The consequences of inbreeding for recognizing competitors

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Extreme inbreeding will compromise an animal's ability to discriminate between individuals and, thus, assess familiarity and kinship with conspecifics. In rodents, a large component of individual recognition is mediated through chemical communication. The counter-marking of competitor males' scent marks provides a measure of discrimination between their own scent and that from other individuals. We investigated whether males in common outbred (ICR(CD-1) and TO) and inbred (BALB/c) strains of laboratory mice could recognize the urinary scents of other individuals by measuring their investigation and counter-marking responses. Dominant males of outbred strains investigated and counter-marked scents from other males, whether of the same or another strain. Dominant inbred BALB/c males investigated but did not counter-mark their own strain scents, counter-marking only those from another strain. They did not use environmentally induced status differences in odours to recognize scents from other males. The inability of the inbred mice to discriminate between their own scent marks and those of other males is likely to alter their competitive behaviour, which could influence responses in experiments and the welfare of caged laboratory mice.

Keywords: individual recognition; inbreeding; scent marking; individual identity odours; mice; animal welfare

1. INTRODUCTION

The effects of inbreeding, particularly among rodents and domestic animals, are of considerable relevance in scientific research and in animal production. Animals are artificially selected and inbred to ensure consistency between individuals, the outcome of which is that they are often virtually genetically identical (Cohen 1999). Inbreeding depression (Festing 1979; Charlesworth & Charlesworth 1987; Lacy 1997) and disease susceptibility (Festing 1980; Kozak 1989; Apanius et al. 1997) are widely recognized outcomes of such extreme inbreeding. Another consequence which has received less attention is the influence of inbreeding on recognition and interaction between animals. Individual recognition, familiarity and kinship play key roles in the modulation of social behaviour, particularly competition and mate choice (Barnard et al. 1991). Inbreeding could reduce the ability to discriminate between individuals and to assess familiarity and/or kinship with other conspecifics (e.g. see Barnard & Aldhous 1991; Barnard et al. 1991). Disturbances in such recognition and interaction processes could affect the welfare of inbred animals (e.g. in laboratory, agricultural and commercial contexts) and should therefore inform approaches to husbandry and management. Further, abnormal behaviour or unstable social relationships could affect responses in experiments and the success of animal production.

Rodents and probably most other mammals identify each other through individually unique odour cues which are determined in part by genetic differences between individuals (reviewed by Boyse et al. 1991; Brown 1995).

Many genes contribute to individual identity odours (Brown 1995; Eggert et al. 1996), but genes of the major histocompatibility complex may make a particular contribution because of the very high polymorphism of these genes (Yamazaki et al. 1992; Apanius et al. 1997). Individual identity odours of mice and rats are carried predominantly in the high mass fraction of their urine in which major urinary proteins may also play an important role, either directly or through differential binding and release of volatile odorant ligands (Singer et al. 1993; Hurst et al. 1998; Humphries et al. 1999). Individuals of highly inbred strains are unable to discriminate between each others' volatile urinary odours when kept under identical conditions (Yamaguchi et al. 1981). Environmental factors, such as food type, bacterial gut flora and social pressure, also induce changes in the volatile odours emitted by animals (Apps et al. 1988; Schellinck et al. 1991, 1992). Untrained changes in odour investigation or trained responses to specific odours show that conspecifics can readily discriminate such environmentally induced odour differences. It is therefore possible that environmentally induced odour differences might be exploited to allow individual recognition when perceived against a common genetic background. However, to assess how animals use such perceived differences in conspecific odours requires a specific test to show that they interpret the source of the odours as a different individual. In this study, we investigated how males in two common outbred and one inbred strain of laboratory mice respond to the urinary scent marks of potential competitors, in order to assess whether they can recognize the scents of different individuals and the likely consequences for competitive behaviour between cage mates. Although the mouse is the

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most common laboratory animal, aggression between male cage mates can cause welfare problems and uncontrolled variation in behaviour and physiology between subjects (Bishop & Chevins 1987; Cane 1988; Jennings *et al*. 1998).

Scent marking and the counter-marking of the scent marks of other males are important components of dominance advertisement among male house mice and have a strong influence on their aggressive interactions (Mugford & Nowell 1970; Hurst 1993). Males can prove their ability to dominate an area by scent marking and ensuring that their scent marks are the freshest in that area (Hurst & Rich 1999). Males which fail to counter-mark any competing scent marks can no longer ensure that their own marks are the freshest and, thus, indicate their inability to prevent or overcome challenges to their dominance. In house mice, counter-marking is achieved by increasing their rate of scent marking in the vicinity of a competitor male's marks (Desjardins *et al*. 1973; Hurst 1990). Potential mates and other competitor males are highly sensitive to such counter-marking, discriminating in favour of males which counter-mark over those which are themselves counter-marked by competitor males (Hurst 1993; Rich & Hurst 1999). This is also exhibited in voles and hamsters (Johnston *et al*. 1995, 1997). Thus, any males attempting to advertise their dominant status should thoroughly scent mark their area and counter-mark scent marks from any male other than themselves, regardless of the status or familiarity of the competitor. Subordinate males, in contrast, suppress such competitive signalling to avoid challenges (Desjardins *et al*. 1973; Bishop & Chevins 1987). Counter-marking of scent marks from other males by dominant or isolated males thus provides a specific test of individual recognition.

We can thus predict that dominant males from outbred strains, in which individuals are genetically distinct, should be able to detect scent marks from competitors as different from their own and counter-mark them. In contrast, if dominant males from inbred strains are unable to discriminate between the odours of genetically identical individuals, they should only be able to discriminate and counter-mark when males are from other, genetically distinct strains. However, if animals of an inbred strain use status-related odour differences to discriminate between each other, dominant males should detect and counter-mark scents from subordinate males since these are of different status to themselves.

2. METHODS

(a) *Experimental subjects*

Two outbred strains, ICR (CD-1) and TO and an inbred strain, BALB/c (H-2^d haplotype) were chosen based on their different level of inbreeding, known aggressive behavioural characteristics (Van Oortmerssen 1971; Nevison *et al*. 1999) and common use in the laboratory (Festing 1979, 1980). Males of these strains show intrastrain aggression and form dominance relationships reliably when housed together in cages (Nevison *et al*. 1999).

We established 16 males in pairs for each strain to give eight dominant and eight subordinate subjects in each strain. The mice were obtained from Harlan UK (Bicester, Oxon) aged 35 ± 2 days. They were housed in cages (12.5 cm $\times 45$ cm $\times 14$ cm) and maintained in the same room on a 12 L : 12 D schedule (white lights off at 09.00) with *ad libitum* food

(TRM 9607 rat and mouse diet, Harlan Teklad, Hull, UK) and water throughout. Each male was marked for individual identification using hair dye (Clairol Nice 'n' Easy Natural Black, Bristol-Myers Co. Ltd, Uxbridge, UK). Stimulus urine came from mice obtained from the same source and housed in pairs under the same conditions.

Males were housed in pairs within strains on arrival, then regrouped within strains into new experimental pairs after four days. Social status was assessed over three separate 5 min periods each day for three days. Observations were made within the first half of the dark period with at least 120 min between successive observations. Dominant status was assigned to the male within each pair which directed the greatest number of aggressive acts towards its cage mate (at least 60% of aggressive acts within the pair) while its cage mate was classed as subordinate. The social status of urine donors was assigned in the same way.

(b) *Urine marking trials*

All trials were carried out during the first 6 h of the dark period, under dim (40 W) red lighting, starting the day after dominance assessment was completed. Males were placed singly into a clean varnished wood arena (29 cm $\times 60$ cm $\times 28.5$ cm) with absorbent paper on the floor (Benchkote, Whatman International Ltd, Maidstone, UK).

The urine marking behaviour of each male was first measured in response to a clean arena with two 30 µl water marks placed on the Benchkote in diagonally opposite corners. A response to urine from an unfamiliar male of their own strain was tested by placing urine in a 30 µl streak in one corner of the arena and an equivalent 30 µl streak of water in the corner diagonally opposite as a within-trial control. Each male was tested with urine from a dominant and a subordinate conspecific in two separate trials, with the test order randomized. In addition, BALB/c and TO males were tested with urine from ICR dominant males to compare their response to dominant male urine from their own strain or from another strain. Trials using the same subject were carried out at least 48 h apart.

The males were placed in the centre of the arena at the start of each 10 min trial. The latency to investigate each stimulus mark was recorded, together with the time spent investigating each stimulus (nose within 2 cm and pointing towards or in contact with the mark).

(c) *Measurement of scent marks*

Urine marks were viewed under ultraviolet light (Desjardins *et al*. 1973). We counted the total number of separate urine marks deposited in each trial. The area covered with urine was estimated by placing a 1 cm^2 Perspex grid with a small dot at the centre of each grid cell over the Benchkote and counting the number of dots which covered urine marks. This was divided by the total number of marks to estimate the mean mark size. Urine marks were described as 'pools' (roughly circular, > 2 cm^2), 'streaks' (narrow lines) or 'dotted' (roughly circular, < 2 cm^2). Dotted marks were often placed in linear series.

(d) *Biochemical assays*

At the end of the study, the males were weighed and humanely killed. Serum testosterone concentration was measured by radioimmunoassay using a Coat-a-Count solid phase ^{125}I total testosterone kit (Diagnostic Products Corporation, Los Angeles, CA, USA) with 50 µl undiluted serum obtained from a postmortem blood sample. Protein concentration in urine excreted post-mortem or obtained from the bladder was assayed

using the Coomassie Plus Protein assay (Pierce Chemicals, Chester, UK) following the procedure in Humphries *et al.* (1999).

(e) *Data analysis*

We first checked for any strain and status differences in aggression (non-parametric ANOVAs), plasma testosterone or urinary protein output (ANOVA tests with body weight as a covariate) which might be related to differences in competitive signalling behaviour, though no strain differences were predicted *a priori*. The protein concentrations approximated normality while the testosterone titres were $\log_{10}$ transformed to meet the assumptions of parametric analyses.

We then checked that the competitive aggression and scent marking behaviour of the outbred strains followed the specific predictions arising from the known behaviour of wild-type mice and that there were no differences between the two strains in such competitive behaviour. The predictions tested were as follows.

(i)   Dominant males are significantly more aggressive than subordinates.
(ii) Dominant males deposit more scent marks than subordinates.
(iii) Dominant but not subordinate males counter-mark the urine of other males of their own strain (dominant or subordinate) more than a clean water control.
(iv) Dominant males counter-mark urine from another individual whether from their own strain or another strain.

We then tested whether inbred BALB/c males showed the same difference in competitive scent marking between dominant and subordinate males but, in this case, tested the prediction that dominant males would only counter-mark the urine of males that were genetically distinct from themselves or, possibly, those of different social status to themselves. Counter-marking is evident when males deposit a greater number of scent marks than their response to a clean arena (Hurst 1990; Humphries *et al.* 1999). Since data describing scent marking and aggression showed considerable individual variation, we used non-parametric tests to assess the overall status differences predicted in scent marking and aggression (Mann–Whitney test) and differences in individual counter-marking responses to different stimuli (Wilcoxon matched-pair or Wilcoxon matched-sets tests) (Meddis 1984). Specific versions of these tests (Meddis 1984) were used when the direction of the response was clearly predicted (see above) and exact versions of the tests where the sample sizes were less than 15.

Within the outbred and inbred strains, repeated measures ANOVAs examined the effects of social status of the subject and urine donor, stimulus type and strain (outbred only) on the duration of stimulus investigation, which approximated a normal distribution within strains (Kolmogorov–Smirnov tests, n.s.). The latency to first investigation of different stimuli was compared by non-parametric Wilcoxon matched-pair tests. The data are expressed as means ± s.e. throughout and *n* gives the sample size.

3. RESULTS

(a) *Aggression, testosterone and urinary protein*

Each pair of males established clear aggressive dominant or subordinate relationships across all three strains. There were no significant strain differences in the levels of aggression shown by dominant males (number of
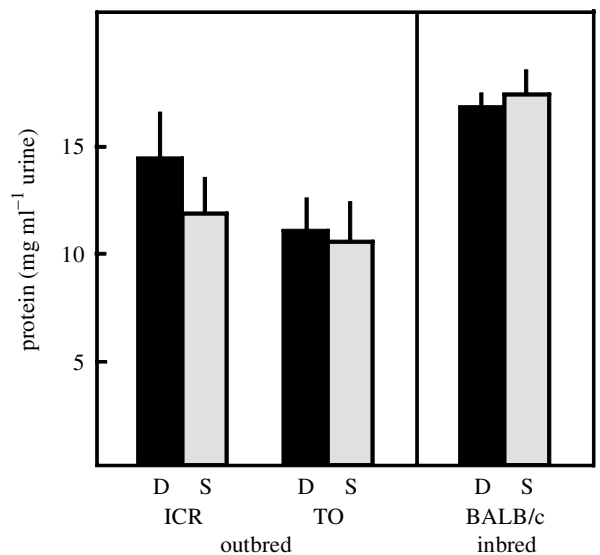

Figure 1. Protein concentrations of urine samples according to mouse strain and status (mean ± s.e.). D, dominant males; S, subordinate males. The protein concentrations differed significantly between the outbred and inbred strains.

aggressive acts by dominant ICR = 10.9 ± 3.2, number of aggressive acts by dominant TO = 7.3 ± 1.3 and number of aggressive acts by dominant BALB/c = 16.0 ± 4.6) ($\chi^2_2 = 2.04$ and n.s.) or by subordinates (number of aggressive acts by subordinate ICR = 0.88 ± 0.61, number of aggressive acts by subordinate TO = 1.25 ± 0.62 and number of aggressive acts by subordinate BALB/c = 0.25 ± 0.16) ($\chi^2_2 = 1.77$ and n.s.), with all dominant males exhibiting considerably more aggression than subordinates (number of aggressive acts by dominants = 11.4 ± 2.0 and number of aggressive acts by subordinates = 0.8 ± 0.3) ($Z = -5.75$, $n_1 = n_2 = 24$ and $p < 0.001$). The plasma testosterone concentrations did not differ between strains ($F_{2,41} = 0.27$ and n.s.) or between dominant and subordinate males ($F_{1,41} = 0.02$ and n.s.).

Since individual identity odours appear to be contained in the high mass fraction of urine (Singer *et al.* 1993) and it is urinary proteins which stimulate counter-marking (Humphries *et al.* 1999), we measured the concentration of protein in the urine samples collected at autopsy. Social status had no effect on the urinary protein concentrations ($F_{1,39} = 0.19$ and n.s.) (figure 1) but there was a significant difference between strains ($F_{2,39} = 4.80$ and $p < 0.025$). Planned contrasts showed that this was because the inbred BALB/c males excreted a higher concentration of urinary protein than the two outbred strains while there was no significant difference between the two outbred strains (figure 1).

(b) *Response to own strain urine*
(i) *Outbred strains*

Dominant males deposited more scent marks than subordinates when placed in a clean area ($T = 95$, $n = 15$ and $p < 0.01$). Although this status difference in scent marking was predicted to occur in both outbred strains (similar to wild mice), it was only apparent in the ICR strain. When placed in a clean arena, TO subordinates deposited as many marks as dominant animals (figure 2).
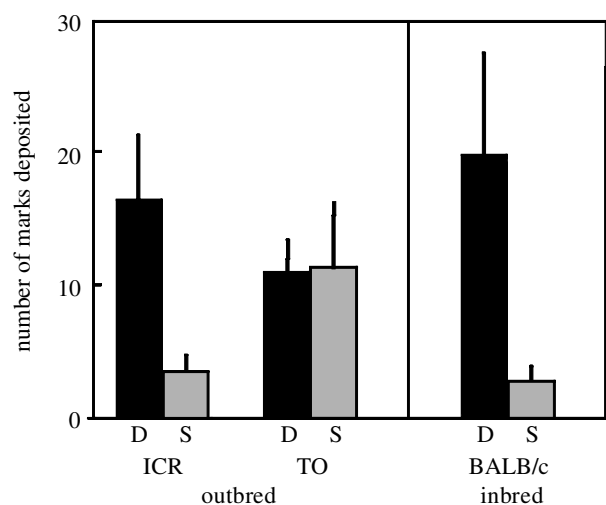
Figure 2. Number of marks deposited by mice in response to a clean arena (mean ± s.e.). D, dominant males; S, subordinate males.

The predicted status difference in counter-marking was shown in both outbred strains (figure 3a). Only dominant males responded to a scent mark from an unfamiliar male of their own strain by counter-marking strongly, depositing more marks in response to their own strain urine than in response to a clean arena ($\mathcal{Z} = 2.6$, $n = 16$ and $p < 0.025$). Subordinate males did not increase their marking ($\mathcal{Z} = 0.47$, $n = 16$ and n.s.) and tended to deposit urine in larger spots than dominant males though the difference was not significant (table 1). As expected, the dominance status of the urine donor had no effect on the counter-marking response of outbred dominant males ($\mathcal{Z} = 0.66$, $n = 16$ and n.s.) (figure 3a).

Despite the difference in readiness to counter-mark an unfamiliar competitor's urine mark, both dominant and subordinate males detected the presence of the stimulus urine mark. All outbred males investigated an unfamiliar own strain urine mark significantly more than the control water mark presented simultaneously ($F_{1,27} = 39.2$ and $p < 0.001$), with no difference in the duration of investigation by the dominant or subordinate males ($F_{1,27} = 0.01$ and n.s.) (figure 3b). The investigatory responses varied according to the status of the urine donor even though counter-marking did not. Males were quicker to investigate urine from a dominant male first than from a subordinate ($H = 7.88$, $n = 32$ and $p < 0.01$). Interestingly, in trials with dominant male urine, the latency to investigate the water mark was also low (dominant male urine 15.7 ± 2.0 s and water 18.1 ± 3.7 s) ($\mathcal{Z} = -0.3$ and n.s.), suggesting that the presence of dominant male urine stimulated a general increase in investigation of the arena. Although it took much longer to investigate subordinate urine, this was still significantly shorter than their latency to investigate water (subordinate male urine 30.7 ± 10.6 s and water 56.7 ± 12.4 s) ($\mathcal{Z} = -2.05$ and $p < 0.05$). After first contact, subordinate male urine stimulated longer investigation than that from a dominant male ($F_{1,27} = 5.9$ and $p < 0.025$) (figure 3b). ICR males spent longer investigating stimulus urine than TO males ($F_{1,27} = 5.73$ and $p < 0.025$), but both strains showed the same differences in response according to the dominance status of the urine donor (figure 3b).
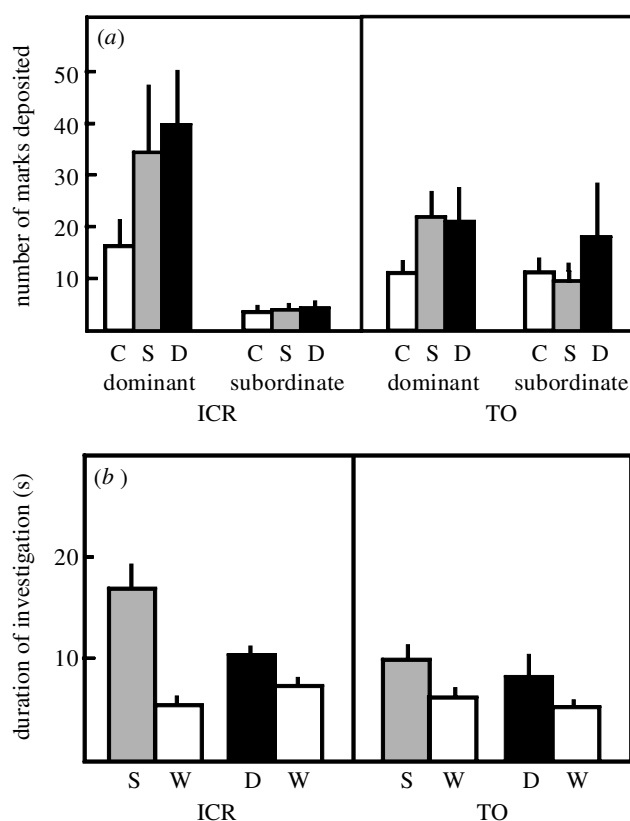

Figure 3. The response of outbred males to their own strain urine (mean ± s.e.). (a) Number of urine marks deposited by dominant and subordinate males in each trial. C, clean arena trial; S, subordinate male urine trial; D, dominant male urine trial. (b) Investigation when presented with a choice between urine and water marks. The data are pooled for dominant and subordinate males within each strain since there was no difference according to status. W, water mark; S, subordinate male urine mark; D, dominant male urine mark. See § 3(b)(i) for the statistical analysis.

(ii) *Inbred males*

The number of marks deposited by dominant and subordinate BALB/c mice was similar to those of the ICR outbred strain, subordinate males depositing many fewer marks than dominant males ($T = 32$, $n = 8$ and $p < 0.025$) (figure 2). Subordinate BALB/c mice deposited larger marks than dominant males in a few large pools (table 1).

When presented with urine marks from their own strain (effectively genetically identical individuals), both dominant and subordinate BALB/c males spent much more time investigating the urine marks than the control water marks ($F_{1,14} = 33.5$ and $p < 0.001$) (figure 4b). Their investigation times were generally longer than those of outbred males, both towards urine and water ($F_{1,43} = 15.97$ and $p < 0.001$). Like outbred males though, BALB/c males spent longer investigating urine from a subordinate male than urine from a dominant ($F_{1,14} = 8.37$ and $p < 0.025$), suggesting that they detected a difference in the donor's dominance status, with no difference in investigation by dominant and subordinate males ($F_{1,14} = 1.47$ and n.s.). Dominant male urine was not investigated more quickly than the water control or subordinate male urine, suggesting that BALB/c males may not have recognized this as a novel stimulus from a distance and, thus, were not attracted to investigate.

Table 1. *Mean size of marks deposited in response to own strain urine (mean ± s.e. in cm² per male)*

status	strain		
	ICR (CD-1)	TO	BALB/c
dominant	1.60 ± 0.62	1.26 ± 0.29	1.57 ± 0.71
subordinate	2.88 ± 0.65	1.47 ± 0.41	2.36 ± 0.77

Despite investigating urine from an unfamiliar male of their own strain, dominant BALB/c males did not counter-mark urine from another male of their own strain more than their response to a clean arena ($Z = 1.08$, $n = 8$ and n.s.), with no difference in their marking response according to the social status of the urine donor ($T = 24$, $n = 8$ and n.s.) (figure 4a). This suggests that they failed to recognize that the mark came from a competitor, even when the donor was of different status to themselves. Subordinate males did not mark in the presence of their own strain urine (figure 4a).

(c) *Response to another strain urine*

Outbred mice should be able to discriminate between urine from different individuals, even those from their own strain. As expected, TO dominant males deposited the same number of marks in response to dominant male urine, whether from their own or another strain (Wilcoxon matched-pair test, $T = 21.5$, $n = 8$ and n.s.) (figure 5a). Both types of urine stimulated more marking than a clean arena though the increase in marking in response to urine from another strain was not quite significant (Wilcoxon specific matched-pair test, own strain $T = 19$, $n = 6$ excluding ties and $p < 0.05$ and other strain $T = 29$, $n = 8$ and $p = 0.07$). This was because three TO males, all of which deposited only small numbers of marks in each test despite their dominant status (one to six marks), deposited even fewer marks in the presence of urine from an ICR (CD-1) dominant male than in clean arena trials. Those males that responded strongly to male urine of their own strain (depositing 25–47 marks) all responded strongly to ICR (CD-1) male urine (depositing 29–50 marks). As expected, TO subordinate males did not counter-mark either urine (figure 5a). TO males did not differ in the time spent investigating marks from their own or another strain ($F_{1,14} = 1.97$ and n.s.), with no difference in investigation by dominant or subordinate males ($F_{1,14} = 0.43$ and n.s.) (figure 5b). Investigation of both types of dominant male urine was very low and was not significantly greater than that of the control water mark ($F_{1,14} = 2.43$ and n.s.) (figure 5b).

In contrast, inbred dominant males were only expected to be able to discriminate between urine from different individuals which were of another strain and genetically distinct from themselves. Accordingly, BALB/c dominant males showed significant counter-marking when presented with urine from an ICR (CD-1) dominant male (figure 5a), depositing more marks than in a clean arena ($T = 33$, $n = 8$ and $p < 0.025$) and more marks than when presented with urine from a dominant male of their own strain ($T = 25$, $n = 8$ and $p < 0.05$). Again, as expected, BALB/c subordinate males showed no significant counter-marking,
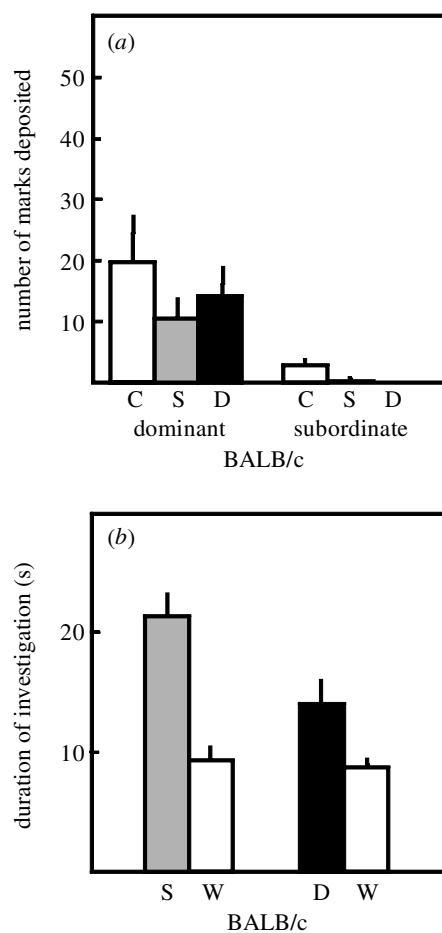

Figure 4. The response of inbred BALB/c males to their own strain urine (mean ± s.e.). (a) Number of urine marks deposited by dominant and subordinate males in each trial. C, clean arena trial; S, subordinate male urine trial; D, dominant male urine trial. (b) Investigation when presented with a choice between urine and water marks. The data are pooled for dominant and subordinate males within each strain since there was no difference according to status. W, water mark; S, subordinate male urine mark; D, dominant male urine mark. See §3(b)(ii) for the statistical analysis.

depositing no marks in response to urine from a dominant male of their own or another strain (figure 5a). Both dominant and subordinate BALB/c males spent much longer investigating urine from a dominant male of another strain than urine from a dominant male of their own strain ($F_{1,14} = 9.35$ and $p < 0.01$) (figure 5b) with no difference in investigation by dominant or subordinate males ($F_{1,14} = 0.35$ and n.s.).

4. DISCUSSION

Males of the two outbred laboratory strains, which have been bred to maintain heterozygosity, clearly recognized urine marks from other males, whether of the same or another strain. Investigation of urine more than water showed detection of an interesting odour. However, this cannot be taken as proof that they recognized that the stimulus mark came from another individual. Mice will investigate social or non-social odours which contrast with the general background environment even when that odour is highly familiar or even if it is their own scent mark
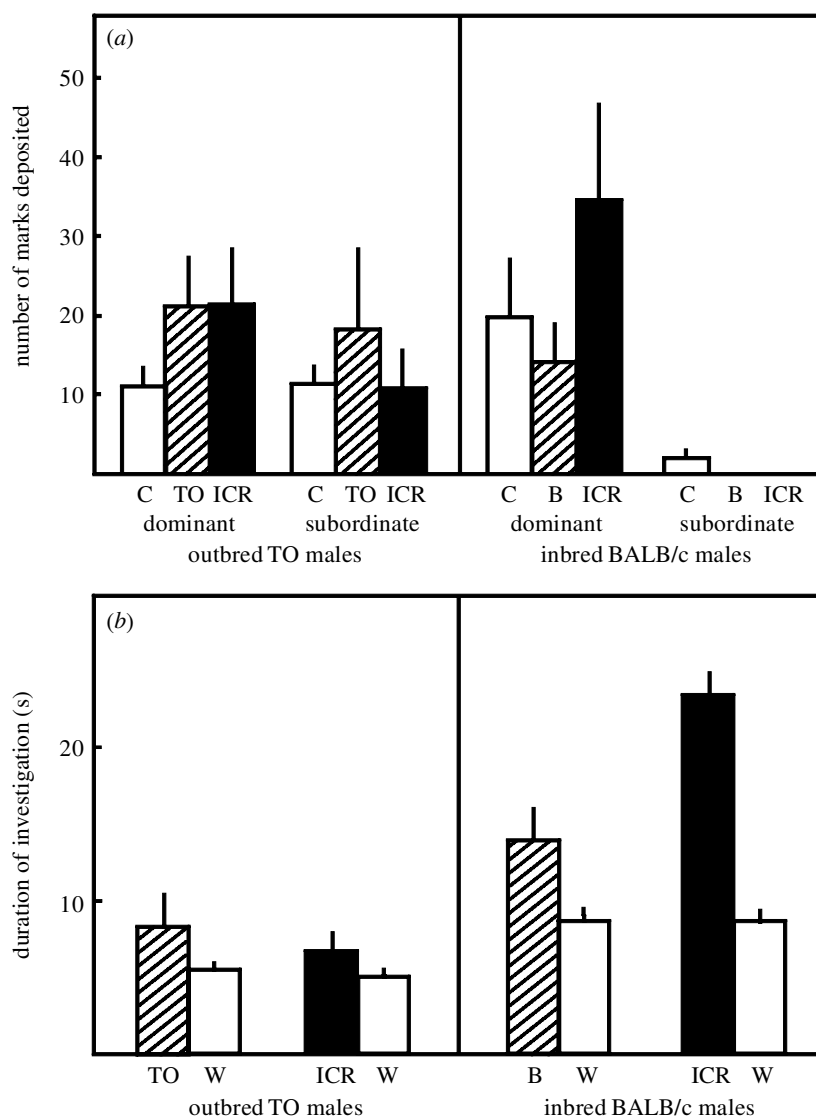
Figure 5. The response of outbred TO and inbred BALB/c males to dominant male urine marks from their own or another strain urine (mean ± s.e.). (*a*) Number of urine marks deposited by dominant and subordinate males in each trial. C, clean arena trial; TO, TO dominant male urine trial; ICR, ICR dominant male urine trial; B, BALB/c dominant male urine trial. (*b*) Investigation when presented with a choice between urine and water marks. The data are pooled for dominant and subordinate males within each strain since there was no difference according to status. W, water mark; TO, TO dominant male urine mark; ICR, ICR dominant male urine mark; B, BALB/c dominant male urine mark.

(Hurst 1989). Thus, simple investigation is inadequate as an index of individual recognition. Counter-marking, on the other hand, is a much more specific response. Any males advertising their dominant status should thoroughly scent mark their area and counter-mark scent marks of other males, regardless of the status or familiarity of the competitor. In agreement with this, dominant males in the two outbred strains scent marked at a high rate and increased the number of marks deposited to counter-mark stimulus urine, regardless of the status of the donor male or whether this came from their own strain or another strain. In contrast, inbred BALB/c dominant males did not counter-mark urine from another male of their own strain irrespective of status, despite extensive investigation. There are three possible explanations for this result.

(i)   BALB/c males do not show normal competitive scent marking and counter-marking behaviour.

(ii) BALB/c male urine is qualitatively different from that of other mice and does not stimulate counter-marking.

(iii) BALB/c males failed to recognize that the stimulus mark came from another male.

The high rate of scent marking by dominant BALB/c males and their clear counter-marking behaviour when they encountered ICR male urine demonstrated normal counter-marking behaviour in response to scent marks from males which were genetically different from themselves, discounting the first explanation. The concentration of urinary proteins, which stimulate male competitive scent marking, was significantly greater in BALB/c male urine than in the outbred strains. Further, Humphries *et al.* (1999) showed that wild-caught house mice readily counter-mark BALB/c male urine and, thus, there appears to be nothing qualitatively different about

BALB/c urine in this respect. Therefore, in agreement with our predictions, the lack of counter-marking signified that BALB/c males failed to discriminate between their own marks or stimulus marks coming from other males which, effectively, were genetically identical to themselves.

The males were able to discriminate between urine from dominant and subordinate males, regardless of the genetic similarity of the donor to themselves. BALB/c males, like males of both outbred strains, spent significantly more time investigating subordinate than dominant male urine. Despite this, when dominant BALB/c males encountered urine from a subordinate BALB/c male, they did not counter-mark. It thus appears that the chemical information signalling individual status is additive to the information used to signal individual identity. Males do not therefore appear to use environmentally induced status differences in odours to discriminate between individuals with a common genetic background. The increased investigation of subordinate-quality urine (by both dominant and subordinate males) may have been stimulated by the novelty of encountering a large subordinate-quality urine mark, since subordinate males generally suppress their scent marking behaviour. In support of this, only BALB/c and ICR males showed prolonged investigation of subordinate male urine. In TO males, where both dominant and subordinate males showed similar basal levels of scent marking in a clean arena, both urine types stimulated relatively low levels of investigation.

The inability to recognize urinary odours from different individuals and, thus, distinguish these from their own has a number of implications for the maintenance of social relationships within aggressive inbred strains such as BALB/c. First, dominant males will not detect any competitive signals on the substrate from other genetically identical males, including those from cage mates. Normally, subordinate male mice mark the substrate, but much less than a dominant, to advertise their presence and maintain tolerance from other group members (Hurst *et al.* 1993). They avoid depositing marks which will compete with those of the dominant male by pooling the remainder of their urine in corners away from areas marked by the dominant (Desjardins *et al.* 1973; Bishop & Chevins 1987). The dominant male's marks thus remain predominant throughout and, in wild mice, any subordinates which attempt to deposit competing marks are fiercely attacked (Hurst 1993). When males are housed together in laboratory cages, changes in this distribution of urinary scent due to mixing of the soiled cage bedding may thus create problems. However, this is unlikely to be a problem in groups of inbred males since the dominant male will not recognize that the subordinate's scent is not their own and potential aggression will be reduced.

Since subordinate males normally avoid depositing competing scent marks in an area scent marked by a dominant male, inbred subordinates may experience a problem in urinating at all within laboratory cages. The high concentration of scent corresponding to the dominant male, which is indistinguishable from their own, may lead to an inability to deposit their urine anywhere in the cage, the consequence being extreme urine reten-

tion among subordinate males. Urine retention is a well-recognized problem among subordinate male laboratory mice and Taylor (1985) reported a particularly acute problem in the inbred MM strain causing nephritis in 49% of the males in their colony. Scent marking was very strongly suppressed among subordinate BALB/c males, despite the fact that their urine marks were unlikely to compete with the dominant male's and were unlikely to induce aggression (see above). Subordinate BALB/c males also seemed to have particularly distended bladders at autopsy (C. M. Nevison, personal observation).

Finally, the results of this study highlight the potential dangers of interpreting responses to volatile scents in terms of individual recognition. Tests of individuality odours have largely used habituation–dishabituation tests or an olfactory discrimination learning paradigm to test for discrimination between different volatile scents. Such tests have suggested that individual identity odours are highly susceptible to disruption by environmental influences (e.g. Schellinck *et al.* 1992). However, as shown here, animals may detect differences in scents which stimulate different investigation, such as status-related odours, but do not necessarily interpret such differences as coming from different individuals. Humphries *et al.* (1999) suggested that volatiles emanating from scent marks may alert animals to the presence of interesting odours in their local environment, stimulating close investigation of the scent source where non-volatile peptides and proteins or protein–ligand complexes provide more reliable information on the species and individual identity of the depositor. As they pointed out, the response when animals encounter volatiles is usually to approach the odour source to investigate more closely and they will usually contact the odour unless prevented from doing so (e.g. see Brown *et al.* 1987; Hurst 1990, 1993; Ninomiya & Brown 1995; this study). One might expect animals to use genetically determined cues as reliable and unchanging indicators of an individual's identity rather than cues which are strongly influenced by the environment.

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