

Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour

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The adaptation of death-feigning (thanatosis), a subject that has been overlooked in evolutionary biology, was inferred in a model prey-and-predator system. We studied phenotypic variation among individuals, fitness differences, and the inheritance of death-feigning behaviour in the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). Two-way artificial selections for the duration of death-feigning, over 10 generations, showed a clear direct response in the trait and a correlated response in the frequency of death-feigning, thus indicating variation and inheritance of death-feigning behaviour. A comparison of the two selected strains with divergent frequencies of death-feigning showed a significant difference in the fitness for survival when a model predator, a female Adanson jumper spider, *Hasarius adansoni* Audouin (Araneomorphae: Salticidae), was presented to the beetles. The frequency of predation was lower among beetles from strains selected for long-duration than among those for short-duration death-feigning. The results indicate the possibility of the evolution of death-feigning under natural selection.

Keywords: anti-predator behaviour; artificial selection; defence; immobility; quantitative genetics; thanatosis

1. INTRODUCTION

Death-feigning (thanatosis), a state of immobility assumed by many animals in response to external stimuli, has been considered a defence mechanism against predators that is shared by mammals (Francq 1969), birds (Sargeant & Eberhardt 1975), fishes (Howe 1991), reptiles (Gehlbach 1970), mites (Ebermann 1991) and insects (Fabre 1900; Frost 1959; Edmunds 1974). In particular, death-feigning has been studied in beetles (e.g. Bleich 1928; Chemsak & Linsley 1970; Allen 1990; Oliver 1996). Studies on death-feigning have not been treated as a subject of evolutionary biology despite many descriptive observations of this curious behaviour.

Although death-feigning behaviour has frequently been the subject of neurophysiological studies (e.g. Godden 1972, 1974; Nishino & Sakai 1996; Nishino *et al.* 1999), there are few experimental studies on its adaptive nature. Endler (1986) has proposed three prerequisites for the evolution of an adaptive trait by natural selection: (i) variation in a trait among individuals; (ii) fitness differences related to the trait; and (iii) inheritance of the trait. We examined the three prerequisites for natural selection (heritable variation in fitness differences) for the death-feigning behaviour using a model prey and a model predator. To date, some descriptive studies have been conducted on individual variations in death-feigning behaviour (DuPorte 1916; Bleich 1928); however, some of its parameters, such as occurrence and duration, are affected by environmental factors, including temperature (Holmes 1906; Larsen

1991), light-dark conditions (Miyatake 2001a), physiological factors including starvation (Acheampong & Mitchell 1997; Miyatake 2001b) and the pre-stimulation status of insects (Miyatake 2001a). Therefore, it is difficult to evaluate the genetic basis of individual variation in death-feigning. Only one experiment on the heritable basis of death-feigning has been conducted by Prohammer & Wade (1981), where geographical and heritable variation in the duration of death-feigning was found in the red flour beetle, *Tribolium castaneum* (Herbst), demonstrating the genetic basis of the duration.

In the present study, we examined the three conditions for natural selection, variation, fitness difference and inheritance of death-feigning in *T. castaneum*. First, we investigated the response to two-way artificial selections for duration of death-feigning using experimental populations of *T. castaneum* to determine inheritance and to establish genetic lineages with different durations of death-feigning. Second, the difference in the fitness for survival between the two selection lines was compared to determine the frequency of death-feigning (as a correlated response to the two-way artificial selection for the duration of death-feigning) by predation tests using a model predator, an Adanson's jumper spider (*Hasarius adansoni* Audouin (Araneomorphae: Salticidae)).

2. MATERIAL AND METHODS

(a) Insects and culture

The *T. castaneum* beetle culture used in this study has been maintained in the laboratory for more than 25 years (T. Suzuki, Tsukuba University, personal communication) according to the rearing method described by Suzuki & Nakakita (1991). The beetles were reared with a mixture of wholemeal (Yoshikura-shokai, Tokyo) enriched with brewer's yeast (Asahi beer, Toyko) as the

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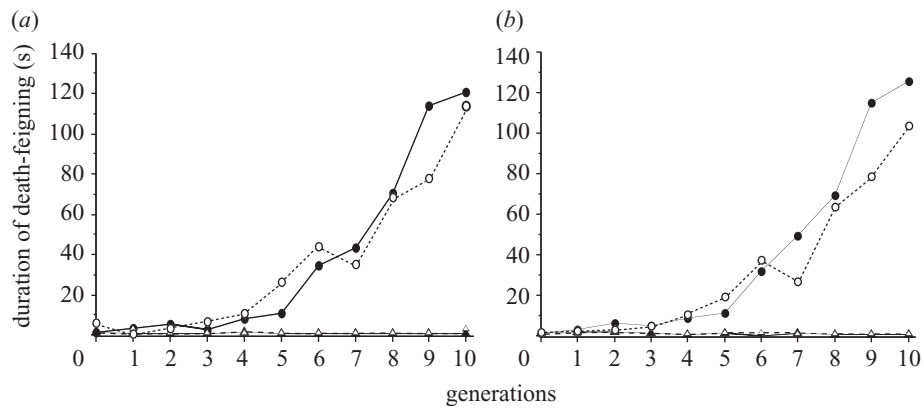


Figure 1. Direct response to selection in (a) male and (b) female *Tribolium castaneum* on short (S)- (triangles) and long (L)- (circles) duration death-feigning. First replicate, filled symbols; second replicate, open symbols.

rearing medium and kept in a chamber (Sanyo, Tokyo) maintained at 25 °C and 60 r.h. under a photoperiod of 16 L:8 D (lights on at 07.00, lights off at 23.00).

(b) Observation of death-feigning

One day before the observation, each beetle was placed in one well of a 48-well tissue culture plate (Falcon, Becton Dickinson and Company, NJ, USA) to avoid disturbance by other beetles. Each beetle was gently moved and turned over onto a small white china saucer (140 mm diameter, 15 mm deep). Death-feigning behaviour was induced by touching the abdomen of the beetle with a wooden stick. A trial consisted of provoking the behaviour and recording its duration with a stopwatch (the behaviour duration was specified as the length of time between touching the beetle and detecting its first visible movement). If the beetle did not respond, the touch was repeated. When the beetle feigned death, the duration was recorded. If the beetle was unresponsive to both stimulations, it was recorded as a non-responsive individual. All the trials were conducted between 13.00 and 17.00 in the chamber previously mentioned (§2a).

(c) Artificial selection

A random collection of 100 males and 100 females (15 days old) was made from the stock culture and their death-feigning behaviour was observed (F_0 generation). Males and females (10 of each) with the shortest duration of death-feigning were selected to propagate short-duration lines (S-lines); similarly, 10 of each with the longest duration were selected to propagate long-duration lines (L-lines). The males and females of each line were placed in a plastic cup (6.5 cm diameter, 2.5 cm height) with 20 g of medium and allowed to lay eggs for one week. The pupae from the eggs were stored in separate-sex groups in other plastic cups and allowed to emerge. When the adults reached 10–15 days old, 100 males and 100 females were randomly collected from each line and their death-feigning was observed again (F_1 generation). The same procedure was carried out in each generation. Two selection replicates for the short and long lines (S-1 and L-1; and S-2 and L-2) initiated at the same time were tested and maintained in the chamber. The selection regimes were continued for 10 generations for each line. Realized heritability was calculated as described by Falconer & Mackay (1996).

(d) Predation tests

Female adults of Adanson's house jumper spider, collected indoors in Okayama City and Naruto City, western Japan, were used as model predators. Each female spider was maintained in a

Table 1. Realized heritability of short (S) and long (L) durations of death-feigning.

(Heritability was calculated as the regression of the population mean on the cumulative selection differential for the first 10 generations.)

replicate	sex	short (S) line	long (L) line
1	male	0.055	0.133***
	female	0.100*	0.164***
2	male	0.336**	0.107***
	female	0.079	0.117***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (ANOVA).

plastic Petri dish (8 cm diameter, 2 cm height) with wet cotton, and provided with adult flies, *Drosophila hydei*. Before the tests, each spider was starved for one week, and then an adult short- or long-line *T. castaneum* was introduced into the Petri dish. Predation behaviour was observed for 15 min in each trial. Fourteen trials were replicated for beetles of the eleventh generation of S-1 and L-1 lines. The sex of beetles used for the predation tests was not recorded. Different individuals of spiders and beetles were used for each trial.

3. RESULTS

(a) Direct and correlated responses to selection

The duration of death-feigning showed a clear direct response to selection and a steady divergence between the two selection regimes with an asymmetrical nature (figure 1). The response of L-lines (long-duration death-feign) was evident and similar in the replicate lines and sexes. Realized heritability was calculated as the regression of population means on the cumulative selection differential for ten generations of each line (table 1). The significance of each regression was determined using ANOVA; the realized heritability was significantly different from zero for all of the L-lines, and for females of replicate 1 and males of replicate 2 for the S-lines.

The frequency of death-feigned beetles showed a clear correlated response to selection for the duration of death-feigning (figure 2). After the tenth generation of selection, a clear divergence was observed between the two selection regimes; almost all L-line beetles feigned death, whereas few S-line beetles did (figure 2).

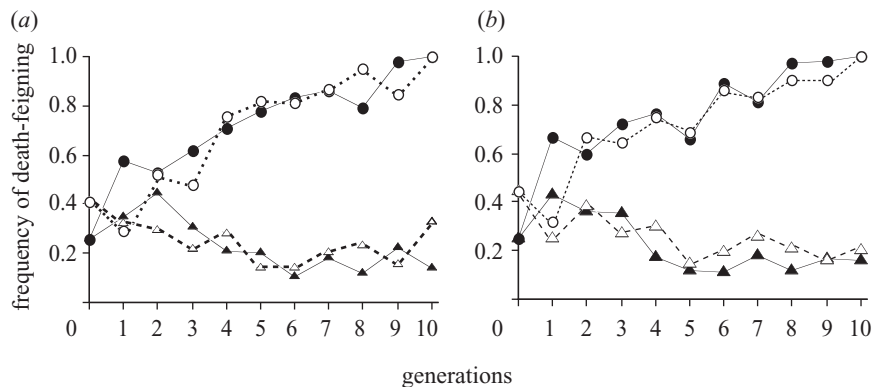


Figure 2. The relation between the frequency of death-feigning in (a) male and (b) female *T. castaneum* (correlated response to selection) and the generations for disrupted selection for duration of death-feigning. Triangles and circles show S- and L-lines, respectively. First replicate, filled symbols; Second replicate, open symbols.

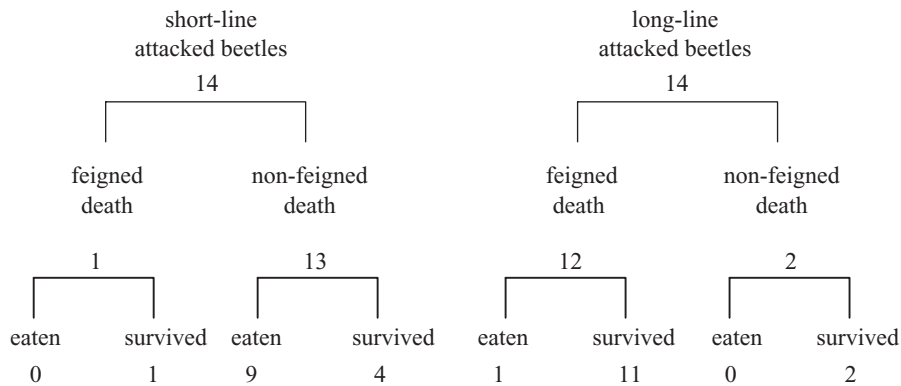


Figure 3. Behavioural sequences of predation by Adanson's jumper spiders against beetles from S- and L-lines. The numerals show the number of beetles observed. See §§3,4 for detailed explanation.

(b) Predation tests

The spider attacked when the prey came closer, but it always set the beetle free once. L-line beetles (85.7%) and S-line beetles (only 7.1%) feigned death (figure 3). The spider attacked again, and if the beetle struggled or moved it was eaten ($n = 9$ for S-line); however, if the prey remained immobile, feigning death, the predator lost interest after several seconds and the prey survived ($n = 11$ for L-line). The frequency of predation was significantly higher in S- than in L-lines (S-line: 64.3%, L-line: 7.1%, $p = 0.0016$, $\chi^2 = 9.956$).

4. DISCUSSION

The inheritance of the duration of death-feigning in *T. castaneum* has been shown by Prohammer & Wade (1981). The direct response to selection on the duration of death-feigning in the present study also supported the heritable variation in death-feigning. The calculated low heritability of the duration of death-feigning in both S and L lines supports the concept that death-feigning has suffered by strong natural selection in *T. castaneum* (Roff & Mousseau 1987). Although our selection was aimed at the duration of death-feigning, the frequency of death-feigning was also markedly influenced by the selection as a correlated response; thus, two *T. castaneum* strains clearly divergent in their frequency of death-feigning were obtained. The fitness for survival differed in the two strains when the model predator was provided to them, as evidenced by the long duration of death-feigning that was adapted to escape from

the attack by the jumper spider. This outcome showed a clear heritable variation in the fitness for death-feigning behaviour, leading us to conclude that death-feigning can potentially evolve by natural selection: 'the evolvability of death-feigning'.

Geographical differences in the duration of death feigning of *T. castaneum* described by Prohammer & Wade (1981) could be the result of different selection pressures caused by predation. Notably, in the Prohammer & Wade (1981) study, the duration of death-feigning was shortest for beetles from the Chicago stock population, which had been maintained in the laboratory without exposure to predators for at least 100 generations. This suggests that, in the absence of predators, death-feigning behaviour is costly and might be selected against or lost.

When a female Adanson's jumper spider was provided with a fly, *Drosophila hydei*, the spider never set the fly free, and immediately ate it in the first attack ($n = 36$; T. Miyatake, unpublished data). By contrast, when the prey was a flour beetle, the spider always relinquished its hold on the prey ($n = 20$). This is probably owing to the hard cuticle of beetles and/or to some chemical compounds which the beetles release as an anti-predator defence (Happ 1968). If the prey struggled or moved after the first attack, it was immediately attacked again and, in several cases, eaten. If, however, the prey feigned death and remained immobile after the first attack, the spider often lost interest and the prey survived. This conduct of the predator probably caused the significant difference in

predation frequency between S- and L-lines of no death-feigning and long-duration death-feigning, respectively (figure 3). The present result also showed the highly significant difference in predation frequencies between death-feigned ($n = 1$ of 13) and non-death-feigned ($n = 9$ of 15) beetles ($p = 0.0040$, $\chi^2 = 8.299$). These observations support the idea that having two sequential defences; releasing chemical substances and feigning death are effective in beetles escaping from jumper spiders. Alternatively, the presence of a chemical predator defence in *T. castaneum* (Happ 1968) might indicate a slightly different explanation for the present results; the results were owing to death-feigning being highly correlated with chemical defences. Studies of a possible correlated evolution between death-feigning and the chemical defences are required for the beetle–spider relationship in view of multivariate evolution (Arnold 1994).

Evidently, the present results do not clarify all aspects of adaptation in the death-feigning behaviour of insects, especially considering that this study was conducted under laboratory conditions using a model prey-and-predator system. We cannot, therefore, speculate, without further natural history data, on death-feigning and predation hazards in nature. Other types of death-feigning behaviour have also been observed. For example, some insects on roosts drop and feign death on the ground, whereby spilling and feigning death might evolve as a behavioural set. We trust the present study will revive interest in the significance of various types of adaptive death-feigning behaviour, which has been bypassed in recent evolutionary studies.

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