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Life history costs of olfactory status signalling in mice

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Abstract Large body size confers a competitive advantage in animal contests but does not always determine the outcome. Here we explore the trade-off between short-term achievement of high social status and longer-term life history costs in animals which vary in competitive ability. Using laboratory mice, *Mus musculus*, as a model system, we show that small competitors can initially maintain dominance over larger males by increasing investment in olfactory status signalling (scent-marking), but only at the cost of reduced growth rate and body size. As a result they become more vulnerable to dominance reversals later in life. Our results also provide the first empirical information about life history costs of olfactory status signals.

Key words Status signals · Olfaction · Life history costs · Laboratory mice

Introduction

Body size is generally believed to reflect competitive ability and has been shown to influence the outcome of fights in a variety of mammals, including mice (van Zegeren 1980; Clutton-Brock et al. 1988; Schüler and Renne 1988; Andersson 1994). Sexual selection for large body size may also be partly responsible for sexual dimorphism in mice and other mammals (Trivers 1972; Andersson 1994). But when pairs of male mice are housed together, the individual that proves to be domi-

inant is sometimes smaller than the subordinate (Collins et al. 1997; this paper). This may be because dominance is determined partly by how hard an animal is prepared to fight at a particular life history stage (Partridge and Harvey 1988; McNamara and Houston 1996; Charnov 1997) in addition to its inherent fighting ability (Gosling et al. 1996). In this respect, distinguishing the short-term effects of differences in fighting ability, and the longer term, sustained effort needed to establish and maintain dominance relationships may be relevant.

Body size may thus confer an advantage in competition but may not always determine the outcome. If this is the case, a male that is relatively smaller than a subordinate partner may need to incur higher costs to maintain dominance than one that is larger. One such cost may arise from the social signals that dominant males use to help maintain their status. Olfaction is the dominant sensory modality in mammals and high levels of scent-marking are known to be associated with the defence of territories and the maintenance of dominance amongst males of numerous species (e.g. Gosling and Wright 1994; Kappeler 1998; reviews in Brown and Macdonald 1985; Gosling 1990; Gosling and Roberts, in press), including mice (Desjardins et al. 1973; Gosling and McKay 1990; Hurst 1990). Mice mark using smears of urine and the preputial glands are the source of some of the active components (Mugford and Nowell 1971; Jones and Nowell 1973). These glands and their products are known to be androgen dependent and are larger on average in dominant than subordinate males (Bronson and Marsden 1973; Hayashi 1987; Even and vom Saal 1991).

While investigation of the costs of signalling is an active area of signalling theory, very little is known about the costs of olfactory signals (Penn and Potts 1998). However, there are both empirical and theoretical reasons for thinking that scent-marking may be costly. The urine of male mice contains major urinary proteins whose function may be to mediate the controlled release of pheromones in scent marks (Hurst et al. 1998). These proteins reach concentrations of 10–20 mg ml⁻¹ (Nevison et al. 2000) and the energetic costs of synthesis may thus be

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substantial. Scent marks are also used by hunting kestrels, *Falco tinnunculus*, and thus lead to increased risk of predation (Viitala et al. 1995). More generally, costly signals of status may evolve because they signal honestly the quality of the signaller to potential opponents (Zahavi 1975). This central tenet of signalling theory remains to be tested for olfactory signals.

Here we explore the costs of olfactory status signalling using a model system in which male mice, *Mus musculus*, vary in competitive ability (body size) in relation to their opponents. Pilot observations suggested that signalling costs might be expressed by life history trade-offs between signalling intensity (as a component of reproduction) and growth. Since body size and competitive ability are often linked, we speculated that there might also be implications for reproductive tenure. To test these ideas, we placed pairs of male mice together and compared the scent-marking rates and growth of dominant males that were either lighter than or heavier than their partner. We tested three main predictions: (1) that since it should be more difficult for a poor competitor to maintain dominance over its partner, dominant males smaller than their partner should signal at higher intensity than males larger than their partner; we initially tested this using estimates of scent-marking rates but, in a further analysis, we also tested the secondary prediction that small dominant males should have relatively large preputial glands; (2) that there should be an energetic cost to scent-marking so that males signalling at high intensity should grow more slowly than those signalling at lower intensity; (3) that dominant males whose size (competitive ability) was thus reduced should eventually be defeated and replaced as a dominant male by their partner.

Methods

Husbandry

We used an outbred strain of laboratory mice (TO, purchased from B & K, Hull, UK). We established breeding pairs which were housed in MB1 cages (45×28×13 cm) and fed ad libitum with food (breeder diet RM3) and water under a reversed 12:12 light:dark cycle and at constant temperature (21±2°C). Cages contained a sawdust substrate and cardboard tunnels for behavioural enrichment. Sires were removed 2 weeks after pairing with the females. Litters were manipulated on day 3 to a 3:2 male:female sex ratio, in order to control for potential influences of social environment on subsequent competitive strategies (Mendl and Paul 1990; Collins et al. 1997); remaining sibs were euthanased. When the mice were aged 5 weeks, females were removed. When males were aged 8 weeks, individuals were randomly assigned to one of 21 pairs (age matched, no sibling pairs). Males remained in these pairs throughout the experiment, under the same husbandry conditions as before. Scent-marking rates, dominance relationships and body weights were measured weekly; we include data up to 25 weeks of age, when asymptotic weight is reached, although pairs were maintained beyond this age for further study.

Techniques

Marking rates were measured by separating subjects using perforated perspex dividers (maintaining olfactory and visual contact during this period) and presenting a standard stimulus (a 5×5×3.5 cm plastic block covered in clean Benchkote filter paper)

for 1 h; scent marks were then stained using ninhydrin spray (BDH, Poole, UK) and percent coverage was calculated by overlaying a transparent grid onto the scent-marked paper. The 1 h test period has been found to be an appropriate measure of scent-marking variation between individuals while minimising the degree of overlap between successive deposits. These data were then arcsine-transformed before analysis (Sokal and Rohlf 1995).

Pairs were observed for 10 min immediately after removal of the perspex divider, which helped to stimulate higher rates of social interactions and facilitated the assessment of relationships (see Brain and Kamal 1989). Males would have been separated if major injuries had occurred but none were seen. We recorded fighting behaviour, biting, persistent following of one individual by another and submissive or defensive posturing. In each week, dominance status was attributed on the basis of the frequency with which individuals displayed these behaviours and the outcome of such interactions within pairs of males. In this paper, we define dominance status from the proportion of weekly observations in which each male was either dominant or subordinate over the initial 5 weeks after pairing. Dominance reversals were defined as the point at which a 5-week running mean of dominance status (based on weekly observations) changed from dominant to subordinate. Relative size was defined by the weight of dominant males relative to their subordinate when pairs were formed in week 9.

To investigate effects on growth and body size during the study period (weeks 9–25), we averaged the rates of scent-marking and interactions over the same period. All interactions are included, regardless of whether they were initiated by the dominant or subordinate male. To investigate growth and body size prior to dominance reversals, we averaged the rates of scent-marking and frequency of interactions from the date of pairing until the week of reversal. For comparison, we use data up to week 16, the median age of reversal, for those pairs in which a reversal did not occur. Proportional growth was calculated by dividing body mass when mice were aged 25 weeks by initial mass at pairing (9 weeks; ±2 weeks in each case). Asymptotic size is body mass at age 25 weeks (the age at which mean population asymptotic size was attained). Change in relative size was calculated by dividing the weight ratio at reversal (dominant/subordinate) by the initial weight ratio; thus indices less than 1 indicate that the dominant has become increasingly small relative to its subordinate.

Preputial glands could not be measured in our main experimental group which was kept alive for further behavioural observation. We therefore raised additional samples of 17 dominance pairs as above, in which 9 dominant males were smaller and 8 larger than their subordinate partner. Dominant males were euthanased and dissected when aged 18 weeks. Glands were opened and the contents removed by gently dabbing them onto filter paper; the weights of empty glands were divided by (femur length)³ to control for variation in body size.

Results

As predicted, when pairs of males were housed together, dominant males that were smaller than their partners marked at higher rates than those that were larger (Fig. 1a; $t_{19}=2.70$, $P=0.014$).

Our prediction that preputial glands should be relatively heavier in small than in large dominant males fell short of the results obtained. Preputial glands were not only relatively heavier (i.e. when controlling for body size) in small dominant males (Fig. 1b; $t_{12}=3.48$, $P<0.01$), they were also absolutely heavier (Fig. 1c; $t_{15}=2.64$, $P<0.02$). Relatively small males thus appear to invest more in olfactory status signalling than large males.

Our results were also consistent with the predictions that high levels of scent-marking should result in reduced

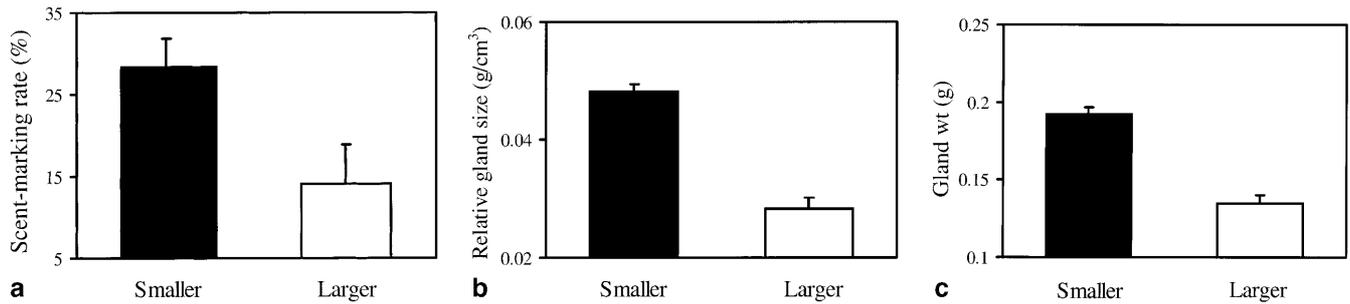
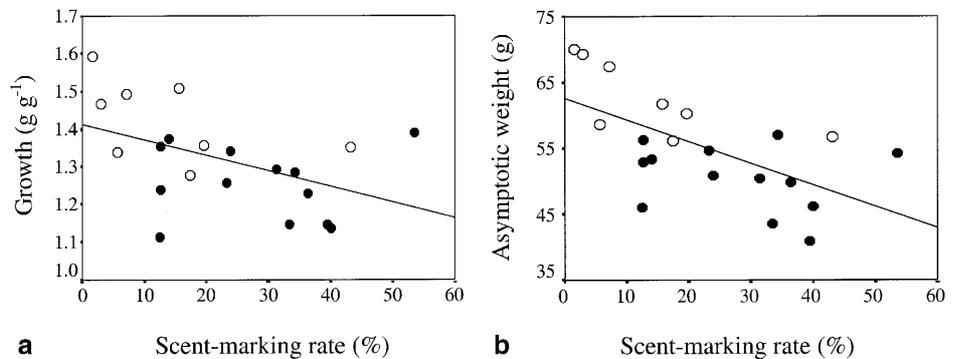


Fig. 1 Investment in olfactory signalling and in growth of signalling structures of dominant male mice kept in pairs and which were either smaller (*closed bars*) or larger (*open bars*) than their subordinate partner. Relatively smaller males have higher signalling frequencies, measured by scent-marking rates (independent

samples *t*-test: $P=0.014$) (a), and both relatively ($P=0.005$) (b) and absolutely ($P=0.019$) (c) larger preputial glands. Preputial gland sizes were obtained from dominant males aged 18 weeks; the weights of empty glands were divided by (femur length)³ to control for body size. *Bars* represent means+SE

Fig. 2 Costs of scent-marking in terms of growth (a) and asymptotic size (b) in dominant male mice. *Closed circles* denote relatively small males. Proportional growth was calculated by body mass at 25 weeks divided by initial mass on pairing (9 weeks; ± 2 weeks in each case; Pearson correlation: $P=0.011$). Asymptotic size is body mass at 25 weeks (the age at which mean population asymptotic size was attained; $P=0.001$)



growth rates and asymptotic size (Fig. 2). There was a linear inverse relationship between scent-marking rate and both growth rate ($r=-0.544$, $n=21$, $P=0.011$) and asymptotic size ($r=-0.675$, $n=21$, $P=0.001$) in dominant males.

These relationships remained strong when we controlled for the number of aggressive interactions within pairs, which we identified as an alternative explanatory variable. Although there were more interactions in pairs in which the dominant male was smaller (mean of 4.14 interactions in 10 min) than in those in which it was larger (2.70), this difference was non-significant ($t_{19}=1.40$, NS). However, scent-marking rates of dominant males and the number of agonistic interactions were intercorrelated ($r=0.541$, $n=21$, $P=0.011$) and interactions were also inversely related to asymptotic weight ($r=-0.604$, $n=21$, $P=0.004$), with a strong tendency towards a relationship with growth ($r=-0.428$, $n=21$, $P=0.053$). We therefore used partial correlations to control for the influence of the other variable; these analyses revealed that scent-marking rate was the best predictor of asymptotic weight ($r=-0.519$, $n=21$, $P=0.019$), while the effect of interactions was reduced ($r=-0.385$, $n=21$, $P=0.094$). In addition, while there remained a tendency towards an inverse relationship between scent-marking and growth ($r=-0.411$, $n=21$, $P=0.072$) when the effects of agonistic interactions were removed, there was no relationship between interactions and growth ($r=-0.190$, $n=21$, $P=0.423$) after controlling for marking rate. Back-

ward stepwise multiple-regression analyses confirmed these results, showing that scent-marking remained the only significant predictor of growth ($F=7.98$, $P=0.011$) and asymptotic weight ($F=10.4$, $P=0.019$).

It could be argued that relatively smaller dominant males grew more slowly because they were initially small; however, further analyses showed this not to be the case. We found no relationship between birth weight and either growth rate after pairing ($r=0.07$, $n=21$, NS) or asymptotic size ($r=0.118$, $n=21$, NS), nor was there a relationship ($r=0.126$, $n=21$, NS) between growth rates before (weeks 1–8) and after (weeks 9–25) pairing. Furthermore, if this argument were correct, we would have expected no difference in the growth rates of the smaller animal in the pair, irrespective of social status. However, our data show that relatively small dominants grew more slowly than small subordinates (data from Fig. 3a; $t_{19}=3.43$, $n=13$ and 8, $P<0.01$).

The results of tests of our third main prediction, that increasing size differences between dominant and subordinate males should result in dominance reversals, were as follows. First, smaller dominant males grew more slowly than their subordinate partners (Fig. 3a; $t_{12}=2.83$, $P=0.015$), while larger dominant males and their subordinates grew at a similar rate ($t_7=0.64$, NS). Next, with increasing scent-marking frequency, the difference in the relative body size of the dominant and subordinate, up to the time when dominance reversals occurred, became pro-

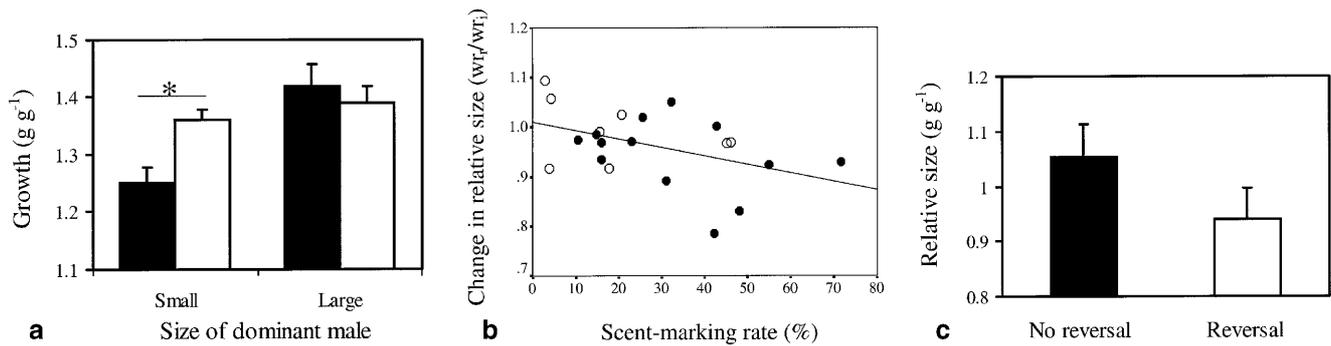


Fig. 3a–c Consequences of signal cost on relative body size and social status in pairs of male mice. **a** Relatively smaller (but not relatively larger) dominant males (*closed bars*) grow more slowly than their subordinate partners (*open bars*; paired *t*-test: $P=0.015$). **b** Dominant males with high scent-marking rates become progressively smaller than their subordinate partner up to the point of dominance reversal (Pearson correlation: $P=0.038$). *Shaded circles* are relatively smaller males. Change in relative size was calculated by the weight ratio at reversal (dominant/subordinate) divided by the initial weight ratio; thus, indices less than 1 indicate that the dominant has become increasingly small relative to its subordinate. **c** Initially dominant males which incurred a reversal were smaller at the point of reversal, relative to their partner, than those which did not (independent-sample *t*-test: $P=0.037$; mean+SE). Reversals occurred in 15 of 21 pairs; if no reversal occurred, data which corresponded with the median age of reversal (16 weeks) were used

gressively greater (Fig. 3b; $r=0.455$, $n=21$, $P=0.038$). Finally, while the occurrence of dominance reversals (in 15 of 21 pairs) was not predicted by either the initial size difference between dominant and subordinate ($t_{5,14}=1.30$, NS) or scent-marking rates up to the point of reversal ($t_{5,14}=0.86$, NS), those dominants which experienced a status reversal were relatively lighter, at the point of reversal, than those which did not (Fig. 3c; $t_{5,14}=2.24$, $P=0.037$; of the 15 initially dominant males that incurred a reversal, 2 were larger and 13 were smaller than their subordinate at the point of reversal, while 3 of the 6 males that remained dominant throughout the study were larger).

Discussion

As predicted, dominant males that were smaller than their subordinate partners scent-marked at higher intensity than dominants that were larger than their partners. The suggestion that these small males were investing relatively more in signalling was reinforced by post-mortem evidence showing that these males had absolutely larger preputial glands, the source of some of the pheromones used in status signalling. But is this higher level of signalling costly? Our experiment suggested that it is. Males marking at high intensity grew more slowly than those marking at low intensity and, as a result, the discrepancy in size between relatively small males and their partners grew larger over time. The cost appeared to be principally energetic and may be linked to the costs of synthesis of high concentrations of major urinary protein, an important component of mouse scent marks, and the enhanced develop-

ment of the preputial gland. Finally, we found that dominance reversals did occur and that they were associated with large negative size differences between dominant males and their partners. Small males that were forced to invest heavily in status signalling became even smaller and were eventually defeated by their partner. Males that maintained dominance over their partners for long periods were on average relatively large.

These findings provide evidence for a life history trade-off between the costs of olfactory signalling when used to help establish and maintain social status, and the time over which dominance can be maintained. Relatively poor competitors (which we equate here with small body size) apparently have to invest more heavily in status signalling to maintain dominance and as a result incur relatively high costs. This is in contrast to related findings in wolf spiders, *Hygrolycosa rubrofasciata*, in which small males are at an advantage due to the relatively lower energetic costs of their drumming display (Kotiaho et al. 1998). In mice, as a result of relatively greater costs of status signalling, poor competitors grow more slowly and can only maintain their dominance for shorter periods. However, this period of dominance could provide critical fitness benefits particularly when the probability of survival is low, as in *r*-selected species, including the wild progenitors of the laboratory mouse, or if small males are relatively vulnerable to some sources of mortality such as predation (Dickman et al. 1991; Koivunen et al. 1998). Under these circumstances, selection would favour early breeding, even if the costs of reproduction adversely affected the chance of long-term survival.

The argument that signalling costs trade off against the opportunity to reproduce assumes that social dominance is equivalent to reproductive tenure. This is supported by naturalistic observations of mouse colonies showing that dominant males achieve all or nearly all copulations (Wolff 1985; Franks and Lenington 1986). But while it thus seems likely that dominant males achieve most reproductive success, alternative strategies could possibly exist in natural situations. This is relevant here because our results also raise the question as to why some larger mice appear to accept a subordinate role early in life. The strategic options appear to be principally some form of sneak breeding or waiting strategies (Kozłowski 1992; Maynard Smith 1996; Pilastro et al. 1997; Kokko and Sutherland 1998). There is no direct

evidence for either of these options, although subordinate male mice do occasionally obtain some matings (Wolff 1985; Franks and Lenington 1986). Life history benefits from delaying reproduction are consistent with the dominance reversals observed in our experiments, but the consequences for lifetime reproductive success have not yet been tested.

Our findings that scent-marking is costly are also consistent with the theoretical notion that selection should favour the evolution of costly status signals because they provide reliable, cheat-proof, information about the quality of the signaller (Zahavi 1975; Grafen 1990). They appear to represent the first empirical information about the life history costs of an olfactory status signal.

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