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# Maternal Aggression Toward Infanticidal Males of Different Social Status in Wild House Mice (*Mus musculus domesticus*)

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**Maternal aggression was examined in wild female mice (*Mus musculus domesticus*) derived from animals trapped in Alberta, Canada. Lactating females were tested for their behavior toward intruder males during the time of postpartum estrus while housed in a two-cage apparatus containing a defensible nest area. Prior to being used as intruders, sexually naive males were screened for their behavior toward a newborn pup (83% exhibited infanticide). Only infanticidal males were then housed in pairs and allowed to establish a dominance hierarchy. Dominance status was further verified by a urine marking test. The dominant and subordinate infanticidal males were then placed into a lactating female's cage and observed for 1 hr. The test was terminated immediately when a male began to attack the pups. Lactating females attacked the males in both groups, but subordinate males received more intense attacks than dominant males. Dominant males elicited significantly more fear/defense behavior than subordinate intruders. All of the dominant males and only one submissive male attacked the pups. Females were thus successful in blocking infanticide only by infanticidal subordinate males. Since females do not persist in attacking males with high fighting ability, one function of maternal aggression could be to assess the fighting, and resource holding, potential of a future mate.** © 1994 Wiley-Liss, Inc.

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## INTRODUCTION

During lactation female house mice become intensely aggressive toward unfamiliar conspecifics, referred to as intruders, which enter into the nest area. Maternal, or post-

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partum, aggression has been proposed to have evolved due to the need to protect the offspring from being killed by conspecifics [Ostermeyer, 1983]. While the proportions vary between genetic stocks, in virtually all stocks of house mice so far examined, infanticide (the killing of nursing pups) by sexually naive males is observed [e.g., Kennedy and Elwood, 1988; Perrigo and vom Saal, 1993; Parmigiani, 1989]. This suggests that variation in the levels of aggression toward male intruders displayed during postpartum estrus can influence a female's reproductive success, since strange males (but not the prior mate) will typically kill the newborn pups being nursed by the female [e.g., vom Saal and Howard, 1982].

During the first night following delivery, postpartum estrus occurs, and females become sexually receptive and ovulate. In some laboratory stocks of mice, postpartum estrus is associated with little or no aggressiveness by females; the intensity of maternal aggression then increases and reaches a peak at the end of the first week postpartum [Svare, 1989]. Infanticide has been proposed to be an adaptive behavior that is exhibited by males of the house mouse, as well of several other species of mammals, in order to increase mating opportunities [Hrdy, 1979; vom Saal and Howard, 1982]. When a strange male enters the nest area of a lactating female prior to postpartum estrus, the act of killing the pups results in the more rapid production of offspring sired by the infanticidal male during the postpartum estrus. This is due to the fact that implantation is delayed (referred to as facultative diapause) if females are nursing so that the delivery of offspring sired by non-infanticidal males is delayed relative to offspring sired by infanticidal males [vom saal and Howard, 1982]. When a strange male enters the nest area of a lactating female after termination of postpartum estrus, infanticide by the male dramatically reduces the interovulatory period of the female whose pups are killed, so that males can mate with the female much sooner than would be the case if the female's pups would have survived [vom Saal and Howard, 1982]. Intense maternal aggression could reduce the likelihood of pups being killed by an intruder male; it has thus been proposed that maternal aggression is a female counterstrategy to male infanticide [Ostermeyer, 1983].

However, the apparent failure of maternal aggression to protect the litter from male infanticide (even after postpartum estrus) has led to questions about this proposal [Parmigiani et al., 1989, 1993]. Specifically, studies with Swiss mice showed that the intensity of maternal aggression and success at protecting the litter were modulated by the degree of aggressiveness of the male intruder (as measured in previous intermale tests), rather than a response to the potential risk posed by the male to the litter, based on whether the male had previously been shown to be infanticidal (Palanza and Parmigiani, submitted). Since female mice prefer to mate with males with good fighting ability (i.e., dominant males) [Parmigiani et al., 1982], maternal aggression could also serve to assess the quality, in terms of fighting ability, of males that may become the females' future mates [Parmigiani et al., 1989].

Given this background, our aim was to investigate the functions of maternal aggression toward male intruders in wild mice by examining: 1) whether the behavior of a male toward a female and her pups, as well as the behavior of the female toward the male, depends on the male's fighting ability, and the effect of postpartum estrus on females' aggressiveness toward males and on the occurrence of infanticide by the male.

## MATERIALS AND METHODS

### Animals

Mice (*Mus musculus domesticus*) were originally trapped from a commensal population near Calgary, Alberta, Canada, in 1979. The stock was maintained in an outbred colony at the University of Texas-Austin and, subsequently, at the University of Missouri-Columbia. After weaning (around 25 days of age), mice were housed with same-sex siblings in polypropylene cages measuring 29 × 18 × 13 cm in rooms maintained at 23°C on a 12:12 hr light:dark cycle, with lights on at 0700 hr. Water and food were available ad libitum. At 90 days of age, females were paired with adult males of the same stock. About 3 days before delivery, each female was individually housed in the experimental apparatus, consisting of two polyethylene cages (each 45 × 25 × 15 cm) connected by a 20-cm tunnel, which could be closed by a removable partition. One of the cages was provided with a polyethylene nest box (10 × 7.5 × 7 cm) containing nest material. The nest box was covered with a removable transparent lid and rested on the Aspen bedding covering the floor of the entire cage. The entrance to the nest box was provided via a 5-cm-diameter, 7-cm-long tunnel, which could also be closed by a removable partition. The objective in designing this test apparatus was to simulate burrows of mice living in natural conditions and to provide the female with a defensible nest area. The second cage was to provide the intruder male with a separate area into which he could escape when attacked. The cages were covered with a stainless steel lid on which food (Purina 5001) and a water bottle were placed.

### Infanticidal Tendency and Dominance Status of Intruder Males

Males to be used as intruders were screened for their behavior toward young. At 90 days of age, sexually naive males were individually housed in 29 × 18 × 13-cm polypropylene cages. One day later, a newborn pup was placed into a corner of each male's cage with a minimum of disturbance. Males that attacked the pup within 30 min were labeled infanticidal. As soon as a pup was attacked, the test was immediately terminated and the pup was removed from the cage and euthanized by $CO_2$ asphyxiation. Only infanticidal males were then used in the following experiment. Of 112 males screened for their behavior toward a pup, 93 (83%) exhibited infanticide and 19 (17%) ignored the pup during the test session.

One day after the test for infanticide, 28 infanticidal males were randomly selected, marked to allow individual recognition, and randomly placed in pairs (n = 14 pairs) into a 30 × 30 × 15-cm cage that was divided into two chambers of equal size by a removable wire-mesh partition. The males were initially placed on either side of the wire-mesh partition for 1 hr, after which they were allowed to physically interact. The result of the physical encounter was that a dominance hierarchy was established in all pairs. Dominance status was assessed on the basis of a variety of measures of agonistic behavior: chasing, fleeing, crouching, and upright posture [van Oortmerssen, 1971]. The males were separated when there was clear submissive behavior exhibited by one male, but prior to the occurrence of severe wounding of the submissive male; these encounters were typically about 20 min in duration. The two males remained separated by the partition that allowed visual, auditory, olfactory, and even tactile communication, thus allowing the maintenance of a dominance hierarchy [D'Amato, 1988]. This was done to avoid a high mortality rate due to prolonged physical contact between the males. For

the next 3 days (days 2–4 after being paired), the males in each dyad were allowed to physically interact during a 10-min session by removal of the partition. There were two test sessions per day at 1000 and 1800 hr.

At 0900 hr on the fifth day after being paired, about 8 hr before being introduced into a lactating female's cage, social status was further verified by a urine marking test. Urine marking patterns vary in relation to male social status [Desjardins et al., 1973]. During testing for urine marking, the two-chambered cages, with the males separated by the wire-mesh partition, were placed on sheets of Whatman filter paper (No. 2) for 1 hr. Discrete urine marks (as well as puddles of urine) deposited on the filter paper by each of the males in a dyad were scored under ultraviolet (UV) light. In 11/14 pairs of males, a concordance between the assignation of dominant/surbordinate status based on fighting and that based on urine marking was found: dominant males mark extensively along the wire-mesh boundary, while subordinate males stay in a far corner and urinate in puddles rather than making many discrete marks. The three dyads where urine marking did not allow a clear-cut discrimination of dominance status were discarded.

Since it has been proposed that social status can influence infanticidal behavior in laboratory mice, the response toward a pup was reassessed 10 min after the urine marking test by placing a pup into a corner of each chamber containing a male (the chambers were still separated by wire mesh). Only one of the subordinate males did not attack the pup within 10 min, and this one male was thus not used in the experiment. As a result of these tests, two categories of intruders were obtained: 1) infanticidal dominant males and 2) infanticidal submissive males.

### Maternal Aggression Test

Lactating females were randomly assigned to one of the two intruder male groups (n = 10/group). Females were tested during the predicted time of postpartum estrus to assess the female's behavior toward a male that would be likely to mate with her and sire the next litter, regardless of whether or not the male were to kill the nursing pups. Pregnant females were examined every hour throughout the day when it was apparent that they were near the end of pregnancy. Females that delivered prior to 1300 hr were tested on the evening of the day of parturition during the dark phase (between 1800–2300 hr). Females that delivered after 1300 hr were tested the next evening at the same time, since postpartum estrus is delayed 1 day when delivery occurs late in the light phase of the light:dark cycle (vom Saal, unpublished observation). Just before the intruder test, the lactating female resident was confined in the nest box containing the litter by placing a partition in the tunnel leading to the nest box. The intruder (marked with ink to facilitate identification) was placed into the test apparatus opposite the nest area. After 2 min, the barrier was raised and the animals were allowed to interact.

Detailed behavioral recording lasted 10 min. Males that had not entered the nest area and attempted to attack the pups within the 10-min test period were allowed to remain in the test apparatus for an additional 50 min. The male was removed as soon as an attack of a pup occurred, and if a pup was injured prior to stopping the test, the pup was immediately euthanized as described above.

The following variables of lactating females' behavior were recorded: 1) proportion of intruders attacked per group, 2) latency to attack, i.e., the time from the initial contact to first biting attack, 3) total duration of biting attacks, 4) intensity of attacks, i.e.,

number of visible wounds received by the intruder during the test, which was determined by a careful examination of its pelage, 5) fear and defense, i.e., total durations of fear-related and defensive behaviors, such as contact-related immobility, upright defensive posture, vocalizations, and startle response, and 6) social investigation of intruders (sniffing and mild grooming). The number and duration of these behaviors exhibited by the lactating females were recorded using an Esterline Angus multichannel event recorder.

The occurence of, and latency to, infanticide by the male, as well as retaliation by the male (attack by the male toward the female), were also recorded in order to assess whether a female's success at protecting her litter would differ as a function of the male intruder's social status. Since the occurrence of infanticide caused the termination of the test, the duration data were calculated as a proportion of the total amount of time of the test session.

## RESULTS

The results presented in Table I show that lactating females attacked most of the males in both groups. No differences based on type of intruder were recorded either in the proportion of females attacking or in the amount of time spent attacking, although there was a tendency for subordinate males to receive more attacks than dominant males ($P = 0.08$, Mann-Whitney test). Subordinate males were also attacked with greater intensity than dominant males: females inflicted visible wounds on 7/10 subordinate males, while only 1 dominant male was found to have a visible wound ($P < 0.02$, Fisher exact probability test). The latency to attack by the lactating females tended to be shorter toward the dominant than toward the subordinate male intruders ($P = 0.1$, Mann-Whitney test). Dominant males elicited significantly more fear/defense behavior by females than did subordinate males ($P < 0.001$, Mann-Whitney test). No differences were found for social investigation displayed by the lactating resident when confronting the intruders.

**TABLE I. Lactating Females' Aggression Toward and Infanticide by Male Intruders of Differing Social Status†**

| Status of intruder | Proportion of intruders attacked | Latency to attack (sec) | Total attacking time (duration) | Fear/ defense behavior (duration) | Proportion of males with visible wounds | Proportion of males retaliating | Proportion of males exhibiting infanticide |
|---|---|---|---|---|---|---|---|
| Dominant males | 9/10 | 4 (1–600) | 0.8 (0–9.8) | 5.3 (0–17) | 1/10 | 5/10 | 4/10 (10 min)* 10/10 (1 hr)** |
| Subordinate males | 8/10 | 37.5 (1–600) | 3.2 (0–23) | 0 (0–3.3) | 7/10 | 0/10 | 0/10 (10 min)* 1/10 (1 hr)** |
| | ns | $P < 0.1$ (Mann-Whitney test) | $P < 0.08$ (Mann-Whitney test) | $P < 0.001$ (Mann-Whitney test) | $P < 0.02$ (Fisher exact probability test) | $P < 0.05$ (Fisher exact probability test) | *$P < 0.05$; **$P < 0.001$ (Fisher exact probability test) |

†Data on latency and duration are presented as medians with ranges in parentheses.

Fifty percent of the dominant males and none of the submissive males counterattacked the lactating resident during the 10-min period of observation ($P < 0.05$, Fisher exact probability test). Within this period, 40% of the dominant and none of the submissive males started to attack the pups. By the end of 1 hr after the beginning of the test, all of the dominant males and only 1 submissive male had attacked the pups ($P < 0.001$, Fisher exact probability test).

## DISCUSSION

The great majority of naive male mice of the stock used in this study exhibit infanticide and thus represent a potential risk for litters produced by other males; mating inhibits infanticide in these males [Perrigo and vom Saal, 1993]. The behavior of a lactating female toward a male, as well as her success in protecting her litter, were affected by male social status. Virtually all males were attacked, regardless of dominance status, although attacks on subordinate males were more intense than attacks on dominant males. In addition, the behavior of lactating females toward dominant males was typical of fear-related behavior [as described by Parmigiani et al., 1988] in a stock of Swiss mice. Specifically, whereas the females vigorously attacked the subordinate males, the pattern of attack toward the dominant male involved tentative thrusts and retreats, with considerable vocalization (squeaking), suggestive of fear. The fear behavior of the lactating female while attacking the dominant male occurs prior to retaliation by the male. After the dominant males retaliated and attacked the females, the females were not successful at protecting their offspring from attack by the male. In contrast, the subordinate males did not retaliate and attack the lactating female, and only 1 of 10 subordinate males attacked the pups during the 1-hr period of observation.

Typically, females are sexually receptive and ovulate during the first night after parturition (see Materials and Methods). Thus, during the first night after parturition (when we conducted this experiment), the females would likely be inseminated by the intruder male, who would then become the sire of her next litter. Intense aggression toward subordinate male intruders would preserve her offspring and prevent reproduction with a male of low fighting ability and thereby possibly optimize her fitness. An important aspect of this hypothesis is that wild female mice, in contrast to some laboratory stocks, are highly aggressive toward intruders on the day of parturition and around the time of postpartum estrus (Franks et al., submitted), a finding which was confirmed in the present experiment.

In the natural environment, females are more likely to live within a deme (or family unit) in which there is a single dominant, territorial male [Crowcroft and Rowe, 1963; Hurst, 1987]. The male is thought to be mainly responsible for territorial defense toward other males [Parmigiani et al., 1989; Palanza et al., 1993], while females may restrict the immigration of unrelated females into the deme [Palanza et al., 1993] (Franks et al., submitted). When the dominant male in a deme that sired a litter dies or is displaced, the cost for a female of defending her current parental investment may be outweighed by the gain of a new mate, but only if the new mate has a good fighting ability (i.e., is a dominant male). Dominant males may, in fact, produce more fit offspring and/or may be more successful in gaining and defending resources (referred to as resource holding potential [Parker, 1974; Maynard-Smith, 1982]) when compared to subordinate males [DeFries and McClearn, 1970]. Thus, the most successful strategy for the

females should be to associate and mate with a male (of good resource holding potential) who is capable of defending the territory and offspring.

Social status of males modulates sexual preferences and subsequent mating in female house mice; females prefer to mate with males that are dominant in agonistic interactions [DeFries and McClearn, 1970; Parmigiani et al., 1982; Hurst, 1986]. The findings presented here with wild mice lend support to the hypothesis, based on other studies with Swiss albino mice [Parmigiani et al., 1989, 1993] (Palanza and Parmigiani, submitted), that aggression by a lactating female toward an intruder male might be a method of the female assessing the male's future potential to assist in nest defense of litters that he sired. Specifically, while the wild females tested here did vigorously (and successfully) defend their pups against subordinate males of low fighting ability, they did not persist in defending their pups (which could have resulted in serious injury to the female) when confronted with a dominant male intruder of high fighting ability. Maternal aggression may thus represent a category of intersexual selection whereby females assess the resource holding potential of intruder males [Trivers, 1985]. This does not imply that maternal aggression is not related to protection of a female's litter, but that both functions, intersexual selection and a counterstrategy to infanticide, could have operated in the evolution of maternal aggression.

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