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Response of the aboriginal house mouse *Mus spretus* Lataste to tunnels bearing the odours of conspecifics

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Abstract. The behaviour of wild-caught *Mus spretus* towards tunnels previously occupied by conspecifics, and to the conspecifics themselves, was examined to test whether (1) willingness to investigate and enter tunnels corresponded to the predictions of territorial avoidance, and (2) exposure to substrate odours primed a defensive response to the odour's donor. Adult males and females were provided with a choice of a clean tunnel versus one that had been occupied by themselves, by a conspecific from the same, a neighbouring or a distant area, or by a woodmouse, *Apodemus sylvaticus*. Mesh caps initially prevented tunnel entry while investigation was measured, then they were removed to record choice of entry. Subsequently, mice met the odour's donor in a neutral arena. Almost all aggression was between mice from different capture areas and affiliative behaviour was between those from the same area. Conspecific odours attracted more investigation than clean tunnels, although odour investigation varied according to the subject and donor's sex and their relative capture areas. Mice investigated then avoided entering a neighbour's tunnels, consistent with predictions of territorial avoidance. Provided with conspecific odours from the same or a distant area versus a clean tunnel, mice generally entered whichever tunnel they had preferred investigating before the tunnels were opened. Regardless of capture area, mice that entered a scented tunnel were significantly more defensive on encountering the donor than those that entered clean tunnels, especially towards female donors. This suggests that mice entering an apparently occupied (scented) area were primed to display submissive behaviour towards a potentially dangerous resident.

Mus spretus Lataste is an aboriginal house mouse living in grassland and woodland in western Mediterranean regions, from Iberia and the south of France to North Africa (Marshall & Sage 1981). Although morphologically very similar to the sympatric commensal house mouse *Mus domesticus*, *M. spretus* is biochemically distinct (Thaler et al. 1981) and lives entirely in vegetational habitats where there is dense ground cover (Cassaing & Croset 1985), in which it creates a system of grass tunnels. Little is known about the behaviour of this species, though trapping studies indicate that it occupies large stable home ranges containing a pair or small group (Cassaing & Croset 1985). Experiments in the laboratory suggest that *M. spretus* are less agile than commensal mice and, though more continuously active, show less intense exploration of a novel environment (Mota 1989). In captive encounters, adult male *M. spretus* are highly aggressive towards both other conspecific and *M. domesticus* males (Cassaing 1984), leading Cassaing & Croset (1985) to suggest that males are territorial, patrolling and defending their large home ranges against conspecifics and against *M. domesticus* where their distributions overlap.

Substrate and body odours are known to play an important role in maintaining the group-territorial social organization of commensal mice (e.g. Hurst 1990a, 1993; Hurst et al. 1993). Using mice caught from small granaries, Cox (1984, 1989) showed that individuals were attracted to the odours of others from the same granary as themselves but avoided the odours of conspecifics caught in neighbouring or distant granaries. Similar attraction and avoidance responses to the substrate marks of dominant territorial mice

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in laboratory populations correspond to the arrangement of mice in different territorial social groups (Hurst 1990b, c, d). However, while commensal mice may use substrate odours to orient to their respective territories and tend to avoid the marks of neighbouring and unfamiliar males, odours do not prevent territory invasion, especially when there are attractive resources within the territory (e.g. Mackintosh 1973; Harrington 1976; Hurst 1987a). Instead, subordinates and intruders appear to use substrate marks to identify a dominant resident and take evasive action (Hurst 1993), or be less inclined to attack (Gosling & McKay 1990), if they encounter the resident within his marked area.

We examined the responses of wild-caught male and female *M. spretus* to tunnels recently occupied by different conspecifics, and to the conspecifics themselves, to test whether their willingness to investigate and enter tunnels corresponded to the predictions of territorial avoidance, or whether the mice use substrate odours to prime their response on encountering a potentially dangerous resident. If mice use odours to maintain their spatial separation and avoid intruding into potentially defended areas, we might expect them to avoid entering tunnels scented by neighbours and unfamiliar mice but to be attracted to the odours of mice sharing their own tunnel systems. However, mice might show an initial attraction to investigate odours, even of territorial neighbours, to identify the donor (Hurst 1990b, c, d). Mice that avoid entering an occupied area to avoid potentially dangerous encounters with residents should be evasive or defensive on suddenly encountering the odour's donor, or aggressive if the donor is a neighbour, but not affiliative or ambivalent. Alternatively, if mice do not avoid tunnels scented by other conspecifics but use odours to prime their behaviour on encountering a resident defending its territory, we would expect them to enter scented tunnels but then show defensive or submissive behaviour on encountering the donor. To test whether any responses to odours were species-specific, we also tested *M. spretus*' responses to the odours of woodmice, *Apodemus sylvaticus*, which were trapped in the same tunnel systems (commensal house mice were present in buildings in the vicinity of the study populations but were never trapped in tunnels used by *M. spretus*, so were not used in our tests).

METHODS

Subjects and donors were seven male and six female adult *M. spretus* trapped on two disused farms near Lisbon, Portugal, over an 8-day period in April 1991. *Mus spretus* could be clearly distinguished from *M. domesticus* by the ratio of their tail to body length (<1) and the contrasting white coloration of fur on the belly and paws (this is grey-brown in *M. domesticus*; Ferns 1979). Nine woodmice caught in the same areas were also used as donors (see below). The farms had not been cultivated for many years and consisted of dense grassland containing small clumps of trees, macchia thicket and gorse bushes. A number of small, well-separated areas of mouse activity were identified by the presence of tunnels and fresh faeces in the long grass of the main study site, the Quinta de Sao Pedro (approximately 4 ha). We set 80 Longworth traps, baited with peanut butter and grain, each night within five separate but neighbouring areas, each covering 10–20 m^2 centred around clumps of trees, bushes or a bank and 50–200 m apart. To provide unfamiliar and unrelated subjects and donors, we set a further 40 traps in another equivalent small area of the Quinta Nisa, 3 km away. A total of 37 captures of *M. spretus* netted nine individuals from four neighbouring areas of the main study site (five woodmice were caught in the fifth area) and a further four individuals from the distant site. Mice caught more than once ($N=9$, 65% of captures) were always recaptured in their original area (including one male released accidentally more than 100 m away). Mice trapped in the same sites the previous year ($N=33$) showed similar site fidelity (J. L. Hurst, personal observation).

Trapped mice were weighed, individually marked by fur-clipping, and housed temporarily in clean individual polypropylene cages (30 × 13 × 12 cm) containing sawdust and hay bedding. They were then transferred to a darkened room under dim red lighting where daytime tests were carried out. One female subject was visibly pregnant and another lactating. At the end of each day mice were released in the same area from which they were caught, to maintain any social relationships within and between neighbouring areas and to give an indication of their site fidelity (care was taken to release the lactating female as quickly as possible). Each dyad of mice was tested first for their response to tunnels containing each other's odours, then for their reaction when encountering each other directly.

Olfactory Tests

In each trial, individual mice were provided with a choice of a clean Perspex tunnel (38 cm long, 3 cm diameter) versus one previously occupied by themselves or another mouse, inserted 42 cm apart in one wall of a clean test arena (60 × 20 × 60 cm varnished plywood). Subjects were thus able to avoid close contact with tunnels containing any aversive odours and had to visit the entrance to a tunnel to investigate. The left–right locations of the Odour and clean tunnels were randomized for each trial. The tunnels, protruding 20 mm into the arena, were covered in black polythene to mimic the darkness of natural tunnels; tunnel ends were blocked with mesh (0·6 mm) caps to allow natural air flow through the tunnels. Dim red illumination was directed over the centre of the arena to avoid any shadow bias and to encourage interest in the darkened tunnels. Before being used in trials, each mouse was accustomed to entering the Perspex tunnels and had been introduced into a clean test arena for 5 min to become acclimatized and locate the tunnel entrances. Olfactory trials were conducted in two parts.

Investigation of tunnels

Since mice usually entered the first tunnel encountered, mesh caps were placed over both tunnel entrances initially, providing them with an opportunity to investigate both tunnels during a 5-min trial. To avoid any stress that might be induced by direct handling, subjects were introduced into the arena by allowing them to enter an uncovered clean tunnel which was then inserted into the arena mid-way between the two test tunnels. Any mice reluctant to leave the introduction tunnel were encouraged very gently from behind with a stick, then the introduction tunnel was quickly replaced with a Perspex stopper. Two observers recorded the number of visits to the entrances of the Odour and clean tunnels, and the cumulative time spent in investigation. A mouse was defined as investigating a tunnel when its nose contacted, or was closely directed towards, the entrance cap (gnawing the mesh included). After 5 min the subject was isolated briefly in a clean neighbouring chamber while the caps to the tunnels were removed.

Tunnel entry

The subject was immediately re-introduced into the test chamber through an introduction tunnel as before, mid-way between the now familiar test tunnels. Two observers recorded the direction first turned on re-entering the arena, the tunnel first visited and first entered, the latency to first entry (whole body in tunnel), and the frequency and duration of any visits when the subject did not enter a tunnel. The trial was terminated once the subject entered a tunnel, or after 3 min (reached in 2/124 trials). The subject was then returned to its holding cage, and all equipment washed with detergent, rinsed and dried, then wiped with 70% alcohol prior to the next trial. Test chambers were similarly wiped down with detergent, then water and alcohol.

Tunnel odours

Odour tunnels were obtained by allowing individual donors to enter a clean tunnel where they were held for 5 min by capping both ends. After this time, mouse odour was clearly detectable even by the human nose, especially if a male had been in the tunnel. Mice did not appear to be stressed by this short confinement and readily entered the tunnels. Odour tunnels were sealed with masking tape until used, always within 24 h.

We tested five types of odours: (1) donor from the same capture area (N=22); (2) donor from a neighbouring capture area (N=39); (3) unfamiliar donor from another farm (N=39); (4) subject's own odour (N=10); (5) *A. sylvaticus* donor from a neighbouring capture area (N=14). We attempted to test the mutual response to odours between as many of our *M. spretus* subjects as possible, testing each dyad of individuals only once. However, tests were constrained by the range of mice caught each day so that only 110 of 169 possible dyadic combinations were achieved. The short duration of tests (8 min), with at least 1 h between trials using the same subject, reduced the chance that subjects would become tired or habituate to the test situation. Test order was randomized subject to these constraints.

Social Interaction Tests

The behaviour of subjects on encountering the individual odour donors themselves was tested in short (3-min) trials. To ensure that encounters did not bias responses to olfactory tests, all interaction trials were conducted in the evening (between 1800 and 2200 hours) under dim red lighting after all odour trials had been completed. Thus all mice had already encountered the odour of their opponents during an olfactory trial.

The two members of a dyad were introduced simultaneously into opposite corners of a clean test arena (60 × 60 × 60 cm) from separate clean tunnels. The tunnels were removed and social behaviour recorded for 3 min, unless aggression other than very brief attacks caused the trial to be terminated immediately. Such behaviour clearly signified intolerance between the opponents and early termination protected the mice from undue stress or damage. Nine interactions were curtailed (five neighbour, four distant) while one neighbour, four distant and one same area dyads involved only brief attacks followed by withdrawal of the opponents to opposite sides of the arena. The occurrence and initiator of any of the following social behaviour patterns was recorded, always by the same trained observer: social investigation (anogenital and body), sitting together (within 3 cm) without directed attention between the mice, allogrooming, aggression (attacks, chases, fights, offensive postures) and defence (defensive postures, squeaking and flight) by each mouse. Two other observers recorded the cumulative time that each mouse spent in active contact with the other, defined when a mouse was within 3 cm and facing the other mouse. Behavioural elements were similar to those shown by *M. domesticus* (see Grant & Mackintosh 1963 and Mackintosh 1981 for detailed definitions). Notably, defensive behaviour was seen frequently and usually involved defensive upright or sideways postures or the more extreme submissive upright posture described by Grant & Mackintosh (1963) for laboratory mice. In this latter posture, the nose was lifted right up in the air and the body held rigid with forelegs outstretched, often pushing at the opponent. In *M. spretus* this posture was usually accompanied by repeated squeaks and closed eyes, with defensive mice occasionally leaning backwards until they were partly resting on their backs. At the end of a trial, mice were returned to their holding cage and the arena cleaned thoroughly as above. Individuals were used in up to three trials per evening, and allowed to rest for at least 15 min between trials.

Data Analysis

Logarithmic transformation of the time spent investigating Odour and clean tunnels, and the frequency of visits, provided distributions that were not significantly different from normal (Kolmogorov–Smirnov tests, NS). We then used matched-pair *t*-tests to test for bias in response to the Odour versus clean tunnel. Two-factor ANOVAs tested whether the subject and donor's gender and their relative capture area had significant effects on the log ratio of investigation (Odour–clean), and whether choice of tunnel entry was related to previous investigatory bias while the tunnels were capped. Within sexes, one-way ANOVAs tested whether tunnel investigation (log ratio) was related to social response when subjects met donors. Since it was not possible to conduct all dyadic combinations of individuals (see above), we could not use a repeated-measures design. However, the individual subject and donor had no significant effects on the responses detailed below. Contingency chi-squared tests examined the effects of gender, relative area of capture and choice of tunnel entered on their social response. Bias in tunnel entry was examined using binomial tests.

RESULTS

Social Behaviour

Summarizing behaviour towards another mouse as aggressive, defensive or ambivalent (showing no aggressive or defensive behaviour), the gender of the mice had a highly significant effect on response ($\chi^2=38{\cdot}4$, $df=3$, $P<0{\cdot}001$). Males were either aggressive (55% of trials) or defensive (41%) on encountering another male, with only one example of ambivalence, but were mostly ambivalent towards females (65%) which they investigated extensively. Females, in contrast, were mostly defensive on encountering conspecifics of either sex (65% of trials with males, 67% with females), although they occasionally attacked when the mouse encountered was

Table I. Effect of capture area on the percentage of trials in which a particular social response was given

	Same	Neighbouring	Distant
Aggressive*	5·9	24·2	27·8
Defensive only	17·6	30·3	41·7
Defensive and affiliative	35·3	9·1	11·1
Ambivalent only	5·9	27·3	19·4
Ambivalent and affiliative	35·3	9·1	0
Number of trials	17	33	36

*Two males that attacked females (neighbouring and distant) showed affiliative behaviour initially.

another female (in 17% of cases). Gender had no significant effects on the frequency with which mice sat together (24%) or groomed each other (9% of all trials).

The occurrence of aggressive and affiliative (sit together, allogroom) behaviour varied according to whether mice were caught from the same or different areas, while defensive behaviour did not (summarized in Table I). As expected, attacks were biased against conspecifics caught from a different area: males attacked only neighbours or unfamiliar opponents ($\chi^2=5{\cdot}58$, $df=1$, $P=0{\cdot}018$). The only instance of aggression between mice caught in the same area was between two females (but note that most same-area interactions were inter-sexual). Despite this bias in aggression, mice were no more likely to be defensive towards conspecifics caught from neighbouring (45·5%) or distant areas (54·1%) than from the same area as themselves (52·9%; $\chi^2=0{\cdot}56$, $df=2$, NS), regardless of the sexes of the mice involved. Defence was not provoked by an attack in most cases (defensive postures were performed in the absence of aggression in 60·4% of cases, with a further 16·7% of mice showing defence before they were attacked).

Despite the wariness between some dyads caught in the same area, the frequency with which mice sat together or groomed each other was strongly related to their respective capture areas (note that mice that eventually sat in close contact often showed defensive behaviour at the initial encounter, see Table I). Mice caught in the same area were much more likely to sit together (64·7%) than were neighbours (22·2%) while few mice from different farms stayed close together for long (6·1%; $\chi^2=20{\cdot}90$, $df=2$, $P<0{\cdot}0001$). Notably, males were never observed to sit together during encounters if they came from different areas, but they sat together in both trials in which they came from the same area. Almost all allogrooming was between mice caught in the same area ($\chi^2=12{\cdot}79$, $df=2$, $P<0{\cdot}001$) and was equally prevalent within and between the sexes.

Response to Odour Tunnels

Tunnel investigation

Mice generally showed a positive bias for investigating tunnels that carried the odour of another conspecific compared with clean tunnels while the tunnels were capped, regardless of whether the odour's donor came from the same or a different area from the subject (total duration of investigation per trial, all conspecific odours, $t=4{\cdot}47$, $N=102$, $P<0{\cdot}0001$). Odour tunnels were visited more frequently ($t=2{\cdot}46$, $N=102$, $P=0{\cdot}016$) and for longer per visit ($t=3{\cdot}77$, $N=102$, $P<0{\cdot}001$). A two-factor ANOVA showed that the ratio of time spent investigating the Odour compared with the clean tunnel varied according to the sex of the subject and donor (effect of dyad sex, $F_{3,90}=2{\cdot}82$, $P=0{\cdot}044$) but there was no overall effect of capture area (same, neighbouring or distant, $F_{2,90}=0{\cdot}74$, NS). Generally, female subjects were more strongly attracted to Odour tunnels than males were (effect of subject's sex, $F_{1,98}=7{\cdot}16$, $P=0{\cdot}009$; Fig. 1), while the importance of the donor's sex varied according to whether the donor came from the same or different area from the subject (effect of donor's sex, $F_{1,98}=0{\cdot}44$, NS; two-factor interaction with capture area, $F_{2,96}=3{\cdot}78$, $P=0{\cdot}026$) as outlined below.

Males were attracted to investigate all types of male odour compared with clean tunnels ($t=2{\cdot}96$, $N=25$, $P=0{\cdot}007$) especially those from a different farm (Fig. 1a). They made the same number of

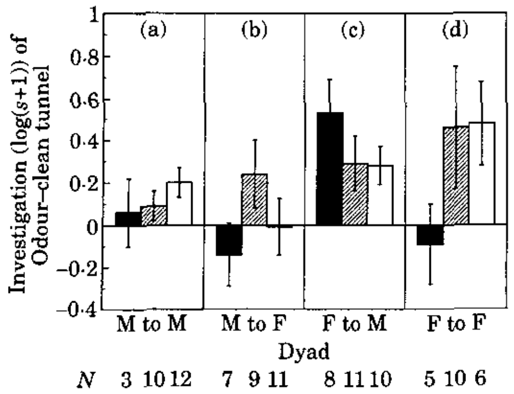

Figure 1. Investigation of capped tunnels ($\bar{X}$ ± SE log ratio of time spent at Odour versus clean). M: Male; F: female. Shading indicates the relative capture areas of subjects and odour donors. ■: Same; ▨: neighbour; □: distant. Differences in bias between these three capture areas were not significant for any of the four dyad types (one-way ANOVAs, NS).

visits to Odour and clean tunnels (t=0·16, N=25, NS) but spent more time per visit to the Odour tunnel (t=2·78, N=25, P=0·01). Bias appeared to be related to their behaviour when they encountered the donor later that day (effect of aggression on differential investigation, $F_{1,18}$=4·02, P=0·06): only males that were not aggressive towards a donor were significantly attracted to investigate a tunnel that bore his odour (t=2·95, N=8, P=0·02), and all but one of these showed clear defensive behaviour. Aggressive males showed neither attraction nor avoidance towards the Odour tunnel (t=1·41, N=11, NS).

Surprisingly, males were not significantly attracted to investigate female Odour tunnels from any of the capture areas (t=0·46, N=27, NS; Fig. 1b), regardless of whether they were aggressive, defensive, showed affiliative behaviour or simply investigated the female donor when they met.

In contrast to males, females were strongly attracted to investigate tunnels that bore the odours of mice of the opposite sex (t=4·72, N=29, P<0·0001; Fig. 1c): females both visited male Odour tunnels more frequently (t=3·4, N=29, P=0·002) and spent longer per visit (t=3·45, N=29, P=0·002). Attraction was particularly strong towards the odours of males caught from the same area (Fig. 1c). Like that of males, preference for a tunnel that bore male odour was related to a female's subsequent behaviour towards the donor himself. Females that were defensive towards a donor (no females were aggressive towards males) had shown a stronger prior preference for investigating his tunnel than those that were not defensive (effect of defence on differential investigation, $F_{1,18}$=5·26, P=0·03), although even non-defensive females had spent significantly longer investigating the Odour tunnel than the clean one (t=3·97, N=8, P=0·005). Females that sat in close contact with a male also showed a stronger attraction to investigate his tunnel than those that kept apart during their interaction trial ($F_{1,18}$=6·02, P=0·02).

Females were significantly attracted to investigate tunnels that bore the odour of another female (all trials, t=2·10, N=21, P=0·049) although Fig. 1d suggests that they were attracted only when the donor came from another area. Attraction mainly resulted from their tendency to visit the Odour tunnels more frequently than clean ones (all trials, t=2·04, N=21, P=0·054), a tendency that was highly significant when females came from different areas (neighbouring or distant, t=2·98, N=16, P=0·009). In contrast to their response to male odours, females that were subsequently defensive on encountering a female donor had not shown significant investigation of the donor's tunnel (t=0·47, N=9, NS). Females that were ambivalent or aggressive, on the other hand, had shown significant investigation of the Odour tunnel (t=2·56, N=7, P=0·043).

Sex differences in the ratio of time spent at a tunnel that contained conspecific-odour versus a clean tunnel were due entirely to the amount of time spent investigating the Odour tunnels (effect of dyad, $F_{3,98}$=7·98, P=0·0001); time spent investigating the clean tunnels did not differ between dyads ($F_{3,98}$=0·98, NS). Neither males nor females showed any tendency to investigate tunnels marked with their own odour more than clean tunnels (t=0·609, N=10, NS) and also failed to respond significantly towards tunnels that contained the odour of woodmice (t=1·52, N=14, NS).

Tunnel entry

Entry to tunnels once they were uncapped followed a different pattern to tunnel investigation. In almost every trial (95·6%), mice entered whichever tunnel was in the direction they first moved towards after their re-introduction into the test

Table II. Percentage of mice entering Odour tunnels

Capture area	Male to male	Male to female	Female to male	Female to female	All mice
Same	66·7 (3)	0 (7)	28·6 (7)	20·0 (5)	22·7 (22)
Neighbouring	40·0 (10)	22·2 (9)	36·4 (11)	33·3 (9)	33·3 (39)
Distant	50·0 (12)	54·5 (11)	70·0 (10)	83·3 (6)	61·5 (39)

Number of trials is given in parentheses.

arena (χ^2=93·86, *df*=1, *P*<0·0001). Considering all trials combined, there was no significant bias in the number of mice that entered a tunnel that bore the odour of a conspecific versus a clean tunnel (58 entered clean, 42 entered Odour, *z*=1·5, NS) and no significant difference according to the subject and donor's sex (χ^2=2·94, *df*=3, NS).

The expectation of territorial avoidance was that mice would be attracted to enter tunnels scented by themselves or by conspecifics caught in the same area, but would avoid tunnels scented by mice caught from a different (neighbouring or distant) area. Willingness to enter a tunnel that contained the odour of a conspecific varied significantly according to whether the donor came from the same area, a neighbouring area, or from another farm (χ^2=10·67, *df*=2, *P*=0·005, Table II), although there was no bias in entering tunnels carrying their own odour (6/10 entered the Odour tunnel, *z*=0·32, NS) or that of a woodmouse (9/14 entered the Odour tunnel, *z*=0·80, NS). Entry to a tunnel containing conspecific odours also appeared to be related to the mouse's prior bias in investigating the capped tunnels (above), but prior investigation led to a different choice of entry according to whether subjects and donors were caught from the same, neighbouring or distant areas (ANOVA examining the effects of capture area and choice of tunnel entered on differential investigation, two-factor interaction, $F_{2,94}$=5·04, *P*=0·008, see Fig. 2).

In agreement with predictions, mice of both sexes tended to avoid entering tunnels previously occupied by mice caught from a neighbouring area (13/39 entered Odour, *z*=1·92, *P*=0·055, Table II). Female odours tended to be avoided more strongly than males (5/18 entered female and 8/21 entered male Odour tunnels). Prior investigation of a neighbour's odours appeared to lead to strong avoidance once the tunnels were uncapped (Fig. 2). Classifying mice according to their choice of tunnel entry, those entering the clean tunnel had shown a highly significant preference for investigating the Odour tunnel (*t*=3·46, *N*=26, *P*=0·002). The smaller number of mice that entered a Neighbour tunnel had shown no significant bias when investigating the capped tunnels (*t*=0·30, *N*=13, NS) thus may not have been aware of the presence of the neighbour's odour when making their choice of entry. Gender made no difference to these responses (two-factor interaction between choice of entry and subject × donor sex on ratio of investigation, NS).

Contrary to expectations, however, mice also appeared to avoid entering a tunnel that bore the odour of another mouse caught from the same area as themselves (5/22 entered Odour, *z*=2·35, *P*=0·02, Table II). This bias was due to significantly fewer mice of either sex entering tunnels

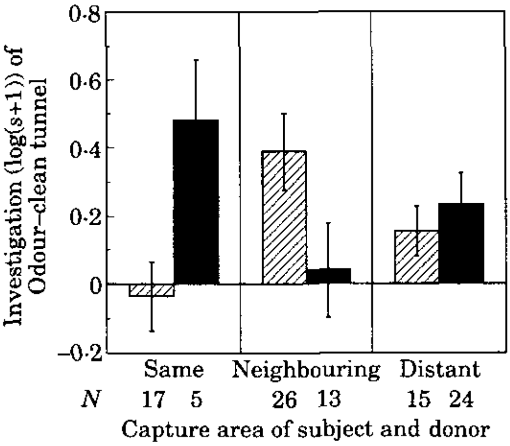

Figure 2. Investigation of capped tunnels according to subsequent choice of tunnel entered ($\bar{X}$ ± SE log ratio of time spent at Odour versus clean). ▨: Entered clean tunnel; ■: entered Odour tunnel.

previously occupied by a female from the same area (only 1/12 entered female and 4/10 entered male Odour tunnels). Again, the choice of tunnel entered was significantly related to investigation of the capped tunnels in the preceding test (effect of entry choice on the ratio of tunnel investigation, $F_{1,15}=5{\cdot}78$, $P=0{\cdot}03$; two-factor interaction between entry choice and the four combinations of subject and donor sex, NS) but, in this case, prior investigation had the opposite effect to a neighbour's odour on tunnel entry (Fig. 2). The small number of mice that entered an Odour tunnel (largely females attracted to own-area males) had previously shown a strong preference for investigating this tunnel while it was capped ($t=2{\cdot}73$, $N=5$, $P=0{\cdot}050$; see Fig. 2). Those that chose to enter the clean tunnel had tended to spend longer investigating the clean rather than the Odour tunnel although not significantly so ($t=-1{\cdot}84$, $N=11$, $P=0{\cdot}096$). This suggests that mice may have been more attracted to the relatively novel clean tunnel than to familiar own-area odours.

Mice did not avoid entering tunnels marked by conspecifics caught from another farm (24/39 entered Odour, $z=1{\cdot}28$, NS). Indeed, females tended to enter Odour tunnels more frequently than clean ones (Table II) although their bias was not significantly greater than chance ($z=1{\cdot}75$, $P=0{\cdot}08$). Choice of tunnel entered was again related to previous investigatory bias towards the capped tunnels, although this depended on the sex of the subject and donor (two-factor interaction, $F_{2,31}=3{\cdot}45$, $P=0{\cdot}03$). Mice entering the clean tunnels had failed to show bias when investigating the tunnels (Fig. 2). Females that entered male or female Odour tunnels had shown a highly significant preference for investigating the odour ($t=3{\cdot}53$, $N=12$, $P=0{\cdot}005$). Males also showed a similar preference before entering tunnels that contained male odours ($t=4{\cdot}48$, $N=6$, $P=0{\cdot}007$) although, corresponding with their lack of interest in unfamiliar female odours (Fig. 1), they entered tunnels that contained female odours after comparatively little investigation ($t=-1{\cdot}85$, $N=6$, NS).

Tunnel Entry and Social Response

Contrary to the prediction of the territorial avoidance hypothesis, mice that chose to enter an Odour tunnel were much more likely to show defensive behaviour when they subsequently encountered the donor than those entering a clean tunnel, while those that chose a clean tunnel were more likely to show ambivalent (neither aggressive nor defensive) behaviour (Table III; $\chi^2=12{\cdot}64$, $df=2$, $P=0{\cdot}002$). Since most mice that responded ambivalently towards a donor had previously entered a clean tunnel, it appeared that mice showing this response avoided entering the donor's tunnel (4/20 entered Odour, $z=3{\cdot}06$, $P=0{\cdot}002$). In contrast, those that were defensive (8/17 entered Odour, $z=0$, NS) or aggressive (13/35 entered Odour, $z=1{\cdot}35$, NS) towards a donor showed no significant bias in entering or avoiding the Odour tunnel. However, such analysis assumes that the social relationship observed between the mice determined their prior response to each other's odours rather than vice versa. The alternative hypothesis was that mice intruding into an occupied area would use substrate odours to prime a defensive response when they encountered the donor. After entering an Odour tunnel, mice showed more frequent defence and less ambivalence towards a donor than those that had entered a clean tunnel regardless of the subject's sex, although this effect was stronger in response to tunnels that contained female odours ($\chi^2=9{\cdot}35$, $df=2$, $P=0{\cdot}009$) than to tunnels containing male odours ($\chi^2=4{\cdot}04$, $df=2$, $P=0{\cdot}13$). The strongest response followed exposure to odours of neighbours: although few mice entered Neighbour tunnels, 75% of those that did were subsequently defensive towards the donor and none was ambivalent ($\chi^2=8{\cdot}47$, $df=2$, $P=0{\cdot}01$).

Table III. Relationship between tunnel entry and behaviour towards donor

	Percentage of tunnels entered	
	Clean	Odour
Aggressive	19·5	25·7
Defensive	31·7	62·9
Ambivalent	48·8	11·4
N	41	35

DISCUSSION

Investigation of tunnels and choice of tunnel entry by *M. spretus* were significantly influenced by the presence of different conspecific odours in the tunnels, depending on the subject and donor's sex as well as their relative areas of capture. While tunnel entry was significantly related to capture area, this did not correspond simply to the expectation that tunnels bearing the odours of neighbours and unfamiliar mice would be avoided while those carrying odours from the same area as the subject would not. Choice of tunnel entry appeared to be complicated by prior investigation of the tunnels while they were capped. When responding to tunnels previously occupied by mice from either the same area as themselves or a different farm, mice generally entered whichever tunnel they had previously spent most time with. Thus, odours from unfamiliar mice generally stimulated investigation followed by entry, except that males failed to be attracted to tunnels containing female odour. Mice attracted to investigate own-area odours (largely females towards male odours) also chose to enter the Odour tunnel. Presented with female odours, however, males and females from the same area tended to spend more time investigating, and then entered, the clean tunnel. This may not have been due to an avoidance of the female odour per se, which was likely to have been familiar, but instead may have signified greater interest in the clean and potentially more novel tunnel when presented with a choice (see below).

While clearly in opposition to the strong attraction to own-area and avoidance of unfamiliar odours measured by Cox (1984, 1989) among *M. musculus* inhabiting small granaries, these responses were remarkably similar to those measured by one of us in captive populations of *M. domesticus* (Hurst 1989, 1990b, c, d). In these latter studies, mice responded more strongly to the novelty of a clean patch of substrate encountered in their home area than to familiar adult female urine marks, while the marks of unfamiliar mice generally stimulated more extensive investigation (Hurst 1990a). Clean areas may not provide a good control stimulus to measure attraction and avoidance to social odours among mice which normally live in a mouse-scented environment (Hurst 1987a, 1989). Like the *M. spretus* reported here, male *M. domesticus* also failed to show more interest in urine from an unfamiliar adult female than in a clean substrate and females were attracted to resident male marks (Hurst 1990d). While it is possible that mouse populations like those studied by Cox differ in their response to social odours compared with *M. domesticus* and the *M. spretus* reported here, a more likely explanation may lie in the strength of the stimulus odours used in these studies. Cox (1984, 1989) measured responses in an olfactometer in which stimulus air was drawn continually from a chamber containing captive mice or their substrate into another small chamber containing the subject; the other experiments measured responses when mice encountered marks on the substrate while exploring a test area. Airborne stimuli in the olfactometer are likely to have been much stronger and signalled the close proximity of the donors of the odours. Avoidance or attraction may thus have signified a response to the likelihood of an imminent encounter with the donor. Encounters with potential aggressors can be avoided by taking evasive action on encountering a resident defender rather than avoiding the aggressor's marked territory completely (Hurst 1993). Experiments within *M. domesticus* populations show that invading and resident subordinate males use odours to recognize resident territory defenders and flee when they encounter the resident after investigating his odour from a distance (Hurst 1993). Such avoidance appears to play an important role in maintaining social relationships, at least between males in *M. domesticus* populations where intrusion into other males' territories is a frequent occurrence.

The relationship between tunnel entry and the social response of *M. spretus* corresponded to the hypothesis that mice that intrude into an occupied area show defensive behaviour on encountering the resident rather than that those submissive to the resident avoid entering occupied (scented) habitat in the first place. Thus, exposure to odours on entering a tunnel appeared to prime a defensive response towards the donor among both male and female *M. spretus*, especially in response to females and to neighbours. In this study, however, defensive behaviour generally consisted of static submissive postures with audible squeaking rather than an attempt to flee. Such defence was not usually provoked by overt aggression and even when one mouse attacked, its opponent had often shown defensive behaviour first. Further, while aggression occurred between mice from different areas and affiliative behaviour between those from the same area, as expected if mice live in territorial pairs or small social groups, defensive behaviour was equally frequent between mice from the same, neighbouring or distant areas. Thus even on encountering a familiar individual that stimulated affiliative behaviour, the initial response was often to show a defensive posture.

Avoidance of, or attraction to, an area after investigating marks on the substrate, on the other hand, signifies willingness to enter a potentially occupied area in the first place. That *M. spretus* avoided tunnels previously occupied by mice from neighbouring areas after clearly investigating them, but did not avoid those from a distant farm, suggests that these mice avoided entering tunnels bearing the odours of potentially familiar (and probably related) neighbours but did not generalize this response to unfamiliar (and unrelated) mice. *Mus domesticus* avoid substrate marked by dominant male neighbours after experiencing aggressive exclusion from a neighbour-group territory but tend to generalize, showing a lesser avoidance of unfamiliar males (Hurst 1990b; see also Jones & Nowell 1973, 1989). The responses of *M. spretus* to potentially known neighbours thus correspond with the expectation of territorial avoidance but, interestingly, the response was particularly strong towards tunnels previously occupied by females. This is somewhat surprising in view of the rarity of female aggression in our social response tests. Social responses were typical of those found in similar tests with *M. domesticus* where adult males are frequently aggressive, especially towards other males (see review by Mackintosh 1981). Females are not generally aggressive when encountering conspecifics in an unfamiliar area (Mackintosh 1981) but dominant breeding females can be highly aggressive in defence of their familiar home area and nest sites (e.g. Crowcroft & Rowe 1963; Gandelman 1972; Hurst 1987b). Female odours may thus have signalled a female-defended territory which should be avoided. Investigations of territorial avoidance in *M. domesticus* have concentrated on response to male territorial odours since males mark the substrate at a much higher rate than females (Maruniak et al. 1974, 1975; Hurst 1989) and are more consistently aggressive. There is some evidence that females avoid substrate marked by female neighbours after brief investigation (Hurst 1990c) and avoid nest sites bearing the odours of isolated pregnant or non-pregnant adult females (Hurst & Nevison 1994) although, as yet, there is no evidence that male mice avoid female substrate odours in commensal mouse populations. The functional significance of these responses remains to be tested.

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REFERENCES

Cassaing, J. 1984. Interactions intra- et interspecifiques chez les souris sauvages du Midi de la France, *Mus musculus domesticus* et *Mus spretus*: consequences sur la competition entre les deuz especes. *Biol. Behav.*, **9**, 281–293.

Cassaing, J. & Croset, H. 1985. Spatial organization, competition and dynamics of wild mice populations (*Mus spretus* Lataste and *Mus musculus domesticus*) in Southern France. *Z. Säugetierk.*, **50**, 271–284.

Cox, T. P. 1984. Ethological isolation between local populations of house mice (*Mus musculus*) based on olfaction. *Anim. Behav.*, **32**, 1068–1077.

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 mice (*Mus musculus* L.). *Proc. zool. Soc. Lond.*, **140**, 517–531.

Ferns, P. N. 1979. Live-trapped small mammals from the Trás-os-Montes region of North-east Portugal. *Bolm. Soc. port. Ciênc. nat.*, **19**, 65–69.

Gandelman, R. 1972. Postpartum aggression elicited by the presence of an intruder. *Horm. Behav.*, **3**, 23–38.

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

Grant E. C. & Mackintosh, J. H. 1963. A comparison of the social posture of some common laboratory rodents. *Behaviour*, **21**, 246–259.

Harrington, J. E. 1976. Recognition of territorial boundaries by olfactory cues in mice (*Mus musculus* L.). *Z. Tierpsychol.*, **41**, 295–306.

Hurst, J. L. 1987a. The functions of urine marking in a free-living population of house mice, *Mus domesticus* Rutty. *Anim. Behav.*, **35**, 1433–1442.

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

Hurst, J. L. 1989. The complex network of olfactory communication in populations of wild house mice

Mus domesticus Rutty: urine marking and investigation within family groups. *Anim. Behav.*, **37,** 705–725.

Hurst, J. L. 1990a. The network of olfactory communication operating in populations of wild house mice. In: *Chemical Signals in Vertebrates V* (Ed. by D. W. Macdonald, D. Muller-Schwarze & S. Natynczuk), pp. 401–414. Oxford: Oxford University Press.

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

Hurst, J. L. 1990c. Urine marking in populations of wild house mice (*Mus domesticus* Rutty). II. Communication between females. *Anim. Behav.*, **40,** 223–232.

Hurst, J. L. 1990d. 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 & Schwarz. *Anim. Behav.*, **45,** 55–81.

Hurst, J. L., Fang, J. & Barnard, C. J. 1993. The role of substrate odours in maintaining social tolerance between male house mice (*Mus musculus domesticus*). *Anim. Behav.*, **45,** 997–1006.

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

Jones, R. B. & Nowell, N. W. 1973. Aversive effects of the urine of a male mouse upon the investigatory behaviour of its defeated opponent. *Anim. Behav.*, **21,** 707–710.

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.

Mackintosh, J. H. 1973. Factors affecting the recognition of territory boundaries by mice (*Mus musculus* L.). *Anim. Behav.*, **21,** 464–470.

Mackintosh, J. H. 1981. Behaviour of the house mouse. *Symp. zool. Soc. Lond.*, **47,** 337–365.

Marshall, J. T. & Sage, R. D. 1981. Taxonomy of the house mouse. *Symp. zool. Soc. Lond.*, **47,** 15–25.

Maruniak, J. A., Owen, K., Bronson, F. H. & Desjardins, C. 1974. Urinary marking in male house mice: responses to novel environmental and social stimuli. *Physiol. Behav.*, **12,** 1035–1039.

Maruniak, J. A., Owen, K., Bronson, F. H. & Desjardins, C. 1975. Urinary marking in female house mice: effects of ovarian steroids, sex experience and type of stimulus. *Behav. Biol.*, **13,** 211–217.

Mota, P. G. 1989. Behavioural analysis of two rodent species from different ecological habitats. In: *House Mouse Aggression* (Ed. by P. F. Brain, D. Mainardi & S. Parmigiani), pp. 69–86. London: Harwood Academic.

Thaler, L., Bonhomme, F. & Britton-Davidian, J. 1981. Processes of speciation and semi-speciation in the house mouse. *Symp. zool. Soc. Lond.*, **47,** 27–41.