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The importance of exposure to other male scents in determining competitive behaviour among inbred male mice

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Abstract

Inbred mouse strains are homozygous at almost all loci, with individuals of the same strain expressing the same genetically determined scents that would normally provide individuals with their own unique scent. As laboratory mice are normally housed with others of the same strain in a simple and constant environment, this will compound the ability of inbred mice to link social status with individual identity within their social group. Further, mice may be exposed accidentally to the scents of others during routine maintenance, or during experiments, which may influence their competitive relationships. We investigated the effect of repeated exposure to soiled bedding from males of either the same or different strain on competitive urine counter-marking and investigation and on aggressive behaviour within same-strain pairs of BALB/c and C57BL/6 males. Males pre-exposed to different strain scents in the home cage had more defined social relationships, in that dominant males were more aggressive while subordinate males suppressed counter-marking near other male urine. Exposure to male urine from the same or different strain outside the home cage stimulated increased aggression when males returned home, an effect that was exacerbated by different strain scents in the home cage. The duration of urine investigation varied according to both strain and experience of home cage scents. Results demonstrate the importance of scent experience in determining competitive behaviour among male mice. To protect welfare, we recommend that males are not exposed to male urine when temporarily removed from their social groups and that care is taken to avoid contamination of home cages with different strain scents, for example, by cleaning cages thoroughly and ensuring that soiled substrate cannot fall into other cages.

Keywords: Laboratory mice; Inbred strains; Scent communication; Aggression

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House mice (*Mus musculus*) are a gregarious species capable of forming complex social relationships and will readily seek social contact (Sherwin, 1996; Van Loo et al., 2001a, 2004). However, competitive males can be highly territorial and extremely aggressive towards unfamiliar mice or familiar males attempting to compete for dominance over the territory (Crowcroft and Rowe, 1963; Rowe and Redfern, 1969; Hurst, 1993). The most important system of communication underlying recognition and competitive advertisement between mice is through scent, particularly through urinary scent marks deposited on the substrate. Resident male territory owners scent mark at a high frequency to advertise aggressive dominance over other resident and intruder males, leaving urine marks all over the territory (Desjardins et al., 1973; Hurst, 1990a; Drickamer, 2001). Subordinate males mark at a much lower frequency with scent of different quality to help ensure familiarity and tolerance by the dominant male (Desjardins et al., 1973; Jones and Nowell, 1973; Hurst, 1990a; Hurst et al., 1993; Drickamer, 2001). Both dominant and subordinate males investigate scent marks to a similar extent to assess status signalling among males in the locality (Hurst et al., 2001a) and to orient themselves towards their resident territory and away from areas marked by other dominant males (Hurst, 1987, 1990a).

The ability to discriminate between conspecifics is important in facilitating the development and maintenance of stable social groups (Hurst, 1990a, 1990b, 1990c; Hurst et al., 1993, 1994). Wild house mice are able to maintain stable groups with relatively little aggression, in part, through the ability to advertise social status through scents that also contain an individual scent signature. For example, a dominant male will seek out, attack and chase a familiar subordinate that deposits competing urinary scent marks (Hurst, 1993), or can recognise a familiar subordinate that stops contributing to group substrate scents (Hurst et al., 1993). If an individual can accurately detect the sex, relatedness, familiarity, social and reproductive status of another through substrate scent cues and associate this information with the appropriate scent owner, then they can modify their behaviour towards this animal accordingly (Hurst, 1987, 1990a, 1990b, 1990c).

The urinary scent cues of wild mice provide individually unique scent signals, which are in part genetically determined. The highly polymorphic loci of the major histocompatibility complex (MHC) play a part in defining the olfactory signal of an individual (Yamaguchi et al., 1981; Eggert et al., 1996; Penn and Potts, 1998; Leinders-Zufall et al., 2004). There is also growing evidence that major urinary proteins (MUPs) are significant mediators of chemical messages between wild mice (Humphries et al., 1999; Hurst et al., 2001b; Beynon et al., 2002; Beynon and Hurst, 2003). MUPs are encoded by a large, multigene complex and exhibit a high degree of polymorphism between individual wild house mice, providing a long-term signature of individual scent ownership in substrate scent marks (Hurst et al., 2001b; Hurst and Beynon, 2004). MUPs bind male signalling pheromones (Robertson et al., 1996; Novotny et al., 1999) and at least two of these pheromones stimulate aggression between males (Novotny et al., 1985). Furthermore, MUPs elicit a counter-marking response from other competitive males that express a different MUP type (Hurst et al., 2001b), even when MUPs are depleted of these natural volatile ligands (Humphries et al., 1999).

Laboratory mice are the most frequently used vertebrate model, making up 67% of animals used in scientific procedures in Great Britain in 2004 (Statistics for Scientific Procedures, 2004). To reduce data variation and improve data reproducibility, hundreds of inbred strains have been established whereby mice within strains are homozygous at almost all loci and are virtually genetically identical (Cohen, 1999). However, since each inbred strain of mouse is genetically homogenous, members of the same strain and sex have identical MHC types (Yamaguchi et al., 1981) and express the same relatively simple pattern of MUPs (Robertson et al., 1996), and thus, share the same individual identity signatures.

Individual scent signatures within a group of mice may contribute to a familiar group odour due to scent transfer between group members that may occur, for example, at times of allo-grooming or through contact with each other and shared substrate scents (Aldhous, 1989). Familiarity of scent cues is a major factor that influences aggression between male mice. Wild male house mice can maintain stable groups so long as each member of the group continues to contribute to the shared substrate odour in order to maintain familiarity and tolerance with each other (Hurst et al., 1993). Since inbred mice of the same strain and sex share individual scent signatures, the extreme familiarity of shared substrate scent cues should contribute to increased tolerance and reduced aggression. Moreover, if an inbred dominant male is unable to detect competing urinary scent marks from cagemates on the home-cage substrate, then potential aggression may be reduced since the subordinate's scent shares the same ownership signal as the dominant's scent (Nevison et al., 2000). However, if a subordinate inbred mouse is unable to advertise his subordinate status clearly, and cagemates have difficulty in recognising the dominant territory owner because they are unable to link social status with identity through substrate scent cues, this might destabilise social relationships as mice are unable to modify their behaviour appropriately towards different owners. Correspondingly, social hierarchies within caged males of aggressive inbred strains have been reported to be unstable with sudden, unpredictable outbreaks of injurious and often fatal aggression occurring within previously harmonious groups (Nevison et al., 2003a; Van Loo et al., 2003b).

Although the scent environment could have strong implications for housing and husbandry of inbred mice, and for behavioural studies dependent on chemical communication or social recognition, the effects of scent exposure on welfare or in stimulating individual variability in behaviour is poorly understood. We therefore investigated how exposure to the scents of genetically different or similar males influences urine marking behaviour, scent investigation and home cage aggression within two common inbred mouse strains of different genetic lineage. We selected BALB/c and C57BL/6 inbred laboratory male mice due to their common use in the laboratory (Festing, 1979; Dean, 1999), their different genetic lineage (Clissold and Bishop, 1982; Robertson et al., 1996) and the reliable formation of dominance relationships in same-strain caged groups (Nevison et al., 1999, 2000, 2003a). Based on the lack of genetic variation within strains in contrast to the normal situation within wild mouse populations, we predicted that inbred males would counter-mark scent marks only if they were from genetically distinct strains. Likewise, if inbred mice respond only to different strain scents as though they were from a competitor, then home cage aggression between males of the same strain would be raised following exposure to odours from a different strain compared to exposure to own strain scents. Furthermore, if the complexity of an animal's scent environment is important in stimulating competitive behaviour, then social status signalling and social relationships would be more clearly defined between same-strain mice with experience of different strain scents.

1. Methods

1.1. Experimental subjects

Thirty-two males of each strain (BALB/c and C57BL/6) were obtained from Harlan, UK (Bicester, Oxon) aged 25 ± 3 days. Males were housed in same-strain pairs in polypropylene cages (13 cm × 48 cm × 15 cm external dimensions, M3 cages North Kent Plastics, Medway, Kent, UK) on sawdust bedding (BCM IPS Ltd., London, UK) with paper wool nest material (BCM IPS Ltd., London, UK). Upon arrival, eight pairs of each strain were randomly assigned to one of two treatment groups in which they

were pre-exposed in their home cages to either same or different strain scents. Throughout, mice were maintained on a reversed 12:12 h light:dark cycle (white lights off at 09:00), at 20 ± 1 °C, with ad libitum food (TRM 9607 rat and mouse diet, Harlan, UK, Bicester, Oxon) and water. One male from each pair was marked for identification using hair dye (Clairol Nice 'n' Easy Natural Black, Bristol-Myers Co. Ltd., Uxbridge, UK for BALB/c mice; Jerome Russell B Blonde, London for C57BL/6 mice). It is interesting to note that marked males were more likely to be subordinate (Mann–Whitney test, $U = 141.50$, $N = 42$, $P = 0.05$), however as males from each treatment group were randomly selected for marking this was deemed not to have a confounding effect on the results or discussion.

Following a seven-day settling period, social status within pairs was assessed by direct observation of home cage aggression during the first half of the dark period over two separate 10 min periods each day for five days prior to the start of treatments and twice a week thereafter on days when substrate odours were not added to the cage. All observations were carried out by one researcher sat approximately two feet away, and the mice appeared not to respond to the presence of the researcher. Dominant status was assigned to the male within each pair that directed the greatest number of aggressive acts towards its cage mate (at least 60% of aggressive acts recorded for a pair) while its cage mate was classed as subordinate. Of the eight pairs within each treatment group, five BALB/c and six C57BL/6 male pairs assigned to same strain scent pre-exposure, and six BALB/c and five C57BL/6 pairs assigned to different strain scent pre-exposure, established clear dominance relationships, with one male from each pair remaining in a dominant social position throughout the course of the experiment. Male pairs that did not demonstrate clear social status relationships were excluded from further analysis; this was due to a lack of aggression in eight pairs and excessive aggression by both males in two pairs. Pairs were immediately separated if any injuries occurred or aggressive interactions were prolonged (>30 s without respite). Two pairs of males were separated when one male of each pair sustained one or two bite wounds to the tail. These wounds were superficial and required no further treatment. The mean frequency of attacks initiated per pair, per 10-min observation, over all observations throughout the experiment was 5.07 ± 0.65 between BALB/c males and 2.72 ± 0.19 between C57BL/6 males. These levels of aggression were in accordance with those recorded by other authors (Van Loo et al., 2001b, 2002; Bolivar et al., 2002).

1.2. Home cage odour pre-exposure

At five to six weeks of age, 10 g samples of soiled bedding were collected from each home cage during the first half of the dark period every other week day (Monday, Wednesday and Friday) throughout the experiment and transferred to another home cage according to the treatment group. Same strain odour pre-exposure pairs swapped bedding samples within the same strain, whilst different strain odour pre-exposure pairs swapped bedding samples with a different strain. Bedding was homogenized within cages before obtaining samples and the cages that swapped bedding were randomised on each occasion. During the experiment, home cages were not washed so as to maintain a familiar group odour but dirty bedding was replaced with clean bedding on a weekly basis on a day when soiled bedding was not swapped between cages.

1.3. Scent marking and urine investigation

All trials were carried out during the first 6 h of the dark period, under dim (40 W) red lighting, starting the week after odour pre-exposure commenced when mice were aged six to seven weeks. Each male was placed into a test arena (a clean MB1 polypropylene cage, 28 cm × 48 cm × 13 cm external dimensions, North Kent Plastics, Medway, Kent, UK) with absorbent paper on the floor (Benchkote, Whatman International Ltd., Maidstone, UK) and a clear perforated acrylic lid. Urine stimuli were different pooled samples from four adult BALB/c or C57BL/6 male mice to reduce any non-genetic variation in scent stimuli. All urine samples were collected within the same month from mice kept in similar environments and were stored at −18 °C until needed.

Urine marking was tested in response to a clean arena with two 10 μl streaks of water on the Benchkote, 50 mm in from the centre of each end wall. Response to urine from unfamiliar adult males was tested by

streaking 10 μl urine at one end of the test arena and an equivalent 10 μl water streak at the opposite end as a within-treatment control. The location of urine and water streaks was counterbalanced between trials. Each male was tested with urine from its own or a different strain in two separate trials, with the test order balanced, and such that mice from the same pair were exposed to the same type of urine stimulus simultaneously in separate test arenas. Both urine tests were repeated at weekly intervals for five weeks while the water control was repeated during weeks one, three and five. Males were thus, continually exposed to odours in their home cages according to the two odour pre-exposure treatments (either same or different strain), while all males were exposed briefly to urine from the same and different strain once per week in a neutral arena.

Each male was placed in the centre of the test arena at the start of a 10 min trial. Odour investigation (nose within 2 cm and pointing towards or in contact with the stimulus mark) was video recorded remotely and later transcribed from tapes using Observer 3.0 © behavioural observation computer software (Noldus Information Technology, Wageningen, The Netherlands). Urine marks deposited during each test were visualised under ultraviolet light using a Fluor-S™ MultiImager with Quantity One 4.2.1® software (Bio-Rad, Hemel Hempstead, Herts, UK). The total number of urine marks deposited in each trial was counted using Scion Image, Release Beta 4.0.2 © software (http://www.scioncorp.com/). The frequency of scent marking rather than the area covered was recorded as although the two measurements are correlated, frequency is the more reliable indicator of competitiveness since the area covered depends on the concentration and volume of urine present in the bladder. Similarly, although the pattern of urine marking is important under natural conditions, under the context of this experiment it was not deemed to be an appropriate measure in a relatively small laboratory cage.

1.4. Post-urine marking interactions

Immediately following each urine or water scent marking and investigation trial, pairs of mice were returned to their home cages and the frequency of aggressive attacks, i.e. biting, tail rattling and chasing within each pair recorded over the first ten minutes (see Grant and Mackintosh, 1962; Smith et al., 1994 for description of aggressive behaviour). Mice were separated immediately if aggressive interactions were prolonged (>30 s without respite) or injurious. This occurred in one out of 352 observations due to prolonged aggression by a dominant BALB/c male pre-exposed to same strain odours in the home cage. This pair of males was separated and removed from the experiment to prevent further aggression.

1.5. Data analysis

We calculated the mean number of urine marks deposited and the mean duration of urine and water investigation over five weeks for each mouse according to the type of urine experienced in scent marking and investigation trials (same or different strain). To control for individual differences in response to a clean arena containing no urine marks, we calculated the difference between the mean response to a urine stimulus minus the water control for each mouse and used this for subsequent analysis. Values equal to zero indicate no difference in response to urine or water; values greater than zero indicate a greater response to urine, while values less than zero correspond to a lower response to urine. A repeated measures ANOVA examined the effect of pre-exposure treatment and strain on the mean number of urine marks deposited, which approximated a normal distribution within strains and pre-exposure type (Kolmogorov–Smirnov tests, NS), with type of urine stimulus and social status as within-subject factors. The duration of stimulus investigation and subsequent aggressive interaction data did not show equality of variances. The effects of pre-exposure type and strain on the investigation of either same or different strain urine stimuli were examined separately by two-way non-parametric ANOVAs; data from dominant and subordinate males were pooled as there were no status differences in urine investigation within the treatment groups (Wilcoxon signed ranks tests, NS; see also Nevison et al., 2000). We calculated both the mean sum of aggressive attacks initiated within each pair when returned to their home cage after each type of scent marking trial (same strain urine, different strain urine or water), and the mean difference in the frequency of

aggressive attacks initiated by the dominant and subordinate male within each pair. Aggression following same and different strain urine trials was pooled to prevent pseudoreplication by calculating the mean response after each trial for each pair, as there were no differences according to urine stimulus type (Wilcoxon tests, NS). The effects of pre-exposure treatment and strain on aggression were then examined by two-way non-parametric ANOVA.

2. Results

2.1. Effect of home cage odours on scent marking

Although it is well established that dominant male mice generally deposit more scent marks than subordinates, this status difference was only apparent among inbred males that had been pre-exposed to different strain odours in the home cage (repeated measures ANOVA: status × pre-exposure interaction, $F_{1,17} = 3.88$, $P = 0.066$; Fig. 1). Since pre-exposure to different strain odour in the home cage had a differential effect on the scent marking behaviour of dominant and subordinate males, further analysis of this behaviour examined dominant and subordinate males separately. Furthermore, as there was no effect of stimulus type on scent marking response (repeated measures ANOVA: $F_{1,17} = 0.45$, $P = 0.512$), and no interaction between stimulus type and other factors, further analyses examined the mean response to same and different strain urine

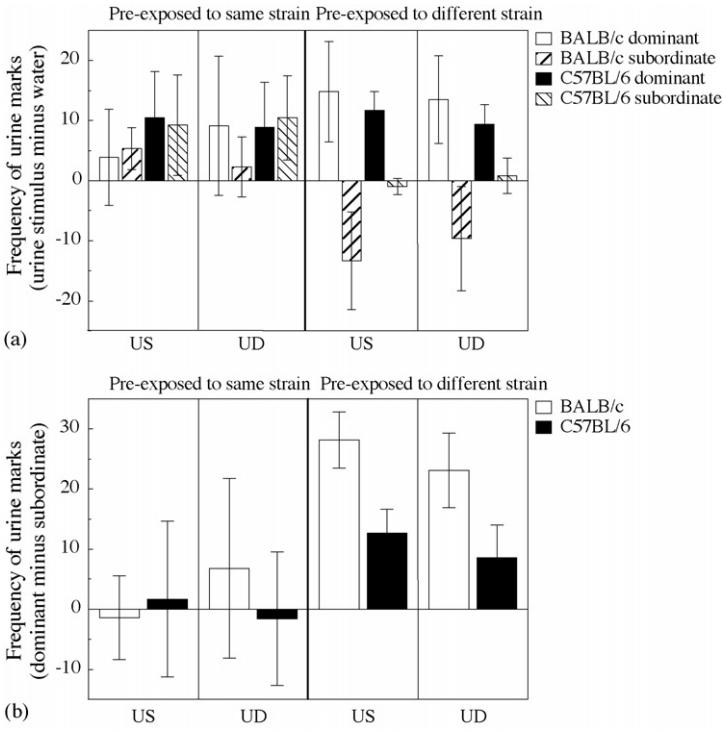

Fig. 1. Mean number of scent marks deposited in response to same (US) or different strain (UD) urine stimuli relative to water control tests by males pre-exposed to same or different strain scents (mean ± S.E.). Frequency of scent marks deposited by dominant and subordinate BALB/c and C57BL/6 males (a). Difference in scent marking between dominant and subordinate BALB/c and C57BL/6 males (b).

stimuli per male. Pre-exposure to odours in the home cage did not influence the number of scent marks deposited by dominant males (non-parametric two-way ANOVA: $Z = 0.99$, $N = 21$, $P = 0.322$). However, subordinate males pre-exposed to different strain odour deposited fewer scent marks in response to all urine stimuli compared to males pre-exposed only to own strain odours in the home cage (non-parametric two-way ANOVA: $Z = 2.11$, $N = 21$, $P = 0.035$; Fig. 1a). This effect was a specific suppression of subordinate scent marking in the presence of other male urine as the scent marking levels of subordinate males were very similar to those of dominant males in the absence of a urine stimulus (mean frequency of scent marks ± S.E. in a clean arena by males pre-exposed to different strain odour: dominant = 17.5 ± 6.3, subordinate = 19.0 ± 7.1, Wilcoxon signed ranks test: $Z = -0.20$, $N = 21$, $P = 0.84$).

Since pre-exposure to different strain odour in the home cage had a differential effect on the number of scent marks deposited by dominant and subordinate males, the difference in scent marking within each dominant-subordinate male pair was analysed. Pairs that were pre-exposed to a different strain odour in the home cage tended to show a greater polarization of scent marking between the dominant and subordinate compared to pairs that had been exposed only to same strain odour in the home cage (repeated measures ANOVA: $F_{1,17} = 3.88$, $P = 0.066$; Fig. 1b). Thus, the distribution of scent marking within male pairs pre-exposed to different strain odour was more typical of that seen among wild house mice, as opposed to male pairs pre-exposed only to same strain odours. This status difference did not depend on whether the urine stimulus in the test arena was from the same or different strain, neither were any differences noted between the two strains examined.

2.2. Effect of home cage odours on scent investigation

Dominant and subordinate mice investigated each urine stimulus to a similar extent. Furthermore, urine from a different strain stimulated similar investigation regardless of the male's strain (non-parametric two-way ANOVA: $Z = 0.71$, $N = 42$, $P = 0.478$) or home cage odour pre-exposure (non-parametric two-way ANOVA: $Z = 0.76$, $N = 42$, $P = 0.447$). However, interest in same strain urine varied according to both strain and odour pre-exposure, such that pre-exposure to different strain odour had opposite effects on C57BL/6 and BALB/c males (non-parametric two-way ANOVA: pre-exposure × strain interaction, $Z = 2.64$, $N = 42$, $P = 0.008$; Fig. 2); thus, further analysis considered each strain separately. Overall, urine investigation increased among BALB/c mice pre-exposed to a different strain odour in the home cage (non-parametric two-way ANOVA: $Z = 2.81$, $N = 42$, $P = 0.005$; Fig. 2). It appears from Fig. 2 that such pre-exposure increased interest in same but not different strain urine. However, it must be noted that the interaction between home cage pre-exposure and urine stimulus type was not statistically significant (non-parametric two-way ANOVA: pre-exposure × stimulus interaction, $Z = -0.39$, $N = 42$, $P = 0.697$). When looking at individual data, variability in response was reduced among BALB/c males pre-exposed to different strain odours (odour investigation variance within BALB/c males: pre-exposed to own strain odours: same strain urine = 44.9, different strain urine = 38.2, pre-exposed to different strain odours: same strain urine = 28.4, different strain urine = 14.9); response to different strain urine among those pre-exposed only to same strain odour was skewed by prolonged investigation by a small number of males. C57BL/6 males pre-exposed to different strain odours spent much less time investigating own strain urine and more time investigating different strain urine compared to those pre-exposed only to own strain odours (non-parametric two-way ANOVA: pre-exposure × stimulus interaction, $Z = 3.16$, $N = 42$, $P = 0.0016$; Fig. 2).

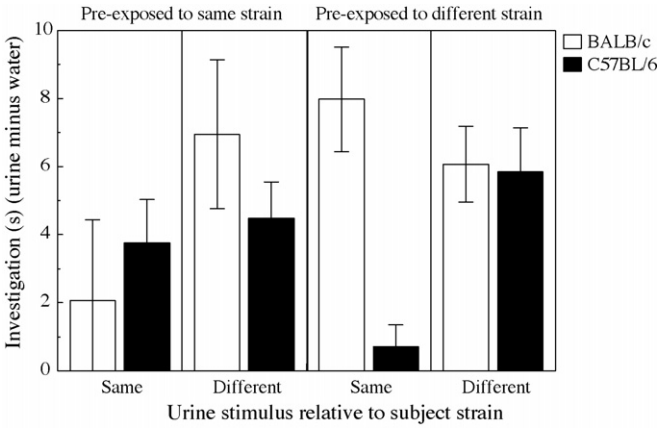

Fig. 2. Mean duration of investigation of same or different strain urine by BALB/c and C57BL/6 males pre-exposed to same or different strain odour (mean ± S.E.). Investigation of urine marks was calculated relative to time spent investigating water marks during the same trial. Data were pooled for dominant and subordinate males as there were no status differences in investigation.

2.3. Effect of home cage odours on aggression

Total aggression was much higher when male pairs were returned to their home cages following urine trials compared to water trials (Wilcoxon signed ranks test: $Z = -3.91$, $N = 21$, $P < 0.0001$). This increase in aggression in response to urine was greater among males that were pre-exposed to different strain odour than among those exposed only to same strain odour in the home cage (non-parametric two-way ANOVA: $Z = 2.32$, $N = 21$, $P = 0.020$; Fig. 3a), with males pre-exposed to different strain scents exhibiting 2–3 times more aggression after urine than after water trials. The type of urine (same or different strain) did not influence the amount of aggression within pairs (BALB/c mean aggression in response to same urine: 5.52 ± 0.65; different urine: 5.08 ± 0.60; C57BL/6 mean aggression in response to same urine: 2.75 ± 0.35; different urine: 2.82 ± 0.32; effect of urine type: $Z = -0.10$, $N = 42$, $P = 0.92$). Pre-exposure to scents in the home cage had no significant effect on aggression following water trials (non-parametric two-way ANOVA: $Z = -0.63$, $N = 21$, $P = 0.529$; Fig. 3a). Although BALB/c male pairs were more aggressive than C57BL/6 male pairs (non-parametric two-way ANOVA: $Z = 3.52$, $N = 21$, $P < 0.0004$), the effects of immediate exposure to male urine and pre-exposure to home cage scents were evident in both strains (Fig. 3a).

Pre-exposure to different strain odour in the home cage tended to have a greater effect on the difference in aggression between dominant and subordinate males within C57BL/6 pairs compared to BALB/c pairs (non-parametric two-way ANOVA: pre-exposure × strain interaction, $Z = 1.42$, $N = 21$, $P = 0.156$; Fig. 3b). A post-hoc analysis of each strain separately suggested that dominant males generally initiated more aggression than subordinates in BALB/c pairs regardless of home cage odour pre-exposure (Mann–Whitney U test: $U = 10$, $N_1 = 4$, $N_2 = 6$, $P = 0.67$; Fig. 3b). However, status differences in aggression were greater among C57BL/6 males pre-exposed to different strain odour in the home cage, with only a small difference in aggressiveness between dominant and subordinate C57BL/6 males pre-exposed only to own strain odour in the home cage (Mann–Whitney U test: $U = 2$, $N_1 = 6$, $N_2 = 5$, $P = 0.018$; Fig. 3b).

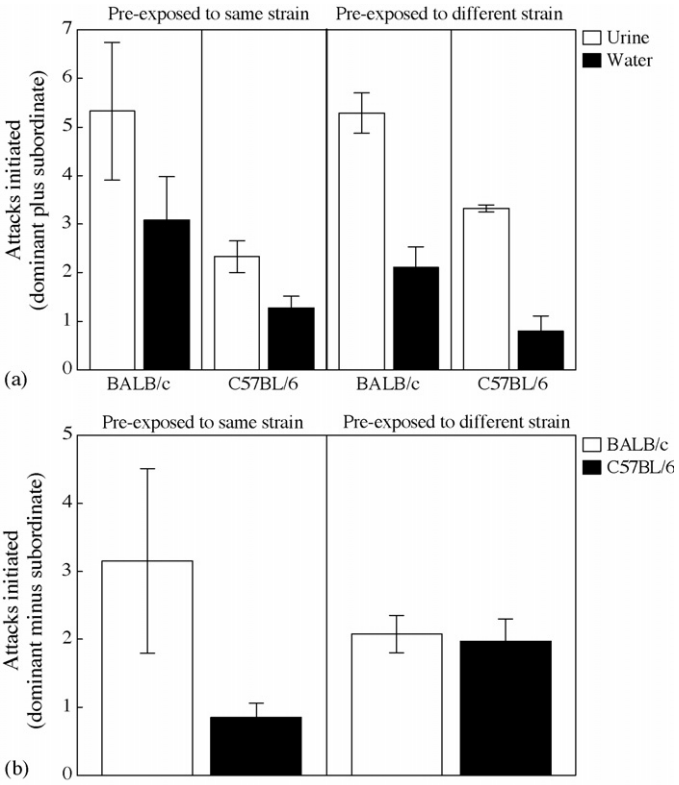

Fig. 3. Mean total aggression within BALB/c or C57BL/6 male pairs when returned to their home cages following urine and water tests (a). Difference in aggression between dominant and subordinate BALB/c and C57BL/6 males according to home cage scent pre-exposure (mean ± S.E.) (b). Data were pooled according to the type of urine stimulus experienced in the test arena as this did not affect the level of aggression (see text).

3. Discussion

We predicted that increasing the complexity of the olfactory environment by repeatedly exposing inbred male mice to genetically distinct odours in the home cage would lead to more defined social relationships and status signalling. Responses were consistent with this hypothesis in that status-dependent scent counter-marking was only apparent among males exposed to genetically distinct scents in the home cage. Whilst dominant males maintained a high rate of scent marking regardless of the type of pre-exposure experience they received, subordinate males failed to show a reduction in scent marking unless pre-exposed to scents genetically distinct from their own. The stimulus to decrease scent marking in the presence of other male urine among subordinates may thus be the long-term detection of scents on the substrate that are clearly not their own. Likewise, status differences in aggressiveness among C57BL/6 males were greater when pre-exposed to different strain odours in the home cage due to an increase in aggressiveness among dominant males. C57BL/6 males exposed only to own strain odours were less aggressive following tests of response to other male urine and there was little status differentiation in aggression due to relatively low levels of aggression and dominance instability during the early stages of the experiment. By contrast, BALB/c males maintained a clear status difference in

aggression regardless of their experience of other odours in the home cage. Thus, the suppression of scent marking among subordinate males (evident only after exposure to different strain scents in the home cage) was independent of status differences in the experience of aggression from a more dominant male, at least among BALB/c males. Compared with C57BL/6 males, males of the BALB/c strain are highly aggressive (Nevison et al., 1999). This was clear from home cage aggression following exposure to urine stimuli in the test arena, with BALB/c males initiating around twice the number of attacks on their cagemates compared to C57BL/6 males.

Wild house mice discriminate between their own scent marks and those of other males through more prolonged investigation of unfamiliar scents and an increased rate of countermarking of another male's scent, whether familiar or unfamiliar (Hurst, 1990a; Hurst et al., 2001b). BALB/c and C57BL/6 males investigate and countermark urine from an unfamiliar male of different strain more than same strain urine (Nevison et al., 2000, 2003a; Hurst et al., 2005) and show greater aggression towards genetically different males when housed with an own strain companion (Nevison et al., 2000, 2003a) but not when housed in isolation (Hurst et al., 2005). Thus, we predicted that inbred males housed in same strain pairs would show stronger competitive behaviour in response to urine from males of a different strain. Contrary to this, both BALB/c and C57BL/6 males failed to differentiate between same and different strain urine in either countermarking or their aggression once returned to their home cage. Even when investigating urine in the test arena, only C57BL/6 males with long-term experience of scents from the different strain within their home cage spent more time investigating different than same strain urine, although, in this case, both types of odour should have been highly familiar. This difference was mostly due to unusually little interest in same strain urine rather than prolonged investigation of different strain urine and the duration of investigation of all stimuli was relatively short (see for example, Nevison et al., 2003b; Hurst et al., 2005). Notably in this experiment, scents introduced into the home cage came from many different individuals. This may have given C57BL/6 males that were pre-exposed to many same strain odours the opportunity to recognise non-genetic differences in scent marks, for example due to social status, health or age, that then promoted responsiveness to same strain odours. By contrast, C57BL/6 males pre-exposed to different strain scents did not have long-term experience of same strain odours from different individuals and were not interested in investigating these. However, BALB/c males pre-exposed to different strain odours spent more time investigating all urine stimuli compared to those pre-exposed only to same strain odours. Perhaps BALB/c males with long-term experience of different strain odours became more sensitive to male scent cues in general, resulting in increased time spent gathering information from other male scents. Nonetheless, this interest in gaining information from urine stimuli in the test arena did not translate into differences in countermarking or subsequent aggression.

There was no indication of habituation in response to repeated exposure to scents. Responses did not show a general increase or decrease over the five-week period (data not shown). Further, relatively novel stimuli did not generally stimulate greater investigation than scents that should have been highly familiar. The lack of discrimination between urinary scent cues by all BALB/c males and those C57BL/6 males pre-exposed only to own strain odours may mean that these mice simply detected an interesting odour that contrasted to the general background environment. This cannot be taken as evidence that they understood the functional significance that these odours came from unfamiliar competitor males, as mice investigate any change in odour that contrasts with the general background environment even if it is highly familiar or if it is their own scent mark (Hurst, 1989) or if a familiar odour is in a novel location (Mayeaux and Johnston, 2002). Thus, simply recording the duration of investigation of urinary scent marks is inadequate as an

index of the information gained from odour stimuli (Thom and Hurst, 2004). Nevison et al. (2000) pointed out that a countermarking response is of much more specific functional significance. Dominant males scent mark at a high rate and countermark any other male scent marks encountered in order to advertise their competitive ability, whilst subordinates reduce their scent marking rate to help ensure tolerance by the dominant male (Desjardins et al., 1973; Hurst, 1990a; Drickamer, 2001).

Pre-exposure to a different strain odour had a greater effect on relative aggressive behaviour within C57BL/6 male pairs compared to BALB/c males and had opposite effects on investigatory behaviour. It is not uncommon for behavioural phenotypes to differ quite dramatically between inbred strains of mice. A number of inter-strain differences have been reported in tests of aggression and of olfactory discriminatory ability (Mihalick et al., 2000; Miczek et al., 2001; Lee et al., 2003; Van Loo et al., 2003a). Such differences highlight the danger of extrapolating from the results of this study to other inbred strains, other than to suggest that exposing inbred males to scents from different strains in the home cage is likely to promote increased competition and status differentiation, though the precise effects will be strain specific. In addition, the increase in aggression when males were returned to their home cages following exposure to male urine was consistent across males of both strains. This effect was apparent even when scents were from the same genetically identical strain and, although the response was exacerbated among males pre-exposed to different strain scents, occurred even among males exposed only to own strain scents. This suggests that detection of male urine of any type when males are removed from their home cages is likely to considerably promote aggression on their return.

The impact of the loss of variability in individual identity cues on social relationships and inter-male aggression is, at present, poorly understood. We have demonstrated that pre-exposure to genetically distinct scents promoted social status differentiation in competitive scent signalling and resulted in increased competitive aggression among males of a strain showing relatively little aggression (C57BL/6). We found no evidence that the lack of clear status-related scent marking behaviour among pair-housed males exposed only to same strain home cage odours resulted in greater fighting. The restricted social olfactory environment within inbred strains is thus, likely to reduce problems of aggression and variability among males and, based on the results of this study, we recommend that care should be taken to avoid contamination of cages with different strain scents, for example, by cleaning cages thoroughly and ensuring that soiled substrate cannot fall into other cages. Most importantly, the strongest effect on aggression was whether or not males encountered urine from other males when temporarily removed from their home cage. Thus, care should be taken to avoid exposing group-housed males to any other male urine scents while they are temporarily removed from their social group, even those from genetically identical males.

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