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ORIGINAL PAPER

Territory ownership and familiarity status affect how much male root voles (*Microtus oeconomus*) invest in territory defence

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Abstract Neighbour–stranger discrimination occurs when individuals respond with more aggression to strangers than to territorial neighbours—a phenomenon termed the "dear enemy phenomenon" (DEP). We investigated the DEP with male and female root voles (*Microtus oeconomus* Pallas 1776) using field dyadic arena tests conducted in enclosures where we could test for the effects of familiarity (familiar versus stranger), ownership (resident versus intruder status) and resource-holding potential (body mass) on territorial behaviours. The results showed that males put more effort into territorial defence than females, and males could discriminate between neighbours and strangers. In males, aggressiveness was influenced by a significant two-way interaction between treatment and ownership. Male residents were more aggressive towards stranger intruders than towards neighbour intruders, while male intruders were less aggressive towards stranger residents than towards neighbour residents. In females, neither treatment nor ownership status had a significant effect on aggressiveness. Familiar males performed more social behaviours but less non-social behaviours than stranger males. Furthermore, there was a clear dominance hierarchy between residents and intruders in stranger dyads, with the male territory holders dominating the intruder in pairwise interactions. To our knowledge, these results demonstrate for the first time DEP in a small mammal with a known pedigree and present the first evidence for "prior resident advantage" in voles. We argue that both ownership status and familiarity status affect how much an individual invests in territory defence. The benefits of neighbour–stranger discrimination for male root voles and the absence of neighbour–stranger discrimination in female root voles are discussed.

Keywords Dear enemy · Neighbour–stranger discrimination · Prior resident advantage · Territoriality

Introduction

One form of social recognition that occurs in territorial species is termed the "dear enemy phenomenon" (DEP), in which territory holders respond less aggressively to intrusions by neighbours than non-neighbours (strangers; Fisher 1954; Ydenberg et al. 1988; Temeles 1994). Consistent with theoretical game models (e.g. Maynard Smith and Parker 1976; Maynard Smith 1982), the DEP hypothesis predicts that a resident should invest more in aggression against a more threatening individual that is capable of inflicting greater losses on the resident (Getty 1987). Unlike neighbours, strangers (i.e. so-called "wanderers"; McGuire

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and Getz 1998) generally do not own a territory or have a mate and hence are more likely to compete with a resident (Temeles 1994). These wanderers might be recently dispersing individuals or individuals that could be exhibiting alternative mating tactics (Solomon and Jacquot 2002). A territory holder learns to recognize or discriminate neighbours from strangers (Ydenberg et al. 1988; Temeles 1994). Furthermore, interactions with less threatening neighbours represent wasted energy that could be better utilized in the defence of the territory from strangers (Jaeger 1981; Temeles 1994). Expressed in terms of game-theoretic payoffs, the owners' potential losses to strangers may be higher than to neighbours (Temeles 1994). Thus, dear enemy recognition is apparently an adaptive strategy that minimizes the energetic costs of territoriality (Jaeger 1981; Ydenberg et al. 1988).

The DEP posits that territorial behaviour should be modulated by neighbour–stranger discrimination (NSD). However, alternative models of territorial behaviour predict an important role for other cues like ownership (Maynard Smith and Parker 1976; Kokko et al. 2006), resource-holding potential (RHP) or the value of the territory (Krebs 1982). For example, traits correlated with fighting ability and RHP, such as body size, is often used to settle contests (Parker 1974; Archer 1987; Kemp and Wiklund 2004). These alternative models imply, respectively, that animals should invest more in territorial defence if they own the resources (the "prior resident advantage", automatic owner status of individuals who arrived first in an area), if they have a high RHP or if they occupy a more valuable territory. Kokko et al. (2006) recently reviewed the empirical literature on animal contests to test whether asymmetries in RHP are required to explain observations of the prior resident advantage. This review indicates that most current evidence for prior resident advantage do not rule out that an underlying power asymmetry has a strong influence on territorial behaviour (e.g. Kemp and Wiklund 2004). For example, the prior-residence effect may be a consequence of ownership status being correlated to asymmetries in competitive ability or value of the resource (e.g. Stokkebo and Hardy 2000). Thus, results will remain inconclusive if experiments are unable to control for the multiple cues that can elicit territorial behaviour. Furthermore, many studies have reported on the outcomes of fights for territories (i.e. who is observed to win) rather than investment in territorial behaviours, which is not sufficient to distinguish between territorial strategies (Kokko et al. 2006). Not all pairwise interactions led to fights, and the outcome of these fights was also not obvious in the current study. We therefore believed that a quantitative assessment of behavioural items provided a better assessment of territoriality than a qualitative assessment of the outcome of pairwise interactions. Finally, another complication arises from kin selection because most models of territorial behaviour are not assuming any benefits in terms of inclusive fitness between related individuals, but neighbours may often be more related. It is therefore inappropriate to test territorial behaviours on animals with an unknown pedigree.

In a review of NSD studies in a variety of taxa (mammals, birds, reptiles, amphibians and insects), Temeles (1994) found that the DEP occurred primarily in species with territories that contain both the breeding site and food supply ('multi-purpose/breeding' territory) but rarely in species with feeding territories or very small breeding territories. Most microtine rodents typically occupy 'multi-purpose/breeding' territories as they breed under and feed upon the meadow or forest vegetation that constitutes their natural habitat (Boonstra et al. 1987). In female-defence polygynous species, like the root voles (*Microtus oeconomus*) of this study (Tast 1966; Ims et al. 1993), females compete for space and food resources, whereas males compete for mating partners (Ostfeld 1985a; Ims 1987). Males are usually more territorial and defend larger areas than females, and females have a high degree of home-range overlap within matrilineal clusters (Andreassen et al. 1998; Gundersen and Andreassen 1998). Thus, the social system of root voles (and other similar *Microtus* species) is characterised by marked territoriality in males and extensive space sharing in females, which is in sharp contrast with the social system of bank voles (and other similar *Myodes* species) where females defend exclusive territories and commit infanticide against each other (Ostfeld 1985a; Ims 1987). In root voles and other similar *Microtus* species, kin clusters of females seem to be associated with defence against aggressive unfamiliar conspecifics, in particular against infanticidal males (Agrell et al. 1998; Ebsensperger 1998; Le Galliard et al. 2006).

In the present study, we investigated the territorial behaviour of unrelated male and female root voles with field dyadic arena tests conducted in outdoor enclosures. We were able to control for kinship by using laboratory-raised animals of known pedigree and run all field arena tests with unrelated animals from the same enclosure (familiar) or from different enclosures (stranger). We tested for the effects of familiarity (familiar versus stranger), ownership (resident versus intruder status) and resource-holding potential (body mass) on territorial behaviour. We hypothesised that male root voles put more effort into territorial defence than females and that only males discriminate between neighbours and strangers. We predicted that males should respond less aggressively to intrusions by their territorial neighbours than intrusions by strangers (non-territorial floaters). If owner status is solely determined by who arrive first in the territory, we further predict prior resident advantage and expect that resident males should be more motivated to fight than intruders. However, if power status is the critical determinant of the outcome of the fight, we predict that larger males should dominate smaller males in pairwise interactions irrespective of ownership. Due to the hypothesised lower territorial behaviour of females, no differences in the above cues (or variables) were expected.

Materials and methods

Study area and study animals

Our study was conducted in spring 2004 at Evenstad Research Station, South-east Norway. The section of the research area used for the present study consisted of six plots (50×50 m) enclosed by steel sheet fences extending 0.4 m below and 0.6 m above ground. The experimental area was additionally surrounded by an anti-mammalian predator fence (1.5-m-high chicken mesh fence supplied with an electric wire). Each plot consisted of one large meadow habitat patch (30×44 m=1,320 m^2) sown, burned and fertilized before this study to fulfil all habitat requirements of root voles (Ims et al. 1993). The rest of the enclosed area surrounding the habitat patches (i.e. the matrix) was kept barren and uninhabitable for root voles by mowing and herbiciding before and throughout the study.

Plots were emptied from overwintering, resident voles by intensive live-trapping in February–March 2004. The field study was initiated by seeding each source habitat with laboratory-raised root voles obtained by breeding one parent originating from Valdres with one parent originating from Finse, southern Norway (Andreassen and Ims 2001), at the animal facility of the University of Oslo (see Gundersen and Andreassen 1998 for the laboratory procedures). All released animals were born in the laboratory and kept with their littermates and their mother until the release day on the 28th of May. Animals were kept in family groups (one breeding pair and one to seven littermates) inside laboratory steel cages (50×30×20 cm) equipped with one nest box provided with nesting material. Cages were supplied daily with water and food ad libitum (rat pellets and oats) and bi-weekly with a handle of dry hay. The cages were located in the same laboratory room maintained at a light/dark cycle of 6 h dark and 18 h light, and a temperature of 20°C.

Populations were seeded with four to eight females and two to four males (approximately 90–100 voles per hectare) marked individually by toe-clipping (Gundersen and Andreassen 1998). Animals were marked in the laboratory by clipping sharply the tip of one or two toes using fine scissors disinfected with alcohol. Toe-clipping was done by a trained investigator; a minimum number of toes was clipped to permit individual identification of the released animals (n=54), and animals were then left undisturbed in their laboratory cages for more than 5 days before release. Bleeding was minimal, and the clipped toes were cleaned with a paper strip and a diluted betadine solution, ensuring the absence of infection and swelling. The decision to use a female biased sex ratio was based on previous observations showing that the adult sex ratio usually stabilises at 60–80% of females in root vole populations (Ims and Andreassen 1999). The released animals were aged 25 days (±5.4 SD) and weighed 19.1 g (±3.6 SD), indicating that they were ready to mate (Ims 1997). The founder groups consisted of unrelated and unfamiliar animals from the laboratory and same-sex littermate pairs (less than one to two littermate pairs per population). However, as pedigrees were known and as the number of pairs of relatives was very small during the study period, we choose to conduct all our behavioural tests with dyads of unrelated animals. After a settlement period of 20 days, a total of 31 females and 11 males were still present in the enclosures and thus constituted the pool of individuals on which we conducted our “dear enemy” behavioural experiments. The settlement period was long enough to permit sexual maturation and territory establishment (Le Galliard et al. 2006).

Trapping procedure

Before the behavioural experiments, we trapped animals with Ugglan multiple capture live traps located in a 6×7 grid in the habitat patch. Traps were baited with carrots and wholegrain oats, placed under the vegetation (to avoid heating), set at midnight and checked at 6:00 A.M. and 12:00 noon for three consecutive days (June 14, 15 and 16). We used this procedure to obtain data on the animals present in the enclosures (i.e. survival from release process) and to get access to the animals available for the behavioural experiment. Each time an individual was trapped, we recorded trap location, identity, sex, mass and reproductive status (i.e. open and perforated vagina for females and scrotal testis for males). Body mass was measured to the nearest *g* to assess resource-holding potential. After each trap inspection at 6:00 A.M., the animal was put back into the same trap at the exact same location. Although captured females were not clearly gravid during the trapping period, all of them were pregnant based on obvious signs of gestation and lactation later in the summer season (Le Galliard, personal observation). All females delivered a litter in the field a few days after the experiment, suggesting that the capture, handling and experimental procedures did not cause pregnancy blocks or abortion. Furthermore, all males were sexually mature with visible scrotal testis. Thus, both females and males were assumed to perform behaviours associated with breeding at the time where we conducted our tests. Using the trapping procedure, we normally have a capture rate near 99% for adults (Aars and Ims 2000). We therefore assumed to know exactly the individuals present in each enclosure.

Experimental procedure

During the 3 days when trapping was performed, we conducted the arena contests between 9:00 A.M. and 12:00 noon. Each contest was performed between one *resident* and one *intruder* animal of the same sex. We ran a total of 28 female contests involving 28 individuals and 12 male contests involving 12 males. The intruder was either an animal living in the same habitat patch as the resident, i.e. a *familiar*, or an animal living in another habitat patch, i.e. a *stranger*. Given the area of the patches and the typical area of a reproducing root vole home range (reviewed in Hansteen et al. 1997), we assumed that all individuals inhabiting a patch were familiar to each other and interacting over mutual territory borders (i.e. neighbours) or within shared home ranges (i.e. overlapping; see Ferkin 1988). The average home-range size of a female in that study has been found to be 50 m^2 (50% kernel estimation), while the average overlap among female home ranges within the same habitat patch was as high as 30% (Hoset et al. 2008). We lack data on space use and spatial overlap for males from this study. However, male root voles usually roam over larger areas than females and therefore were even more likely to be familiar to each other when they lived in the same patch. The contests were performed in the field with an arena (25 cm wide × 20 cm high × 50 cm long) situated adjacent to the trap of the resident in a distance of approximately 20 cm. The bottom and the lower parts of the arena were made of netting wire to allow olfactory and visual contacts with the ground and the outside. The whole arena was protected by a 1.70-m-high steel frame with white fabric “walls and roof” to avoid disturbance and create as similar light conditions as possible for all trials. Transport of animals from their trap site to the arena was done by hand carriage of the trap with the vole inside, immediately before they were released into the arena. The residents were also carried around for an equal amount of time as the intruders to create as identical conditions as possible between all animals. Immediately after the contest was terminated, both individuals were again put into their respective traps and carried back to their trapped position. The contest voles and all other voles trapped were released from the traps when all behavioural tests were completed for the day, i.e. around 12:00 noon, and after an extra check of their health condition. No signs of injury were observed.

Due to shortage of animals, some voles were used more than once. Animals were never used twice the same day, animals were never confronted with the same individual twice and animals used more than once were always alternated between groups (resident versus intruder and stranger versus neighbour; see below). Voles were also distributed equally according to their body mass between groups (all $P > 0.21$).

Behavioural observations

Each trial of the behavioural contests started with the release of both the resident and the intruder into the arena, one on each side (randomly selected) of a non-transparent wall separating the arena in two equal-sized parts. To distinguish the two individuals from each other, animals were marked with randomly chosen colours of fluorescent powder (yellow or red). The fluorescent powder contained non-toxic pigments (Radiant color, Richmond, CA, USA) and was brushed on the back of each vole with a cotton swab before each trial. The powder was carefully removed after each behavioural trial with another cotton swab. Fluorescent powders previously have been used successfully in this species with no detrimental effects (Aars et al. 1994), even when the powder was used as dye in bait (Hovland and Andreassen 1995). During 2003, we carried out a pilot experiment to determine how the animals reacted to each other and the arena. We designed our main experiment in 2004 based on our findings from our pilot experiments. During these test trials, an observer was close to the arena during the acclimation period and during the observation period. That person was ready to interact if any signs of injury were observed. However, during no occasion did we feel that it was necessary to intervene (this was later also confirmed when we observed the videos from the main experiments). No observer was therefore close to the arena during our main experiments, either during the acclimation period or during the observation period, except for the one careful approach to remove the wall and turn on the camera. After a pre-observation period of 5 min for acclimation, the wall was carefully removed so that interaction among the contestants could occur, and the 5-min-long observation period started. The time duration of the pre-observation experiment was one short option to permit full acclimation (assessed by loss of distress signs), while the observation period was chosen to be short enough to avoid stress of prolonged social interactions but properly assess most aspects of the territorial behaviour repertoire (see results below). During the 5-min observation period, most of the aggressive interactions lasted not more than 10–20 s for males (maximum was 40 s), and they were less than 10 s for females. Only short bouts of fights occurred throughout the experiment. The contestants were filmed by a digital video camera (Sony DSR 200P, Media System AS, Larvik, Norway) positioned on the top centre of the arena. Between each trial, the arena was washed first with 70% alcohol to remove all trace of vole odour and then rinsed with clean water and dried.

The raw data analysed in this paper are the total times that each vole spent in each behavioural category. The data were first computed from a videotape by scoring for each second the behaviour of both the resident and the intruder voles of each dyad (see Table 1 for scoring techniques). This first step gave us continuous time series of behavioural data from which we could calculate the total time of a specific behaviour by summing over all bouts of that behaviour. Thus, a high score of a specific behaviour could mean more bouts of that behaviour (higher frequency), longer bouts of that behaviour (longer duration) or a combination of both more and longer bouts of that behaviour. According to descriptions of social behaviours given elsewhere (e.g. Wolff et al. 1983; Luque-Larena et al. 2002), we classified the different types of behaviours into five main categories (Table 1): (1) aggressive behaviours, (2) subdominant behaviours, (3) investigative behaviours, (4) avoidance behaviours (the animal is ignoring the other animal) and (5) approach behaviour (the animal is moving in a precise direction towards the other animal). We assumed that behaviours 1, 2 and 4 were non-social behaviours, and that 3 was a social behaviour (see below for behaviour 5). Before the investigation of treatment effects on behaviours, we performed an explorative correlation analysis at the individual level. Aggressive behaviours were positively correlated with subdominant and approach behaviours (Kendall's τ coefficient, τ=0.35 and 0.31, respectively), and negatively with avoidance behaviours (τ=−0.40). Subdominant behaviours were correlated positively with approach behaviours (τ=0.25) and negatively with avoidance behaviours (τ=−0.31). Furthermore, investigative behaviours were negatively correlated with avoidance behaviours (τ=−0.45), while avoidance behaviours were negatively correlated with approach behaviours (τ=−0.59). Thus, animals that spent more time “ignoring” their opponent engaged less often in contact behaviours like approach, investigative, subdominant and aggressive. Approach behaviours were positively correlated with territorial behaviours (subdominant and aggressive), while investigative behaviours showed a distinct variation.

We next removed the approach behaviour from our analyses because it may be aggressive or non-aggressive (see correlation analysis above) and was difficult to interpret as a vole may approach another for aggressive reasons but abandon the approach without any obvious sign of aggressiveness. Furthermore, the total time recorded was the same for all trials, and therefore, the sum of the time spent in each behaviour equals the same value (5 min), and behavioural variables were not independent. By analysing all behaviours except one, we could reduce this problem of statistical dependency.

Data analysis

We included data from all contests conducted in June 2004 and from the two male contests of the pilot study performed the previous year. The habitat patches of the pilot study were twice the size of those used in 2004. However, the basic definition that individuals living in the same continuous habitat patch are considered as *familiars* (see above), whereas individuals living in different patches are considered as *strangers*, is the same for both years. Data from the pilot study did not stand as outliers, and their exclusion did not change the effects' size and statistical significance.

The total time duration (seconds) was calculated for each of the four behavioural categories (Table 1). We then compared the total duration of each behavioural category between sexes, treatments (familiars versus strangers) and ownership (resident versus intruder status). To this aim, we modelled the total duration of each behavioural category as function of sex, treatment, ownership and the interactions between these factors. To control for potential non-

Table 1 Description of the behavioural items recorded for each animal in the arena test and for each second of recording (total recording time is 300 s)

Behavioural response	Description
Aggressive behaviours	
Attacking	Tries to bite the other animal in a fast movement towards it
Chasing	Running after the other animal
Threatening/upright position	Standing in an upright position close to the other animal
Subdominant behaviours	
Attacked	Being attacked by the other animal
Escaping	Escaping from a chasing or attacking animal
Investigative behaviours	
Sniff rear	The animal is sniffing on the rear end of the other animal
Sniff front	The animal is sniffing on the head/snout of the other animal
Avoidance behaviours (ignorance)	The animal ignores the other animal; e.g. grooming itself or sniffing along the arena floor or walls

The total duration of each behavioural item were thereafter calculated for each of the five main behavioural categories for statistical analyses (see “Materials and methods” for further details).

independence among dyads, the model also included a random effect of dyad identity. Furthermore, as the data conformed to a Poisson distribution, we fitted a generalised model using a log-link function and Poisson error terms. The parameters of the model were estimated using a penalised quasi-likelihood method implemented in the glmmPQL procedure in R software 2.2.1 (Venables and Ripley 2002). This model fitted well the data, and the goodness-of-fit tests based on a Pearson chi-squared statistic were satisfactory in all cases (McCullagh and Nelder 1989). Model selection was done by backward elimination of the non-significant terms. To correct for multiple comparisons in the analyses of the four behavioural categories (Table 1), we calculated critical α values from a sequential Bonferroni procedure (Sokal and Rohlf 1995).

In addition to these analyses, we calculated the difference in the total duration of aggressive behaviours for the resident and the intruder (i.e. the total duration for the resident minus the total duration for the intruder within each dyad). This difference was significantly negatively correlated with a similar difference calculated for subdominant behaviours (Pearson $r=-0.47$, $t=-3.26$, $P=0.002$). Thus, a positive value of this difference indicates investment in aggressive rather than submissive behaviours in the resident relative to the intruder. We called this difference the “prior-residency index” (sensu Kokko et al. 2006) and analysed it with a linear model involving the effects of sex, treatment (familiars versus strangers), difference in resource-holding potential (body mass) between the resident and the intruder and the interaction between these factors. The linear model was fitted with the lm procedure in *R*, assuming normally distributed error terms (Venables and Ripley 2002). The normality and homoscedasticity of the residuals was checked and satisfied in this model (Fox 2002).

The same voles were potentially tested more than once (mean number of repeats=2; see above). However, none of the recorded behaviours showed significant inter-individual variation (mixed-effect models, all $P>0.05$). None of the recorded behaviours showed significant change with the number of repeats (mixed-effect models involving 30 animals tested more than once, all $P>0.05$). These results indicate that prior experience did not affect the future behaviour of root voles. Furthermore, we reached similar conclusions than the ones reported below with a repeated-measures analysis that explicitly accounted for dependency within the data. We are therefore confident that the analyses reported below were only slightly affected by pseudoreplication in the data.

Results

Animals did not engage in social interactions in as much as 86.5% of the recorded time and thus often avoided each other. Non-passive behaviours ranked according to their frequency from investigative (6.7% of the observations), subdominant (1.4%) to aggressive interactions (1.3%). The effects of sex, treatment (strangers versus familiars) and ownership (resident versus intruder status) on social and non-social interactions depended on the behavioural category examined (see Table 2 for a summary of selected models). First, there were significant sexual differences for aggressive, subdominant and avoidance behaviours (Table 2): Male dyads performed more aggressive and subdominant behaviours but less avoidance behaviours than females (Fig. 1). Second, the treatment had no detectable effect on the behaviour of the females, but significant effects of familiarity were observed for some behaviour in males. More precisely, males that were strangers performed, though not significantly, less investigative behaviours, but they performed significantly more avoidance behaviours than when they were familiars (Table 2 and Fig. 1). Third, the duration of aggressiveness, a typical territorial behaviour, was influenced by a significant three-way interaction between sex, treatment and ownership. In females, neither treatment nor ownership status had a significant effect on aggressiveness (all $P>0.22$). In males, aggressiveness was influenced by a significant two-way interaction between treatment and ownership ($t=2.66$, $df=10$, $P=0.02$). Male residents were more aggressive on average towards stranger intruders than towards neighbour intruders (log-scaled contrast=0.51 ± 0.18, $P=0.004$), while male intruders were less aggressive on average towards stranger residents than towards neighbour residents (log-scaled contrast=-1.03 ± 0.23, $P<0.001$).

The “prior-residency index” (see “Materials and methods” for the definition of the index) was influenced by a significant interaction between the sex and the treatment effects (sex× treatment effect: $F_{1,36}=10.26$, $P=0.003$; see Fig. 2). There was no clear prior-resident effect in females, and the treatment had no effect on prior-residency in female dyads (Fig. 2). For males, there was no obvious prior-residency within dyads of familiar animals, while the resident invested more in aggressive behaviours than the intruder within dyads of strangers [prior-residency index=11.28 s (−1.0, 24.6) 95% CI, see also Fig. 2]. The difference in body mass between the resident and intruder did not influence the prior-residency index ($F_{1,35}=0.03$, $P=0.86$). The mean mass of males was 34.4 g (±4.86 SD), the mean mass of females was 29.0 g (±4.19 SD) and the difference in body mass between the resident and the intruder ranged from −10 to +9 g.

Discussion

Our behavioural experiments relied on field dyadic arena tests in natural habitats and provided evidence for the DEP (Fisher 1954) in root vole males but not in females. In

Table 2 Best selected models describing the effects of sex, treatment (familiars versus strangers) and ownership (resident versus intruder status) on the behavioural profile of root vole dyads

Factor	Estimate±SE	Df	t-value	P-value
Aggressive behaviours				
Sex	2.48±0.72	36	3.45	0.001
Treatment	0.60±0.69	36	0.87	0.39
Ownership	0.00±0.64	36	0.00	1.00
Sex×treatment	−1.67±0.97	36	−1.73	0.09
Sex×ownership	−0.24±0.73	36	−0.33	0.74
Treatment×ownership	−0.90±0.89	36	−1.01	0.31
Sex×treatment×ownership	2.44±1.02	36	2.39	0.02
Subdominant behaviours				
Sex	1.86±0.37	38	4.96	<0.001
Investigative behaviours				
Sex	0.18±0.25	36	0.72	0.47
Treatment	0.23±0.18	36	1.30	0.20
Sex×treatment	−0.90±0.35	36	−2.58	0.01
Avoidance behaviours				
Sex	−0.18±0.03	36	−5.69	<0.0001
Treatment	−0.03±0.02	36	−1.19	0.24
Sex×treatment	0.16±0.04	36	3.63	0.009

The behavioural profile was described by calculating the total duration of behaviours in each category as described in Table 1. Estimates (±SE) are obtained from a generalised linear mixed-effects model and are given on a log scale with female dyads and familiar treatment group as baseline reference for contrasts. The model controlled for non-independence among dyads by inclusion of a random dyad effect (see "Materials and methods"). Italicized terms indicate significant factors after correction for multiple comparisons using a sequential Bonferroni procedure.

accordance with our hypothesis, males put more effort into territorial defence than females, and males responded to the presence of the stranger, while females were insensitive to the manipulation. Furthermore, there was a clear dominance hierarchy between resident and stranger males, with the territory holders dominating the intruder in pairwise interactions. To our knowledge, our study is the first experimental demonstration of NSD in a small mammal with a known pedigree. NSD in root vole males was probably performed directly through association or indirectly via exposure to a conspecific's olfactory signals. Unfortunately, the cues used were not elucidated by this study.

Our results are also in agreement with the study of Wolff et al. (1983) of white-footed mouse (*Peromyscus leucopus noveboracensis*) and cloudland deer mouse (*P. maniculatus nubiterrae*), and with Vestal and Hellack (1978) study of *P. leucopus*. Wolff et al. (1983) found that both species showed strong defence in the centre of their home ranges and lower levels of aggression on the periphery. These findings suggest that both species have defended core areas (territories) with peripheral areas of home range overlapping with neighbours of either species. Social organization of these species is apparently also based on mutual recognition of neighbours and intolerance, and aggression toward strangers. Furthermore, previous studies that have examined sex differences in owner–neighbour relationships have also found that males and females can differ in their responses to neighbours and strangers, and have proposed that different classes of intruders pose different threats to males and females (e.g. Ferkin 1988; Bard et al. 2002). For example, Ferkin (1988) studied meadow voles (*M. pennsylvanicus*) in which the sex-specific social structure is opposite to the root voles, as males have overlapping territories and females have small exclusive territories. Resident male meadow voles responded with less aggression towards strangers than neighbours because strangers are often transient males, whereas neighbours compete for females within each other's territories. Females, however, responded more aggressively to strangers than to neighbours. Females are philopatric, hence, a neighbouring female is likely to be a close relative and pose less of a threat than a stranger. Therefore, the degree of territoriality between neighbouring individuals depended on kinship between individuals. Temeles (1994) suggested that the DEP is not a permanent feature of all territory holders from the same species but rather may vary depending upon conditions, i.e. the relative threat presented by neighbours relative to strangers.

NSD in male root voles

Familiar males performed more social behaviours (investigative; however, only marginally significant) but less non-social behaviours (ignoring the other animal) than stranger males. Stranger males therefore tended to "avoid" each

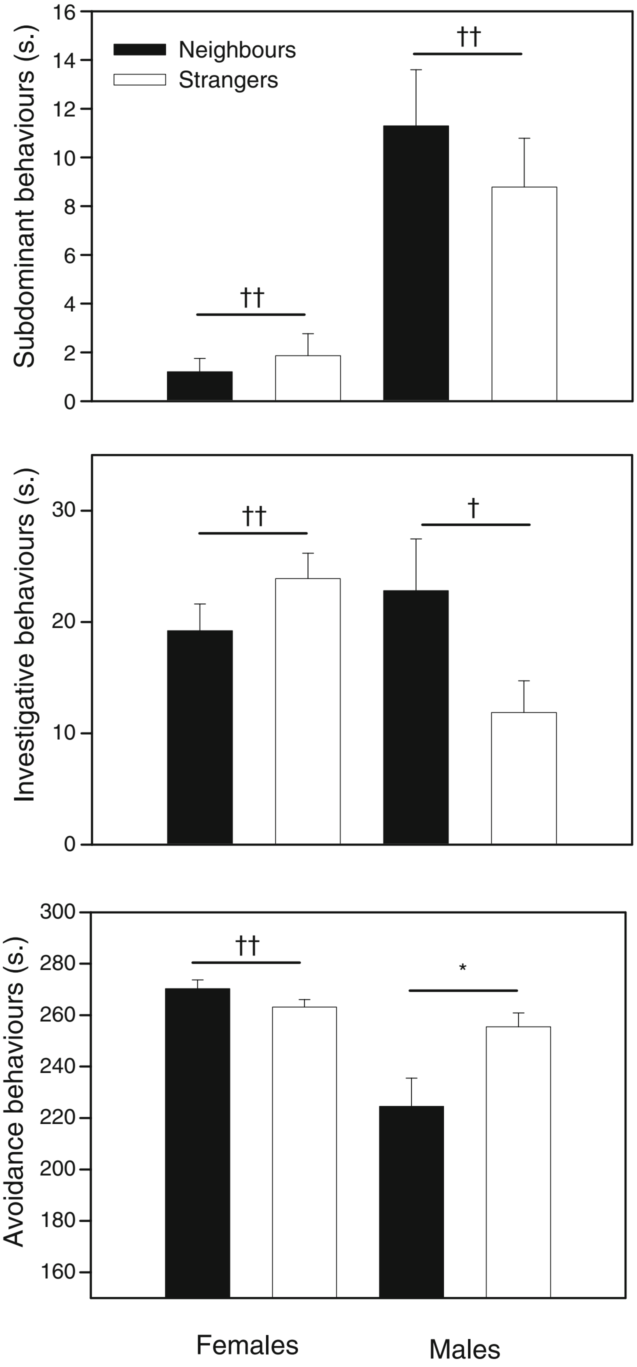

Fig. 1 Mean (±SE) duration (seconds) of each behavioural category per treatment group (neighbours versus strangers) and sex. Behavioural observations were determined for each individual according to the classification in Table 1, except for aggressive behaviours for which results are reported in Fig. 2. Statistics for contrasts between treatments are indicated: *$P<0.05$, $^{\dagger}P<0.10$, $^{\dagger\dagger}P>0.10$

other more. In fact, spacing behavior in small mammals is largely determined by occurrence of non-aggressive avoidance between individuals (Ostfeld 1985b; Wolff 1985). In root vole males, interactions with neighbours may occur several times per day, whereas strangers are rarely encountered. Therefore, neighbours repeatedly assess each others' RHP (Ydenberg et al. 1988), and increased familiarity with neighbours may result in a decreased response to their intrusions. Male root voles may benefit from NSD by conserving energy and reducing the risk of injury from fighting with neighbouring individuals (familiar). Excessive expenditure of time and energy in territorial defence may reduce the fitness of a territory holder. With a reduction in the amount of time and energy spent responding to neighbouring individuals, territorial males can devote more time to attract mates, forage and defend the territory from strangers, and they may also reduce predation risk (Krebs 1982; Temeles 1994). These statements may also be true for the subordinate if he knows he would lose an escalated contest. Furthermore, established neighbours of male root voles have less to gain by taking another vole's territory than strangers because they already maintain and defend territories of their own. Thus, neighbour males represent little threat to each other for territory ownership. Unfamiliar males (strangers), however, could be intruders looking for a territory, thus constituting a stronger potential threat (Stoddard 1996). Male root voles probably responded more aggressively to unfamiliar individuals because strangers are dispersing individuals searching for a breeding territory (Steen 1994; Aars and Ims 2000). Thus, one benefit of NSD for male root voles appears to be for established breeders to direct attention towards real enemies and avoid constant stimulation from other established males.

In addition, we found a clear dominance hierarchy between the resident and the intruder when males were strangers but not when males were familiar. This domi-

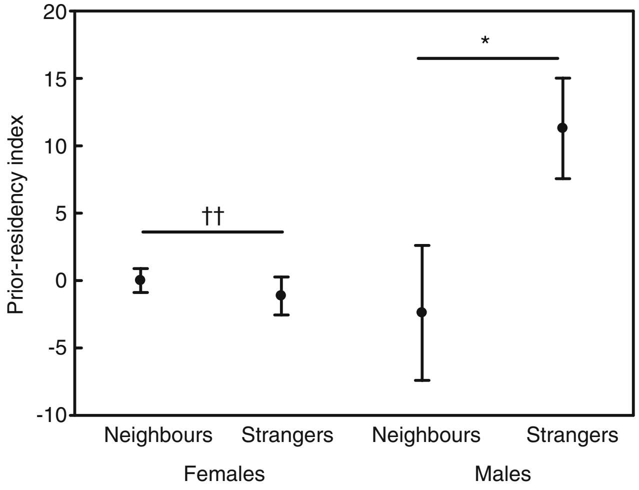

Fig. 2 Prior-residency index in dyads of male and female root voles from familiar (neighbours) and stranger treatment groups. The prior-residency index was calculated as the difference between the total duration of aggressive behaviours of the resident and the total duration of aggressive behaviours of the intruder within the same dyad. Positive values of this index indicate that the resident invested more in aggressive behaviours than the intruder. Data shown are means ± SE per group. Statistics for contrasts between treatments are indicated: *$P<0.05$ and $^{\dagger\dagger}P>0.10$

nance behaviour could be explained by differences in body mass between the resident and the intruder (see Kokko et al. 2006). However, our experimental tests relied on animals of known history and body mass, and we could demonstrate that the prior-resident advantage for male root voles was not due to correlated asymmetries in RHP. Nevertheless, we cannot entirely rule out the possibility that residents valued their territory more than would intruders (Krebs 1982). In microtine rodents such as root voles, an intruder male can cause pregnancy blocks in females and commit infanticide, thereby damaging both current and future mating of the resident male (e.g. Andreassen and Gundersen 2006). Therefore, the resident has more to lose than the benefits for the intruder, and the resident male may give more value to its territory than the intruder (Krebs 1982). Furthermore, in this study, the body mass of root voles was the only measure of their quality. We can therefore not rule out that several other cues may be important and determine the winner between the contests. For example, Mills et al. (2007) found that testosterone level rather that body mass determined mating and reproductive success of male bank voles (*Myodes glareolus)*.

Notwithstanding these difficulties, this study is the first to provide evidence for the prior resident advantage in a vole (the fifth in mammals; see Table A1 in Kokko et al. 2006). However, contrary to the assumptions of game theory models (Kokko et al. 2006), both ownership status and familiarity status affected how much a male root vole invested in territorial defence. Male residents were more aggressive towards stranger intruders than towards neighbour intruders, while the reverse pattern was found for male intruders. This result suggests that territorial defence in animals can be influenced by multiple interacting established conventions. The absence of a prior resident effect in familiar males is probably a consequence of the DEP, as resident males may treat opponents as intruders only when they are strangers. In addition, males might be confused about their residency status in familiar pairs because a "resident" male shared part of his range with a neighbour or could be captured during an occasional sally-out of his normal range. Future studies could try to disentangle these alternative explanations by testing for aggression against intruding neighbours at the border and at the centre of the range of a resident.

NSD in female root voles

In accordance to our hypothesis, the manipulation (familiar or stranger) had no effect on the behaviour of root vole females, and there was no clear dominance hierarchy between females. Thus, females do not appear to exhibit NSD and a prior resident advantage. This finding differs from the general pattern of increased aggression towards stranger individuals reported for numerous species (Temeles 1994). However, the absence of aggression and NSD in females agrees well with the fact that female root voles are less territorial than males (see Andreassen et al. 1998 for comprehensive data on space use by female root voles). There was a substantial home range overlap between females in this study (Hoset et al. 2008) like in other studies with female root voles (e.g. Lambin et al. 1992; Andreassen et al. 1998). In polygynous species, such as microtine rodents with female-defence mating systems, female offspring usually disperse less or shorter distances than males and increased tolerance and space-sharing among females is thought to be a result of kin selection for amicable interactions among relatives (Dobson 1982; Le Galliard et al. 2006). This result cannot explain why females kept on behaving amicably towards strangers in our study, however. Another explanation for the absence of the NSD in female root voles is that females might be more sensitive to intrusion by unfamiliar male root voles than by unfamiliar females. Male intruders are known to commit infanticide and matricide in root voles, while infanticide by females has never been reported in this species (Andreassen and Gundersen 2006). In addition, intruding females may pose no threat during this specific period of the reproductive cycle (early gestation). In the female-territorial species bank vole, Koskela et al. (1997) reported lower female aggressiveness during early gestation than during late gestation and lactation. Unfortunately, no data exists on how territorial behaviour of root voles changes during the reproductive cycle. Additional work will be required to confirm the absence of NSD in female root voles, and readers should bear in mind that a lack of behavioural discrimination does not necessarily imply the absence of a perceptual discrimination.

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