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Social interaction alters attraction to competitor's odour in the mouse *Mus spretus* Lataste

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Abstract. When animals defend territories that are large and structurally complex, scent marks alone are unlikely to be reliable signals of a resident's dominance and competitors should require initial proof through direct interaction. This was tested using freshly captured *Mus spretus* which occupy large non-overlapping ranges in grassland but are strongly attracted to substrate odours from unfamiliar competitors. Choice tests measured time spent investigating and chewing to gain access to paired nestboxes when the entrances were blocked with mesh. Experiment 1 established that mice of both sexes were more strongly attracted to their own odour than to a clean site. Experiment 2 examined choice between the subject's own odour and that of an unfamiliar same-sex competitor both before and after meeting the competitor in a neutral (clean) arena. Prior to interaction, males exerted much effort to gain access to both their own and their unfamiliar competitor's odour. Once relative dominance had been established through agonistic interaction, subordinates avoided their dominant competitor's odour in favour of their own while dominants continued to be attracted to both. There was little aggressive competition between unfamiliar females and relative status did not affect their attraction to a competitor's odour. Females tended to be more attracted to a competitor's odour than to their own prior to interaction but showed less attraction to a competitor's odour post-interaction. A third experiment showed that the odour of an unfamiliar male was more attractive than that from an unfamiliar female, especially to males. The consequences of these responses for maintaining spatial dispersion in this species are discussed.

In a recent study of the behavioural mechanisms underlying the spatial dispersion of the aboriginal mouse *Mus spretus* Lataste living in grassland, Hurst et al. (1996) concluded that individuals do not attempt to force competitors away from defended territories by aggression. The brief attacks and chases and static defensive postures shown between two unfamiliar males and, less frequently, between females are more consistent with mice competing for dominance over suitable sites. This differs significantly from the aggressive pursuit of competitors shown, for example, by the well-studied commensal house mouse, *Mus domesticus* (Rowe & Redfern 1969; Gray & Hurst 1997). However, both trapping (Cassaing & Croset 1985; Hurst et al. 1994, 1996) and radiotelemetry studies (unpublished data) show that the ranges of individual males are mutually exclusive, whilst trapping ranges also suggest that adult females are similarly dispersed but overlap with males (Cassaing & Croset 1985; Hurst et al. 1994, 1996). Individual ranges can be quite large (up to 924 m^2) and may border closely with neighbours (unpublished data). If animals do not force intruders to leave their territory, how do they maintain such large non-overlapping ranges?

A resident's odour in the environment will provide a signal to intruders that a site is occupied. Territorial animals may deliberately deposit scent marks to provide competitors with a cheat-proof signal of the resident's ability to dominate a territory (Gosling 1982). In support of this, commensal house mice use urine marks on the substrate to assess the competitive ability of a resident male and avoid potentially costly encounters (Jones & Nowell 1989; Gosling & McKay 1990;

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Hurst 1993). Gosling (1990) further suggested that intruders would not require direct experience of a resident's competitive ability to use this signal, as they need only match the odour of any individuals they encounter with the predominant scent marks in the territory. Hurst et al. (1996) argued, however, that odours alone would not provide a reliable advertisement of dominance when territories are large and structurally complex, such as those apparently defended by mice in grassland, where they live in loose networks of tunnels that cover large areas. Under these conditions, cheats could easily enter or hide within the territory and deposit marks while the dominant territory owner was elsewhere, or could lay claim to an unoccupied area by depositing scent marks without proving their dominance. In support of this, Hurst et al. (1996) found that recently captured *M. spretus* are strongly attracted to recently occupied nest sites of unfamiliar competitors and will compete strongly for occupied sites. They suggested that spatial dispersion might result from the avoidance of known dominant competitors and their scent marks, since a dominant individual would have priority of access to the most suitable sites within a territory, but only after dominance is established by direct interaction between the individual competitors. This learnt association between an individual's proven competitive ability and its substrate odour would provide a reliable signal of dominance even over large complex areas, at least in the short term. Evidence supporting the importance of familiarity and competition between individuals in determining their response to substrate odours comes from the finding that recently captured *M. spretus* avoid entering tunnels previously occupied by conspecifics caught from neighbouring sites (and thus potentially familiar) but do not generalize this response to the odours of unfamiliar mice originating from a distant population (Hurst et al. 1994).

Our aim was thus to test whether the establishment of dominance in direct interaction alters the attractiveness of a competitor's substrate odour in this spatially dispersed grassland species. Our prediction was that mice temporarily displaced from their home area will initially be attracted to substrate odour from an unfamiliar mouse, as this will indicate a suitable, and thus desirable, site (Hurst et al. 1996). However, once dominance has been established through agonistic interaction, the subordinate should subsequently avoid sites bearing the odour of a familiar dominant. The dominant, on the other hand, should be unaffected or even more strongly attracted to sites bearing the odour of a familiar subordinate that it can displace.

To test these predictions, we developed a different bioassay to that used in previous studies, to measure (1) the effect of substrate odour in determining spatial location, and (2) the attractiveness of a competitor's odour in a choice between two sites bearing mouse odour and thus both potentially suitable for mice. The standard test paradigm to assess attraction to, or avoidance of, a stimulus is to provide animals with a choice between the test stimulus and a clean control, with the assumption that a clean control will stimulate a neutral response (e.g. Jones & Nowell 1973; Drickamer 1989; Hurst et al. 1994). However, as Hurst et al. (1996) pointed out, in an artificial test situation a clean site may not be recognized as suitable habitat, and animals might show a relative attraction to conspecific odour that they would not show in their home area. Furthermore, a clean (i.e. unused) site very near to one bearing a conspecific's odour could indicate a site judged unsuitable by conspecifics already using the area. A choice between conspecific odour and clean may not represent the assumed choice between 'occupied and unavailable' versus 'unoccupied and available' but instead represent 'occupied and desirable' versus 'unoccupied and probably unsuitable'. It is commonly found, for example, that rodents of a variety of species will enter traps soiled by conspecifics more frequently than clean traps (e.g. Boonstra & Krebs 1976; Stoddart 1982; Drickamer et al. 1992; Gurnell & Little 1992). In this study, therefore, we provided mice with a choice between a sheltered site bearing their own odour versus that of a competitor (a same-sex conspecific), after first establishing that, like their response to odour from an unfamiliar competitor (Hurst et al. 1996), *M. spretus* are much more attracted to their own odour than to a clean site. We also tested whether mice would prefer a site bearing the odour of an unfamiliar conspecific of the opposite sex, which might be a potential mate, to odour from a conspecific of the same sex, which would be more likely to attack (Hurst et al. 1994).

Odour choice tests usually measure investigatory preferences or other non-specific behavioural responses (Albone 1984) but these are notoriously difficult to interpret. Investigation may be

strongly affected by the novelty of an odour, for example, or by the need to gain social information from scent marks without necessarily reflecting a preference for that location (e.g. see Hurst et al. 1994). In this study, we measured how hard mice would work to gain access to the two odour sites (see above) before and after they interacted with the competitor in a neutral (clean) arena. We achieved this by placing a mesh barrier in front of each odour site and measured how long the mice chewed at the mesh in an attempt to gain access to each site. Many of the mice expended considerable time and effort in trying to chew through these barriers, a response that was shown spontaneously and needed no training. However, subjects were first familiarized with the apparatus without barriers to ensure that they were aware of the presence of shelter at the two sites where the odour choices were presented.

METHODS

We used 27 male ($\bar{X} \pm SE = 14.3 \pm 0.4$ g) and 21 female (15.8 ± 0.5 g) adult *M. spretus* recently captured from a 4–5 ha area of grassland (a disused farm) in Sobreda, Portugal, during April 1995 as both subjects and odour donors in these experiments. We selected mice at random for use in each of the experiments, subject to the constraint that mice paired in experiment 2 were caught from separate areas. We captured the mice in 120 Longworth traps set out in two main areas that were separated by a small cliff and an area of open woodland. Previous trapping studies on this farm (Hurst et al. 1994, 1996; J. L. Hurst, unpublished data) and a simultaneous radiotracking study in one of the areas (unpublished data) indicated that individual ranges would not encompass both areas. In all trials, mice were tested for their response to odours from conspecifics caught from the other area (>100 m apart), and thus were very unlikely to be recently familiar with the test odour or donor. All trials were conducted within 10 days of capture, with experiments 1 and 3 conducted before experiment 2.

After capture, we sexed and weighed the mice (± 0.25 g), and clipped small patches of their dorsal fur for individual identification. Eight females (38%) were visibly pregnant, six giving birth during the study. The mice were housed singly in clean polypropylene cages ($44 \times 26 \times 12$ or $30 \times 13 \times 12$ cm) on sawdust substrate with grass for nest material, ample food (laboratory pellets, Banton & Kingman, Hull, U.K. and wheat grain) and water. All mice were housed in a darkened room at ambient temperature and humidity where tests were carried out. A small amount of light penetrating the room during the daytime (0730–2130 hours) provided a dim light: dark cycle, supplemented by dim red lighting over arenas during tests. At the end of the study, all mice were released at their original capture site, together with any pups born in captivity which were placed under shelter in grass nests.

Test Apparatus and Procedure

We modified the test apparatus used by Hurst et al. (1994) by using nestboxes as odour sites rather than Perspex tunnels. In all odour-choice trials, we provided mice with a choice between two nestboxes containing a handful of grass (collected from sites uninhabited by mice) in which odour donors had been confined prior to the trial (see separate experiments below). The nestboxes (10×15 cm diameter, blackened Perspex) were attached, 42 cm apart, along the front wall of a test arena ($60 \times 18 \times 60$ cm varnished plywood) by two short tunnels (3×3 cm diameter). During trials, mice were prevented from entering the nestboxes by clean mesh caps (6.5 mm galvanized steel) on the inner end of the tunnels. These allowed mice to insert their heads into a nestbox but no more; thus mice could not contact the odour source. To avoid any stress that might be induced by direct handling, we transferred mice between cages and arenas by allowing them to run into clean Perspex tunnels (19×3 cm; Hurst et al. 1994). We introduced subjects into a test arena from a handling tunnel through a hole in the front wall of the arena, mid-way between the two nestboxes; the entrance hole was then plugged by a Perspex stopper. One observer sat on either side of the arena to record the response to each nestbox. We measured the total time spent at each tunnel entrance (timed when the tip of the nose entered the tunnel), together with the number of visits and the time spent chewing at the mesh caps in an attempt to enter the nestbox (clearly detectable from the sound of chewing), during 10-min trials. We calculated investigation time as the difference between total contact time and the duration of chewing. Between trials, we

thoroughly washed nestboxes and tunnels in detergent, rinsed and dried them and wiped down arenas and mesh caps with 70% ethanol.

Prior to each odour-choice trial, subjects were familiarized with the clean test apparatus and the presence of shelter within the nextboxes. We attached a grass-filled clean nestbox by an open tunnel to the left or right side of the arena entrance hole in a balanced design (the other nestbox entrance was temporarily plugged). We introduced the subject into the arena through the central entrance hole and allowed it to investigate until it entered the uncapped nestbox. After 30–60 s, we removed the mouse and repeated the procedure with the nestbox attached on the opposite side of the entrance hole. This ensured that, prior to a test, the mouse was aware that boxes to both the left and right side of the arena provided shelter, and had established that there was no other escape route.

Experiment 1: Response to Own Odour versus Clean

To check that mice showed a significant attraction to their own substrate odour relative to a clean site, we presented 12 male and 12 female mice with a choice between a nestbox in which they had been confined for 30 min immediately prior to a 10-min trial versus a clean nestbox. After their confinement, subjects were familiarized with the test apparatus using another clean nestbox, as detailed above. We then placed the nestbox containing their own odour on the right or left in alternate trials with the clean box on the opposite site. Clean mesh caps prevented subjects entering the test boxes.

Experiment 2: Response to a Competitor's Odour versus Own

To test the prediction that interaction with a conspecific of the same sex alters the attractiveness of their substrate odour, we gave 32 male and 32 female subjects a choice between a nestbox containing their own odour versus one containing the odour of a conspecific caught from a different area (thus presumed previously unfamiliar) before and after interacting with the odour donor in a clean arena. Owing to the limited availability of animals from different areas, we used 13 (68%) individual males and 12 (60%) females twice as subjects. Their second trial was at least 36 h after their first and involved a different conspecific (repeated measures analyses of variance confirmed that social experience in their first trial had no significant long-term effects on pre-interaction response in their second trial). We confined two unfamiliar mice of the same sex to separate clean nestboxes for 60 min prior to their pre-interaction test, to generate strong stimulus odours. Each subject was then familiarized with the clean apparatus and we measured their preference between their own and their conspecific's nestbox (entry prevented by clean mesh caps) over a 10-min trial, as detailed above, before they interacted with the conspecific odour. Own odour was placed alternately on the left or right between trials and we replaced each subject in its home cage while we tested the other subject's response.

Immediately after the second subject had completed the pre-interaction choice test, we introduced both mice into a clean arena ($60 \times 60 \times 60$ cm varnished plywood) via their Perspex handling tunnels for a 10-min trial, starting from their first interaction. The interaction arena contained a clean open-topped nestbox (supplied with a small handful of grass for cover) and wheat grain in a ceramic pot. Two observers recorded the frequency of attack, chase and fight (aggressive acts); defend, roll, shove and flee (defensive acts); approach, retreat, close and distant investigation, squeak, eyes closed, allogroom and sit by, shown by each individual, together with the number and duration of each separate interaction (following definitions in Hurst et al. 1996).

For analysis, we classified the mice in each dyad as Neutral if they initiated and received no aggression, or as Dominant and Subordinate depending on whether they initiated more aggressive acts than they received. Where both initiated a similar amount of aggression (one female trial) both were classified as Dominant. Further details are given in the Results.

Immediately after the interaction, we confined mice in their own odour nestbox for 10 min to refresh their substrate odour cues. We then tested again the response of both subjects towards their own versus their competitor's odour in a post-interaction test. To ensure that the location of the stimuli had no effect on preference, we placed their own odour nestbox either on the same side as in their pre-interaction test, or on the opposite side, alternating between trials. Since subjects were already familiar with the apparatus, the

familiarization procedure was not carried out prior to the post-interaction odour-choice test.

Ethical note

During interactions the nestbox provided a retreat for the mice without inhibiting observation. However, to ensure that competitors did not physically damage one another or suffer undue distress on being confined together, we had decided to separate any dyads immediately if they showed persistent chasing or biting, or if mice made frantic attempts to escape. Only one trial was terminated prematurely (after 32 s), owing to a female persistently attacking when a subordinate female rolled onto her back in a defensive posture. As found in previous studies (Hurst et al. 1994, 1996), aggressive acts were very brief in this species and aggressors generally retreated when their opponent showed defensive postures. Defenders frequently chose to approach and investigate their aggressor after an attack and sometimes sat in close contact, suggesting that their level of social stress was low. The mean number of interactions per trial that involved aggression (attacks or chases) ± SE was 8.5 ± 1.2 among males and 0.9 ± 0.3 among females. The duration of aggression per se was not measured but all attacks were very brief (<1 s); the total duration per trial of interactions that involved chasing ± SE was 8.8 ± 3.2 s among males and 0.06 ± 0.06 s (one chase) among females, although this also included time spent in investigation and defensive postures prior to the chase.

Experiment 3: Effect of Donor's Sex

We gave 16 male and 16 female subjects a choice between two capped nestboxes containing odours from a male versus a female conspecific, caught from an area different from the subject (thus presumed unfamiliar). Odour donors were confined to clean grass-filled stimulus nestboxes for 60 min and subjects familiarized with the clean test apparatus prior to each trial. The left/right location of the male and female test odours was alternated between replicates in a balanced design.

Data Analaysis

We transformed the total time spent chewing and investigating each nestbox entrance, together with the frequency of visits per trial, by natural logarithms to meet the assumptions of parametric analyses. The distributions of the transformed variables did not differ significantly from normal (Kolmogorov–Smirnov tests: NS). Repeated measures analyses of variance examined the effect of odour source on preference measures within subjects, together with the effect of relative social status (experiment 2) or sex (experiments 1 and 3) between subjects. A significant interaction between odour source and status or sex would thus indicate a significant difference in odour preference according to the subject's status or sex. A significant main effect of status or sex would indicate a general difference in responsiveness to both odour sites within a test. Since reproductive status is known to have a strong effect on odour preference among *M. domesticus* females (e.g. Drickamer 1989; Hurst & Nevison 1994), a further repeated measures ANOVA checked whether the breeding status of female subjects and donors (pregnant/lactating or with no visible signs of breeding, although the latter may have included some females in early pregnancy) affected their chewing preference. To illustrate the chewing response within separate classes of mice, figures show the mean bias in response between the two odour choices ± 95% confidence intervals. Confidence intervals that do not cross the line of zero bias thus indicate a significant bias in response within that class of mice.

RESULTS

Attraction to Own Odour

First we confirmed that mice of both sexes would work harder to try to get to a site bearing their own odour than to an equivalent clean site. Given the choice, mice spent significantly longer chewing the mesh that prevented access to their own odour than that covering the clean site ($F_{1,22}=5.98$, $P<0.01$, one-tailed test). There was no significant difference between the sexes in this bias (interaction between sex and odour: $F_{1,22}=0.02$, NS). Mice of both sexes spent much more time investigating their own odour ($F_{1,22}=13.9$, $P<0.0005$, one-tailed test) and visited the entrance more frequently than the clean site ($F_{1,22}=3.42$, $P<0.05$, one-tailed test). Again, there were no significant differences between the sexes in these measures of preference (sex × odour

interaction for investigation time: $F_{1,22}=0.02$, NS; frequency of visits: $F_{1,22}=0.05$, NS). Untransformed durations and frequencies are given in Table I.

Male's Response to a Competitor's Odour

In most cases when two unfamiliar males met in a neutral arena ($N=10$), only one of the dyad showed any aggression and was clearly dominant. When both males initially showed aggression ($N=5$), this was quickly resolved with the subordinate of the two showing much defensive posturing (see Hurst et al. 1996). There was no aggression in one trial and so both males were classified as neutral for analysis of their prior and subsequent response to their competitor's odour.

Prior to interacting, males showed no bias in behaviour when given a choice between their own odour or that of their unfamiliar competitor, with no significant differences in response according to their subsequent social status on meeting the competitor. Males of all three status categories spent as much time chewing to gain access to the other male's odour as they did towards their own (Fig. 1a; effect of odour: $F_{1,29}=0.15$, NS) with no difference according to their status when subsequently interacting with that male (status and odour interaction: $F_{2,29}=0.53$, NS). This was not because the males were not responsive to the presence of the nestboxes as they spent appreciable time chewing at both mesh caps (Table II), similar to that stimulated by their own odour rather than the clean nestbox in the previous test (Table I). The novelty of the other male's odour did not stimulate significantly longer investigation than their own ($F_{1,29}=0.52$, NS; Table II), again with no difference according to their subsequent status when interacting with the donor (status × odour interaction: $F_{2,29}=0.21$, NS). There was thus no bias in the total time spent in contact

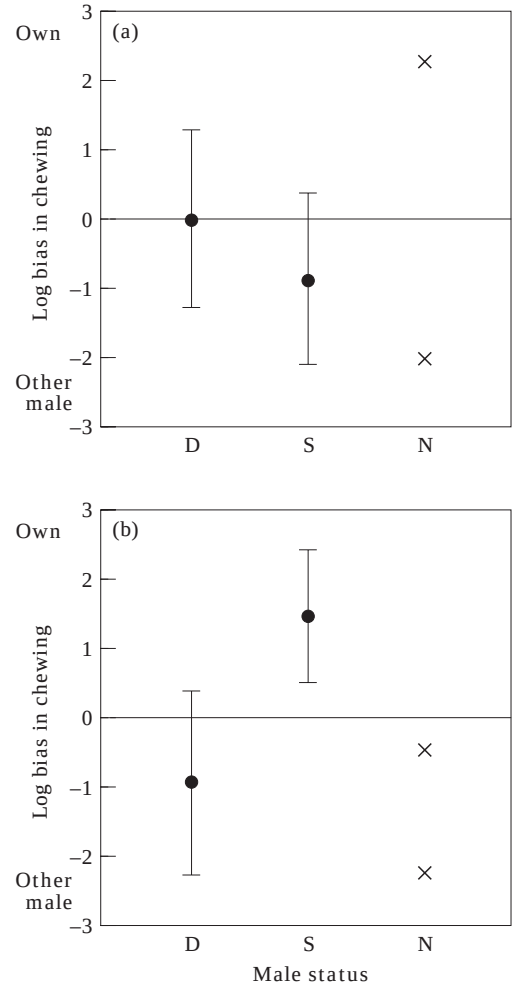

Figure 1. Difference in time (ln s+1) males spent chewing to gain access to paired nestboxes containing their own or a competitor's odour ($\bar{X}\pm 95\%$ confidence intervals), (a) before and (b) after meeting the competitor odour donor. Male status is the subject's status on meeting the odour donor (D: dominant; S: subordinate; N: neutral). Responses of the two neutral males are shown individually.

Table I. Male and female behaviour towards paired nestboxes containing their own or no mouse odour ($\bar{X}\pm$ SE)

	Males		Females	
	Own odour	Clean	Own odour	Clean
Chewing (s)	81.3 ± 23.3	28.3 ± 11.7	81.6 ± 38.2	26.0 ± 22.3
Investigation (s)	22.9 ± 4.5	11.7 ± 1.9	15.3 ± 4.2	8.5 ± 2.6
Visit frequency	7.0 ± 1.2	5.3 ± 0.7	5.6 ± 1.4	3.8 ± 0.9

Table II. Male behaviour towards paired nestboxes containing their own or a competitor's odour before and after meeting the competitor odour donor ($\bar{X} \pm$ SE)

	Dominant males		Subordinate males	
Odour choice	Own	Other male	Own	Other male
Pre-interaction				
Chewing (s)	102.9 ± 28.6	71.8 ± 16.7	80.4 ± 26.3	90.1 ± 21.9
Investigation (s)	18.3 ± 4.9	19.3 ± 3.5	15.4 ± 2.8	17.7 ± 3.4
Visit frequency	6.5 ± 0.8	6.6 ± 0.9	6.3 ± 1.0	6.7 ± 1.2
Post-interaction				
Chewing (s)	65.7 ± 17.5	139.4 ± 33.6	125.1 ± 22.8	45.1 ± 13.2
Investigation (s)	16.9 ± 3.7	18.8 ± 2.6	26.8 ± 7.2	12.8 ± 2.6
Visit frequency	6.6 ± 1.2	6.3 ± 0.9	6.6 ± 0.6	4.7 ± 0.7

with the two odours, or in their frequency of visits.

As predicted, interacting with a competitor altered a male's subsequent response to his competitor's odour according to the relative social status established in their interaction. Given a choice between own odour and that of the now familiar competitor post-interaction, a male's status had a significant effect on the bias in time spent chewing to gain access to the two odours ($F_{2,29}=5.10$, $P<0.05$). Subordinate males now spent significantly longer attempting to get to their own odour than to their dominant competitor's odour, while dominant males tended to show the opposite response (Fig. 1b). The two neutral males each showed an opposite preference. Repeating the analysis with these two males excluded confirmed that there was a highly significant difference in the chewing bias shown by dominant and subordinate males ($F_{1,28}=8.95$, $P<0.01$) owing to subordinates directing most of their effort towards their own odour and much less towards the dominant's odour (Table II). Note though that subordinates still spent 45 ± 13 s chewing to gain access to the dominant's odour compared with only 28 ± 12 s shown towards a clean nestbox when this was matched against their own odour (Table I, see above). Dominants continued to show no significant bias. This was not because dominant males failed to chew at the mesh caps; dominants continued to show a strong response to both nestboxes and the total duration of chewing by dominants and subordinates was very similar (Table II; $F_{1,28}=0.01$, NS). Comparison of individual bias post-interaction with that shown prior to interacting with the odour donor confirmed that interaction failed to induce any change in a dominant male's odour preference (matched-pair $t_{14}=-1.22$, NS) but stimulated a highly significant change in that of subordinates ($t_{14}=4.33$, $P<0.001$). Dominance status had a similar, although less significant, effect on their bias in investigation time (status × odour interaction: $F_{1,28}=4.92$, $P<0.05$) as subordinates spent more time investigating their own odour than that of the now familiar dominant male (Table II).

Although mice were classified into distinct classes of social status for analysis, the strength of their chewing bias after interacting with a donor appeared to vary continuously with the difference in the number of aggressive acts initiated and received during the interaction ($F_{1,30}=7.71$, $P<0.01$; $R^2=20.4\%$; Fig. 2). It appears from Fig. 2 that most dominant males that initiated frequent aggression against a defensive subordinate were more strongly attracted to the subordinate's odour than to their own, while all subordinates showed a consistent preference for their own odour. The difference in aggressive acts initiated and received during the interaction explained 26.5% of the variance in the change in individual chewing bias from the pre- to the post-interaction test ($F_{1,30}=10.81$, $P<0.005$).

Female's Response to a Competitor's Odour

As found in previous studies, there was much less aggression when two females met in a neutral arena ($\bar{X} \pm$ SE; males: 17.4 ± 2.3; females: 1.75 ± 0.5 aggressive acts per trial; effect of sex: $F_{1,30}=42.6$, $P<0.0001$). No aggression was shown in seven trials, these females being classified as

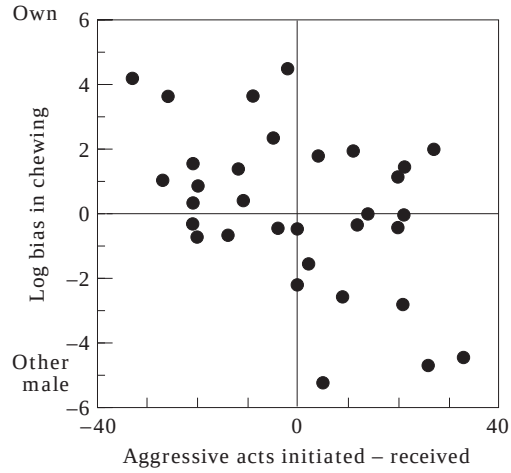

Figure 2. Difference in time (ln s + 1) males spent chewing to gain access to paired nestboxes containing their own or a competitor's odour during post-interaction trials according to the difference in aggressive acts (attacks, chases, fights) they had initiated and received on meeting the competitor odour donor. See text for regression statistics.

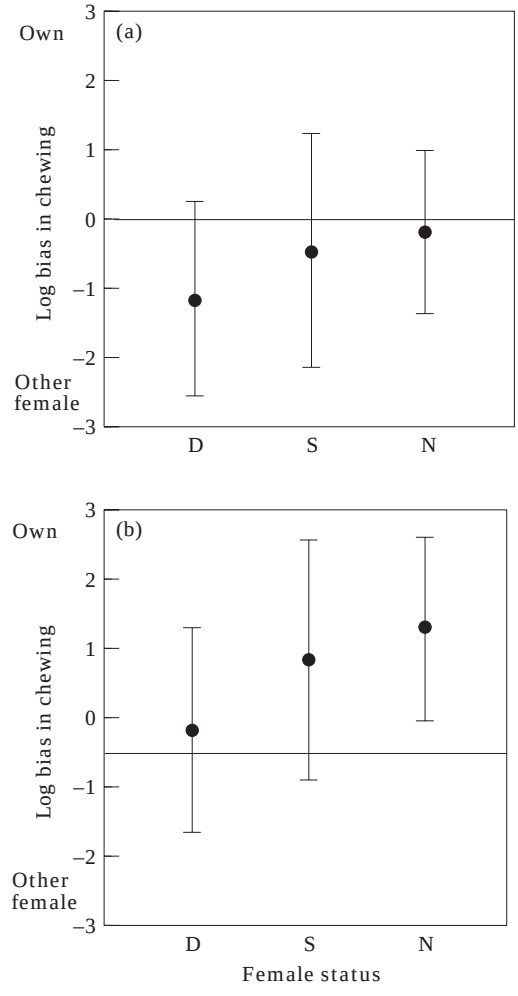

Figure 3. Difference in time (ln s + 1) females spent chewing to gain access to paired nestboxes containing their own or a competitor's odour ($\bar{X} \pm 95\%$ confidence intervals), (a) before and (b) after meeting the competitor odour donor. Female status is the subject's status on meeting the odour donor (D: dominant; S: subordinate; N: neutral).

neutral. In the remaining trials ($N = 9$), females were classified as dominant or subordinate on the basis of the difference in aggression initiated and received, although fewer aggressive acts were involved than in male trials ($\bar{X} \pm$ SE; males 18.5 ± 2.2; females 3.5 ± 0.6; $F_{1,22} = 24.7$, $P < 0.0001$) and flight among females was less frequent ($F_{1,22} = 5.67$, $P < 0.05$), although static defensive postures did not differ ($F_{1,22} = 2.31$, NS). In one trial, both females were equally aggressive and defensive and were both classified as dominants for analysis of their odour responses.

Prior to interacting, females in general showed no significant bias in chewing to gain access to their own odour or to that of an unfamiliar competitor ($F_{1,29} = 2.27$, NS) with no difference in response according to their subsequent status ($F_{2,29} = 0.50$, NS; Fig. 3a). The odour of an unfamiliar female did not induce greater investigation than their own either ($F_{1,29} = 0.77$, NS) and there was no difference in the frequency of visits or total time spent at the two nestbox entrances or any effect of status on these responses. However, taking the breeding status of subject and donor into account instead of subsequent social status revealed a pre-interaction tendency to prefer a competitor's odour over their own (effect of odour source on chewing: $F_{1,28} = 4.04$, $P = 0.054$). This was largely because non-breeding females showed a very strong attraction to the odour of another non-breeding female ($t_3 = 8.3$, $P < 0.005$). No significant bias was apparent in trials involving breeding female subjects or their odours. Females were generally less responsive than males when presented with a choice between their own and a competitor's odour, spending less time in total

Table III. Female behaviour towards paired nestboxes containing their own or a competitor's odour before and after meeting the competitor odour donor ($\bar{X} \pm$ SE)

	All females	
	Own	Other female
Pre-interaction		
Chewing (s)	48.6 ± 15.0	57.9 ± 11.3
Investigation (s)	13.7 ± 2.1	12.4 ± 1.4
Visit frequency	4.8 ± 0.6	5.3 ± 0.5
Post-interaction		
Chewing (s)	96.9 ± 19.2	44.0 ± 10.0
Investigation (s)	16.3 ± 3.1	14.8 ± 3.0
Visit frequency	4.9 ± 0.5	4.3 ± 0.4

chewing to gain access to the nests ($F_{1,62} = 8.06$, $P < 0.01$). Despite this, they still spent 107 ± 17 s chewing to gain entry to the nestboxes (Table III).

Interacting with the odour donor appeared to induce a small but significant change in the preference shown by all females towards their own and their now familiar competitor's odour. A female's aggressive status when interacting with a competitor had no significant effect on her subsequent chewing preference ($F_{2,29} = 1.13$, NS; Fig. 3b). Females overall tended to spend longer chewing to gain access to their own odour than to that of another female in post-interaction trials (Table III), although this was not statistically significant ($F_{1,29} = 2.34$, NS). This post-interaction preference was not significant even when the breeding status of subject and donor was taken into account ($F_{1,28} = 0.55$, NS) since non-breeding females no longer showed a preference for a similar conspecific's odour. However, when post-interaction chewing was compared with each individual's pre-interaction bias, the change in their bias was significant ($F_{1,31} = 5.08$, $P < 0.05$) as females spent more time attempting to gain access to their own odour relative to their competitor's after social interaction. They showed no significant bias in investigation or in frequency of visits to the two nestbox entrances, with no effects of status on the lack of bias in these behaviours. The bias in chewing shown by individual females post-interaction was not related to the small difference in aggressive acts that they initiated and received when interacting with the donor ($F_{1,30} = 1.05$, NS).

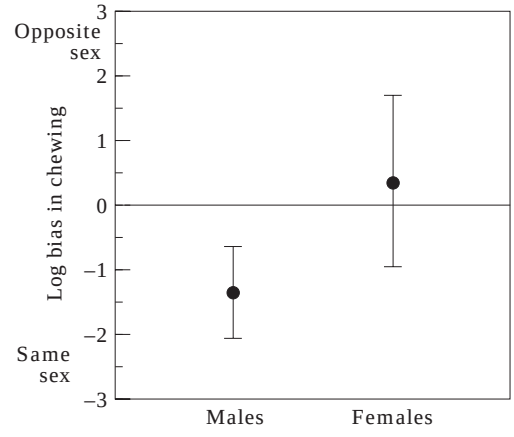

Figure 4. Difference in time (ln s + 1) spent chewing to gain access to paired nestboxes containing odour from an unfamiliar conspecific of the opposite or their own sex ($\bar{X} \pm$ 95% confidence intervals).

Preference for Male versus Female Odour

Given a choice between the odours of an unfamiliar male and female conspecific, we might expect mice of both sexes to be more attracted to odour from their opposite sex. However, males and females differed significantly in their chewing bias towards odour from an opposite versus same sex conspecific ($F_{1,30} = 5.50$, $P < 0.05$). Contrary to expectations, males spent significantly longer attempting to gain access to the odour of an unfamiliar male than to the odour of a female, while females showed no significant bias (Fig. 4). The untransformed durations in Table IV indicate that female odour stimulated no more chewing from males than a clean nestbox while stimulating

Table IV. Male and female behaviour towards paired nestboxes containing odour from conspecifics of the same or opposite sex ($\bar{X} \pm$ SE)

	Males		Females	
	Own odour	Clean	Own odour	Clean
Chewing (s)	27.9 ± 6.4	77.3 ± 16.4	77.5 ± 31.2	40.8 ± 12.7
Investigation (s)	19.8 ± 4.4	20.1 ± 2.4	23.4 ± 4.9	18.9 ± 3.9
Visit frequency	11.3 ± 2.8	9.3 ± 1.1	8.9 ± 0.9	9.3 ± 1.4

only a little more from females. Female odour, however, stimulated as many visits and as much investigation as male odour (Table IV), with no significant biases shown by either sex or difference between the sexes for these measures. Thus mice, particularly males, made more effort to gain access to the male odour site, although a post hoc test suggested that there was no significant sex difference in this preference for male odour ($F_{1,30} = 1.80$, NS).

DISCUSSION

Our results confirmed that both sexes of *M. spretus* were strongly attracted to gain access to their own substrate odour, and were as strongly attracted to the substrate odour of an unfamiliar conspecific competitor as to their own. This is consistent with previous findings of (1) a strong investigatory preference for artificial tunnels bearing mouse odour (own or a conspecific's) over clean tunnels in this species (Hurst et al. 1994), and (2) much greater time spent inside a nest recently occupied by an unfamiliar competitor than inside a clean nest (Hurst et al. 1996). The considerable effort that mice expended in chewing to gain access shows that their general attraction to a conspecific's odour is not simply to investigate an interesting or novel odour. Substrate odours appear to play an important role in determining their choice of location, mice preferring sites recently occupied by themselves or by unfamiliar conspecifics. Hurst et al. (1996) found that *M. spretus* appeared to prefer an unfamiliar competitor's nest site even over their own, evident from a bias in the time spent inside nests among males and in the frequency of visits to nests among both sexes when given free access. This was not matched by a significant bias in chewing when mice could not gain access in our study, even though their post-interaction bias showed that they were clearly capable of discriminating between individual odours without physical contact with the odour cues. This suggests that the bias found in the earlier study reflected a greater desire to investigate the novel conspecific's odour rather than a genuine preference for the conspecific's nest site over their own.

The significant change in attractiveness of a competitor's substrate odour relative to their own, found among males post-interaction, precisely matched predictions from the hypothesis that these grassland mice would require direct proof of a donor's superior competitive ability before they would use its substrate odour as a reliable signal of dominance (see Introduction). The subsequent avoidance of a dominant competitor's odour by a subordinate male in favour of his own could be due to (1) qualitative or quantitative differences in the substrate odour of dominant compared with subordinate males, (2) a change in the subject's response to substrate odours induced by its experience in a competitive interaction, or a combination of both (1) and (2). In captive studies of small mammals, males can often be divided into distinct dominant and subordinate classes according to their general willingness to compete or be submissive towards competitors. This has been studied most thoroughly in the commensal house mouse where the experience of defeat stimulates a wide variety of behavioural (e.g. Desjardins et al. 1973; Andrade et al. 1987; Hurst 1987) and physiological (e.g. Brain 1972; Bronson 1973; Jones & Nowell 1974; Rodgers 1987) changes among subordinates. Qualitative differences in their urine (e.g. Harvey et al. 1989; Novotny et al. 1990) are easily detected both by competitors (e.g. Jones & Nowell 1973; Hurst 1990a, 1993) and by females (e.g. Jones & Nowell 1974; Lombardi &

Vandenbergh 1977; Hurst 1990b; Drickamer 1992). Only urine from dominant males, not from subordinates, induces avoidance by conspecifics (Bishop & Chevins 1987; Jones & Nowell 1989; Hurst 1990a), while a dominant male's substantially greater rate and distribution of urine marking also leaves a much stronger odour in the environment (Desjardins et al. 1973; Bishop & Chevins 1987; Hurst 1990a). Males that emit odours signalling their subordinate quality suffer a considerable cost as they are not selected as mates by females (e.g. Parmigiani et al. 1982a, b; Hurst 1987), although they benefit from being relatively tolerated by dominant males (Jones & Nowell 1973; Hurst 1990a) and can often remain within another male's territory where they will have access to food and shelter (e.g. see Crowcroft & Rowe 1963; Hurst 1987). Jones & Nowell (1989) showed that subordinate *M. domesticus* produce urine that signals their subordinate quality only if they are maintained in continuous close olfactory/visual contact with a dominant male, when there would be a strong advantage to avoiding competition with a higher quality male. Males with the same experience of defeat but housed in isolation produce the same aversive signal in their urine as dominant males, and they are strongly attacked even by familiar dominants (Andrade et al. 1987). It is not yet known whether there are such distinct dominant and subordinate classes among male *M. spretus*, reflected by qualitative differences in their odour cues. However, if individuals of low competitive ability are easily able to evade dominant competitors in a complex environment, it seems unlikely that they would emit odour cues that are likely to reduce their reproductive potential (through female mate choice) without any mitigating advantages, such as increased access to resources within a dominant male's territory. It is notable that individual male *M. spretus* in our study responded alternately as 'dominant' and 'subordinate' in four out of 19 replicate trials in which a male was used twice. Relative dominance thus may be determined in interactions between particular males, altering their response to the odour cues of known individuals, without any effect on the quality of the odour cues they emit. This deserves further study.

It is likely that experience of being attacked reduced a subordinate's willingness to approach sites bearing the odour of their aggressor since this would help males (1) avoid a further attack and (2) avoid potential displacement from a protected site by a dominant individual, which might expose them to the very serious danger of predation. Substrate odours may thus provide a mechanism for maintaining the separation of males in large non-overlapping territories once a resident has proven his superior competitive ability in direct interaction with the respondent, a mechanism that could also apply to many other small mammal species that show similar dispersion. This may involve the deliberate deposition of scent marks by residents to advertise their use of particular sites, although the source of the active odour and any associated marking behaviour remain to be established. In contrast, both dominant and defeated subordinate *M. domesticus* living at high density in much smaller territories show strong evasion of body odour or urine marks from a dominant male in favour of clean substrate, even when they are unfamiliar with the individual odour donor (Jones & Nowell 1974; Cox 1989; Hurst 1990a).

Experience of aggressive competition had no effect on choice between odour sites among females, which is not surprising given the low levels, if any, they generally experienced on encountering another female (see also Hurst et al. 1994). The tendency for all females to show an increase in preference for their own odour post-interaction may have been a consequence of repeated exposure to their competitor's odour, which was no longer novel in the post-interaction test, rather than a response to the interaction. The low strength of their change, which was not sufficient to cause a significant bias away from the conspecific's odour and towards their own, suggests that this was not a serious attempt to avoid re-encountering a competitor. The significant effect of aggressive competition on male post-interaction odour choice, in contrast, shows that the males' response could not be explained by decreased novelty, and resulted in an adaptive avoidance of a dominant competitor's odour. Both the low level of aggression between females in our study, and their equal attraction to own and another female's odour, suggest that they do not avoid areas inhabited by other females and, generally, are tolerant of each other. However, trapping ranges indicate that adult females are as widely dispersed as males (Cassaing & Croset 1985; Hurst et al. 1994, 1996). As in many rodents, aggressive behaviour appears to be very

variable among females and may be shown only in defence of their offspring. When pregnant or lactating, some *M. spretus* females can be just as aggressive as males (see Hurst et al. 1996 and the one trial stopped early in the present study to prevent possible injury). It remains to be seen whether *M. spretus* females would use substrate odour to avoid defended nest sites after experiencing strong attack from a defending female.

Female *M. spretus*, in contrast to males, produce very low quantities of major urinary proteins (Sampsell & Held 1985) which are thought to play a role in the slow release of olfactory signals in the environment (Robertson et al. 1993). If females do not gain an advantage from continuously advertising their location to conspecifics, it may be more advantageous to avoid leaving behind strong odour cues which might be used by predators. Males, on the other hand, may gain much from advertising their recent use of a site both to known competitors and to potential mates and they invest a great deal in such signals (Sampsell & Held 1985). This sex difference in investment and use of substrate odour for signalling their presence in an area may explain our rather surprising finding that the odours left behind by unfamiliar males were more attractive than those left by females to both male and female mice.

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REFERENCES

Albone, E. S. 1984. *Mammalian Semiochemistry*. Chichester: John Wiley.

Andrade, M. L., Kamal, K. B. H. & Brain, P. F. 1987. Effects of positive and negative fighting experiences on behaviour in adult male mice. In: *House Mouse Aggression* (Ed. by P. F. Brain, D. Mainardi & S. Parmigiani), pp. 223–232. London: Harwood.

Bishop, M. J. & Chevins, P. F. D. 1987. Urine odours and marking patterns in territorial laboratory mice (*Mus musculus*). *Behav. Proc.*, **15,** 233–248.

Boonstra, R. & Krebs, C. J. 1976. The effect of odour on trap response in *Microtus townsendii*. *J. Zool., Lond.*, **180,** 467–476.

Brain, P. F. 1972. Endocrine and behavioral differences between dominant and subordinate male house mice housed in pairs. *Psychonom. Sci.*, **28,** 260–262.

Bronson, F. H. 1973. Establishment of social rank among grouped male mice: relative effects on circulating FSH, LH, and corticosterone. *Physiol. Behav.*, **10,** 947–951.

Cassaing, J. & Croset, H. 1985. Organisation spatiale, compétition et dynamique des populations sauvages de souris (*Mus spretus* Lataste et *Mus musculus domesticus* Rutty) du Midi de la France. *Z. Saugetierk*, **50,** 271–284.

Cox, T. P. 1989. Odor-based discrimination between non-contiguous demes of wild *Mus*. *J. Mammal.*, **70,** 549–556.

Crowcroft, P. & Rowe, F. P. 1963. Social organization and territorial behaviour in the wild house mouse (*Mus musculus* L.). *Proc. zool. Soc. Lond.*, **140,** 517–531.

Desjardins, C., Maruniak, J. A. & Bronson, F. H. 1973. Social rank in the house mouse: differentiation revealed by ultraviolet visualization of urinary marking patterns. *Science*, **182,** 939–941.

Drickamer, L. C. 1989. Odor preferences of wild stock female house mice (*Mus domesticus*) tested at three ages using urine and other cues from conspecific males and females. *J. chem. Ecol.*, **15,** 1971–1987.

Drickamer, L. C. 1992. Oestrous female house mice discriminate dominant from subordinate males and sons of dominant from sons of subordinate males by odour cues. *Anim. Behav.*, **43,** 868–870.

Drickamer, L. C., Mikesic, D. G. & Schaffer, K. S. 1992. Use of odor baits in traps to test reactions to intra- and interspecific chemical cues in house mice living in outdoor enclosures. *J. chem. Ecol.*, **18,** 2223–2250.

Gosling, L. M. 1982. A reassessment of the function of scent marking in territories. *Z. Tierpsychol.*, **60,** 89–118.

Gosling, L. M. 1990. Scent marking by resource holders: alternative mechanisms for advertising the costs of competition. In: *Chemical Signals in Vertebrates V* (Ed. by D. W. Macdonald, D. Muller-Schwarze & S. E. Natynczuk), pp. 315–328. Oxford: Oxford University Press.

Gosling, L. M. & McKay, H. V. 1990. Competitor assessment by scent-matching: an experimental test. *Behav. Ecol. Sociobiol.*, **26,** 415–420.

Gray, S. J. & Hurst, J. L. 1997. Behavioural mechanisms underlying the spatial dispersion of competitors: a comparison between two species of *Mus* inhabiting different environments. *Anim. Behav.*, **53,** 511–524.

Gurnell, J. & Little, J. 1992. The influence of trap residual odour on catching woodland rodents. *Anim. Behav.*, **43,** 623–632.

Harvey, S., Jemiolo, B. & Novotny, M. 1989. Pattern of volatile compounds in dominant and subordinate male mouse urine. *J. chem. Ecol.*, **15,** 2061–2072.

Hurst, J. L. 1987. Behavioural variation in wild house mice (*Mus domesticus* Rutty): a quantitative assessment of female social organization. *Anim. Behav.*, **35,** 1846–1857.

Hurst, J. L. 1990a. Urine marking in populations of wild house mice *Mus domesticus* Rutty. I. Communication between males. *Anim. Behav.*, **40,** 209–222.

Hurst, J. L. 1990b. Urine marking in populations of wild house mice *Mus domesticus* Rutty. III. Communication between the sexes. *Anim. Behav.*, **40,** 233–243.

Hurst, J. L. 1993. The priming effects of urine substrate marks on interactions between male house mice, *Mus musculus domesticus* Schwarz & Schwartz. *Anim. Behav.*, **45,** 55–81.

Hurst, J. L. & Nevison, C. M. 1994. Do female house mice, *Mus musculus domesticus*, regulate their exposure to reproductive priming pheromones? *Anim. Behav.*, **48,** 945–959.

Hurst, J. L., Hayden, L., Kingston, M., Luck, R. & Sorensen, K. 1994. Response to the aboriginal house mouse *Mus spretus* Lataste to tunnels bearing the odours of conspecifics. *Anim. Behav.*, **48,** 1219–1229.

Hurst, J. L., Hall, S., Roberts, R. & Christian, C. 1996. Social organization in the aboriginal house mouse, *Mus spretus* Lataste: behavioural mechanisms underlying the spatial dispersion of competitors. *Anim. Behav.*, **51,** 327–344.

Jones, R. B. & Nowell, N. W. 1973. Aversive and aggression-promoting properties of urine from dominant and subordinate male mice. *Anim. Learn. Behav.*, **1,** 207–210.

Jones, R. B. & Nowell, N. W. 1974. A comparison of the aversive and female attractant properties of urine from dominant and subordinate male mice. *Anim. Learn. Behav.*, **2,** 141–144.

Jones, R. B. & Nowell, N. W. 1989. Aversive potency of urine from dominant and subordinate male laboratory mice (*Mus musculus*): resolution of a conflict. *Aggress. Behav.*, **15,** 291–296.

Lombardi, J. R. & Vandenbergh, J. G. 1977. Pheromonally induced sexual maturation in females: regulation by the social environment of the male. *Science*, **196,** 545–546.

Novotny, M., Harvey, S. & Jemiolo, B. 1990. Chemistry of male dominance in the house mouse, *Mus domesticus*. *Experientia*, **46,** 109–113.

Parmigiani, S., Brunoni, V. & Pasquali, A. 1982a. Behavioural influences of dominant, isolated and subordinate male mice on female socio-sexual preferences. *Boll. Zool.*, **49,** 31–35.

Parmigiani, S., Brunoni, V. & Pasquali, A. 1982b. Socio-sexual preferences of female mice (*Mus musculus domesticus*): the influence of social aggressive capacities of isolated or grouped males. *Boll. Zool.*, **49,** 73–78.

Robertson, D. H. L., Beynon, R. J. & Evershed, R. P. 1993. Extraction, characterization, and binding analysis of two pheromonally active ligands associated with major urinary protein of house mouse (*Mus musculus*). *J. chem. Ecol.*, **19,** 1405–1416.

Rodgers, R. J. 1987. Physiological consequences of defeat. In: *House Mouse Aggression* (Ed. by P. F. Brain, D. Mainardi & S. Parmigiani), pp. 233–252. London: Harwood.

Rowe, F. P. & Redfern, R. 1969. Aggressive behaviour in related and unrelated wild house mice (*Mus musculus* L.). *Ann. appl. Biol.*, **64,** 425–431.

Sampsell, B. M. & Held, W. D. 1985. Variation in the major urinary protein multigene family in wild-derived mice. *Genetics*, **109,** 549–568.

Stoddart, D. M. 1982. Does trap odour influence estimation of population size of the short-tailed vole, *Microtus agrestis*? *J. Anim. Ecol.*, **51,** 375–386.