

Research



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Animal behaviour

Latitudinal cline of death-feigning behaviour in a beetle (*Tribolium castaneum*)

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Death-feigning behaviour is a phenomenon in which a prey is rendered motionless due to stimulation or threat from a predator. This anti-predator defence mechanism has been observed across numerous animal taxa and is considered adaptive in nature. However, longer durations of death feigning can result in decreased opportunities for feeding and reproduction, and therefore is often associated with fitness costs as compared to environments without predators. Differences have also been observed in the frequencies and durations of death feigning within populations, and these differences are thought to be influenced by the balance between survival and other fitness costs. Furthermore, this balance is predicted to vary in response to changes in environmental conditions. In this study, we examined the death feigning in 38 populations of the red flour beetle (*Tribolium castaneum*). Our results demonstrate that frequencies and durations of the death feigning in *T. castaneum* show geographical variations and a latitude cline, indicating that this behaviour is influenced by location as well as latitude. This study is the first to demonstrate the existence of a latitudinal cline in death feigning and suggests that death-feigning behaviour might have evolved in response to environmental factors that vary with latitude.

1. Introduction

Predation is one of the key selection pressures that influences the evolution of anti-predator strategies [1–3]. Behaviours characterized by immobility, such as death feigning and tonic immobility, which involve a state of lack of motion as a response to external stimulus, are widely recognized as anti-predator defence mechanisms across a wide range of animal taxa [4–7]. The act of death feigns, when the prey is at a risk of predator attack, can lead to the loss of sight or interest of the predator towards the prey. Empirical studies indicate that individuals with longer durations of death-feigning behaviours have higher survival rates during encounters with predators [4–6,8]. Thus, selection pressure may drive an organism towards longer death-feigning durations in environments with higher predation pressure. Conversely, lengthy and extended death feigning durations may increase fitness costs. For example, individuals with longer durations of death feigning, have lower tolerance to physical stress [9]. Moreover, the fact that individuals with longer death-feigning durations remain inactive for longer periods of time, adversely effects the number of encounters they can have with potential mates [10,11]. Therefore, it is hypothesized that the duration of death feigning is regulated by the balance between various selection pressures. Thus, it is expected that death-feigning behaviour may differ among populations of the same species, living in diverse environmental conditions [12].

Body size and life-history traits often vary in accordance with varying geographical conditions, in several animal taxa (e.g. [13–20]). These differences are often influenced by latitude at which the animal lives; animals inhabiting

higher latitudes exhibit larger body sizes than those living at lower latitudes [21]. These latitudinal variations are often affected by environmental factors such as temperature that changes with latitudes. Conversely, predation pressure can also be considered to vary with latitude, and not only temperature [22], because of which, latitudinal clines can be observed in anti-predator behaviours. As a matter of fact, several studies have previously reported latitudinal clines of behavioural traits associated with predation avoidance [22–24]. Since predation pressure is thought to gradually decrease with latitude, a latitudinal cline may also be observed in death-feigning behaviour affected by the balance of advantages and disadvantages. If predation pressure varies with latitude, duration of death feigning should also vary accordingly, and should be longer at latitudes with higher predator pressure and shorter in latitudes with lower predator pressure. Since previous studies have reported genetic variation in death-feigning behaviour [7,12,25], it can be postulated that if predation pressure is the cause of the latitudinal clines in the discussed trait, the genetic variation in the latitudinal clines is possibly a result of natural selection. However, no studies to our knowledge have previously investigated the genetic basis for geographical variation in anti-predator behaviours.

In the present study, we have examined the frequency and duration of death-feigning behaviour in the red flour beetle *Tribolium castaneum*, a common cereal storage pest existent worldwide [26]. Since a previous study has reported that the intensity of circadian rhythms of *T. castaneum* collected across Japan is weaker at higher latitudes than at lower latitudes [27], there is a possibility that the behaviour and responses of this organism also vary in accordance with the geography. In this study, we have investigated the death-feigning behaviour in 38 populations of *T. castaneum*. To test whether there is a genetic basis for immobile geographical variation and/or latitudinal cline, we procured some red flour beetles from the field and then allowed them to breed in a controlled and unified laboratory environment. We used these laboratory-bred beetles for our experiment. This can help us to establish the fact that while death-feigning behaviour differs between populations, environmental factors definitely play a part; however, it is possible that there is a genetic link as well. If predation pressure in *T. castaneum* also varies with latitude, latitudinal cline may show in frequency and duration of death-feigning behaviour in this beetle.

2. Materials and methods

(a) Insects

Tribolium castaneum beetles were collected from 38 locations in Japan between 2016 and 2021 (figure 1). We predominantly collected these beetles from rice bran storage areas, adjacent to rice polishing machines. These sites are common throughout Japan, but we chose localities that had a similar environment to maintain uniformity in our experiments. The latitudes and longitudes of each sampling site are given in the electronic supplementary material, table S1. These rearing environments were determined on the basis of some previously conducted studies (e.g. [7,25,28]). The beetles were reared in an incubator (Sanyo, Tokyo, Japan). The temperature of these incubators was maintained at 25°C and 16 : 8 h light/dark cycle (light on at 07.00 h, switched off at 23.00 h) was maintained. The beetles were fed a mixture of whole meal (Nisshin Seifun, Tokyo, Japan) with brewer's yeast (Asahi Beer, Tokyo). The beetles we used for this

experiment, where the third generation of the beetles obtained from the fields. After pupation, the male and female beetles were separated on the basis of morphological characteristics. We used virgin male and female insects (21–28 days old, around 40 to 176 individuals per population) selected randomly from each population. The experiment was conducted with each population separately between 2016 and 2021.

(b) Measurement of death-feigning behaviour

One day before the experiment, each beetle was placed in a well of a 48-well tissue culture plate to prevent interaction with other beetles. In the experiment, each of the beetles was gently placed on its back in a saucer (diameter 140 mm, height 15 mm), and death-feigning behaviour was induced by gently touching the abdomen of the beetle with a wooden stick. This was done to provoke death-feigning behaviour and its duration was measured with a stop watch and readings were recorded. The duration of the behaviour was defined as the length of time between the stick touching the beetle and detection of its first visible movement. If the beetle did not respond, the touch was repeated. The number of stimuli until death-feigning behaviour was displayed was not recorded. While conducting the experiments, the identity of the beetles (population, year, latitude and location) was not revealed to the observer. All trials were conducted between 12.00 h and 18.00 h in the laboratory where temperature was maintained at 25°C.

(c) Statistical analysis

Death-feigning duration was analysed using a generalized linear model (GLM) with population, sex, and the interaction between population and sex as explanatory variables. In GLM analyses, the Akaike information criterion (AIC) values of the Gaussian, Poisson and gamma distributions were compared, and the gamma distribution was chosen, because it had the lowest AIC values (electronic supplementary material, table S2). We added one to all values to test with the GLM with gamma distribution before calculating the AIC values. The frequency of death feigning was analysed using a GLM with a binomial distribution. To test the effect of latitude, we used GLMs with gamma and binomial distributions for the duration and frequency of death feigning, respectively. To test the relationship between duration and frequency of death feigning, we used the Pearson product-moment correlation coefficient. All analyses were performed using R v. 3.4.3 [29].

3. Results

There were variations in frequency (0.91 ± 0.01) and durations (mean \pm s.e. = 114.99 ± 3.89) of death-feigning behaviour among populations (figure 1). The GLM results showed the significant effect of population in the frequency and duration of death feigning (table 1). Moreover, the GLM results showed a significant effect of latitude in the frequency and duration of death-feigning behaviour (table 2). Thus, a latitudinal cline was observed, with the proportion of beetles with a longer duration of death-feigning behaviour increasing with increasing latitude (figure 1). In the correlation between death-feigning duration and latitude, the regression slope was observed to be significantly higher for female beetles as compared to male beetles (figure 2a, table 2). The death-feigning frequency was significantly higher in male beetles than in female beetles (figure 2b, table 1 and 2). Duration and frequency of death feigning showed a significant positive correlation ($r = 0.33$, $p < 0.0001$; figure 2c).

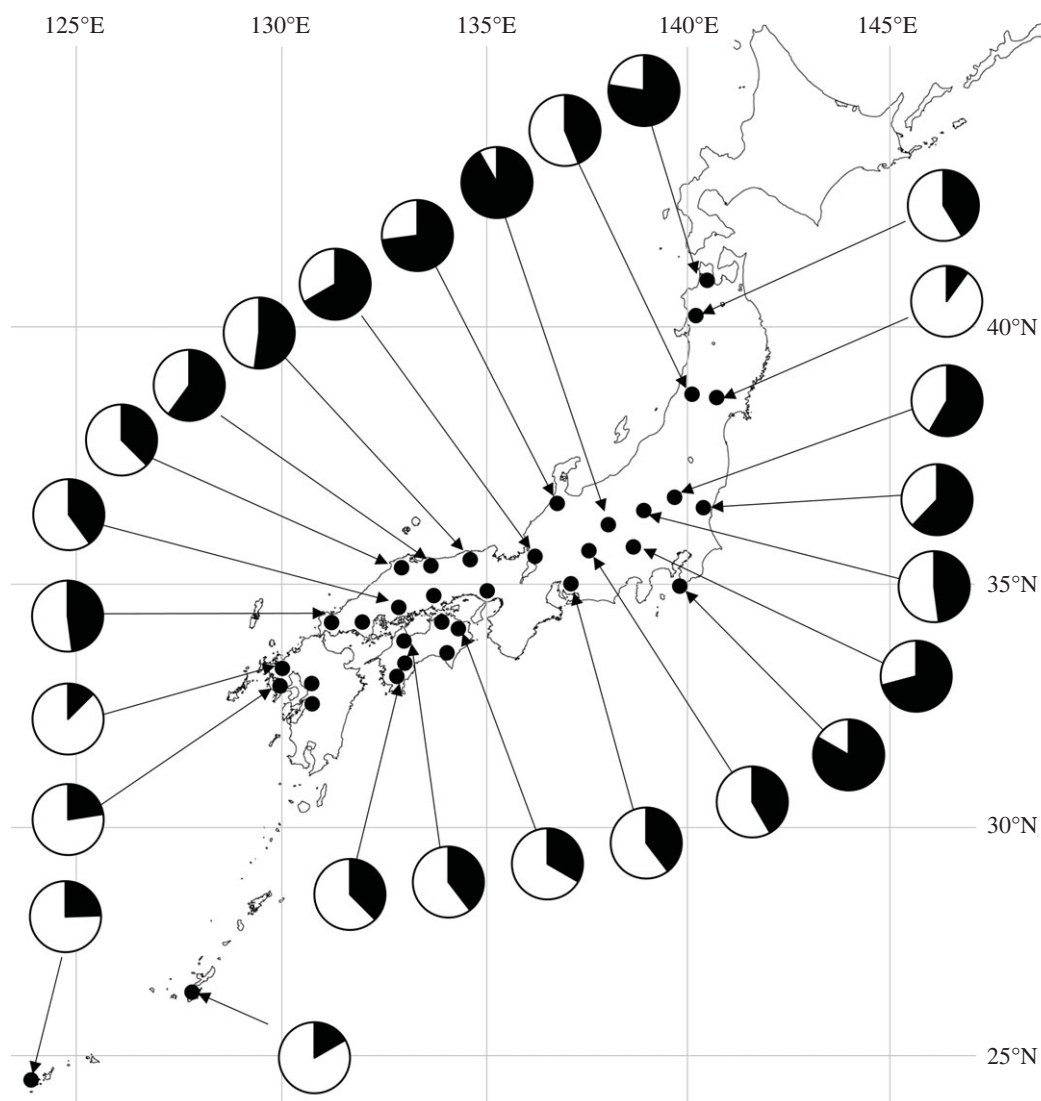


Figure 1. Locations from where the populations of *T. castaneum* were collected (beetles were collected at 38 locations (a), but if collection sites were adjacent to each other, these are depicted by a single point). Pie charts show the proportion of longer (black) and shorter (white) durations of death-feigning behaviours (long and short durations were divided by 61.78 s, the median of the overall data including individuals with an immobility time of zero seconds, as a boundary).

Table 1. Results showing GLM values for the effects of population and sex on death-feigning behaviour.

death feigning	factor	d.f.	χ^2	p
duration	population	37	479.66	$<2 \times 10^{-16}$
	sex	1	0.36	0.5462
	population \times sex	37	38.89	0.3845
	error	2194		
frequency	population	37	165.96	$<2 \times 10^{-16}$
	sex	1	4.82	0.0282
	population \times sex	37	28.46	0.842
	error	2194		

Table 2. Results showing GLM values for the effects of latitude and sex on death-feigning behaviour.

death feigning	factor	d.f.	χ^2	p
duration	latitude	1	47.89	4.50×10^{-12}
	sex	1	0.1	0.7582
	latitude \times sex	1	5.17	0.023
	error	2266		
frequency	latitude	1	57.82	2.87×10^{-14}
	sex	1	4.38	0.0364
	latitude \times sex	1	0.19	0.6635
	error	2266		

4. Discussion

Both the frequency and duration of death-feigning behaviour showed continual variation with latitude. These findings suggest that death-feigning behaviour is significantly affected by environmental conditions that vary with latitude, and/or

genetic differences among these reproductively isolated populations. Although previous studies have reported geographical variations in death-feigning behaviours in some insect species [30], including *T. castaneum* [31], our study is the first to demonstrate a latitudinal cline with regard to this behaviour. This study used individual beetles that had

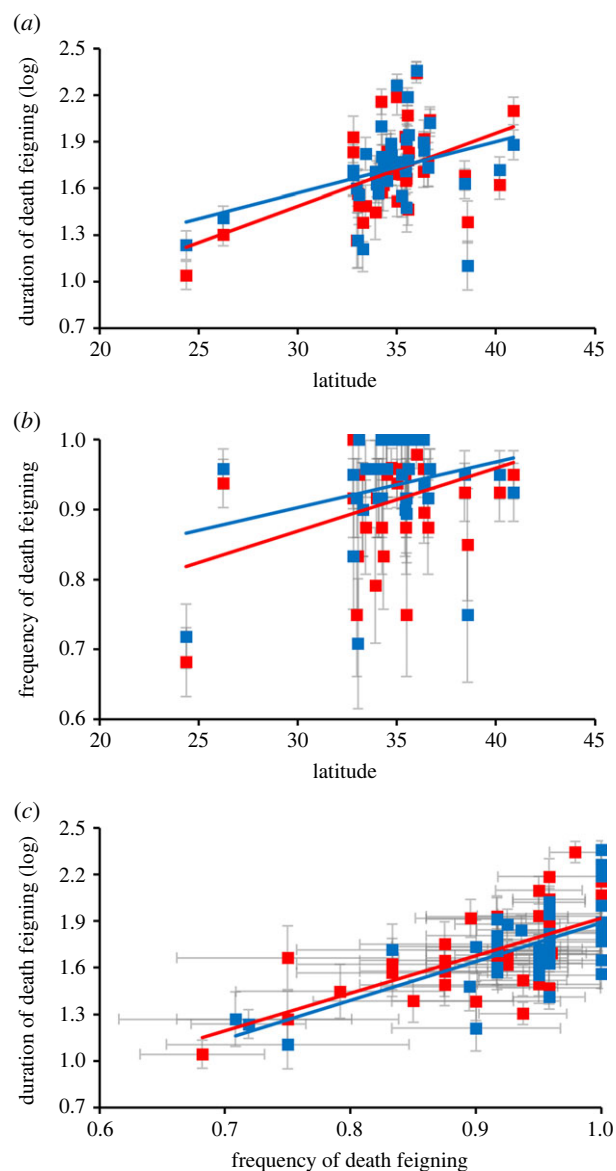


Figure 2. Relationship between latitude and death-feigning behaviour ((a) duration, (b) frequency), and frequency and duration of death feigning (c), respectively. Blue and red colours show males and females of each population. Error bars show standard errors (s.e.).

been reared in the laboratory for generations, suggesting that the latitudinal clines observed in death-feigning behaviours were not due to plasticity to environmental factors, but rather could be attributed to geographical variations with a genetic background. This result is very important, because it reveals for the first time the genetic–latitude–line in anti-predator behaviour.

In this study, beetles from higher latitudinal areas showed a significantly longer duration and higher frequency of death-feigning behaviour than beetles from lower latitudinal areas. These results suggest that the intensity and/or direction of predation pressure varies with latitude. It has been reported that, predator density and abundance tends to decrease with increasing latitude [22]. This prompts the speculation that predator size may increase with increasing latitude, thereby influencing the evolution of anti-predator strategies. However, since the predation pressure of the red flour beetles procured from the fields was not examined in this study, further investigation is needed. In addition, predator foraging patterns may also play a critical role in shaping the evolution

of anti-predator strategies in prey animals. Previous studies have reported that individual creatures with a longer duration of death-feigning behaviour may have a higher survival rate when they encounter actively hunting predators and a lower survival rate when they encounter sit-and-wait predators [32]. Therefore, it is plausible that there are more sit-and-wait predators in the lower latitudinal areas and more active hunting predators at higher latitudes. Moreover, previous studies have suggested death-feigning behaviours of higher frequencies and longer durations in the beetles is greater when conspecific beetle density is high [33]. Future studies should investigate the conspecific density and foraging types of predators in each population.

In many animals, latitude clines are found with regard to body size and other traits. Many of these latitude clines are thought to be influenced by environmental factors, such as temperature. If behaviour can be altered by environmental factors such as temperature, latitudinal clines may also be observed in traits such as anti-predator behaviour. Indeed, previous studies involving other insect species have reported a significantly longer duration of death-feigning behaviour at lower temperatures than at higher temperatures [34,35]. In addition, studies conducted on ladybugs of different body colours revealed, that the relationship between temperature and death-feigning duration differed among individuals having different body colours [36]. Conversely, in this study, we tested death-feigning behaviours under controlled and uniform temperature conditions to explore other possible reasons influencing the frequency and duration of death feigning in *T. castaneum*. Therefore, although the results of this study suggest that the death feigning of *T. castaneum* from geographical lineages is influenced by genetic factors, it is important to also investigate the plastic change in death feigning due to temperature differences.

In our study, although there were no differences between the sexes in the duration of death feigning, it was observed that male beetles feigned death more frequently than female beetles. In addition, female beetles had a greater slope of the regression line than male beetles, in the correlation between death-feigning duration and latitude (see interaction between latitude and sex in table 1). While this study represents the first evidence of latitudinal clines of death feigning related to sex difference, observed in *T. castaneum*, the underlying cause of this phenomenon remains to be determined and this represents an area of focus for future research.

Data accessibility. Data are available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.ksn02v78j> [37].

The data are provided in the electronic supplementary material [38].

Authors' contributions. K.M.: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing—original draft and writing—review and editing; T.M.: conceptualization, funding acquisition and writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. The authors declare no conflict of interest.

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References

- Lima SL, Dill LM. 1990 Behavioral decisions made under the risk of predation: a review and prospectus. *Can. J. Zool.* **68**, 619–640. (doi:10.1139/z90-092)
- Sih A. 1992 Prey uncertainty and the balancing of antipredator and feeding needs. *Am. Nat.* **139**, 1052–1069. (doi:10.1086/285372)
- Lima SL. 1998 Stress and decision-making under the risk of predation: recent developments from behavioral, reproductive, and ecological perspectives. *Adv. Stud. Behav.* **27**, 215–290. (doi:10.1016/S0065-3454(08)60366-6)
- Humphreys RK, Ruxton GD. 2018 A review of thanatosis (death feigning) as an anti-predator behaviour. *Behav. Ecol. Sociobiol.* **72**, 1–16. (doi:10.1007/s00265-017-2436-8)
- Ruxton GD, Allen WL, Sherratt TN, Speed MP. 2019 *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry*. Oxford, UK: Oxford university press.
- Sakai M. 2021 *Death-feigning in insects: mechanism and function of tonic Immobility*. Berlin, Germany: Springer Nature.
- Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M. 2004 Is death-feigning adaptive? Heritable variation in fitness difference of death-feigning behaviour. *Proc. R. Soc. Lond. Ser. B* **271**, 2293–2296. (doi:10.1098/rspb.2004.2858)
- Konishi K, Matsumura K, Sakuno W, Miyatake T. 2020 Death feigning as an adaptive anti-predator behaviour: further evidence for its evolution from artificial selection and natural populations. *J. Evol. Biol.* **33**, 1120–1128. (doi:10.1111/jeb.13641)
- Kiyotake H, Matsumoto H, Nakayama S, Sakai M, Miyatake T, Ryuda M, Hayakawa Y. 2014 Gain of long tonic immobility behavioral trait causes the red flour beetle to reduce anti-stress capacity. *J. Insect. Physiol.* **60**, 92–97. (doi:10.1016/j.jinsphys.2013.11.008)
- Nakayama S, Miyatake T. 2010 Genetic trade-off between abilities to avoid attack and to mate: a cost of tonic immobility. *Biol. Lett.* **6**, 18–20. (doi:10.1098/rsbl.2009.0494)
- Nakayama S, Miyatake T. 2010 A behavioral syndrome in the adzuki bean beetle: genetic correlation among death feigning, activity, and mating behavior. *Ethology* **116**, 108–112. (doi:10.1111/j.1439-0310.2009.01721.x)
- Ohno T, Miyatake T. 2007 Drop or fly? Negative genetic correlation between death-feigning intensity and flying ability as alternative anti-predator strategies. *Proc. R. Soc. B* **274**, 555–560. (doi:10.1098/rspb.2006.3750)
- Timofeev SF. 2001 Bergmann's principle and deep-water gigantism in marine crustaceans. *Biol. Bullet. Russian Acad. Sci.* **28**, 646–650. (doi:10.1023/a:1012336823275)
- Cushman JH, Lawton JH, Manly BFJ. 1993 Latitudinal patterns in European ant assemblages: variation in species richness and body size. *Oecologia* **95**, 30–37. (doi:10.1007/bf00649503)
- Sota T, Takami Y, Kubota K, Ishikawa R. 2000 Geographic variation in body size of some Japanese *Leptocarabus* species (Coleoptera, Carabidae): the 'toppled-domino pattern' in species along a geographic cline. *Entomol. Sci.* **3**, 309–320.
- Belk MC, Houston DD. 2002 Bergmann's rule in ectotherms: a test using freshwater fishes. *Am. Nat.* **160**, 803–808. (doi:10.1086/343880)
- Estlander S et al. 2017 Latitudinal variation in sexual dimorphism in life-history traits of a freshwater fish. *Ecol. Evol.* **7**, 665–673. (doi:10.1002/ece3.2658)
- Adams DC, Church JO. 2008 Amphibians do not follow Bergmann's rule. *Evolution* **62**, 413–420. (doi:10.1111/j.1558-5646.2007.00297.x)
- Ashton KG. 2002 Do amphibians follow Bergmann's rule? *Can. J. Zool.* **80**, 708–716. (doi:10.1139/z02-049)
- Ashton KG, Feldman CR. 2003 Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. *Evolution* **57**, 1151–1163.
- James AC, Azevedo RB, Partridge L. 1997 Genetic and environmental responses to temperature of *Drosophila melanogaster* from a latitudinal cline. *Genetics* **146**, 881–890. (doi:10.1093/genetics/146.3.881)
- Díaz M, Möller AP, Flensted-Jensen E, Grim T, Ibáñez-Álamo JD, Jokimäki J, Markó G, Tryjanowski P. 2013 The geography of fear: a latitudinal gradient in anti-predator escape distances of birds across Europe. *PLoS ONE* **8**, e64634. (doi:10.1371/journal.pone.0064634)
- Zhang L, Liu J, Zhang H, Wan D, Liang W, Möller AP. 2020 Fight or flight: geographic variation in antipredator defenses by cinereous tits. *Glob. Ecol. Conserv.* **24**, e01207. (doi:10.1016/j.gecco.2020.e01207)
- Laurila A, Lindgren B, Laugen AT. 2008 Antipredator defenses along a latitudinal gradient in *Rana temporaria*. *Ecology* **89**, 1399–1413. (doi:10.1890/07-1521.1)
- Miyatake T, Tabuchi K, Sasaki K, Okada K, Katayama K, Moriya S. 2008 Pleiotropic antipredator strategies, fleeing and feigning death, correlated with dopamine levels in *Tribolium castaneum*. *Anim. Behav.* **75**, 113–121. (doi:10.1016/j.anbehav.2007.04.019)
- Sokoloff A. 1977 *The biology of tribolium with special emphasis on genetic aspects*, vol. 3. Oxford, UK: Clarendon Press.
- Abe MS, Matsumura K, Yoshii T, Miyatake T. 2021 Amplitude of circadian rhythms becomes weaken in the north, but there is no cline in the period of rhythm in a beetle. *PLoS ONE* **16**, e0245115.
- Matsumura K, Miyatake T. 2015 Differences in attack avoidance and mating success between strains artificially selected for dispersal distance in *Tribolium castaneum*. *PLoS ONE* **10**, e0127042. (doi:10.1371/journal.pone.0127042)
- R Core Team 2021. R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. See <http://www.R-project.org/>.
- Ohba SY, Matsuda I. 2021 Geographical variation of death-feigning in *Appasus japonicus* (Heteroptera: Belostomatidae). *Rostraria: Trans. Hemipterol. Soc. Japan* **66**, 22–25.
- Prohammer LA, Wade MJ. 1981 Geographic and genetic variation in death-feigning behavior in the flour beetle, *Tribolium castaneum*. *Behav. Genet.* **11**, 395–401. (doi:10.1007/BF01070822)
- Honma A, Oku S, Nishida T. 2006 Adaptive significance of death feigning posture as a specialized inducible defence against gape-limited predators. *Proc. R. Soc. B* **273**, 1631–1636. (doi:10.1098/rspb.2006.3501)
- Miyatake T, Nakayama S, Nishi Y, Nakajima S. 2009 Tonicly immobilized selfish prey can survive by sacrificing others. *Proc. R. Soc. B* **276**, 2763–2767. (doi:10.1098/rspb.2009.0558)
- Holmes S. 1906 Death-feigning in *Ranatra*. *J. Comp. Neurol. Psychol.* **16**, 200–216. (doi:10.1002/cne.920160305)
- Miyatake T, Okada K, Harano T. 2008 Negative relationship between ambient temperature and death-feigning intensity in adult *Callosobruchus maculatus* and *Callosobruchus chinensis*. *Physiol. Entomol.* **33**, 83–88. (doi:10.1111/j.1365-3032.2007.00607.x)
- Kudo T, Hasegawa E. 2022 Relationship between the duration of death-feigning and temperature in black and red elytral types of a polymorphic ladybug, *Harmonia axyridis*. *J. Insect Behav.* **35**, 11–16. (doi:10.1007/s10905-022-09795-3)
- Matsumura K. 2023 Data from: Latitudinal cline of death-feigning behaviour in a beetle (*Tribolium castaneum*). Dryad Digital Repository. (doi:10.5061/dryad.ksn02v78j)
- Matsumura K, Miyatake T. 2023 Latitudinal cline of death-feigning behaviour in a beetle (*Tribolium castaneum*). Figshare. (doi:10.6084/m9.figshare.c.6472261)