the breeding season in wild populations than is reported for fur farm mink (Sundqvist et al. 1988) or introduced populations in Europe and Asia (Dunstone 1993). To investigate these aspects of reproductive biology in wild mink, I conducted a study of captive *Mustela vison evagor*, a subspecies endemic to Vancouver Island, British Columbia, Canada.

In species that have broad geographic distributions, the reproductive seasons of different populations may vary with local conditions (Bronson 1988). Among temperate zone populations, the correlation between latitude and the onset of breeding seasons may be mediated by changes in photoperiod (Bronson 1989). Near the southern extent of their range (~30° N latitude), wild mink in North America have been reported to breed as early as January (Svihla 1931) and give birth in March (Hamilton 1948, Humphrey & Zinn 1982). Near the northern extreme (~60-66° N latitude), however, they breed as late as April and give birth in early June (Harbo 1958, Burns 1964). One exception to this pattern is a population of mink (*M. v. evagor*) on the west coast of Vancouver Island, B.C. (49° N latitude), which breeds in late May and early June (Hatler 1976), although mainland populations of mink at similar latitudes breed in March (Mitchell 1961, Eberhardt 1974, Whitman 1981, Askins & Chapman 1984). Hatler (1976) speculated that late breeding in *M. v. evagor* might be an adaptation to local conditions including unfavorable spring weather and summer peaks in food abundance.

Hatler's (1976) study of *M. v. evagor* also provides some circumstantial evidence for both multiple mating and delayed implantation in wild mink. He reported male *M. v. evagor* grouping near a mating pair and pursuing females that had recently mated. He also speculated that female *M. v. evagor* multiply mate after he observed neck wounds on females during the breeding season which may have resulted from the neck bites of many males. Hatler was unable to measure gestation length for individual females, but he suggested that the observed interval between the

height of mating activity and of births could include a 10-15 day period of delayed implantation.

In fur farm mink, the period of delayed implantation includes cyclical periods of multiple mating (Hansson 1947). After their initial mating induces ovulation, female mink will multiply mate with the same or a different male during the 48 hour period between the first coitus and ovulation (Hansson 1947, Enders 1952). Females then experience a 1-5 week period of delayed implantation during which they again become receptive, remate, and ovulate at approximately 8-day intervals (Hansson 1947). Delay of implantation ends shortly after the vernal equinox (Allais & Martinet 1978) whereupon females give birth to litters in late April or early May (Hansson 1947; Enders 1952). Single litters may contain kits that were sired weeks apart, but 85% of kits result from copulations around the last ovulation (Johansson & Venge 1951, Shackelford 1952).

My study had two goals: first, to validate the report of late breeding in *M. v. evagor*, and second, to determine whether female *M. v. evagor* will show a pattern of multiple mating similar to fur farm mink and suggestive of delayed implantation. To reduce the potentially inhibiting effects of stress on reproduction (both physiological and behavioral aspects), I handled the animals as little as possible and restricted my study to behavioral measures of sexual activity. To determine the breeding season of *M. v. evagor*, I compared male sexual interest, female avoidance and aggressive behavior, and successful mating in fur farm mink and *M. v. evagor* during their reported breeding seasons (March and May/June, respectively). Because both sexes may not be identically responsive to environmental

cues (Bronson 1988), I tested the boundaries of the breeding season in each population by pairing them with individuals from the other population. To determine whether wild females will multiply mate, I provided female *M. v. evagor* with opportunities to mate 2-4 times and compared the timing of their acceptance of multiple males to that of fur farm mink. In addition, I recorded mating dates and gestation lengths for 4 years to confirm the timing of breeding and to look for evidence of delayed implantation.

METHODS

I captured three female and five male *M. v. evagor* (wild mink) in October and November 1989 along Barkley Sound on Vancouver Island, B.C. (~49° N latitude). I captured an additional wild male and pregnant wild female in June 1990. In January of 1990, I obtained two male and two female mink from a local fur farmer (hereafter called 'domestic' mink) in western Washington (~47° N) and relocated all the animals to a private mink farm (~46° N) where they were maintained under conventional fur farm conditions.

The mink were housed in a covered, open-sided enclosure which permitted natural changes in temperature and photoperiod. Individual mink were housed in cages of polyvinyl-coated wire, 77.5 cm by 50 cm by 35 cm, and provided with a fiberglass nest box (30 cm by 20 cm by 25 cm) with pine shavings and wool fabric for bedding. The mink were fed a diet of unground marine fish, beef liver, and chicken. Each animal was fed daily in late afternoon and water was provided ad libitum.

Comparison of breeding season in wild and domestic mink

To reveal any differences in the timing of mating behavior in wild males and females, I paired wild (W) and domestic mink (D) in four combinations: D X D, D X W, W X D, and W X W (male X female). These pairings took place during the height of the domestic mink breeding season, between 10-20 March 1990, and over a longer period likely to encompass the wild mink breeding season, between May 16-June 21, 1990. In March, pairings involved two of the wild males, two of the wild females, and the four domestic mink. In May/June, the same animals were used, but one wild male was injured so I replaced him with a third male. In June, I also tested a third wild female and two more wild males that had previously been in quarantine. Thus, in May/June, I used a total of three wild females and five wild males to generate 11 W X W pairings, five D X W pairings, and four W X D pairings in addition to the four D X D pairings. Eight weeks prior to the experiments, the two domestic males were vasectomized under Vetalar anesthesia (Ketamine HCl; 100 mg/ml; Parke-Davis, Morris Plains, NJ; dosage delivered to effect) to insure that they would not impregnate the wild females.

The pairings took place over 4 days in March and in May/June such that each female was paired with four different males for 2-4 hours (unless fighting occurred) each month. I introduced males to females' cages to minimize stress to wild females and to mimic the natural situation (males are reported to roam during the breeding season in search of the territorial females; Hatler 1976). I transported wild males by coaxing them out of their nest boxes (typically by blowing on them) and into carry cages. As wild

males began to associate the carry cages with mating opportunities, they entered them readily.

I identified male sexual interest as the occurrence of "chuckling," a low, repetitive vocalization known to indicate sexual excitement in domestic mink (Enders 1952) and "grabbing," in which the male grasps the nape of the female's neck with his jaws, the preliminary step in positioning the female for mating. Female interest is more difficult to quantify behaviorally and there are no reliable physiological indicators of receptivity during the breeding season (Travis *et al.* 1978). Thus, I recorded non-receptivity, defined as aggressive face-biting by the females, resulting in males' turning away to protect their faces. I defined successful mating as intromission lasting for at least 6 minutes as described by Adams (1981) accompanied by at least 2-4 deep thrusts thought to be associated with sperm transfer (Enders 1952, J. Adair, Oregon State University Experimental Fur Farm, personal communication).

Onset of breeding season and gestation lengths of wild mink

To determine more precisely the onset of the breeding season of *M. v. evagor*, I paired wild mink between March and June 1991-93 and recorded the date of first mating for seven females. Three of the females had been involved in the previous experiment, the fourth was captured pregnant in June 1990, and the remaining three were her offspring, raised in captivity. I introduced the seven males (the five from the previous experiment, the male captured in June 1990, and a male from the litter born in 1990) to females every few weeks in March and/or April and every few

days in May and/or June. In 1991, I delayed pairing the wild mink until May (the predicted height of sexual activity) because I was concerned that the poor breeding success of the wild mink in 1990 might be due to the stress of frequent pairings too far in advance of the actual breeding season. Thereafter, I gradually started pairing earlier each year to pinpoint the onset of breeding.

Because females multiply mated, I measured gestation length from both the first and last mating to parturition. I determined date of birth by listening daily for the squeals of kits in the nest box of each female.

Multiple mating by female wild mink

The occurrence of multiple mating in wild mink was determined by pairing males and mated females repeatedly in 1990-93. After their first mating, I gave wild females opportunities to remate during known receptive periods in domestic mink, i.e., within 48 hours and 8-10 days later on consecutive days. I was not able to pair all females at all mating opportunities in each of the three years, but I tested all but two of the females at each interval in at least two of the years. One year, I also paired females 3-4 days after their first mating, when domestic mink are typically nonreceptive (Hansson 1947).

I could not statistically analyze differences in the behavior of wild and domestic mink of both sexes during March and May/June due to the small number of subjects involved. Thus, the results presented are purely descriptive. I used Wilcoxin signed-ranks tests to compare wild females'

receptivity to initial and multiple matings, and to multiple mating opportunities during and outside the receptive periods for domestic mink.

RESULTS

Comparison of breeding season in wild and domestic mink

In both wild and domestic male mink, the first stage of sexual activity (grabbing) was always preceded by a display of interest (chuckling). During their March breeding season, male domestic mink chuckled in response to every introduction to domestic females and two of their introductions to wild females (Figs. 4.1a, b). D X D pairings in March always involved grabbing as well as chuckling, while only one of the D X W pairings did (Figs. 4.1a, b). Although male domestic mink also chuckled and grabbed females upon introduction in May/June (Figs. 4.1a, b), they released them without attempting intromission regardless of the females' response. In contrast, male wild mink showed no sexual interest toward either female domestic or wild mink in March (Figs. 4.1c, d). Sexual interest by male wild mink was higher during May/June, toward both wild and domestic females (Figs. 4.1c, d).

Mating acceptance by females most clearly delineated the difference in breeding season between the wild and domestic mink. The two domestic females mated the same domestic male in March and two wild females mated three wild males in May/June. Neither female domestic nor wild mink were aggressive towards males in March (Figs. 4.2a-d). In May and June, female domestic mink became increasingly aggressive towards males (Figs. 4.2a, c) and interactions between domestic mink became so

violent that pairs had to be separated to prevent injury. Contrary to expectations, female wild mink were also more aggressive to both wild and domestic males during May/June (Figs. 4.2b, d). However, in pairs involving wild females, fighting never escalated to the extent that pairs had to be separated. Wild females tended to flee after the initial confrontation and avoid the male thereafter, rather than engage in a prolonged fight.

Onset of breeding season and gestation lengths of wild mink

The earliest first mating by a female occurred on April 22 and the latest on June 19 (Fig. 4.3). Therefore, the mating season of the wild mink began at least 1 month after the end of the mating season of domestic mink. In 1991-93, six of seven females mated each year and each female mated in at least two of the three years. Six litters were produced by four of the wild females between June 29 and July 19. Gestation lengths ranged between 43-55 days from the date of the female's first mating and 41-45 days from the date of her last mating (Fig. 4.3).

Multiple mating by female wild mink

Figure 4.4 shows the mean percentage of mating opportunities accepted initially (a female's first mating) and after three intervals (remating opportunities) between 1991-93 by seven females. Because I did not test all females at every mating interval every year, I calculated the acceptance rate for each female for each interval as a percentage to represent the 3 years combined (e.g., a female that accepted a multiple mating opportunity after an 8 day interval in 1991, but not in 1992 or 1993, had an acceptance

rate of 33%) and used these percentages in subsequent analyses. I derived the mean percentages in Figure 4.4 by averaging the percentages for each female. Also, I based the acceptance rates on each female's first opportunity to mate at a particular interval each year because, as time permitted, I gave some females multiple opportunities to accept a mating at a particular interval during a single year. Thus, while females only accepted 31.9% of first mating opportunities, repeated introductions insured that 85.7% of females mated at least once every year. Because I conducted introductions prior to the hypothesized breeding season to determine its onset, the acceptance rate for first matings included only introductions that were made less than a week before the actual first mating date.

During the four years of the study only one female never multiply mated; the remaining six females mated both on consecutive days and at 8 day intervals but also rejected mating opportunities at both of these intervals. Females were just as likely, if not more likely, to mate multiply within 1-2 days of or >8 days after a previous mating as they were to mate the first time (Fig. 4.4). On the other hand, six of seven wild females rejected males during introductions that took place 3-4 days after their initial mating (putative "mid-cycle," when domestic females are nonreceptive), even though three of those females (and the one that mated) remated 4-6 days later (8-10 days after their initial mating). Females were significantly less likely to remate 'mid-cycle' than on consecutive days (Wilcoxin signed-rank test $W = 2$, $P < 0.05$) or at a >8 day interval (Wilcoxin signed-rank test $W = 1.5$, $P < 0.05$; Fig. 4.4).

DISCUSSION

This study demonstrates that, under identical conditions of diet and photoperiod, the mating season of *M. v. evagor* begins more than one month after the mating season for domestic mink. Sexual interest of male mink in both populations is potentially overlapping, with the interest of the wild males increasing as the interest of the domestic males decreases. Male domestic mink initiated sexual behavior in May/June as well as March, but failed to mount or achieve intromission. On the other hand, female behavior strongly delineated the boundaries of the mating season. Domestic females tolerated male advances only in March, and wild females, only in May/June.

The increased aggression of domestic females in May/June may be explained by female intolerance of male attention outside the breeding season. However, wild females were also more aggressive in May/June (their supposed breeding season). One possible explanation for the wild females' behavior in both months may relate to their enduring furtiveness during their first year in captivity. While wild males became bolder in spending more time outside their nest boxes in May/June, wild females remained secretive. Wild males also began to show sexual interest in wild females in May/June. Because copulation attempts inevitably brought pairs outside of the nest boxes (there was not sufficient room within), males' increased persistence and females' furtiveness may have led to more conflicts between the sexes in May/June that females could no longer resolve simply by fleeing from males.

Although the breeding season of *M. v. evagor* is shifted relative to that of domestic mink, its timing still appears to be under photoperiodic control. *M. v. evagor* exhibit a similar shift in timing of fur growth which is also associated with photoperiod changes in domestic mink. In domestic mink, the 'spring molt' occurs after breeding: in late April and early May (Duby & Travis 1972). In the present study, the loss of winter fur also became apparent in the captive wild mink post-breeding—in July. Hatler (1976) described 'summer molt' in wild *M. v. evagor* as peaking in July for males and in late May for females.

From 1991-1993, the breeding season began progressively earlier each year (Fig. 4.3), but this apparent shift may have been due, in large part, to our delaying pairing the animals until May 1991 and then only gradually attempting more March and April pairings in 1992 and 1993. It is interesting to note that the shift in onset of breeding was not closely tracked by a shift in parturition dates; successful breeders were those that either mated for the first time or remated in late May and early June and births remained concentrated in July (Fig. 4.3).

One of the effects of delayed implantation on domestic mink is to restrict the birth season to approximately two weeks while the mating season may be four or five. This is because all females are stimulated to implant by the same photoperiod cue, regardless of whether they first mated four days or four weeks before (Allais & Martinet 1978). Thus, the greater variation in gestation length as measured from the date of first mating as opposed to the date of last mating (43-55 vs. 41-45 days, respectively) is

also typical of domestic mink when the last mating is shortly before the termination of diapause.

Although I have no direct evidence for delayed implantation in *M. v. evagor*, the multiple mating pattern I observed is perhaps most readily explained by its presence. Not only did wild females mate two or three days in a row, but they experienced another peak in receptivity after 8-10 days like domestic females. In domestic mink, both female multiple mating and subsequent ovulations ('superfetation', Shackelford 1952) are made physiologically possible by delayed implantation. In most mammals, new follicles could not mature and additional ovulations could not be induced because progesterone from the corpora lutea of the ovary would inhibit pituitary production of the hormones that stimulate both follicle development and ovulation—FSH and LH, respectively (Knobil & Neill 1988). During delayed implantation, the mink ovary does not produce sufficient progesterone during delay (Canivenc & Bonnin-Laffargue 1967 in Sundqvist et al. 1988) so the pituitary-gonadal axis continues to function as though the female was not pregnant.

The fact that one wild female also mated during the putative mid-cycle period does not preclude *M. v. evagor* from having an ovarian cycle similar to domestic mink. Enders (1952) paired mated mink repeatedly at 0-16 day intervals but had difficulty identifying the cycle that Hansson (1947) reported because some 10-13% of females remated 3-6 days after their initial mating, during what was subsequently identified by Shackelford (1952) as a period of follicular development.

Relevance of comparisons between wild and domestic mink

Although *M. vison* is native to North America, much of the information on mink behavior in the wild has been gathered from feral domestic populations in the British Isles, Sweden, Norway and Western Europe, and introduced and feral populations in Eastern Europe, Finland and Russia (Dunstone 1993). Because information on the ecology and behavior of feral domestics in Europe is consistent with that from studies of native wild mink in North America, information on reproductive physiology from fur farm populations has also been widely assumed to be similar to that of wild mink (Linscombe et al. 1982, Eagle & Whitman 1987, Dunstone 1993).

Mink farming was firmly established in North America by the 1920's (Hodgson 1945). The fact that variable gestation lengths (Hodgson 1931) and female receptive periods at weekly intervals (Patton 1925, Hodgson 1945) were considered typical of mink early in the process of domestication suggests that these aspects of mink reproduction are not simply products of domestication. Similarly, the fact that split litters were observed early in the development of mutation coat colors (Shackelford 1952) suggests that the mechanisms allowing for superfetation are not recent mutations and are quite likely to occur in native wild mink as well. Furthermore, intensive inbreeding is unlikely to be responsible for these phenomena as it is far from certain that inbreeding was an issue in mink farming prior to 1950 (Shackelford

1950), and the studies scientifically documenting these aspects mink reproduction were done prior to this.

Shackelford (1950) reported that domestic mink were derived from most if not all of the 11 subspecies recognized in North America and that it was common practice for farmers to introduce wild-caught mink into their herds. The development of 'mutant color phases' in the 1930's and 1940's further decreased the tendency for excessive inbreeding by 1) increasing the transfer of mutant mink between farms and 2) by actually increasing the influx of wild mink into the fur farm population via attempts to develop other, lighter coat colors (Shackelford 1950).

Adaptive significance of multiple mating and delayed implantation in mink.

From his observations of *M. v. evagor*, Hatler (1976) hypothesized that the short variable delay of implantation in mink functions to prolong pregnancy, thus allowing females more time to recover from the rigors of the mating season before experiencing the rigors of lactation. A 1-5 week extension of pregnancy would only provide the needed rest, however, if females were not attracting males during that time. If, as suggested in the present study, females are receptive to multiple mating during the delay period, then delayed implantation would actually be encouraging the prolonged male attention that it was supposed to be ameliorating.

The period of delayed implantation in domestic mink is quite short compared to the months' long delay reported for other mustelids, e.g., long-

tailed weasel, *Mustela frenata*, 7-9 months (Wright 1948) and western spotted skunk, *Spilogale gracilis*, 6-7.5 months (Mead 1968). Most hypotheses for the adaptive significance of delayed implantation invoke the advantages of being able to mate at some more favorable time (in terms of food or weather) than one gestation length before the optimal birth season (e.g., Fries 1880, Lack 1954; reviews in Mead 1989b, Sandell 1990). In temperate climates, this optimal birth season is argued to be as early as possible in the spring to allow the longest possible time for the young to grow and gain experience under favorable conditions. However, applying this hypothesis to mustelids with a short delay of implantation is difficult: why should March be a better breeding season than April for mink that give birth in May? It is even more difficult to understand why late-breeding *M. v. evagor* would delay implantation so that offspring conceived in May are born in July rather than June.

Addressing the problem of delayed implantation in mammals in general, Sandell (1990) proposed that delayed implantation evolved specifically so that the breeding season could occur at the most favorable time for female mate choice, i.e., when male movement was easiest. Considering solitary carnivores specifically, Stenson (1985) pointed out that the potential difficulty of locating widely-dispersed mates should favor the evolution of a long breeding season. Suspension of embryonic development coupled with photoperiod control of implantation would allow the breeding season to be extended without extending the birth season, meaning that the offspring of later-breeding females would no longer be at a competitive disadvantage. Taking into account the unusual physiology of

female mink, a combination of Sandell's and Stenson's ideas may apply: delayed implantation prolongs both the breeding season and the opportunity for mate choice for mink.

Regardless of the timing of the breeding season and the duration of delayed implantation, having a prolonged period of receptivity could be advantageous when females are solitary and widely-dispersed, as in mink. Not only would prolonging the mating season insure an encounter with a male, but having additional ovulations and a paternity advantage to males mating during a females last periovulatory period could allow female mink to exercise a strategy termed "fertilization assurance/exertion of preference" by Schwagmeyer (1984). Female mink could mate indiscriminately early in the breeding season to insure fertilization, but then exert post-copulatory mate choice by discriminating among any males they might encounter thereafter. If other mustelids with delayed implantation (32 species) typically arrest embryo implantation without disrupting the ovarian cycle for a time, then the reproductive physiology for multiply mating and mate choice may be available to them as well. At present, there are only scattered reports of prolonged periods (>2 weeks) of multiple mating in other mustelids with delayed implantation (*Martes americana*—Enders and Leekley 1941;*Lutra canadensis*—Woolington 1984; *Martes sp.*—reviewed in Mead 1994) and no reports of multiple ovulation during the delay period.

From this study, I am reluctant to draw any conclusions about differences in *M. v. evagor* females' willingness to mate so as to induce initial or later ovulations. If anything, females appeared more likely to remate after 8 days than they had been to mate initially (64.1% vs. 31.9%),

but with the small sample size (seven females and seven males), this difference was not significant. But because one goal of the study was to determine the onset of breeding, the seeming reluctance of some females to mate initially could be attributable to their not being sexually receptive at the time of pairing.

In conclusion, *M. v. evagor* breeds later than domestic and other wild populations at the same latitude (Enders 1952, Mitchell 1961, Eberhardt 1974, Whitman 1981, Askins & Chapman 1984) and mates multiply in a pattern indicative of delayed implantation in domestic mink. The physiological changes underlying the shift in breeding season, and possibly in photoperiod-stimulated implantation, remain to be determined. Confirmation of delayed implantation must also wait for studies with larger sample sizes (so that variation in gestation length could be noted in singly mated mink, i.e., Hansson 1947) or that are more invasive (to record multiple ovulations, i.e., Shackelford 1952). Finally, I suggest that any advantage to short-term delayed implantation for mink may be associated with prolonged, cyclical receptivity during the delay period, not with the timing of breeding.

SUMMARY

Wild American mink (*Mustela vison*) show latitudinal variation in breeding season. One exception is a population (*Mustela vison evagor*) from Vancouver Island, British Columbia, Canada that mates 2-3 months later than other mink populations at similar latitudes. Multiple mating during delayed embryonic implantation is typical of domestic mink, but has not

been reported for wild populations. I compared the breeding season and mating behavior of captive wild *M. v. evagor* with domestic mink by pairing animals within and between populations from March through June. Multiple mating was identified by repeatedly pairing females after mating. Dates of matings and births were recorded over 4 years. Sexual interest of male domestic mink declined between March and June, during which time sexual interest of wild males increased. Similarly, female domestic mink accepted mates only in March, while wild female mink mated 1-3 months later. Gestation length in wild mink ranged from 41-55 days. Female wild mink multiply mated and the pattern of receptivity was similar to that of domestic mink. Variable gestation length and multiple mating pattern were consistent with the presence of delayed implantation in *M. v. evagor.*

Figure 4.1: Percentages of males showing sexual interest, measured as chuckling and grabbing, in pairings of (a) domestic males and domestic females, (b) domestic males and wild females, (c) wild males and domestic females, and (d) wild males and wild females ($n = 4$ except where noted above the bars). Because male chuckling always preceded grabbing, percentages of both behaviors are shown together. Asterisks indicate the timing and number of males mating (one per mated male).

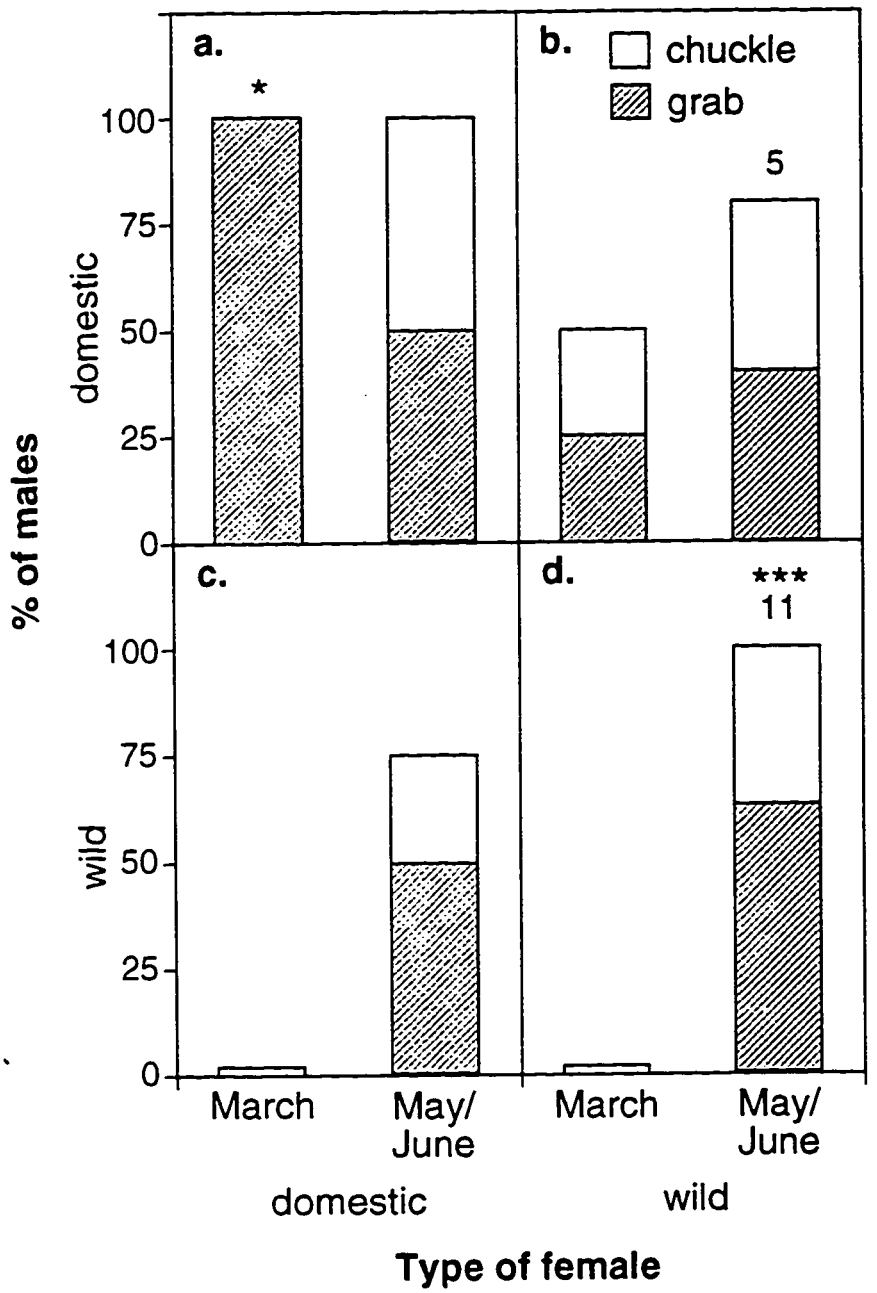

a.
b.
chuckle
grab
*
5
c.
d.
***
11
100
75
50
25
0
domestic
wild
% of males
March
May/
June
March
May/
June
domestic
wild
Type of female

Figure 4.2: Percentages of females showing aggressive behavior in pairings of (a) domestic males and domestic females, (b) domestic males and wild females, (c) wild males and domestic females, and (d) wild males and wild females ($n = 4$ except where noted above the bars). Asterisks indicate the timing and number of females mating (one per mated female).

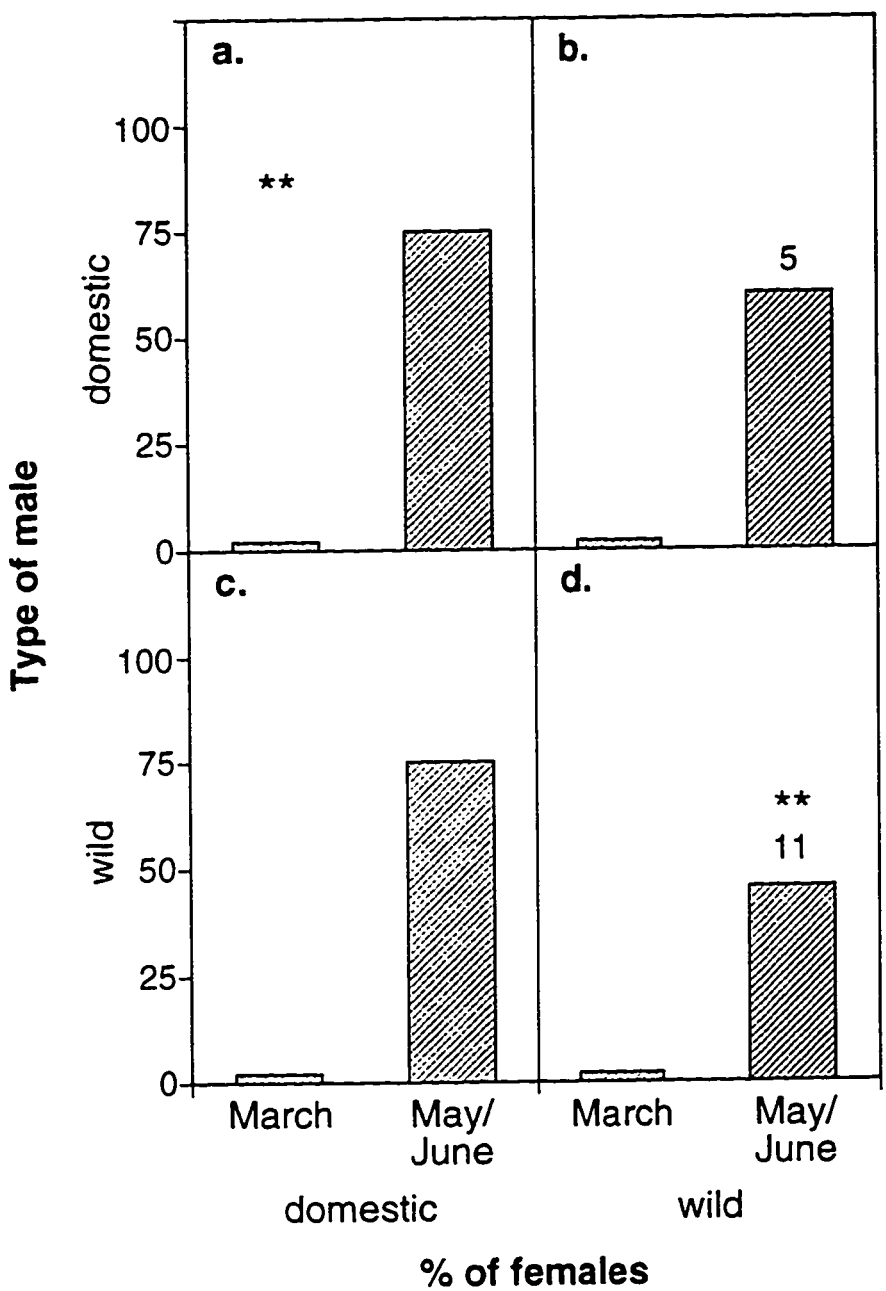

a.
b.
c.
d.
100
75
50
25
0
domestic
wild
**
5
**
11
March
May/
June
March
May/
June
domestic
wild
Type of male
% of females

Figure 4.3: Numbers of pairings and the dates of first matings for females are shown for the four breeding seasons. The horizontal lines indicate gestation length measured from date of a female's last mating. Above the horizontal lines are the gestations lengths calculated from both first and last matings (first/last).

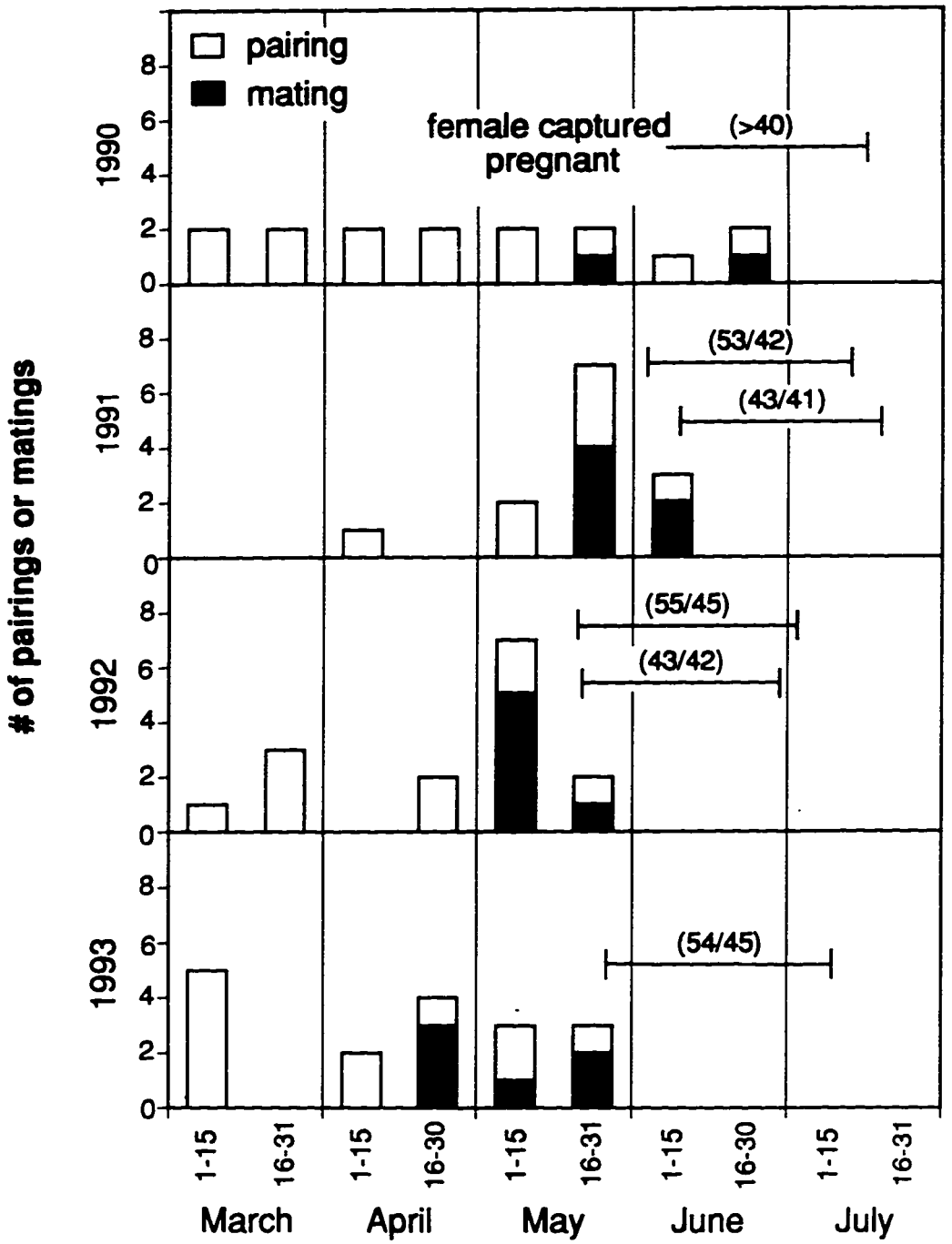
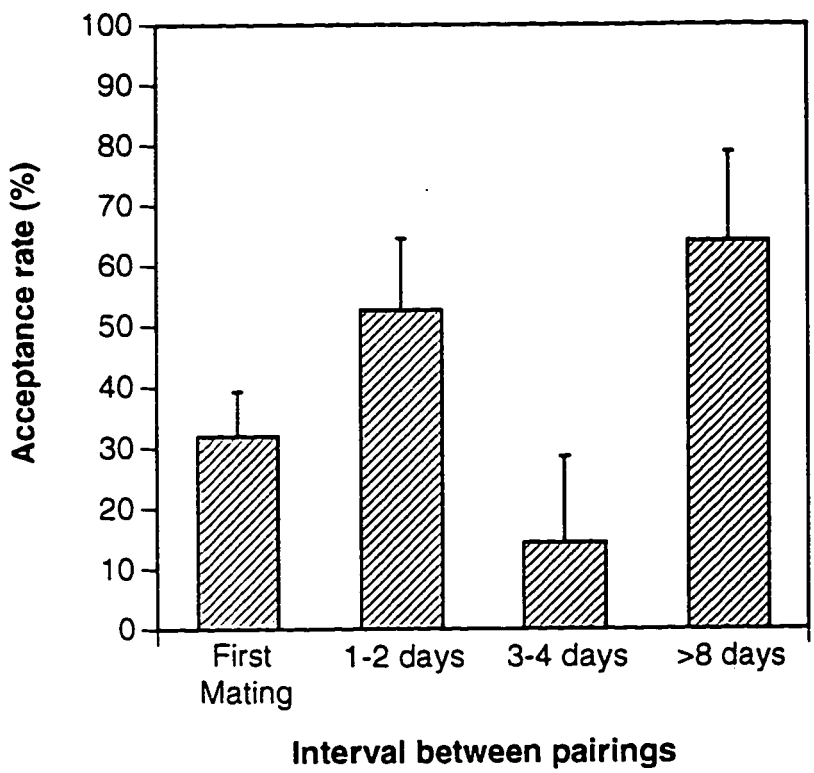

pairing
mating
female captured pregnant
(>40)
(53/42)
(43/41)
(55/45)
(43/42)
(54/45)
1990
1991
1992
1993
# of pairings or matings
0
2
4
6
8
1-15
16-31
March
April
May
June
July

Figure 4.4: The mean (± SE) percentage of first matings and multiple mating opportunities accepted by seven females. Means (± SE) were calculated from individual female acceptance rates, determined by percentage of matings upon initial introduction at a given interval each year over 3 years.


100
90
80
70
60
50
40
30
20
10
0
Acceptance rate (%)
First Mating
1-2 days
3-4 days
>8 days
Interval between pairings

REFERENCES

Adair, J., Scott, R. & C. Thompson. 1988. Single versus multiple mating mink. Mink Farmers' Research Foundation Progress Reports 1988.

Adams, C. E. 1981. Observations on the induction of ovulation and expulsion of uterine eggs in the mink, *Mustela vison*. Journal of Reproduction and Fertility 63: 241-248.

Adams, C. E. & A. A. Reitveld. 1981. Duration of copulation and fertility in the mink, *Mustela vison*. Theriogenology 15: 449-452.

Adler, N. T. 1969. Effects of the male's copulatory behavior on successful pregnancy of the female rat. Journal of Comparative and Physiological Psychology 69: 613-622.

Adler, N. T. & S. R. Zoloth. 1970. Copulatory behavior can inhibit pregnancy in female rats. Science 168: 1480-1482.

Aebersold, P. B., Winans, G. A., Teel, D. J., Milner, G. B. , & F. M. Utter. 1987. Manual for starch gel electrophoresis: a method for the detection of genetic variation. NOAA Technical Report NMFS No. 61.

Alatalo, R. V., Gottlander, K., & A. Lundberg. 1987. Extra-pair copulations and mate guarding in the polyterritorial pied flycatcher, *Ficedula hypoleuca*. Behaviour 101: 139-155.

Alcock, J. , Eickwort, G. C., & K. R. Eickwort. 1977. The reproductive behavior of *Anthidium maculosum* (Hymenoptera: Megachilidae) and the evolutionary significance of multiple copulations by females. Behavioral Ecology and Sociobiology 2: 385-396.

Allais, C. & L. Martinet. 1978. Relation between daylight ratio, plasma progesterone levels and timing of nidation in mink (*Mustela vison*). Journal of Reproduction and Fertility 54: 133-141.

Arnold, S. J. & T. Halliday. 1988. Multiple mating: natural selection is not evolution. Animal Behavior 36: 1547-1548.

Askins, G. R. & J. A. Chapman. 1984. Age determination and morphological characteristic of wild mink from Maryland, USA. Zeitschrift fur Saugeteirkund 49: 182-189.

Backus, G. B. C. 1982. Relation between mating system and reproduction performance of female mink. de Pelsdierenfokker 2: 46-48.

Baker, R. R. & M. A. Bellis. 1989. Number of sperm in human ejaculates varies in accordance with sperm competition theory. Animal Behavior 37: 867-869.

Birkhead, T. R. 1991. Sperm depletion in the Bengalese finch (*Lonchura striata*). Behavioral Ecology 4: 267-275.

Birkhead, T. R. & F. M. Hunter. 1990. Mechanisms of sperm competition. Trends in Ecology and Evolution 5: 48-52.

Birkhead, T. R., Johnson, S. D., & D. N. Nettleship. 1985. Extra-pair matings and mate guarding in the common murre *Uria aalge*. Animal Behavior 33: 608-619.

Birkhead, T. R. & A. P. Moller. 1992. Sperm competition in birds: evolutionary causes and consequences. Academic Press, London.

Birkhead, T. R. & A. P. Moller. 1993. Female control of paternity. Trends in Ecology and Evolution 8: 100-104.

Birkhead, T. R., Moller, A. P., & W. J. Sutherland. 1993. Why do females make it so difficult for males to fertilize their eggs? Journal of Theoretical Biology 161: 51-60.

Boissin-Agasse, L. & J. Boissin. 1985. Incidence of a circadian cycle of photosensitivity in the regulation of the annual testis cycle in the mink: a short-day mammal. General and Comparative Endocrinology 60: 109-115.

Brinck, C., Gerell, R., & G. Odham. 1978. Anal pouch secretion in mink *Mustela vison*: chemical communication in Mustelidae. Oikos 30: 68-75.

Brock Fenton, M. 1984. Sperm competition? The case of Vespertilionid and Rhinolophid bats. Pp. 573-587, *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London.

Bronson, F. H. 1988. Seasonal regulation of reproduction in mammals. Pp. 1831-1872, *in* The physiology of reproduction. First ed. (E. Knobil and J. D. Neill, eds.). Raven Press, New York.

Bronson, F. H. 1989. Mammalian reproductive biology. University of Chicago Press, Chicago.

Burley, N. T., Enstrom, D. A., & L. Chitwood. 1994. Extra-pair relations in zebra finches: differential male success results from female tactics. Animal Behavior 48: 1031-1041.

Burns, J. J. 1964. The ecology, economics, and management of mink in the Yukon-Kuskokwim Delta, Alaska. M.S. thesis, University of Alaska, College.

Canivenc, R. & M. Bonnin-Lafargue. 1967. Luteal asthenia in species with delayed implantation (*Meles meles, Mustela vison*). Review of European Endocrinology 4:29.

Carter, C. S. 1973. Stimuli contributing to the decrement in sexual receptivity of female golden hamsters (*Mesocricetus auratus*). Animal Behavior 21:827-834.

Clutton-Brock, T. H. & G. A. Parker. 1995. Sexual coercion in animal societies. Animal Behavior 49: 1345-1365.

Cohen, J. 1971. The comparative physiology of gamete populations. Advances in Comparative Physiology and Biochemistry 4: 267-380.

Cox, C. R. & B. J. LeBoeuf. 1977. Female incitation of male competition: a mechanism in sexual selection. American Naturalist 111: 317-335.

Clapperton, B. K., Minot, E. O., & D. R. Crump. 1988. An olfactory recognition system in the ferret, *Mustela furo* L. (Carnivora: Mustelidae). Animal Behavior 36: 541-553.

Daly, M. 1978. The cost of mating. American Naturalist 112: 771-774.

Daniel., J. C. 1971. Termination of pregnancy in mink by repeated injections during the period preceding implantation. Journal of Animal Science 22: 659-663.

Dewsbury, D. A. 1982. Ejaculate cost and male choice. American Naturalist 119: 601-610.

Dewsbury, D. A. 1984. Sperm competition in muroid rodents. Pp. 547-571, *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London.

Dewsbury, D. A. 1985. Interactions between males and their sperm during multi-male copulatory episodes of deer mice (*Peromyscus maniculatus*). Animal Behavior 33: 1266-1274.

Dewsbury, D. A. 1988. Copulatory behavior as courtship communication. Ethology 79: 218-234.

Dewsbury, D. A. & D. J. Baumgardner. 1981. Studies of sperm competition in two species of muroid rodents. Behavioral Ecology and Sociobiology 9: 121-133.

Dewsbury, D. A. & D. K. Sawrey. 1984. Male capacity as related to sperm production, pregnancy initiation, and sperm competition in deer mice (*Peromyscus maniculatus).* Behavioral Ecology and Sociobiology 16: 37-47.

Duby, R. T. and H. F. Travis. 1972. Photoperiodic control of fur growth and reproduction in the mink (*Mustela vison*). Journal of Experimental Zoology 182: 217-226.

Dunstone, N. 1993. The mink. T & AD Poyser Ltd., London.

Dzuik, P. J. 1965. Double mating of rabbits to determine capacitation time. Journal of Reproduction and Fertility 10: 389-395.

Eaton, R. L. 1978. Why some felids copulate so much: a model for the evolution of copulation frequency. Carnivore 1: 42-51.

Eagle, T. C. & J. S. Whitman. 1987. Mink. *in* Wild furbearer management and conservation in North America (M. Novak, J. A. Baker, M. E. Obbard & B. Malloch, eds.) Ministry of Natural Resources, Ontario.

Eberhard, W. G. 1985. Sexual selection and animal genitalia. Harvard University Press, Cambridge, Massachusetts.

Eberhard, W. G. 1996. Female control: sexual selection by cryptic female choice. Princeton University Press, Princeton.

Eberhardt, L. E. 1974. Food habits of prairie mink (*Mustela vison*) during the waterfowl breeding season. M.S. Thesis, University of Minnesota, St. Paul.

Elofson, L., Lagerkvist, G., Gustafsson, H., and S. Einarsson. 1989. Mating systems and reproduction in mink. Acta Agricultura Scandinavica 39: 23-41.

Enders, R. K. 1952. Reproduction in the mink. Proceedings of the American Philosophical Society 96: 691-755.

Enders, R. K. & J. R. Leekley. 1941. Cyclic changes in the vulva of the marten (*Martes americana*). Anatomical Record 79: 1-5.

Erlinge, S. & M. Sandell. 1986. Seasonal changes in the social organization of male stoats *Mustela erminea*: an effect of shifts between two decisive resources. Oikos 47: 57-62.

Friend, D. W. & E. W. Crampton. 1960. Observation on the effect of four mating routines for female mink. Canadian Journal of Comparative Medicine 24: 9-11.

Fries, S. 1880. Über die Fortpflanzung von *Meles taxus*. Zoologischer Anzeiger 3: 486-492.

Gerell, R. 1970. Home ranges and movements of mink (*Mustela vison*) in Sweden. Oikos 21: 160-173.

Gerell, R. 1971. Population studies on mink, *Mustela vison* Schreber, in southern Sweden. Viltrevy 8: 83-114.

Ginsberg, J. R. & U. W. Huck. 1989. Sperm competition in mammals. Trends in Ecology and Evolution 4: 74-79.

Ginsberg, J. R. & D. I. Rubenstein. 1990. Sperm competition and variation in zebra mating behavior. Behavioral Ecology and Sociobiology 26: 427-434.

Gittleman, J. L. & P. H. Harvey. 1982. Carnivore home range size, metabolic needs, and ecology. Behavioral Ecology and Sociobiology 10: 57-64.

Gomendio, M. & E. R. S. Roldan. 1993. Mechanisms of sperm competition: linking physiology and behavioural ecology. Trends in Ecology and Evolution 8: 95-100.

Goodall, J. 1986. The chimpanzees of Gombe: patterns of behavior. Harvard University Press, Cambridge, Mass.

Gray, G. D., Zerylnick, M., Davis, H. N., & D. A. Dewsbury. 1974. Effects of variations in male copulatory behavior on ovulation and implantation in prairie voles, *Microtus ochrogaster.* Hormones and Behavior 5: 389-396.

Halliday, T. & S. J. Arnold. 1987. Multiple mating by females: a perspective from quantitative genetics. Animal Behavior 35: 939-941.

Hamilton, W. J., Jr. 1948. A new mink from the Florida Everglades. Proceedings of the Biological Society of Washington 61: 139-140.

Hammond, J. 1951. Control by light of reproduction in ferrets and mink. Nature 167: 150-151.

Hansson, A. 1947. The physiology of reproduction in mink. Acta Zoologica 28: 1-136.

Harbo, S. J. 1958. An investigation of mink in interior and southeastern Alaska. M.S. thesis, University of Alaska, College.

Harcourt, A. H., Harvey, P. H., Larson, S. G. & R. V. Short. 1981. Testis weight, body weight and breeding system in primates. Nature 293: 55-57.

Hartung, T. G. and D. A. Dewsbury. 1978. A comparative analysis of copulatory plugs in muroid rodents and their relationship to copulatory behavior. Journal of Mammalogy 59: 717-723.

Harvey, P. H. & A. H. Harcourt. 1984. Sperm competition, testes size and breeding systems in primates. Pp. 589-600, *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London.

Hatler, D. F. 1976. The coastal mink on Vancouver Island, British Columbia. Ph.D. dissertation, University of British Columbia, Vancouver.

Hodgson, R. G. 1931. A scrap book of mink raising. Fur Trade Journal of Canada, Toronto.

Hodgson, R. G. 1945. The mink book. Fur Trade Journal of Canada, Toronto.

Huck, U. W. & R. D. Lisk. 1986. Mating-induced inhibition of receptivity in the female golden hamster: I. short-term and long-term effects. Behavioral and Neural Biology 45: 107-119.

Huck, U. W., Lisk, R. D., & C. L. Guyton. 1989. Multiple-male mating increases littering rates in golden hamsters mated late in the receptive period. Animal Behavior 37: 334-336.

Huck, U. W., Lisk, R. D., Parenti, E. J., & D. E. Principato. 1985. Determinants of mating success in the golden hamster (*Mesocricetus auratus*): female acceptance of multiple mating partners. Journal of Comparative Psychology 100:128-136.

Humphrey, S. R. & T. L. Zinn. 1982. Season habitat use by river otters and Everglades mink in Florida. Journal of Wildlife Management 46: 375-381.

Ireland, M. C. 1990. The behaviour and ecology of the American mink (*Mustela vison* Schreber) in a coastal habitat. Ph.D. dissertation, Durham University.

Jeppson, B. 1986. Mating by pregnant water voles (*Arvicola terrestris*): a strategy to counter infanticide by males? Behavioral Ecology and Sociobiology 19: 293-296.

Jochle, W. 1975. Current research in coitus-induced ovulation: a review. Journal of Reproduction and Fertility (supplement) 22: 165-207.

Johansson, I. & O. Venge. 1951. Relation of the mating interval to the occurrence of superfetation in the mink. Acta Zoologica, 32: 255-258.

Johnson, C. B. 1985. Use of coastal habitat by mink on Prince of Wales Island, Alaska. M.S. thesis, University of Alaska, Fairbanks.

Jorgensen, G. 1985. Mink production. Scientifur, Hilleroed, Denmark.

Keller, L. & H. K. Reeve. 1995. Why do females mate with multiple males? The sexually selected sperm hypothesis. Advances in the Study of Behavior 24: 291-315.

Kenagy, G. J. & S. C. Trombulak. 1986. Size and function of mammalian testes in relation to body size. Journal of Mammalogy 67: 1-22.

Knobil, E. & J. D. Neill (eds.). 1988. The physiology of reproduction, first ed. Raven, New York.

Knowlton & Greenwell. 1984. Male sperm competition avoidance mechanisms: the influence of female interests. *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London.

Lack, D. 1954. The natural regulation of animal numbers. Oxford University Press, Oxford.

Lanier, D. L., Estep, D. Q. & D. A. Dewsbury. 1979. Role of prolonged copulatory behavior in facilitating reproductive success in a competitive mating situation in laboratory rats. Journal of Comparative and Physiological Psychology 93: 781-792.

Linscombe, G., N. Kinler, & R. J. Aulerich. 1982. Mink. Pp. 629-643, *in* Wild mammals of North America; biology, management and economics (J. A. Chapman & G. A. Feldhamer, eds.). Johns Hopkins University Press, Baltimore.

Lott, D. F. 1981. Sexual behavior and intersexual strategies in American bison. Zeitschrift fur Tierpsychologie 56: 97-114.

Maciejowski, J.. Slawon, J. & S. Brzozowski. 1973. The influence of various mating systems upon fertility of female minks. Materialy Zootechnicze 4: 55-68.

Martan, J. & B. A. Shepherd 1976. The role of the copulatory plug in reproduction of the guinea pig. Journal of Experimental Zoology 196: 79-84.

Martin, P. A. & P. J. Dzuik. 1977. Assessment of relative fertility rates of males (cockerels and boars) by competitive mating. Journal of Reproduction and Fertility 49: 323-329.

Matthews, M. & N. T. Adler. 1977. Facilitative and inhibitory influences of reproductive behavior on sperm transport in rats. Journal of Comparative and Physiological Psychology 91: 727-741.

McKinney, F., Cheng, K. M., & D. J. Bruggers. 1984. Sperm competition in apparently monogamous birds. Pp. 523-545, *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London

McKinney, F., Derrickson, S. R., & P. Mineau. 1983. Forced copulation in waterfowl. Behaviour 86: 250-294.

Mead, R. A. 1968. Reproduction in western forms of the spotted skunk (genus *Spilogale*). Journal of Mammalogy 49: 373-390.

Mead, R. A. 1989a. Reproduction in mustelids. Pp. 124-137 in Conservation biology and the black-footed ferret (U.S. Seal, E. T. Thorne, M. A. Bogan, & S. H. Anderson, eds.). Yale University Press, New Haven.

Mead, R. A. 1989b. The physiology and evolution of delayed implantation in carnivores. Pp. 437-464, *in* Carnivore behavior, ecology, and evolution (J. L. Gittleman, ed.) Cornell University Press, Ithaca.

Mead, R. A. 1994. Reproduction in *Martes*. Pp. 404-422, *in* Martens, sables and fishers: biology and conservation (S. W. Buskirk, A. S. Harestad, M. G. Raphael, and R. A. Powell, eds.). Cornell University Press, Ithaca.

Mead, R. A. & P. L. Wright. 1983. Reproductive cycles of Mustelidae. Acta Zoologica Fennica 174: 169-172.

Mitchell, J. L. 1961. Mink movements and populations on a Montana river. Journal of Wildlife Management 25: 48-54.

Moller, A. P. 1989. Ejaculate quality, testes size and sperm production in mammals. Functional Ecology 3: 91-96.

Moller, A. P. 1990. Sexual behavior is related to badge size in the house sparrow *Passer domesticus*. Behavioral Ecology and Sociobiology 27: 23-29.

Moller, A. P. & T. R. Birkhead. 1989. Copulation behavior in mammals: evidence that sperm competition is widespread. Biological Journal of the Linnean Society 38: 119-131.

Oglesby, J. M., Lanier, D. L. & D. A. Dewsbury. 1981. The role of prolonged copulatory behavior in facilitating reproductive success in male Syrian golden hamsters (*Mesocricetus auratus*) in a competitive mating situation . Behavioral Ecology and Sociobiology 8: 47-54.

Packer, C. & A. E. Pusey. 1983. Adaptations of female lions to infanticide by incoming males. American Naturalist 121:716-728.

Park, R. L., Richards, D. O., & R. S. Crapo. 1988. Effects of mating sequence, sire order, and polyandry on 24-hour litter size in black mink: a three-year study. Mink Farmers' Research Foundation Progress Reports 1988.

Parker, G. A. 1970. Sperm competition and its evolutionary consequences in the insects. Biological Review 45: 525-567.

Parker, G. A. 1974. Assessment strategy and the evolution of fighting behavior. Journal of Theoretical Biology 47: 223-243.

Parker, G. A. 1979. Sexual selection and sexual conflict. Pp. 123-166, *in* Sexual selection and reproductive competition in insects (M. S. Blum and N. Blum, eds.). Academic Press, New York.

Parker, G. A. 1982. Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. Journal of Theoretical Biology, 96:281-294.

Parker, G. A. 1984. Sperm competition and the evolution of animal mating strategies. Pp. 2-60, *in* Sperm competition and the evolution of mating systems (R. L. Smith, ed.). Academic Press, London.

Patton, H. 1925. Raising fur-bearing animals. C. V. Ritter & Hardison Patton, Chicago.

Pelletier, R-M. 1986. Cyclic formation and decay of the blood-testis barrier in the mink (*Mustela vison*), a seasonal breeder. American Journal of Anatomy, 175: 91-117.

Powell, R. A. 1979. Mustelid spacing patterns—variations on a theme by *Mustela*. Zeitschrift fur Tierpsychologie 50: 153-165.

Ramirez, V. D. & C. Beyer. 1988. The ovarian cycle of the rabbit: its neuroendocrine control. Pp. 1873-1892, in The physiology of reproduction (E. Knobil & J. D. Neill et al. eds.). Raven Press, New York.

Robinson, I. H. 1987. Olfactory communication and social behaviour in the mink (*Mustela vison*). Ph.D. dissertation, University of Aberdeen.

Sandell, M. 1990. The evolution of seasonal delayed implantation. Quarterly Review of Biology, 65: 23-42.

Schwagmeyer, P. L. 1984. Multiple mating and intersexual selection in thirteen-lined ground squirrels. Pp. 275-293, *in* Biology of ground-dwelling squirrels (J. O. Murie and G. R. Michener, eds.). University of Nebraska Press, Lincoln.

Schwagmeyer, P. L. & D. W. Foltz. 1990. Factors affecting the outcome of sperm competition in thirteen-lined ground squirrels. Animal Behavior 39: 156-162.

Schwagmeyer, P. L. & S. J. Wootner. 1986. Scramble competition polygyny in thirteen-lined ground squirrels: the relative contributions of over conflict and competitive mate searching. Behavioral Ecology and Sociobiology 19: 359-364.

Shackelford, R. M. 1950. Genetics of the ranch mink. Pilsbury Publishers, Inc., New York.

Shackelford, R. M. 1952. Superfetation in the ranch mink. American Naturalist 86: 311-319.

Sherman, P. W. & D. F. Westneat. 1988. Multiple mating and quantitative genetics. Animal Behavior 36: 1545-1547.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw-Hill, New York.

Smith, R. L. 1984. Sperm competition and the evolution of mating systems. Academic Press, London.

Stenson, G. B. 1985. The reproductive cycle of the river otter, *Lutra canadensis*, in the marine environment of southwestern British Columbia. Ph.D. dissertation, University of British Columbia, Vancouver.

Sundqvist, C., Ellis, L. C., & A. Bartke. 1988. Reproductive endocrinology of the mink (*Mustela vison*). Endocrine Reviews, 9:247-266.

Sundqvist, C. & T. Sundqvist. 1986. Some observations on semen quality in mink of different fur quality. Scientifur 10: 117-119.

Svihla, A. 1931. Habits of the Louisiana mink (*Mustela vison vulgivagus*). Journal of Mammalogy 12: 366-368.

SYSTAT for the Macintosh, Version 5.2. 1992. SYSTAT, Inc. Evanston, Illinois.

Travis, H. F., Pilbeam, T. E., Gardner, W. J., & R. S. Cole. 1978. Relationship of vulvar swelling to estrus in mink. Journal of Animal Science 46: 219-223.

Trivers, R. L. 1972. Parental investment and sexual selection. Pp. 136-179, in Sexual selection and the descent of man 1871-1971 (B. Campbell, ed.). Aldine, Chicago.

Tung, K. S. K., Ellis, L. C., Teuscher, A., Meng, A., Blaustein, J. C., Kohno, S. & R. Howell. 1981. The black mink (*Mustela vison*) —a natural model of immunologic male infertility. Journal of Experimental Medicine 154: 1016-1033.

Tutin, C. E. G. 1979. Mating patterns and reproductive strategies in a community of wild chimpanzees (*Pan troglodytes schweinfurthii*). Behavioral Ecology and Sociobiology 6: 29-38.

van Tienhoven, A. 1968. Reproductive physiology of vertebrates. W. B. Saunders, Philadelphia.

Venge, O. 1956. Experiments on forced interruption of the copulation in mink. Acta Zoologica 37: 1-18.

Voss, R. 1979. Male accessory glands and the evolution of copulatory plugs in rodents. Occasional Papers of the Museum of Zoology, University of Michigan 689: 1-27.

Walker, W. F. 1980. Sperm utilization strategies in nonsocial insects. American Naturalist 115: 780-799.

Westneat, D. L., Sherman, P. W., & M. L. Morton. 1990. The ecology and evolution of extra-pair copulation in birds. Current Ornithology 7: 331-369.

Whitman, J. S. 1981. Ecology of the mink (*Mustela vison*) in west-central Idaho. M.S. thesis, University of Idaho, Moscow.

Williams, G. C. 1975. Sex and evolution. Princeton University Press, Princeton.

Woolington, J. D. 1984. Habitat use and movements of river otters at Kelp Bay, Baranof Island, Alaska. M.S. thesis, University of Alaska, Fairbanks.

Wright, P. L. 1948. Breeding habits of captive long-tailed weasels (*Mustela frenata*). American Midland Naturalist 39: 338-344.

Wright, P. L. 1963. Variations in reproductive cycles in North American mustelids. Pp. 77-97 *in* Delayed Implantation (A. C. Enders, ed.) University of Chicago Press, Chicago.

Zarrow, M. X. & J. H. Clark. 1968. Ovulation following vaginal stimulation in a spontaneous ovulator and its implications. Journal of Endocrinology 40: 343-352.

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Melissa Fleming knew she wanted to study animal behavior as soon as she learned of the profession through the books of Jane Goodall and George Schaller in her early teens. After two years as a Zoology major at the University of Massachusetts, Amherst, she transferred to Cornell University where she received a B.A. in Biology, with a concentration in Neurobiology and Behavior. During her last year at Cornell and a subsequent year off prior to graduate school, she gained experience by assisting in a number of lab and field projects involving naked mole rats, salamanders, butterflies, zoo primates, and acorn woodpeckers. She came to the University of Washington's Animal Behavior Program for graduate school to work with Eric Fischer on the evolution and maintenance of simultaneous hermaphroditism in coral reef fishes. For her Ph.D. research, she applied her long-term interest in mustelids and her growing appreciation of evolutionary and reproductive biology to the study of female multiple mating behavior in mink. In addition to her Psychology major, she completed a Zoology minor and a concentration in Conservation Biology while at the University of Washington. She plans to pursue postdoctoral training in molecular phylogeography in order to better understand the evolution of intraspecific differences in reproductive mechanisms and behavior.