

## Scent marking by common voles *Microtus arvalis* in the presence of a same-sex neighbour

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Several vole species use scent marked runways radiating from their burrows for foraging and dispersion. These marks are probably used for social communication. This 4-day laboratory study investigated the environmental and social causations of marking inside pre-existing corridors in male and female common voles *Microtus arvalis* (Pallas, 1778). Firstly I tested the novelty and the reinforcement hypotheses in isolated voles, predicting respectively a habituation or a continuous increase in mark deposition. I then confronted with each other two same-sex voles for two days to investigate the differences between males and females in the pattern of marks inside three corridors, one of which runs along the common partition with the neighbour. I tested the self-advertisement and territorial-defence hypotheses, respectively predicting in the presence of a neighbour either a similar marking between the three corridors or a greater marking in the corridor close to the neighbour than in the two other corridors. The results showed no habituation in marking, even in a familiar environment, confirming the reinforcement hypothesis. After the addition of a neighbour, only the females left more marks in the corridor that ran alongside the common border than in the two other corridors. The territorial-defence hypothesis was thus confirmed in (territorial) females while the self-advertisement was supported in (non-territorial) males. Finally, I tested the competitive-ability hypothesis in females, stating that the abundance of scent marks of an individual before a social interaction can predict its degree of intolerance in a future social interaction. The results from female pairs physically interacting for four days support the hypothesis.

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### Introduction

Scent marking is a frequent mechanism for olfactory communication in mammals. It is characterised by the deposition of urine, faeces and/or secretions from specialized glands onto a substrate (Eisenberg and Kleiman 1972, Macdonald 1985). It has been widely studied in rodents, where it is known to have several social functions such as reproductive competition (Eisenberg and Kleiman 1972), territorial defence (Gosling and McKay 1990), communication of reproductive condition and even individual identity (Gosling 1982, Thomas and Wolff 2002).

The runways radiating from the burrows of several rodent species are well marked, at the least with urine (Jamon 1994, Galef and Buckley 1996). In rats,

these trail networks are essential in foraging (Galef and Buckley 1996). The runways are also critical (1) for escape behaviour, even in an unfamiliar environment (in *Microtus ochrogaster* Jacquot and Solomon 1997) and (2) for dispersal, even by strangers (in *M. californicus* Lidicker 1980). Raptors use the marks of urine and faeces on these networks to detect patches that contain high densities of voles (Viitala *et al.* 1995). In the laboratory *M. townsendii* prefers to use pathways with the most cover (Harestad and Shackleton 1990), which could diminish the risk of aerial predation (Koivunen *et al.* 1998).

Besides this possible function in spatial orientation, the scent marks on runways are used to transmit social and sexual information to conspecifics. As different rodents have the ability to discriminate between individuals on the basis of their marks (Johnston and Jernigan 1994, Gheusi *et al.* 1997), they are able to maintain neighbouring relationships despite spatial segregation (Hurst *et al.* 1994). The reduction of physical contact minimises the risk of aggressive encounters and facilitates indirect social interactions with neighbours via odours (Hurst *et al.* 1993).

While scent marking has been investigated in rodents for years (for review see Brown 1985), the environmental and social causations of the marking on runways remain largely unknown. For this study, I chose to work on common voles *Microtus arvalis* (Pallas, 1778), as they use such runways for foraging and dispersion above ground and also for long daily excursions (Boyce and Boyce 1988). In *M. arvalis* living on meadows, these networks are often trails without vegetation; in crops or fallow, they are corridors covered by vegetation (Dienske 1979, Pelikán 1982). While marking is not mandatory for spatial orientation of common voles (Dobly 2001), runways facilitate their movements (Boyce and Boyce 1988).

Male common voles are solitary and promiscuous while females live in territorial groups and can be solitary at high densities (Mackin-Rogalska 1979, Boyce and Boyce 1988). Although scent glands were not precisely studied in this species, one can assume that they are similar to those of other promiscuous *Microtus* species, ie they possess at least anal, prepuccial, hip and angulus oris glands (Quay 1962, Jannett 1986, Ferkin *et al.* 1994). In the laboratory the marks deposited along a route do not seem to be composed exclusively of urine but probably also of anal gland secretions (F. M. Rozenfeld and D. Van Avermaet unpubl.). Indeed *M. montanus* and *M. xanthognathus* can scent mark by dragging their anus on the floor (Jannett 1978, Wolff and Johnson 1979). In a matriarchal group of common voles reared in the lab, mothers mark more than daughters (Heise and Rozenfeld 1999). Females prefer non-sibling male odour, especially when in oestrus (Bolhuis *et al.* 1988). They are also able to detect the availability of empty burrows without entering them. In fact, females never enter a conspecific nest if the burrow is occupied, but they detect the disappearance of neighbouring females and invade their burrows very quickly (< 3 h, Boyce and Boyce 1988). This reluctance to enter an occupied nest is probably linked to the high aggression that residents show to intruders (Dobly and Rozenfeld 2000), as in the case of mice (Jones and Nowell 1973).

This laboratory study investigated the temporal and spatial deposition of scent marks of male and female common voles in pre-existing covered runways in an experimental enclosure. The first part studied the marking dynamics for four days in order to discriminate between two competing hypotheses about the environmental causation of marking. According to the first hypothesis, the marking is only a response to a new environment. This novelty hypothesis predicts that the voles will deposit scent marks in a new environment and will then show a decrease in the rate of mark deposition due to their habituation to a known environment (Maruniak *et al.* 1974). This would result in an asymptotic deposition curve, which is consistent with an economic use of resources. Conversely, according to the reinforcement hypothesis, the scent marks are deposited not only in a new environment but also regularly in a known unchanged environment. This hypothesis predicts that the marking in corridors will continue to increase even in a familiar environment. This behaviour would allow the voles to maintain the efficiency of the chemical message over time. It could counter a possible over-marking, which would mask the original odour (as in bank voles and hamsters, Rozenfeld and Rasmont 1991, Johnston *et al.* 1994). A resident vole advertising its repeated presence in an area via odour communication to conspecifics could then diminish the risk of direct social interactions (Hurst *et al.* 1993).

The second part of the study dealt with the communicative functions of marking by confronting two same-sex voles through wire mesh. As rodents typically show an increase in marking in the presence of a conspecific (Maruniak *et al.* 1974, Reasner and Johnston 1987), I aimed to test how differences in social organisation between males and females would influence the marking pattern. I tested two hypotheses: the self-advertisement (Johnston 1983) and the territorial-defence hypotheses (Wolff 1993). The self-advertisement hypothesis states that scent marks mainly indicate individual identity. According to this hypothesis, we can expect that, in males and females, the increase in marks consequent to the appearance of a same-sex neighbour would be of similar proportion in all corridors, independent of their distance from the neighbour. In contrast, according to the territorial-defence hypothesis, territorial individuals, like female common voles (Boyce and Boyce 1988), defend their territory by scent marking. We can thus expect that, in the presence of a same-sex neighbour, females will increase their marks more in the corridor closest to the neighbour than in the other two corridors. In both controls without neighbour and in males, which are socially intolerant but not territorial, we should observe a comparable increase in all corridors.

Finally, I investigated the influence that the amount of marks can have on social interactions. Males are known to mark more than females and juveniles (eg in taiga voles and mice, Wolff and Johnson 1979, Drickamer 1989). I tested the competitive-ability hypothesis, according to which the abundance of marks deposited by an individual reflects the intensity of its intrasexual aggressiveness (Drickamer 2001). The hypothesis predicts that females depositing an abundance

of scent marks before the upcoming social interaction should be less tolerant than females depositing scent marks in lower quantities.

## Material and methods

### Subjects and rearing conditions

I used 22 males and 16 females, which were the third laboratory generation of voles captured in different localities in southern Belgium. Individuals were 12 months old ( $SD = 3$ ) and had sexual experience. Each animal was used only once. At least 35 days before experimentation, each individual was isolated from its social group (composed of one adult male and one or more females) and placed in individual polycarbonate cages ( $36 \times 25 \times 15$  cm). The cage contained wood shavings, soft paper and a  $20 \times 4$  cm PVC tube as shelter. Water and pellets for rodents were available *ad libitum*. When outside of experiments, animals were supplemented twice a week with fresh carrots. Rearing and experiments were performed at room temperature ( $18\text{--}21^\circ\text{C}$ ) and humidity 50–70% under a photoperiod of 16L:8D. Dim conditions were a faint yellow light and were on from 17:30 to 1:30. The techniques conformed to guidelines for capture, handling and care of mammals as approved by the Belgian Bioethical Group.

### Experimental enclosure

Each individual enclosure ( $60 \times 60 \times 50$  cm, Fig. 1) consisted of an aluminium floor and aluminium walls. It contained three yellow PVC corridors (A, B and C,  $33 \times 6 \times 4$  cm) each with an aluminium floor. In each enclosure, corridor 'A' led to the food, which was on the right when coming from the burrow. The enclosure also contained 15 pellets of commercial rat, mouse and hamster food (nr 105, UAR, France) in a small earthenware pot ( $6 \times 4 \times 4$  cm) along the aluminium support of a water bottle (support shaped in a reversed U,  $9 \times 8 \times 11$  cm). The enclosure was connected to a 6-module burrow in vitrified stoneware with aluminium floor, a circular gallery and a nest chamber. At the beginning of the experiment, three pieces ( $10 \times 13$  cm) of soft paper were placed in the middle of the enclosure to serve as nesting material.

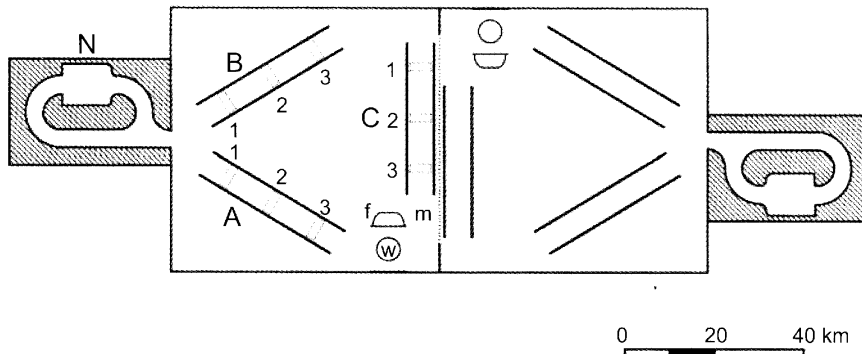


Fig. 1. Two individual enclosures in contact through a common partition with a wire-mesh window (m). Each compartment contained three PVC corridors (A, B and C), a food pot (f) and a water bottle (w) on a support. Each compartment was connected to a burrow with a nest chamber (N). The three zones of scent mark recording are shown in each corridor of the left enclosure (1, 2 and 3); they are labelled according to the letter of the corridor and the number of the zone (eg 'C1'). The corridors, the burrow and the support of the water bottle provided cover for the voles (these covered areas are delimited by thin dotted lines).

### Experimental procedure

On day 0, each of the 38 voles was transferred from its rearing cage to its new individual enclosure inside its shelter tube. The enclosures were set 1 m apart. After two days (Fig. 2), I moved 20 enclosures closer together in order to obtain five random pairs of each sex involving unrelated and unfamiliar individuals. I put the distal walls in contact, both of which were removed and replaced by a common partition with a wire mesh window at floor level ( $54 \times 20$  cm, Fig. 1: m). The enclosures of 12 males and 6 females remained isolated on day 4 for control. At the end of this marking experiment, on day 4, the common partition of the experimental females was removed and both females could physically interact in the common enclosure for four more days (until day 8, Fig. 2). On day 4 in the former female controls, I formed pairs of enclosures with a common wire mesh partition for two days (as in the experimental group but 2 days later). Then, on day 6, I removed the partition for a 4-day social interaction (until day 10). With reference to the contact experiment, the only difference between experimental and former control females was that the former received two days of isolation prior to the wire-mesh confrontation whereas control females received four days. After having verified that both types of social relationships (association and separation, see next paragraph) occurred under both conditions, I compared these relationships in eight female pairs over four days (Fig. 2: dashed squares). For both groups of males, the experiment was concluded on day 4, as males are known to be highly intolerant of each other (de Jonge 1983, Doby and Rozenfeld 2000).

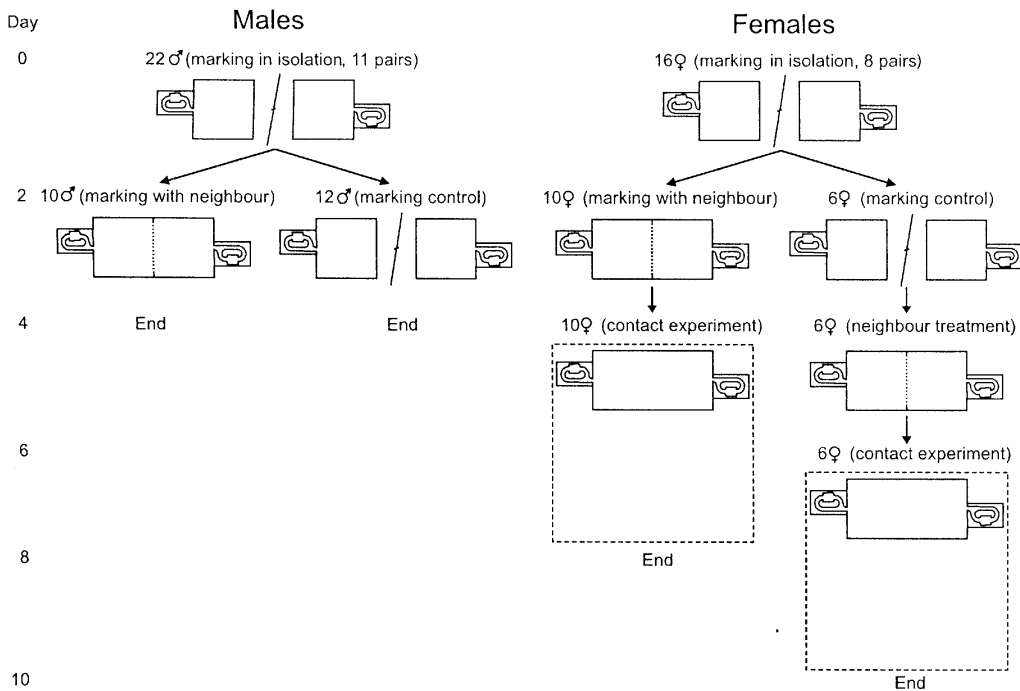


Fig. 2. Experimental procedure for males and females. Boxes represent individual enclosures with nest chambers as described in Figure 1. Inclined lines indicate isolated enclosures (1 m apart) and median dotted lines represent wire mesh between two enclosures. The first step compared marking from day 0 to day 4 in four groups. In a second step, the females underwent a 4-day physical contact experiment (delimited by dashed squares). For these tests, the six former control females during marking received a 2-day neighbour treatment (from day 4 to day 6) to be comparable to the ten experimental females (after adequate verification, see text for details).

### Data recording and analysis

From day 1 to day 4, at 14:00, I removed the corridors from the enclosure, one by one, to record marks on their floor. In each corridor, I observed three zones of  $5 \times 2$  cm ( $1000 \text{ mm}^2$ , Fig. 1). In each zone, I recorded the number of marked square millimetres, appearing dark on the clear aluminium through a commercial transparent paper printed with  $1 \times 1$  mm squares. The centres of the zones were 5.5, 16.5 and 27.5 cm away from the corridor entrances; the zones were labelled according to the letter of the corridor and their position inside the corridor (eg 'B3', Fig. 1). The corridors, still bearing the marks, were then replaced in the enclosure as before. I present the difference between the three recorded surfaces inside each corridor, as well as the analysis of each corridor treated as a single passage using the mean of its three recordings (for the variation in time and the variation between the three corridors). For each corridor, the number of faecal droppings was also recorded. Between day 2 and day 3 (corresponding to the addition of a neighbour in the experiments), besides the absolute increase of the total marked surface, I also assessed the increase of marked surface by the ratio day 3 / day 2 (a ratio of 2 corresponds to twice more marks on day 3 than on day 2, while a value of 1 would mean no change, see Table 1). The treatment without a neighbour present was used to determine marking in a known, consistent environment.

Social interactions between females of each pair were determined during three observation periods, each lasting 2 hours, with a lapse of 1 hour between each period (total time window = 8 hours). They were performed after 4 days of interaction. These interactions were classified as either associated (the two females were non-aggressive and huddled in a communal nest for more than 50% of the observation time) or separated (the two females each lived in their own nest and all interactions between them were aggressive, with at least two interactions observed).

I used Mann-Whitney *U*-tests for two independent variables (ie males vs females, neighbouring vs isolated and tolerant vs intolerant). I used Friedman Two-Way ANOVA for three dependent variables (the three recordings inside a corridor and the three corridors). The temporal dynamics of the marks were assessed over four days by linear regressions (adjusted  $r^2$  are provided). I used Chi-Square tests to compare the number of replicates with faeces present between males and females. All tests were two-tailed except when a clear relationship was expected, ie for the larger marked surface (1) in the presence of a neighbour than in isolation, (2) in males than in females and (3) in intolerant females than in tolerant females. As they underwent the same treatment during the first two days, experimental and control animals of each sex were pooled for statistical analysis during days 1 and 2.

Table 1. Median ratio of the quantity of marks on day 3 divided by the quantity of marks on day 2 in presence and in absence of a same-sex neighbour. The range is shown in square brackets. ns – not significant, \*  $p < 0.05$ , \*\*  $p < 0.025$  with one-tailed Mann-Whitney *U*-test (males and females, significant  $U \leq 34$  and 14,  $n = 22$  and 16, respectively). In neighbouring voles, the average absolute marking as well as the marking in corridors 'A' and 'B', but not corridor 'C', increased more in male groups than female groups (last column,  $U \leq 27$ ,  $n = 20$ ).

Corridor	Males			Females			Males/females difference in neighbouring voles
	Neighbouring ( $n = 10$ )	Isolated ( $n = 12$ )		Neighbouring ( $n = 10$ )	Isolated ( $n = 6$ )		
'A'	2.5 [1.0–6.9] *	1.2 [1.0–2.5]		1.6 [1.0–8.8] *	1.0 [0.8–7.9]		*
'B'	3.2 [1.2–13.8] **	1.6 [1.2–3.3]		2.1 [0.9–20.4] ns	1.4 [0.8–2.0]		**
'C'	1.9 [0.7–9.5] ns	1.4 [1.0–3.9]		1.8 [1.0–12.0] **	0.9 [0.8–8.5]		ns
All	2.8 [0.7–13.8] *	1.4 [1.0–3.9]		1.9 [0.9–20.4] *	1.0 [0.8–8.5]		*

## Results

### Time dynamics and spatial distribution of marks in both sexes

Voles used the distal end of their burrow as a place for urination; in this area urine was deposited in spots 1–2 cm in diameter. Faeces were also found in this place. This was not analysed in the present study, which is focused on corridor scent marking. Most marks were found in the corridors and were mainly long streaks with a more oily consistency than urine. These may have been secretions from specialised glands (eg anal or prepuccial gland in males). During the four days of mark recording, no habituation was detected in experiments or controls. The total surface area marked continued to increase daily (Linear Regression: experiments and controls,  $r^2 = 0.92$  and  $0.94$ ,  $n = 5$ ,  $p = 0.006$  and  $0.004$ , respectively; Fig. 3 showing the average of the three corridors for each group). I obtained the same result for each corridor analysed separately in all four groups (Linear Regression: A, B and C corridors,  $r^2 = 0.82$ ,  $0.81$  and  $0.87$ ,  $n = 5$ ,  $p = 0.021$ ,  $0.024$  and  $0.012$ , respectively). This confirms the reinforcement hypothesis in the experimental groups as well as the controls.

Furthermore, the males marked significantly more than the females in every corridor with or without a neighbour (one-tailed Mann-Whitney  $U$ -test: days 1 and 2,  $U \leq 100$ ,  $n = 38$ ,  $p \leq 0.012$ ; days 3 and 4 respectively for experiment and controls,  $U = 21$  and  $14$ ,  $n = 20$  and  $18$ ,  $p = 0.014$  and  $0.02$ ; Fig. 3). After day 2, the

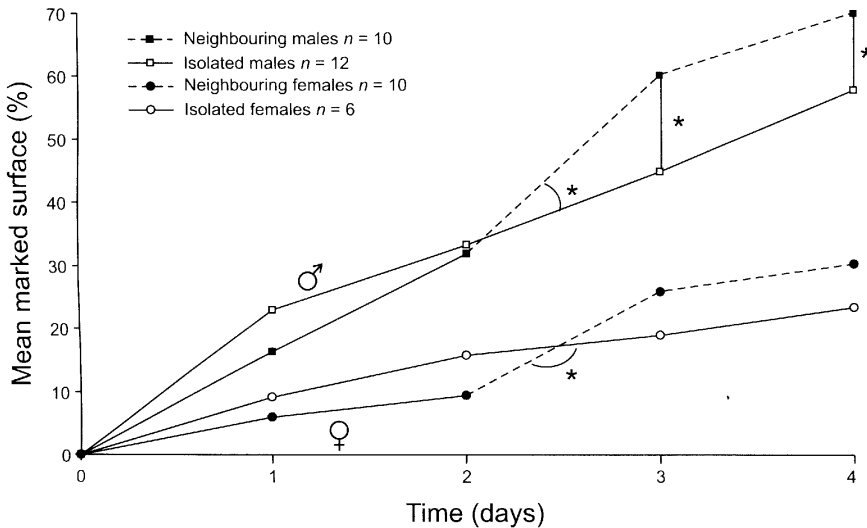


Fig. 3. Changes over time of the mean marked surface inside three corridors in males and females. All individuals were isolated until day 2, then, some of them were confronted through wire mesh with a same-sex conspecific (dashed lines). The increase of marks between day 2 and day 3 was sharper in the presence of a neighbour than in isolation. The difference between males and females was significant on all days. \* one-tailed Mann-Whitney  $U$ -test,  $p < 0.05$ .

control males increased their total marks more than the control females (days 3 and 4,  $U = 11$ ,  $n = 18$ ,  $p = 0.019$  in both comparisons).

On days 1 and 2, in both males and females, the marks were most abundant in corridor 'A', leading to the food, followed by 'C' and finally in corridor 'B' (Two-Way ANOVA: males,  $F_{2,22} \geq 24.8$ ,  $p \leq 0.001$ ; females,  $F_{2,16} \geq 14.0$ ,  $p \leq 0.001$ ). In fact, with the exception of experimental males, corridor 'A' was more marked than corridor 'B' under all conditions (Two-Way ANOVA: experimental males,  $F_{2,10} \leq 5.6$ , NS; control males,  $F_{2,12} \geq 9.5$ ,  $p \leq 0.009$ ; experimental females,  $F_{2,10} \geq 8.6$ ,  $p \leq 0.014$ ; control females,  $F_{2,6} \geq 6.3$ ,  $p \leq 0.042$ ).

During the four days of mark recording, in both female groups zone 'A3', close to the food, showed more marks than 'A1' (Two-Way ANOVA: days 1 and 2,  $F_{2,16} \geq 11.4$ ,  $p \leq 0.003$ ; experiments days 3 and 4,  $F_{2,10} \geq 7.8$ ,  $p \leq 0.02$ ; controls days 3 and 4,  $F_{2,6} \geq 11.6$ ,  $p \leq 0.003$ ). Similarly, on day 4 in neighbouring males, 'A3' and 'B3', away from the burrow, were more marked than 'A2' and 'B2', respectively (Two-Way ANOVA:  $F_{2,10} \geq 7.2$ ,  $p \leq 0.027$ ). Likewise, 'C3', close to the food, was more marked than 'C2' (Two-Way ANOVA:  $F_{2,10} = 7.8$ ,  $p = 0.02$ ).

There were faeces in the corridors of 13 males out of 22 and of 3 females out of 16 (59% vs 19%,  $\chi^2 = 6.18$ ,  $n = 38$ ,  $df = 1$ ,  $p = 0.013$ ). In males, they were mainly present in corridor 'C' (66% of occurrences). In females, they were exclusively present in corridor 'C', even without a neighbour.

The enclosure floor outside the corridors showed elongated marks. They were not precisely quantified but looked identical to those observed inside the corridors. They went through three corners of the enclosure (not through the corner to the left when exiting the burrow, Fig. 1). They thus linked the corridors together through both distal corners (between 'B3'-'C1' and between 'A3'-'C3'). Through the corner close to corridor 'A', they skirted around corridor 'A' and reached the food along the walls from the burrow. Finally, marks linked the entrances of corridors 'A' and 'B' to that of the burrow.

#### **Influence of a neighbouring conspecific on the pattern of marking**

In both sex groups, marking increased more between day 2 and day 3 in the presence of a neighbour than in isolation (ratio day 3 / day 2, one-tailed Mann-Whitney  $U$ -test: males and females,  $U = 31$  and  $14$ ,  $n = 22$  and  $16$ ,  $p = 0.029$  and  $0.046$ , respectively; Table 1). The separate analysis of each corridor showed that males did not mark more along the border in the presence of a neighbour than in isolation. The mark increase was not significantly different in corridor 'C' between neighbouring and isolated males (Table 1). The same happened in the female groups in corridor 'B'. This supports the self-advertisement hypothesis in males.

The absolute increase in marks (day 3 – day 2) was comparable throughout the three corridors except in neighbouring females where corridor 'C' showed a bigger increase than the two other corridors (Two-Way ANOVA: neighbouring males,  $F_{2,10} = 1.8$ ,  $p = 0.41$ ; control males,  $F_{2,12} = 0.17$ ,  $p = 0.92$ ; neighbouring females,

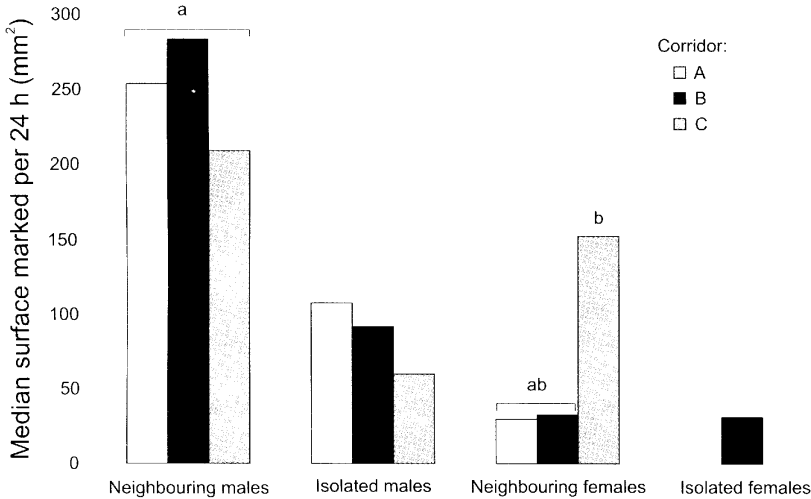


Fig. 4. Median marked surface added between days 2 and 3 inside three corridors by males and females, with or without a neighbour. Neighbouring voles were confronted through wire mesh with a same-sex conspecific. The corridor positions are shown in Fig. 1. Each individual measure was the mean of three zones of 1000 mm<sup>2</sup>. Only the main significant relationships are indicated. In neighbouring voles, the increase in marks was greater in males than in females except in corridor 'C' (one-tailed Mann-Whitney *U*-test:  $p < 0.05$ , 'a' on the graph). The marks added to the three corridors were comparable in each group except in neighbouring females (Two-Way ANOVA:  $p = 0.02$ , 'b' on the graph).

$F_{2,10} = 7.8$ ,  $p = 0.02$ ; control females,  $F_{2,6} = 4.3$ ,  $p = 0.11$ , Fig. 4). In addition, in neighbouring voles, males showed a greater mark increase than females in corridors 'A' and 'B' but not in corridor 'C' (one-tailed Mann-Whitney *U*-test: A, B and C:  $U = 27, 20$  and  $44$ ,  $n = 20$ ,  $p = 0.048, 0.01$  and  $0.34$ , respectively). These last two results, showing the important marking along the border in the neighbouring female group, are in agreement with the territorial-defence hypothesis for the females.

On days 3 and 4, corridors 'B' and 'C' were more marked in the neighbouring male group than in the isolated male group (one-tailed Mann-Whitney *U*-test: B and C:  $U \leq 35$  and  $33$ ,  $n = 22$ ,  $p \leq 0.046$  and  $0.04$ , respectively). As already mentioned, corridor 'A' was always marked more than 'B' except in the experimental male group. The increases between day 3 and day 4 were similar with or without a neighbour.

#### Social interactions in female dyads

After 4 days of physical interaction within eight pairs of females, four pairs were associated, living in the same nest and showing no aggression during the observation. The other four pairs lived separately and two of them showed a clear dominance hierarchy. The pairs of formerly experimental females ( $n = 5$ ) as well as the pairs of formerly control females ( $n = 3$ , Fig. 2) showed the two types of

social relationships (associated and separated). I thus pooled both treatments. Although pairs were too small in numbers to perform relevant statistics, the average discrepancy of age between tolerant females was lower than that between separated females (30.2 vs 134.0 days of age difference). The eight females from the four pairs that remained separated had marked more before the encounter (recorded on day 2 in both treatments) than the future tolerant ones (15.2 vs 7.7% of total marked surface, Mann-Whitney  $U$ -test:  $U = 13$ ,  $n = 16$ ,  $p = 0.050$ ). This adds support to the competitive-ability hypothesis in females.

## Discussion

This study showed that a new environment is quickly and abundantly marked, as predicted by the novelty and the reinforcement hypotheses. However, the marking in corridors increased over days in a linear way in all four groups studied. This absence of significant habituation, even in a familiar environment without changes as experienced by control animals, supports the reinforcement hypothesis and rejects the novelty hypothesis. This is contrary to what happens in male mice even when they receive a clean substrate every day (Maruniak *et al.* 1974) but is comparable to what has been observed in bank voles in the absence of covered runways (Rozenfeld and Rasmont 1991). As the intensity of a given chemical signal declines over time, it has to be regularly renewed. The linear increase of marking could mean that, in this study, the marking investment was increased daily. Indeed, the recording technique used here could have underestimated the quantity of marks added, as a previous marking could have partially masked the surface of the urine added later. Anyway, the costs of such a costly reinforcement could be overbalanced by the diminished risk of intra-specific aggressive encounters (Hurst *et al.* 1993) that repeated marking provides.

As expected, males marked in greater quantities than females even before the presence of a neighbour. This could be partially due to the greater weight of males, to their higher mobility (Boyce and Boyce 1988) and to their known intolerance towards same-sex neighbours. This intolerance is higher than that of females and can be harmful in males (de Jonge 1983, Dobly and Rozenfeld 2000). This result is consistent with the competitive-ability hypothesis stating that the abundance of marks could be a measure of intrasexual aggression. The sexual difference was confirmed by the presence of more faeces in males. Faeces allow individual discrimination in hamsters (Lai and Johnston 1994), possibly sex recognition in some voles (Ferkin *et al.* 1994, Ferkin and Johnston 1995), and also territorial defence in different Muridae (Wolton 1985, Zuri *et al.* 1997). The presence of this pattern of faeces deposition in both sexes before the presence of a neighbour does not match with the territorial-defence hypothesis.

The analysis of the marking pattern showed that in all groups the marks were more abundant in the corridor leading to the food. This was not predicted by the hypotheses of this study. This could simply be a consequence of the more frequent

trips through this corridor if a proportion of the marks is deposited passively, which is probable as suggested by the hair tube technique (Dickman 1986) and the correlation between the number of trips and the abundance of marking in various areas of a maze (Dobly 2001). The higher quantity of marks at the distal end of corridors compared with the end closer to the burrow or the centre of the corridor could be consequent to numerous sessions of vigilance from the end of corridors leading to an uncovered place (eg the food). Such anti-predatory behaviour would not take place at the proximal end of corridors as the inside of the burrow is probably a safer place. This abundance of marks in areas where voles spent more time could help them to detect safer places at a certain distance. As rats use marks to find their bearings in darkness (Lavenex and Schenk 1998), in a future study it would be valuable to test if such a directional marking pattern could help the voles orientate in darkness.

In both sexes, the increase of the marks was greater in the presence of a neighbour than in isolated voles, as already observed in marking outside runways in other species (Maruniak *et al.* 1974, Reasner and Johnston 1987). The presence of different corridors allowed an analysis of the deposition pattern by diminishing the edge effect. The marking in corridor 'B' remained scarce in both sexes, confirming spatial preferences in corridor marking. Furthermore, clear differences were observed between males and females. After the addition of a neighbour, the males showed a higher increase in marking than the females, except in the corridor close to the common partition, which was increased as if without a neighbour in males. Moreover, after the confrontation with a neighbour, the males increased their marks in comparable quantities in the three corridors. These two observations are in agreement with the self-advertisement hypothesis in males. In females confronted with a new neighbour for 24h, the increase in marks was greater in corridor 'C' than in the other two, as predicted by the territorial-defence hypothesis, which is thus supported in females. These results are consistent with the different social organisation in males and females (respectively non-territorial and territorial). The territorial-defence (as well as same-sex competition) in females is supported by results from *M. pennsylvanicus* and *M. ochrogaster*, in which territorial females concentrate their mark in areas scented by females (Ferkin *et al.* 2001).

I finally tested the competitive-ability hypothesis by allowing physical interaction between two females. First of all, the proportion of 50% of tolerant females after a 4-day interaction was comparable to what was observed in pairs of sexually naive females, which were completely unfamiliar to each other (60%,  $n = 10$  pairs, A. Dobly, unpubl.). The current results suggest that, under these laboratory conditions, familiarity does not imply social tolerance in the long term, contrary to what is commonly observed, at least in the short term (Kareem and Barnard 1982, Ferkin 1988).

Secondly, as expected from the competitive-ability hypothesis and already shown for dominance in male mice (Drickamer 2001), the abundance of marks of

females in social isolation can predict their future social intolerance. Besides the information known to be provided by the quality of urine, this abundance of marking could indirectly inform a conspecific about the competitive ability of a competitor (through its activity level or its weight). This result is consistent with the observation that, in unfamiliar *M. arvalis* and *Mus spretus*, marks trigger reluctance to stay close to the odour, as well as defensive behaviour of an intruder (de Jonge 1980, Hurst *et al.* 1994). In common voles, this hostile effect of odours could be related to the fact that in the field, females do not enter an occupied foreign burrow (Boyce and Boyce 1988). Thus, my study highly suggests that scent marks are used by females in competitive ability signalling.

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