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Male urinary cues stimulate intra-sexual aggression and urine-marking in wild female mice, *Mus musculus domesticus*

PAOLA PALANZA*†, STEFANO PARMIGIANI* & FREDERICK S. vom SAAL‡
**Dipartimento di Biologia e Fisiologia Generali, Università degli Studi di Parma, viale della Scienze, 43100 Parma, Italy*
†Dipartimento di Scienze Ambientali, Università degli Studi di Venezia, 30123 Venezia, Italy
‡Division of Biological Sciences and John M. Dalton Research Center, University of Missouri, Columbia, Missouri 65211, U.S.A.

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The female mouse has been commonly regarded as non-aggressive to conspecifics except when engaging in parental care, referred to as maternal or postpartum aggression, in order to defend her parental investment (Mackintosh 1981; Svare 1989). In contrast, it has been traditionally maintained that males compete among themselves for space, which they mark with urine and defend by aggressively excluding unfamiliar conspecifics; females have been thought not to exhibit territorial behaviour, thus suggesting a 'non-territorial nature' (Mackintosh 1981). Recent evidence, however, suggests that this view of females may not be correct. Female mice do mark their substrate with urine although at lower rates than males do (Maruniak et al. 1975); this is probably to advertise territorial occupancy or their breeding status, or both, to other females and males (in laboratory mice: Powell & Wolff 1982; in wild mice: Hurst 1987b, 1990). Females also help the male with which they have mated (the dominant, territorial male) to defend their shared area from intruding conspecifics and they can play an important role in determining social organization and regulating population density (e.g. Yasukawa et al. 1985; Hurst 1987a; Parmigiani et al. 1989; Palanza et al. 1993). These studies suggest that females are territorial when a male is present, and that territorial behaviour is related to breeding.

In previous studies, we observed that female laboratory mice, which are usually non-aggressive as virgins, may become aggressive towards unfamiliar same-sex conspecific intruders when housed for 12–24 h with a male (in R-S and CF-1 females: F. vom Saal, unpublished data; CD-1 females: Parmigiani et al. 1989). This stimulation of the aggressive behaviour of a female by a male may be due to pheromonal cues present in mouse urine. These cues are important in the regulation of the social behaviour of mice (Bronson 1976; Hurst 1987b), and female mice increase their rate of urine deposition when exposed to a male or his urine (Maruniak et al. 1975). Given this background, our aim in the present study was to assess, by examining both aggressive behaviour towards conspecifics and urine marking, whether exposure to male urinary cues would influence the territorial behaviour of wild female mice.

We used the first generation bred in the laboratory derived from wild mice originally captured near Capalbio (Grosseto, Tuscany, Italy) during July 1991–October 1992. After weaning (around 30 days of age), mice were housed with same-sex litter-mates (two to four animals per cage) in Plexiglas cages measuring 40 × 25 × 20 cm. Room temperature was maintained at 22 ± 2°C on a 12:12 h light:dark cycle, with lights on at 0700 hours. When 90 days old, virgin females were randomly assigned to an experimental condition or a control condition. In the experimental condition, females ($N=31$) were exposed to a strange male odour by placing them individually in cages (40 × 20 × 20 cm) which had been inhabited (for 48 h) by a male of the same wild stock. After 24 h, these females were further divided into two sub-groups. One sub-group of females ($N=18$) was exposed to an unfamiliar and genetically unrelated female intruder, which was placed into each female's cage for 15 min. Thirty minutes after the completion of this intruder test, a sexually naive male was also introduced into each female's cage for 15 min; this was done to assess whether male cues can stimulate female aggression

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non-specifically towards all unfamiliar conspecifics or rather, selectively towards same-sex intruders. A second sub-group of females ($N=13$) was tested for their rate of urine marking. The cages containing this second group were divided in half by a removable polypropylene barrier. The floor of the half housing the female remained covered with the soiled bedding from the prior male inhabitant, while the bedding in the other half was removed and replaced with a sheet of Whatman (No. 2) filter paper. The barrier was raised and the test female was allowed to enter the half of the cage containing the filter paper, and the barrier was replaced. After 1 h the filter paper was removed and discrete urine marks were counted under UV light.

In the control condition, females ($N=29$) were individually housed for 24 h in cages ($40 \times 20 \times 20$ cm) containing clean sawdust and subjected to an intruder test ($N=16$) or the urine-marking test ($N=13$) following the same procedures described above.

The experiment was performed in accordance with ASAB guidelines governing animal behaviour research (see also Elwood & Parmigiani 1992). As a rule, we stop the tests if fighting escalates, but in the present study the level of aggression was low, the number of bites was small and no instances of observable physical injury occurred within the experimental time.

None of the females isolated in cages with clean sawdust attacked or showed any agonistic behaviour (such as tail-rattling) or mounting-like behaviour towards female intruders. After exposure to male bedding, nine of 18 (50%) resident females attacked same-sex intruders (versus unsoiled bedding 0/16; $\chi^2=8{\cdot}4$, $df=1$, $P=0{\cdot}004$). Attacks ($\pm$ SEM number of bites $=7{\cdot}9 \pm 1{\cdot}3$) were mostly directed towards the back and flanks of the opponent's body, and six of the nine attacking females displayed tail-rattling before and/or after the attacks. Females thus exhibited patterns of intra-sexual attack similar to those normally seen in inter-male encounters, indicative of a competitive form of aggression (Brain 1981).

The mean ($\pm$ SEM) amount of time spent by resident females in social investigation of same-sex intruders did not differ between the two treatment groups (soiled bedding group: $23 \pm 2{\cdot}6$ s versus no soiled bedding group: 20 ± 4 s).

In both experimental conditions, females were never aggressive towards male intruders. In contrast, the male intruder generally started to attack the resident female within a few minutes; as a result the test was immediately terminated.

Exposure to male soiled bedding significantly elevated urine-marking rates of females above the levels of females exposed to clean bedding ($\bar{X} \pm$ SEM number of urine spots: $71 \pm 12{\cdot}3$ versus $21{\cdot}8 \pm 3{\cdot}5$, respectively: $t=4{\cdot}0$, $df=24$, $P<0{\cdot}001$), confirming the results of Maruniak et al. (1975) in CF-1 laboratory mice.

Increased urine-marking in the presence of a male has generally been interpreted as being due to females advertising their impending sexual receptivity via chemical signals (Eisenberg & Kleiman 1972). A recent study by Coquelin (1992), however, showed that when tested in the presence of a male, female CF-1 mice did not vary their urine marking during their reproductive cycle, thus suggesting that receptivity or breeding status is likely to be signalled by qualitatively different pheromonal cues rather than quantitative differences in urine marking.

Increased urine marking in response to male odour may thus function to advertise territory occupancy to other females. On the basis of these results, we propose that competition among females is induced by the presence of stimuli emanating from a male. We suggest that females compete among themselves not simply for space, but for a space held by a male (a defined territory). In other words, females appear to compete for access to a male that has taken possession of a territory. Male pheromones are known to induce oestrus (referred to as the Whitten effect: Whitten 1958). Thus, increased female aggressiveness in response to male urinary pheromonal cues could enhance the probability of a dominant female mating by subordination (and suppression of reproduction) or dispersal of female rivals. An important aspect of this hypothesis is that in most laboratory stocks of mice, females have been subjected to intense selection for non-aggressiveness, so that groups of females could breed within a single small cage. In contrast, wild female mice show a high level of aggressiveness (i.e. intrasexual and infanticidal attack) when associated with males, and dominance status appears to influence female, as well as male, reproductive success (Lloyd & Christian 1969; vom Saal 1983; Hurst 1987a; Parmigiani et al. 1989; Palanza et al. 1993).

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