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Olfactory contacts mediate plasticity in male aggression with variable male density

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Males typically adjust their reproductive strategies based on the perceived density and relative abilities of nearby competitors. In high-density populations, repeated encounters facilitate reliable, learned associations between individuals and their relative competitive abilities. In contrast, opportunities to form such associations are limited when densities are low or in flux, increasing the risk that individuals will unintentionally engage in potentially costly interactions with higher-quality or aggressive opponents. To maximize their fitness, individuals in low-density and fluctuating populations therefore need a general way to assess their current social environment, and thus their relative competitive ability. Here, we investigate how olfactory social signals (scent marks) might perform this function. We manipulated the perceived social environment of isolated, male house mice (*Mus domesticus*) via their periodic contact with scent marks from 3 or 9 male conspecifics, or a control of no scents, over 15 days. We then paired them with an unknown opponent and examined how the diversity of recent scent contact mediated their behavior towards dominant or subordinate opponents. There was an overall pattern for increasing scent diversity to significantly reduce male mice's aggression (tail rattling and lunging) towards their opponents, and also their willingness to engage in reciprocal investigation. Such cautiousness was not indicative of perceived subordination, however; the diversity of recent scent contact did not affect mice's investigation of their opponent's scents, and some measures of aggression were greater when mice faced dominant opponents. These results suggest that house mice can use scent signals to assess their current social environment in the absence of physical interactions, modifying their behavior in ways that are predicted to reduce their risks of injury when the likelihood of encountering unknown opponents increases.

Key words: animal husbandry, dyadic encounter, house mice, olfaction, population density, social communication, social isolation, territorial aggression

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The reproductive strategy that a male adopts should balance his perceived benefits of accessing females and other resources against the potential costs of defending these resources. Such costs are related to the local density of potential competitors, a male's own competitive ability, and that of his opponents, and most studies have demonstrated that competition increases with density (Nelson 1995; Jirotkul 1999) and male-biased sex ratios (Randall 1991). In the face of increasing competition, males can adopt a number of strategies to potentially increase their fitness, such as investing more in traits that females prefer (e.g., ornaments or weaponry—Preston et al. 2003), in mating rituals (e.g., Griskevicius et al. 2012), or in sperm production (e.g., Delbarco-Trillo 2011; but see Engqvist 2012). Males may

also increase their investment in aggressive behaviors to either guard mates (Sherman 1989) or the resources used to attract or support them (Shonfield et al. 2012).

The relationship between the fitness benefits of such investment and the costs of doing so are rarely linear, however, with varying consequences for mating strategies and behavior across a range of competitor densities (Kokko and Rankin 2006). The potential costs of female or territory defense (e.g., lost opportunities, injury) increasingly outweigh the benefits when the number of competitors is very high, for example, such that territoriality and intermale aggression often peak at intermediate population densities (Chapman and Kramer 1996). The proportion of individuals adopting alternative mating strategies can also be

density dependent (e.g., “sneaking” fertilizations—Parker 1998; forced copulations—Rivera and Andrés 2002), as can switches between different mating systems. Male fallow deer (*Dama dama*) are nonterritorial when population densities are low, for instance, but form leks at high densities to limit their frequency of aggressive encounters with competitors (Pélabon et al. 1999). Thus, because the relative fitness of different mating strategies is predicted to vary with density (Kokko and Rankin 2006), assessing the density and relative competitive ability of potential competitors is a strong driver of male behavior.

In many species, social signals are employed to provide such competitor assessments. In addition to signaling competitor densities (Kasumovic et al. 2011), some song or call frequencies are considered reliable indicators of an individual’s willingness to fight (Templeton et al. 2012), and the intensity of particular visual signals can play a similar role (Pryke et al. 2001). For olfactorily communicating species, an individual’s relative competitive ability is signaled in both the chemical profile of their scent marks (urine, feces, and other secretions—Brown and MacDonald 1985), and also the scents’ abundance, distribution, and age (see Hurst and Beynon 2004 for a review).

Scent marks are used by a wide variety of species to mediate intrasexual competition, and they are particularly common in terrestrial mammals (Brown and MacDonald 1985; Gosling and Roberts 2001). Dominant or resource holding animals generally deposit a greater number of marks than individuals of lesser status (Brown and MacDonald 1985; Gosling and Roberts 2001), and their scents are also qualitatively different, with high concentrations of androgen-dependent volatile compounds in the scents of dominant males (Harvey et al. 1989). These volatile compounds play an important role in an individual’s ability to assess the relative competitive ability of conspecifics, although how a male responds to a scent mark and its owner varies with his own social status and that of the urine donor, and often also the individuals’ familiarity with one another. These relationships have been particularly well studied in high-density populations of commensal and laboratory house mice (*Mus domesticus*), where adult males compete aggressively to dominate other males within their territory and to exclude all other males (Hurst 1987). Under these conditions, home ranges are typically small, and there is a high probability that individuals will repeatedly encounter a potentially aggressive opponent. As such, males typically avoid scent-marked areas, especially if they are of low competitive ability (Gosling et al. 1996) or if scents are from dominant males (Hurst 1993). Although dominant males typically tolerate known subordinate males within their territories, they significantly increase their aggression towards subordinates (and intruders) if they detect the urine of an unknown subordinate in their territory (Hurst 1993). As a consequence, male mice are more reluctant to engage in fights with conspecifics whose scents indicate that they are territory owners (Gosling and McKay 1990). There is evidence that such competitor assessments can be based on olfactory cues alone (Gosling et al. 1996) or on learned associations between scents and the competitive abilities of their owners (i.e., scent matching—Gosling and McKay 1990). Similar processes of

scent-based competitor assessment have been reported in a number of other taxa (e.g., Luque-Larena et al. 2001; Lopez and Martin 2011).

In contrast with captive or commensal populations, home ranges in wild populations are generally much larger, direct contact between individuals can be rare or irregular, individual turnover is high, and social systems can change over relatively short time periods (Bronson 1979). Under such conditions, it is unlikely that resident individuals could exclude intruders from occupied areas, or that scent marks are reliable, cheat-proof indicators of competitive ability (Gray and Hurst 1997). Given the large size of occupied areas, low-quality competitors could mark or occupy an area while dominant individuals are elsewhere, without the need to prove their dominance via competitive interactions (Gray and Hurst 1997).

Although scent marks are unlikely to accurately signal conspecifics’ competitive abilities in wild, low-density populations, individuals in such populations still investigate social scents (Pastro and Banks 2006), and the general diversity of scents that they encounter should be indicative of the number of nearby conspecifics and competitors. We hypothesize that individuals within such populations would benefit from a generalized response to unknown conspecifics based on the diversity of scents they have recently encountered, with a positive correlation between the diversity of scents encountered and the perceived population density. Because the costs of aggression typically increase with competitor densities (Brown 1964), we predict that males will modify their behavior towards competitors based on their own perceived competitive ability, and also on their perceived costs of aggression given the current population density. As such, we predict that males that perceive themselves as dominant or highly competitive will invest more in aggressive behaviors as perceived competitor densities increase. In contrast, if males are unsure of their relative competitive abilities or do not consider themselves highly competitive, then we predict that they will be cautious of unknown conspecifics. For these males, the potential costs of aggression are likely to increasingly outweigh the potential benefits as competitor densities increase, and we therefore predict that aggression towards opponents will decline as perceived competitor densities increase.

In this study, we examine if and how social scents moderate aggressive behavior in olfactorily communicating wild house mice (*M. domesticus*), independent of physical contact with conspecifics. In this species, both sexes can change their social and reproductive tactics to maximize their fitness (Schrading et al. 2012), and wild mice’s social systems consequently range from solitary and nomadic (i.e., not site-attached) at low densities, to small territorial family groups, or large family groups with communal breeding as densities increase (Chambers et al. 2000; Sutherland and Singleton 2006). Drawing on studies that suggest wild mice deposit and inspect scents even when densities are low (Hughes et al. 2009; Hughes and Banks 2010), we manipulated the perceived social environment of temporarily isolated, wild-caught adult male mice by exposing them to the scent marks of 3 or 9 adult male conspecifics, or to a control (no conspecific scents). We then examined how recent olfactory

experience altered their interactions with an unknown male opponent of either dominant or subordinate status. In doing so, we link laboratory findings that short-term physical isolation simulates territory ownership (and associated aggressive behavior—e.g., [Benton and Brain 1979](#)), with our prediction that aggression should be moderated by the perceived density of conspecifics. Specifically, if males perceived themselves as highly competitive, we predicted that:

1. Males will be increasingly motivated to investigate opponents as perceived population densities increase, and thus the investigation of opponents and their scents will increase in a step-wise fashion with the diversity of recent scent exposure;
2. Males will invest more heavily in aggressive behavior as perceived population densities increase, with aggression towards opponents increasing in a step-wise fashion with the diversity of recent scent exposure;
3. Males will perceive greater benefits from challenging dominant than subordinate opponents and will therefore be more aggressive towards dominant than subordinate opponents.

Alternatively, if males are unsure of their relative competitive abilities or do not perceive themselves as competitively dominant, then we predict that their behavior will be the opposite of that outlined above.

MATERIALS AND METHODS

We used wild, adult male mice trapped from 2 agricultural locations in south-eastern Australia during the breeding season (34°48'S, 146°06'E in March 2005 and 35°7'S, 142°1'E in September 2005 and February 2007). When conditions are favorable, reproductive male mice in these agricultural landscapes form territories that overlap with several breeding females; male territories may or may not overlap with those of other reproductive males whereas breeding females' territories are largely exclusive ([Chambers et al. 2000](#); [Sutherland and Singleton 2003](#)). Due to a prolonged drought, all mouse populations were at low densities (< 5 mice/100 trap nights) at the time of capture, and mice were therefore captured in a range of habitats, including roadside vegetation, the edges of crop fields, and around farm buildings. Resources were presumably scarce and widely distributed, although possibly locally clumped. Thus, while breeding females should still have been territorial at the time of capture, male mice are likely to have been nomadic or had relatively large home ranges with low to moderate overlap ([Chambers et al. 2000](#); [Sutherland and Singleton 2006](#)).

Mice were trapped using clean Type B Elliot traps (30×10×10 cm; Elliot Scientific Equipment, Upwey, Victoria, Australia) baited with sunflower seeds and provided with non-absorbent cotton-wool bedding. Traps were placed in a plastic bag and under vegetation or other cover for further protection. Traps were set at sunset and checked at 1st light. Juveniles and adult females were released at the point of capture, while adult

males (testes descended, > 12 g) were transported to the laboratory in groups of up to 4 males trapped from the same location (cages measured 48×26×15 cm and contained wood shavings, shredded paper, sunflower seeds, apple, and water). In the laboratory, mice were housed in groups (2–3 individuals from the same location; cages as above) for approximately 1 month before the experiment began. Mice were kept in a constant temperature room (24±2°C) on a reverse light cycle (lights on at 2200 and off at 1000h). Food (rodent pellets) and water were provided ad libitum.

Scent exposure phase.—At the onset of the experiment (the scent exposure phase), we allocated mice to one of 4 categories: a scent donor category or one of 3 scent recipient categories.

Scent donors.—Mice in the scent donor category were housed in groups of 3 (cages, food, and water as above), and scent collection began 4 days after mice were group-housed. We collected scent marks from donor mice by placing them individually in clean cages (37×60×20 cm) containing 36 clean tiles (5×5 cm) for approximately 2h, after which mice were returned to their home cage. Mice spontaneously scent mark clean surfaces: dominant mice deposit many long, thin trace marks, whereas subordinate mice concentrate their urine in pools ([Desjardins et al. 1973](#)). We used these stereotypical differences in marking patterns in combination with behavioral observations (chasing and fighting) and physical signs of aggression (bite marks on the rump or tail) to rank the dominance status of the 3 mice within a group relative to one another. In each group, 1 mouse was clearly dominant over the other 2 and was therefore classified as dominant; the other 2 mice were both classified as subordinate.

Ethical note.—As some aggression is typical in group-housed mice, we took precautions to prevent injury and minimize stress. Mice were monitored daily for signs of aggression, especially upon first being housed together; where possible, mice were initially housed with individuals trapped from the same location to reduce the stress of being housed with unfamiliar individuals. Fighting was generally observed to decline rapidly after grouping, and mice readily used the refuges provided to them. In those groups where fighting did not decline and superficial bite marks were observed (wounds did not require treatment), the subordinate individual under attack was isolated for the remainder of the experiment (maximum of 10 days; < 15% of groups). The distinctive scent marking patterns of these subordinate mice did not change after isolation, conforming with previous reports that marking rates are reduced in subordinate mice for at least 6 days post-isolation ([Matthews 1981](#)). All mice were euthanized with CO₂ at the completion of the experiment. Our procedures conform to the guidelines of the American Society of Mammalogists for the use of wild mammals in research ([Sikes et al. 2011](#)) and were approved by the University of New South Wales Animal Care and Ethics Committee (Permit Number 05/23A).

Scent recipients.—At the onset of the scent exposure phase, scent recipient mice were housed individually (conditions as described above) and randomly allocated to one of 3 scent diversity treatments: a 9-, 3-, or no (control)-scent donors treatment.

Mice in the 9-scent donors treatment received 1 scent-marked tile from each individual in 3 donor groups, while mice in the 3-scent donors treatment received 3 scent-marked tiles from each individual in 1 group (Fig. 1a). Thus, mice from the 9- and 3-scent donor treatments were exposed to the same number of scented tiles from dominant (3 tiles) and subordinate (6 tiles) individuals, and only the number of scent donors (the diversity) differed between the 2 groups. This range of scent donors is representative of the number of individuals wild house mice from the source areas commonly encounter over a similar time period (Sutherland and Singleton 2003). Most scent recipients shared some scent donors, although the full complement of donors was never identical. The same donors contributed scents to the same recipient in every round of scent exposure. The control group was not exposed to scented tiles; we placed 9 clean tiles in their cages (Fig. 1a).

To simulate intermittent encounters with conspecific scents, scent-marked and clean tiles were distributed to scent recipients 4 days after they were housed individually, and every 2nd day thereafter. Clean and scent-marked tiles were placed in the recipients' cage immediately after the scent collection period and removed 24 h later. The exception to this was the final scent exposure when the tiles were removed after 12 h, ensuring that scent recipients had recent experience with fresh conspecific scents prior to the social interaction trials (see below). Social interaction trials were conducted on the day after the final (8th) round of scent exposure.

Social interaction trials.—We assessed the effect that the diversity of scent donors had on social interactions between a scent recipient mouse and an unfamiliar conspecific (previously group-housed) following a modified version of the methods described in Gosling and McKay (1990). On the

evening before the social interaction trials, we collected scent marks by placing all scent recipient and scent donor mice in individual, paper-lined cages (40×60×20 cm). Each cage contained food, water, and a plastic tube with shredded paper for bedding. Overnight, all scent recipient mice deposited marks and scent donor mice deposited scent marks consistent with their previously determined social status. The following morning, scent-marked paper was removed and cut to fit 1 (scent recipient) or 2 (scent donor) sections of the experimental arena (Fig. 1b).

Social interaction trials were conducted in a purpose-built 60×60×60-cm arena divided vertically by a mesh partition into 2 unequally sized compartments (Fig. 1b). A single opponent mouse was placed in the smaller compartment (section C in Fig. 1b), and a single scent recipient mouse was placed onto section A approximately 30 s later. For each trial, section A of the arena floor was lined with paper scent marked by the scent recipient the previous night, while sections B and C were lined with paper scent marked by the opponent (Fig. 1b). We considered scent recipients to be territory holders when on their self-marked paper (section A), but an intruder when they moved onto section B. Scent recipients had not had olfactory or physical contact with their opponents and were therefore considered unknown. Each mouse participated in the social interaction trials only once.

A small fan was set to deliver steady airflow through the experimental arena from the opponent to the scent recipient, allowing the scent recipient to detect the body and urinary scents of its opponent and compare these scents with those on section B. The airflow also prevented the opponent from smelling the scent recipient or sections A or B, reducing the influence of these scents on the opponent's behavior.

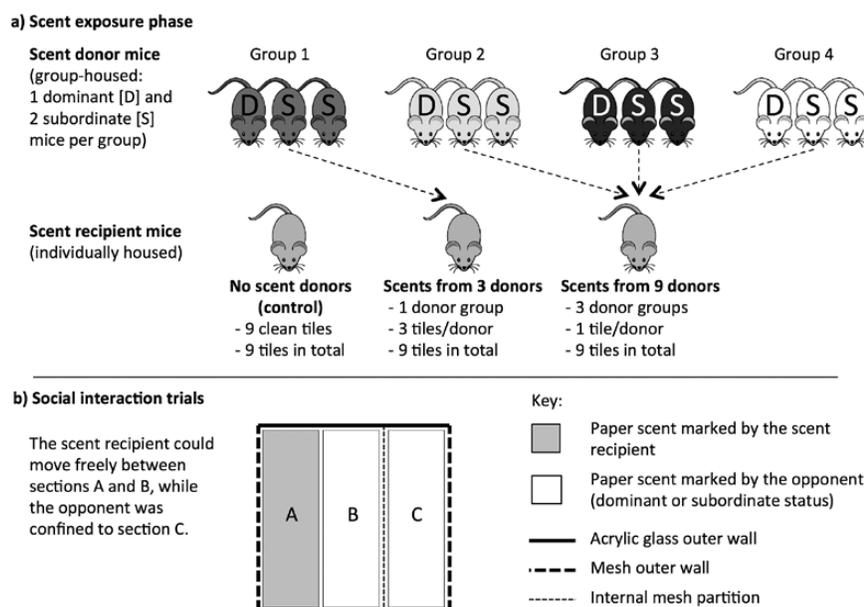


Fig. 1.—During the a) scent exposure phase, scent marks were collected on tiles from mice in scent donor groups (colors distinguish separate scent donor groups; only 4 groups shown for clarity) and presented to scent recipient mice according to their treatment group. In b) social interaction trials, the scent recipient had access to sections A and B, while the opponent was confined to section C. Section A was lined with paper scent marked by the scent recipient, while sections B and C were lined with paper scent marked by the opponent.

We conducted trials in darkness during the mice’s normal activity period and recorded them using infrared cameras. Recording began upon the scent recipient’s entry to the experimental arena and continued for the next 30 min. Trials were stopped if either mouse chewed through the mesh during the social interaction trial ($n = 4$), but earlier time periods were included in analyses. In total, the number of social interaction trials in each treatment varied between 5 and 6 (33 total); refer to [Supporting Information S1](#) for the final sample size within each time period. Experimental arenas were thoroughly cleaned with hot soapy water and 70% ethanol and dried before re-use.

We tested our predictions that scent diversity (i.e., perceived competitor density) would moderate the behavior of scent recipients by recording the following behaviors:

1. Opponent investigation (in seconds): the time spent on section B, measured from when the back of the scent recipient’s head crossed the division between sections A and B; an index of the scent recipient’s willingness to leave its self-marked area (section A) and to investigate the odors of its opponent (on sections B and C).
2. Reciprocal investigation (in seconds): the scent recipient and opponent sniffed directly at one another through the mesh.
3. Tail rattling (in seconds): a rapid, sideways movement of the scent recipient’s tail. An aggressive threat behavior ([Hurst 1993](#)).
4. Lunging (count): an attack behavior by the scent recipient consisting of short, rapid movements towards the opponent.

Statistical analyses.—We assessed the effect of scent diversity (9, 3, or no scents), and opponent status (dominant or subordinate), and their interaction on the scent recipients’ behavior during the first 5 min, between 10 and 15 min, and between 20 and 25 min (hereafter referred to as the 1st, 2nd, and 3rd time

periods, respectively). We chose these time periods to examine the mice’s initial response to their opponent, and then to assess how their behavior changed over time and with increasing exposure to their opponent and their scents. Separate models were constructed for the time spent in opponent investigation, reciprocal investigation, and tail rattling, and for the number of lunges. Of these 4 response variables, only the time spent in opponent investigation was normally distributed. Data were therefore analyzed using generalized linear models with a normal distribution and identity link function for the time spent in opponent investigation, and a Poisson distribution and a log link function for all other response variables. We used planned contrast analyses to identify the cause of significant effects. To aid interpretation, we calculated the magnitude of treatment effects using Cohen’s d , $(M_1 - M_2)/SD_{\text{pooled}}$, where M_1 and M_2 are group means, and $SD_{\text{pooled}} = \sqrt{\frac{SD_1^2 + SD_2^2}{2}}$, where SD_1 and SD_2 are the SD s of groups ([Cohen 1988](#)). Refer to [Supporting Information S2](#) and [S3](#) for all effect sizes. All analyses were performed in JMP (version 11; [SAS Institute 2013](#)).

RESULTS

Scent and opponent investigation.—Neither scent diversity nor opponent status had a significant effect on the time that scent recipients spent investigating their opponent’s scents ([Table 1](#); [Fig. 2a](#); measured as the time on section B). In contrast, mice initially avoided close, reciprocal investigation of one another, before dramatically increasing their time in reciprocal investigation ([Fig. 2b](#)). Overall, there was an increasingly strong negative relationship between the scent diversity treatment and the time that males spent in reciprocal investigation throughout the trial, but this relationship often varied with opponent status ([Table 1](#)). Initially, reciprocal investigation

Table 1.—Results of generalized linear models on the effect of scent diversity and opponent status (and their interaction) on the mean time scent recipients spent a) investigating their opponent, b) in reciprocal investigation with their opponent, c) tail rattling, and d) the mean number of lunges scent recipients made towards their opponent during the 1st, 2nd, and 3rd time periods (0–5, 10–15, and 20–25 min, respectively). Significant results ($P < 0.05$) are shown in bold.

| | <i>d.f.</i> | 1st time period | | 2nd time period | | 3rd time period | |
|-------------------------------------|-------------|-----------------|----------------|-----------------|----------------|-----------------|----------------|
| | | χ^2 | <i>P</i> | χ^2 | <i>P</i> | χ^2 | <i>P</i> |
| a) Time investigating opponent | | | | | | | |
| Scent diversity | 2 | 2.208 | 0.332 | 4.499 | 0.106 | 3.700 | 0.157 |
| Opponent status | 1 | 1.1672 | 0.280 | 2.597 | 0.107 | 1.216 | 0.270 |
| Interaction | 2 | 0.862 | 0.650 | 3.092 | | 0.343 | 0.843 |
| b) Time in reciprocal investigation | | | | | | | |
| Scent diversity | 2 | 6.079 | 0.048 | 31.266 | < 0.001 | 33.208 | < 0.001 |
| Opponent status | 1 | 22.139 | < 0.001 | 0.003 | 0.954 | 13.359 | < 0.001 |
| Interaction | 2 | 50.553 | < 0.001 | 12.151 | 0.002 | 5.450 | 0.066 |
| c) Time tail rattling | | | | | | | |
| Scent diversity | 2 | 37.923 | < 0.001 | 97.587 | < 0.001 | 43.485 | < 0.001 |
| Opponent status | 1 | < 0.001 | 0.999 | 27.319 | < 0.001 | 20.813 | < 0.001 |
| Interaction | 2 | 34.013 | < 0.001 | 1.261 | 0.532 | 4.812 | 0.090 |
| d) Number of lunges | | | | | | | |
| Scent diversity | 2 | 2.807 | 0.246 | 4.769 | 0.092 | 31.152 | < 0.001 |
| Opponent status | 1 | < 0.001 | 0.998 | 2.313 | 0.128 | 0.221 | 0.638 |
| Interaction | 2 | 0.930 | 0.629 | 2.891 | 0.236 | 3.273 | 0.195 |

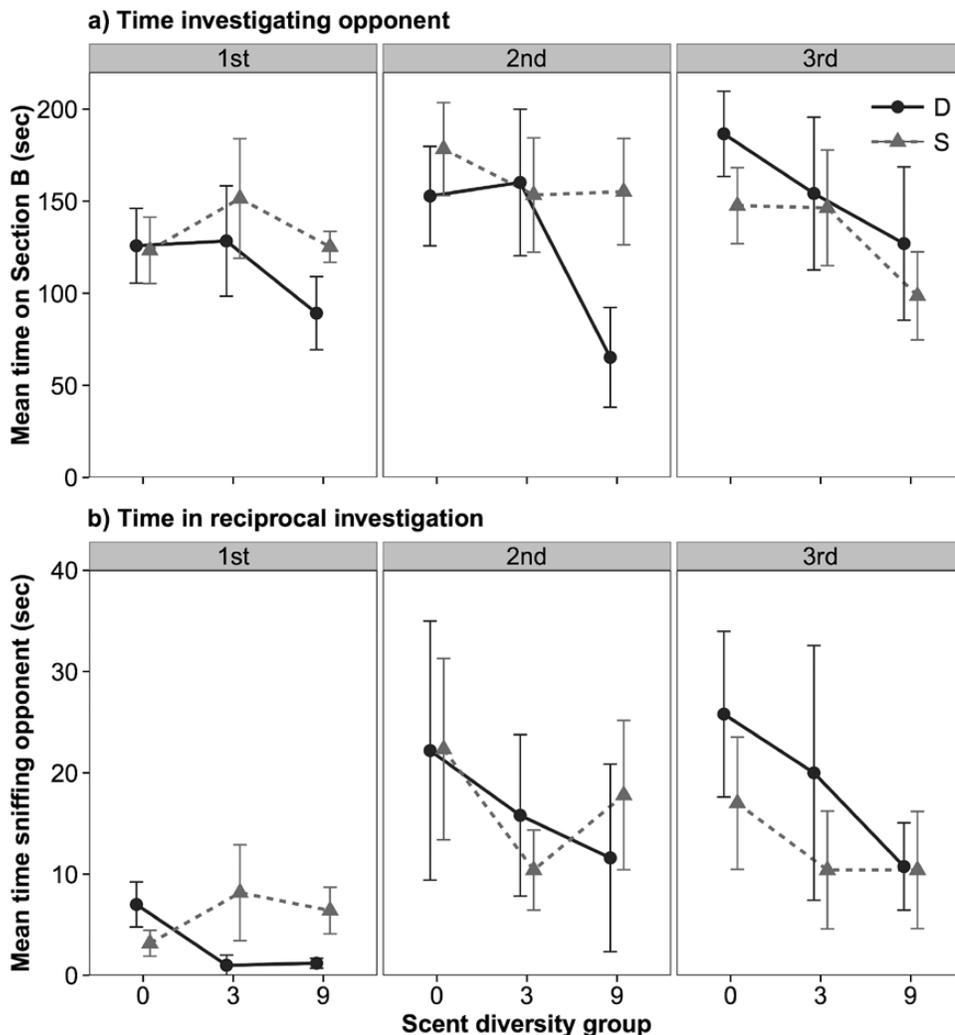


Fig. 2.—The effect of scent diversity (exposure to 0, 3, or 9 scents) and opponent's status (dominant [D] or subordinate [S]) on investigation. The mean (\pm SE) time scent recipient mice from different scent diversity treatments spent a) investigating their opponent's scents and b) in reciprocal investigation with their opponent in the 1st, 2nd, and 3rd time periods (0–5, 10–15, and 20–25 min, respectively).

with dominant opponents was significantly greater in mice that were exposed to no scents (controls) than to 3 or 9 conspecific scents ($\chi^2_1 = 38.658$, $P < 0.001$; Fig. 2b, left panel), and the strength of this negative relationship became more pronounced during the 2nd time period (no scents > 3 and 9 scents: $\chi^2_1 = 14.480$, $P < 0.001$; strong trend for 3 scents > 9 scents: $\chi^2_1 = 3.232$, $P = 0.072$; Fig. 2b, middle panel). In the final time period, increasing scent diversity had a consistently significant, negative effect on the time mice spent in reciprocal investigation (no scents > 3 scents: $\chi^2_1 = 11.666$, $P < 0.001$; 3 scents > 9 scents: $\chi^2_1 = 5.446$, $P = 0.020$; Fig. 2b, right panel). In contrast, the relationship between scent diversity and reciprocal investigation of subordinate opponents was inverted in the 1st time period (no scents < 3 and 9 scents: $\chi^2_1 = 12.085$, $P < 0.001$) and remained less predictable than for dominant opponents in the 2nd time period (no scents > 3 scents: $\chi^2_1 = 24.017$, $P < 0.001$; no scents versus 9 scents: $\chi^2_1 = 2.789$, $P = 0.09$; 3 scents < 9 scents: $\chi^2_1 = 9.824$, $P = 0.002$).

Aggression and attack.—Patterns of aggressive tail rattling and lunging attacks associated with scent diversity treatments

were different to those of scent and opponent investigation (Table 1; Fig. 3). Scent diversity significantly affected the mean time that scent recipients spent aggressively tail rattling towards their opponents, with mice exposed to 9 scents having particularly low levels of tail rattling (Fig. 3a). There was also a significant interaction between scent diversity and opponent status in the 1st time period (Table 1). Tail rattling towards dominant opponents was initially greater in mice that had not been exposed to conspecific scents (no scents > 3 and 9 scents: $\chi^2_1 = 82.850$, $P < 0.001$; Fig. 3a, left panel), while mice facing subordinate opponents rattled their tails less if they had been exposed to 9 conspecific scents than to 3 or no scents (no and 3 scents > 9 scents: $\chi^2_1 = 14.073$, $P < 0.001$). Differences in the aggressive behavior of the scent diversity treatment groups became more pronounced in the 2nd and 3rd time periods (Fig. 3a, middle and right panels), with significantly less tail rattling in groups exposed to increasing numbers of scents (Table 1; 2nd time period: no scents > 3 scents > 9 scents: $\chi^2_1 = 50.914$, $P < 0.001$ and $\chi^2_1 = 4.60$, $P = 0.035$, respectively; 3rd time period: no scents > 3 scents > 9 scents: $\chi^2_1 = 13.529$, $P < 0.001$ and

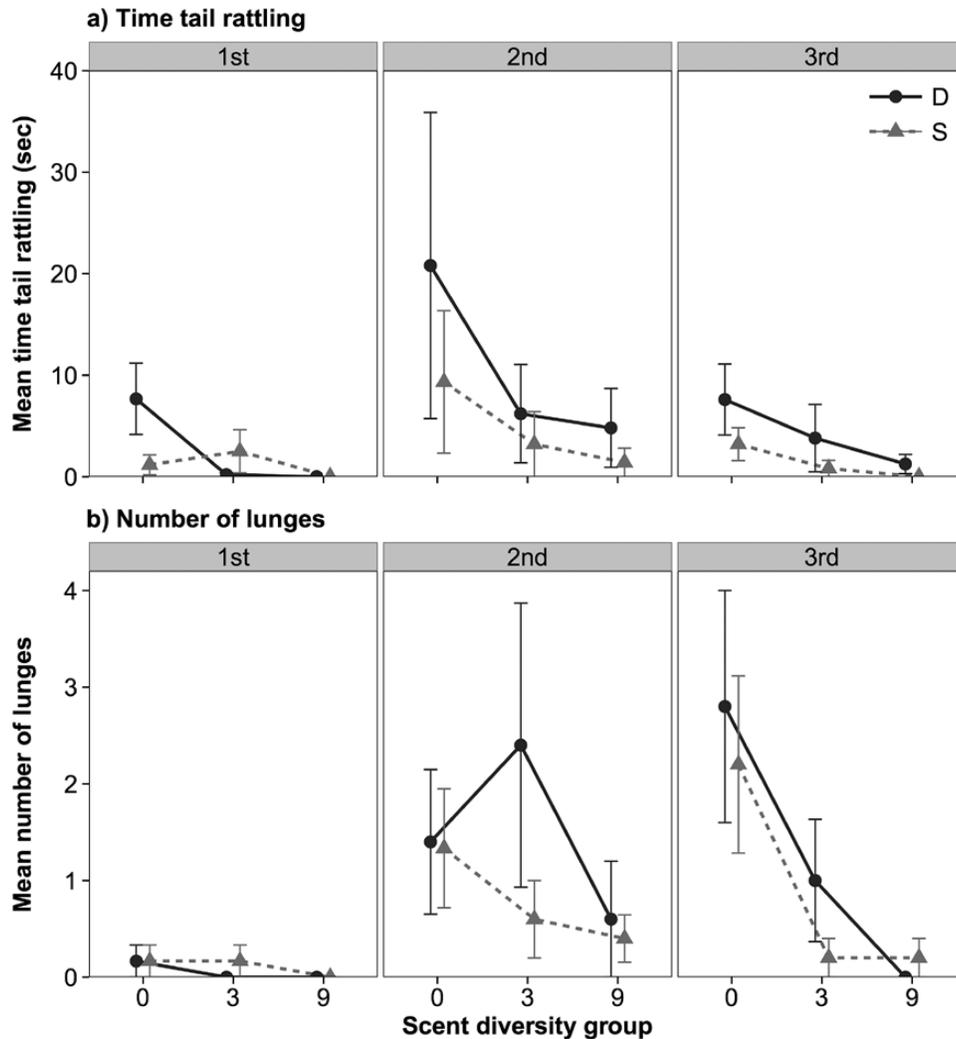


Fig. 3.—The effect of scent diversity (exposure to 0, 3, or 9 scents) and opponent's status (dominant [D] or subordinate [S]) on aggression and attack behaviors. a) The mean (\pm SE) total time scent recipient mice from different scent diversity treatments spent aggressively tail rattling towards their opponents and b) the mean (\pm SE) number of lunges they made towards opponents in the 1st, 2nd, and 3rd time periods (0–5, 10–15, and 20–25 min, respectively).

$\chi^2_1 = 9.428$, $P = 0.002$, respectively). Periods of tail rattling were significantly longer against dominant opponents than subordinate opponents in the 2nd and 3rd time periods (Table 1).

Lunging attacks were initially infrequent across all treatments but increased as the trials progressed. The frequency of lunges was particularly low in mice exposed to 9 scents (Fig. 3b), although a significant effect of scent diversity was not observed until the 3rd time period (Table 1). In this time period, mice in the control group lunged more often than those exposed to both 3 ($\chi^2_1 = 14.158$, $P < 0.001$) and 9 ($\chi^2_1 = 25.977$, $P < 0.001$) scents, and there was also a trend for more lunging in mice exposed to 3 versus 9 scents ($\chi^2_1 = 2.659$, $P = 0.103$). In contrast to patterns of aggressive tail rattling, rates of lunging did not vary with an opponent's status (Table 1).

DISCUSSION

We have demonstrated that the diversity of conspecific scents to which wild-caught mice are exposed does not affect

their willingness to investigate conspecific odors, but there was a significant negative relationship between the diversity of recent scent experience and individuals' willingness to engage in reciprocal investigation with an opponent (Prediction 1). Furthermore, we have shown that aggressive behavior in wild-caught mice is moderated by the diversity of recent scent exposure, with rates of aggressive tail rattling and lunging attacks lower in more scent-experienced mice (Prediction 2). While these results suggest that more scent-experienced mice behaved more cautiously than their less scent-experienced conspecifics upon meeting an unknown opponent, rates of aggressive tail rattling were generally greater when mice faced dominant rather than subordinate opponents (Prediction 3), and this did not vary with scent experience. There was no effect of opponent status on attack behaviors. Taken together, these results demonstrate that mice moderate their aggressive behavior towards unknown competitors based on the number of conspecifics whose scents they have recently encountered and suggest that socializing

via scent signals may therefore act as a proxy for direct physical interactions.

Our results suggest that wild house mice in low-density wild populations (i.e., with limited scent encounter rates) will respond aggressively towards unknown conspecifics. This conflicts with previous suggestions that the benefits of aggressively defending large territories are exceeded by the costs (e.g., Hurst et al. 1996). Instead, we suggest that individuals from low-density populations would still benefit from vigorously excluding conspecifics from resource patches if these patches are rare, localized, and only temporarily available. Strong competition for limited resources has also been proposed to drive the negative relationship between aggression and commensalism in *M. musculus* subspecies (Frynta et al. 2005). If valuable resources are patchy, the simultaneous presence of both scent marks and their owner may be indicative of valuable local resources and therefore encourage high levels of aggressiveness, although levels of aggression may vary if an unknown individual's scents are also present (Gosling and McKay 1990; Hughes 2009). Furthermore, because scent cues are unlikely to be reliable signals of status in large territories, direct interactions to establish a hierarchy may be necessary each time 2 individuals meet (see also Hurst et al. 1996). If true, a brief aggressive encounter might be more profitable if it discourages repeated or prolonged interactions.

A possible alternative explanation is that the mice exposed to conspecific scents considered their territories to be under sustained attack, and thus perceived themselves as subordinates. We find this unlikely for 2 reasons. Firstly, laboratory studies have previously demonstrated that male mice exposed to olfactory, but not physical, contact with other males were less aggressive than those that did not have olfactory contact with other males, yet they did not display other physiological characteristics of subordination (Parmigiani et al. 1989). Secondly, and more tellingly, the mice exposed to conspecific scents in our experiment did not behave as subordinates, with levels of aggression towards dominant opponents not lower in mice exposed to a greater number of scents, and rates of investigation of opponents' scent no different across scent diversity treatments. Mice were also given ample opportunity to counter-mark the scent-marked tiles that we introduced to their cages, enabling them to re-assert their territorial dominance to any potential receivers.

We have assumed that male mice exposed to odors from an increasing number of male conspecifics perceived themselves to be in the presence of an increasing number (or density) of competitors. However, as we did not also expose our mice to female scents, it is not possible to determine from our experiment whether they perceived a high diversity of male scents as indicative of a high population density in general (including a high density of females) or as a male-biased sex ratio. The effects of sex ratio and density on male competition are likely to be closely intertwined, and both are typically reported to increase intrasexual competition (Randall 1991; Nelson 1995). But the fitness strategies that males (and females) employ may vary under these differing social conditions and will also likely

vary with a species' mating system (Kokko and Rankin 2006). Thus, while we discuss our results in relation to male strategies with increasing male density, we note that future studies should aim to determine whether manipulations of sex ratio generate similar results to those of density.

Our results have 3 important implications for free-living populations. Firstly, the relationship between social behavior and the diversity of conspecific signals previously encountered points to a mechanism through which olfactorily communicating species can assess competitor densities and modify their behavior accordingly. Although this remains to be tested in wild populations with differing densities and sex ratios, we suggest that the behavioral changes that we report should increase male fitness in the simulated social environments. Such a generalized use of scent marks should be particularly useful for house mice and other species with fluctuating densities and/or social systems (e.g., prairie voles, *Microtus ochrogaster* [Getz and Hofmann 1986]; striped mice, *Rhabdomys pumilio* [Schoepf and Schradin 2012]), and also in species where social aggression has an element of density dependence (e.g., density-dependent dispersal). Furthermore, while the perceived quality and density of olfactory (Drickamer 1977), auditory (Kasumovic et al. 2011), visual (Walling et al. 2007), and tactile (Gage 1995) signals is known to shape developmental plasticity in juveniles, our results demonstrate that signal density also shapes behavioral plasticity in adults. Combined, these results suggest that social signals have the potential to alter social, behavioral, and reproductive strategies across a wide range of species and communication modes, and that this can function, at least to some degree, independently of physical interactions.

Secondly, our finding that social aggression is higher in animals exposed to low/intermediate numbers of scents may have consequences for population processes in a range of species. Our results suggest that male mice in low-density populations will react aggressively towards subsequently encountered males. If this aggression also extends to females then reproductive rates may be affected: highly aggressive males have reduced behavioral and neuroendocrine responses to estrous females (Kudryavtseva et al. 2004), and females experience lower reproductive success with such males (Ensminger and Meikle 2005). Male aggression at low densities might therefore provide a mechanism for prolonged periods of low population growth (Krebs et al. 1995), although we note that the effect of increasing scent exposure (or population density) on male responses to females remains to be tested. In contrast, sustained favorable conditions that allow mice to remain site-attached might be the trigger for higher population growth (Sutherland and Singleton 2006). Greater site fidelity should increase both olfactory and direct physical interaction rates between individuals, and our results suggest that intermale aggression is lowered under these conditions (over the range of "densities" tested). As female mice prefer the odors of less aggressive males (Ensminger and Meikle 2005), and male odors accelerate female sexual maturation in mice (Vandenbergh 1969), sociobiological changes that emerge because of increased site fidelity may promote reproductive success, and subsequent population growth. Thus, while our results

do not exclude the possibility that there are intrinsic differences in individuals throughout population cycles (Chitty 1960), we show that nuanced behavioral changes can occur over a very short time period, even without physical contact between individuals, and suggest that this could moderate the social interactions that alter population growth.

Thirdly, these results provide an additional explanation for sustained olfactory communication under a perceived elevated risk of predation (e.g., Hughes et al. 2009; Hughes and Banks 2010; Hughes et al. 2010). While previous research has shown that individuals who reduce their scent marking rates are aggressively targeted by conspecifics (Hurst et al. 1993), our study indicates that low rates of scent inspection also increases aggression initiated by individuals. Thus, these combined findings suggest that the potential costs of overly aggressive interactions, especially with a large number of opponents, may compel olfactorily communicating species to maintain social scent inspection, even under an increased risk of predation.

Finally, our findings have significant implications for the management of laboratory populations. Generalizing earlier work that suggested that scent exposure is a means to reduce social aggression in isolated animals (e.g., Kimelman and Lubow 1974; Harmatz et al. 1975), our results show that it is the diversity, and not just the volume, of scents that is effective. Thus, we suggest that the use of social enrichment via exposing individually housed laboratory animals to the scents of a large number of group-housed conspecifics should be investigated for a range of laboratory-housed species, in conjunction with other methods known to reduce aggression when individual housing is unavoidable.

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SUPPORTING INFORMATION

Supporting Information S1.—The final sample size used within all analyses.

Supporting Information S2.—Effect sizes for all response variables, for all combinations of scent diversity treatments and opponent status.

Supporting Information S3.—Effect sizes for all response variables, for all combinations of scent diversity treatments pooled across opponent status.

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