Genetic trade-off between abilities to avoid attack and to mate: a cost of tonic immobility

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Consistent individual differences in correlated behaviours across contexts or situations, that is, behavioural syndromes, have recently been identified as an important factor shaping the evolution of behavioural traits, because of their potential for explaining trade-offs in behavioural responses. We examined a genetic link between abilities to mate and to avoid predation from the viewpoint of two genetically correlated behavioural traits; tonic immobility (TI), which is considered to be an antipredator behaviour, and activity levels in the red flour beetle, *Tribolium castaneum*. Males derived from two strains artificially selected for divergent durations of TI were used in the present study: the L strain (with longer duration and higher frequency of TI) and the S strain (shorter duration and lower frequency of TI). We found that males of the L strain had higher survival rates in predatory environments than those of the S strain, and lower mating success even in predator-free environments. To our knowledge, this is the first empirical study showing a genetic trade-off between abilities to mate and to avoid predation in relation to behavioural syndromes, using individuals exhibiting different behavioural strategies.

Keywords: tonic immobility; death feigning; locomotor activity; behavioural syndromes; genetic trade-offs

1. INTRODUCTION

Consistent individual differences in correlated behaviours across contexts or situations, that is, behavioural syndromes, have recently been identified as an important factor shaping the evolution of behaviour and have attracted attention in evolutionary and behavioural ecology (Sih *et al.* 2004; Bell 2007). Although behavioural syndromes may be adaptive, they may also function as evolutionary constraints on behavioural traits if different behaviours are genetically correlated with each other. For example, individuals that routinely hide in predatory conditions generally exhibit lower activity levels even in predator-free conditions (Sih *et al.* 2003). This correlation between antipredator behaviour and general activity could potentially result in a trade-off between survival under predatory conditions and mating success under

predator-free conditions. General activity when encountering mates is likely to be particularly important in species such as the flour beetle (Fedina & Lewis 2008), which does not exhibit male–male competition or male courtship before mating. To our knowledge, however, no empirical study has identified a genetic basis to such a trade-off, by actually comparing survival rates and mating success rates using individuals that exhibit different behavioural tendencies.

Animals often become immobile, exhibiting tonic immobility (TI), when attacked by predators (Edmunds 1974; Miyatake *et al.* 2004). Recently, the number of studies focusing on the variously adaptive significance of this behaviour in defence has increased (Miyatake *et al.* 2004; Honma *et al.* 2006; Ruxton 2006; Cassill *et al.* 2008; Hansen *et al.* 2008).

The red flour beetle, *Tribolium castaneum*, which exhibits TI, is a suitable model organism for close examination of the genetic correlation between anti-predator and mating behaviours. In this species, strains with longer duration and higher frequency of TI (L strains) and strains with shorter duration and lower frequency of TI (S strains) were established by two-way artificial selection for duration of TI for more than 20 generations (Miyatake *et al.* 2004, 2008). In addition, Miyatake *et al.* (2008) have shown that males of L strains had lower levels of locomotor activity compared to those of S strains, that is, a negative genetic correlation exists between the intensity of TI and activity levels.

In the present study, we examined the following points to identify a genetic correlation between anti-predator behaviour and mating behaviour using the two strains of *T. castaneum* described above. First, we tested whether individual differences in activity levels correlated with TI reflect individual difference in mating success under predator-free conditions. Second, we examined the adaptive significance of TI under predatory conditions using a predator, Adanson's house jumping spider (*Hasarius adansoni*).

2. MATERIAL AND METHODS

(a) Insects and selected strains

Beetles (virgins and 2–4 weeks old) derived from two replicate lines of two strains (L-1 and S-1, L-2 and S-2) of *T. castaneum* were used; L strains exhibited longer duration and higher frequency of TI, and S strains exhibited shorter duration and lower frequency. For a detailed description of the selection protocol see Miyatake *et al.* (2004). Briefly, 100 males and 100 females (F_0 generation) were randomly selected from the stock culture. Males and females (10 of each) with the shortest duration of TI were selected to propagate short-duration strains, and 10 of each with the longest duration were selected to propagate long-duration strains. All experiments were conducted in an experimental chamber maintained at 25°C and 60 per cent relative humidity, under a photoperiod cycle of 16 L:8 D (lights on at 07.00, lights off at 23.00). Observations were conducted between 13.00 and 18.00.

(b) Mating success

Males of the 35th generation of each strain were randomly selected. Each male was placed in a well of a 48-well tissue culture plate (Greiner Bio-One) containing a mixture of wholemeal (Yoshikura Shokai) enriched with brewer's yeast (Asahi Beer), until the time of the test. One day before testing, the elytra were marked with paint (white, Paint Marker PX-1, Mitsubishi) to distinguish the sexes during observations of mating behaviour. The next day, each male was placed gently into a plastic dish (9.0 cm diameter $\times$ 1.5 cm height) that had a piece of paper (9.0 cm diameter) fixed to its bottom. We then waited until the males had recovered from TI, to exclude the possibility that the duration of TI causes a direct

loss of time for mating. Five virgin females were then introduced to the male, and the number of matings was recorded for 15 min. After one mating ended, the female was replaced with another virgin. To measure precisely a male's ability to mate, we controlled the female condition (TI level) by using females from the population that were the base of L and S strains. This experiment was replicated 20 times for each strain.

(c) Avoiding predation

Two species, the red flour beetle and the Adanson's house jumping spider, commonly coexist in grain storehouses in western Japan (T. Miyatake 2007, unpublished data). Males of the 26th generation of each strain were randomly selected and then maintained as described above, until the time of the test. One day before testing, the elytra of one strain were marked with paint to distinguish the strains. The next day, 14 males (seven S males and seven L males) and an adult female of *H. adansoni* were placed together in a plastic container (diameter 10 cm, height 4.5 cm) and the number of surviving beetles recorded every day for six days. A small plastic cup (diameter 3.5 cm, height 1.0 cm), holding the food (5 g) described above, was put in each plastic container for the beetles. The trial was replicated three times for each marking treatment, in both replicates of the two strains, using different prey and predator individuals; that is, 12 trials were set up in total (two replicate lines of the two strains $\times$ L or S strain marking $\times$ three trials). The adult female *H. adansoni* spiders, used as the model predators, were collected indoors in Okayama City and Naruto City, in western Japan, and maintained in the chamber. Before the test, each female spider was starved for 10 days.

(d) Statistical analysis

A generalized linear model (GLM) with replicate line and strain as independent variables was used for comparison of the number of matings using a log-link function because the data are counts. In the comparison of survival rates, GLM (with identity link), with survival days (1–6 days) as a dependent variable and replicate line, marking, trial (nested in marking) and strain as independent variables was also used. All statistics were performed using JMP 7 (SAS Institute 2007).

3. RESULTS

The GLM for the number of matings (figure 1) showed that the effect of strain was highly significant ($\chi^2_1 = 7.80$, $p < 0.01$), but there was no significant effect of replicate or interaction ($p > 0.1$). Similarly, the GLM for survival (in days; figure 1) showed that the effect of strain was highly significant ($\chi^2_1 = 11.09$, $p < 0.001$), but that the effects of replicate, marking, trial and interactions were not ($p > 0.1$). These findings show that S males had a higher mating success than L males, and L males had a higher ability to avoid predation than S males.

4. DISCUSSION

In assessing the adaptive value of an antipredator behaviour, it may not be adequate to study only the resultant probability of survival or death, because antipredator behaviours may be correlated to non-antipredator behaviours. Our present results provide clear evidence that there is a genetic link between survival rates and mating success, which is caused by a behavioural syndrome. Males with a genetically higher intensity of TI showed higher survival rates in predatory environments; however, they had reduced mating success, even in a predator-free environment, owing to genetically lower activity levels. This could represent a serious trade-off for males, as male reproductive success generally increases with the number of mates (Bateman 1948).

Our results should be interpreted with some caution. We cannot completely rule out the possibility

Figure 1. (a) Comparison of the number of matings and (b) survival days between the L strain (longer duration and higher frequency of TI) and the S strain (shorter duration and lower frequency of TI) of *Tribolium castaneum*. Filled bars, L strains; open bars, S strains. Error bars indicate s.e.

that the correlation was produced by random effects such as genetic drift as we only have two replicates of each strain. Ideally, we also need to examine correlated responses to selection for locomotion and mating success, to directly examine the genetic correlation in future.

Prohammer & Wade (1981) showed a geographical difference in duration of TI in *T. castaneum*. Beetles derived from a Chicago stock population, maintained in the laboratory without exposure to predators for at least 100 generations, had the shortest duration of TI compared with individuals derived from five other populations, including field populations. This suggests that TI is costly and selected against in predator-free environments. That is, our results imply that males with a genetically higher intensity of TI may be strongly selected against because of decreased mating success owing to genetically lower activity.

Correlated behaviours may share a common proximate mechanism, such as genes with strong pleiotropic effects (Van Oers *et al.* 2005; Bell 2007). Bell (2007) suggested that approaching the study of behavioural correlations from a proximate perspective is particularly powerful, as it not only explains why syndromes occur, but also allows clear predictions to be made with regard to which behaviours are likely to be correlated.

In *T. castaneum*, the negative genetic correlation between the intensity of TI and locomotor activity is associated with a pleiotropic effect of the expression of brain dopamine, a neurotransmitter (Miyatake *et al.* 2008). Beetles derived from S strains (which have constantly higher levels of brain dopamine) flee, and beetles from L strains (which have lower levels of

brain dopamine), when stimulated, become tonically immobile to avoid predation. The results of the present study demonstrate that the biogenic amines, including dopamine, that regulate insect activity are important not only as an alternative trigger of fleeing or TI, but also as a factor determining mating success, especially in males. To our knowledge, this is the first empirical study showing a genetic link between the abilities to mate and to avoid predation in relation to behavioural syndromes for which the proximate mechanism has been already clarified.

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