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Animal behaviour

Anti-predator behaviour depends on male weapon size

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Tonic immobility and escape are adaptive anti-predator tactics used by many animals. Escape requires movement, whereas tonic immobility does not. If anti-predator tactics relate to weapon size, males with larger weapons may adopt tonic immobility, whereas males with smaller weapons may adopt escape. However, no study has investigated the relationship between weapon size and anti-predator tactics. In this study, we investigated the relationship between male weapon size and tonic immobility in the beetle *Gnathocerus cornutus*. The results showed that tonic immobility was more frequent in males with larger weapons. Although most studies of tonic immobility in beetles have focused on the duration, rather than the frequency, tonic immobility duration was not affected by weapon size in *G. cornutus*. Therefore, this study is the first, to our knowledge, to suggest that the male weapon trait affects anti-predator tactics.

1. Introduction

Sexual selection is selection pressure for traits related to reproduction [1,2]. Because male–male combat occurs for copulation with females, sexual selection often favours males with larger weapons, which lead to victory in combat with rival males [2,3]. However, weapon size varies among males, and males may adopt alternative reproductive tactics depending on weapon size. For example, males with larger weapons often fight rival males, while males with smaller weapons adopt sneaking or dispersal strategies, for copulation with females [4–6].

Sexual dimorphism often affects predation risk, and males suffer higher predation pressure than do females in many animals [7–9]. For example, male Japanese rhinoceros beetles (*Trypoxylus dichotomus*) with larger weapons fall prey to more predators than do males with smaller weapons [9]. Moreover, males with larger weapons may be more aggressive against predators, not only rival males. Therefore, anti-predator strategies or tactics (phenotypic) may have coevolved with male weapon size. However, few studies have investigated the relationship between male weapon size and anti-predator tactics (but see [10]).

Tonic immobility, sometimes called thanatosis or death feigning, and escape are adaptive anti-predator strategies used by many animals [11,12]. More mobile individuals often adopt escape strategies, whereas less mobile individuals adopt tonic immobility [13]. That is, alternative anti-predator strategies may depend on mobility [13,14]. In males, alternative anti-predator tactics may be associated with weapon size. Because movement is often costly for males with larger weapons [4,15], these males may adopt tonic immobility rather than escape, as compared with males with smaller weapons. However, no study to our knowledge has investigated the relationship between male weapon size and anti-predator behaviour.

As an anti-predator behaviour, the frequency and duration of tonic immobility are important. Many studies of various beetle species have focused on the duration of tonic immobility [16–18]. However, the adaptive duration of tonic immobility may vary by predator species or density, and individuals that remain motionless for long durations will be at increased risk of being carried off by ants and predated [19]. Therefore, predation pressure may be affected by the frequency, and not only the duration, of tonic immobility.

This study examined male broad-horned flour beetles, *Gnathocerus cornutus* (Fabricius). Male *G. cornutus* have an exaggerated mandible that is often used as a weapon in male–male combat for copulation with females [20]. Males adopt alternative reproductive strategies based on mandible size: males with larger mandibles often combat rival males, whereas males with smaller mandibles often adopt dispersal to search for females [6]. Furthermore, male weapon size is correlated with mobility measured using a tracking system, such that males with larger mandibles are less mobile than males with smaller mandibles [20]. First, we conducted predation experiments using the jumping spider (*Hasarius adansoni* Audouin) as a model predator to test whether tonic immobility of *G. cornutus* is an adaptive anti-predator behaviour. We also investigated the relationship between weapon size and tonic immobility in *G. cornutus*.

We hypothesized that males with larger mandibles and lower mobility are more likely to adopt tonic immobility, whereas males with smaller mandibles and greater mobility are more likely to escape. We tested this hypothesis in male *G. cornutus*.

2. Material and methods

The *G. cornutus* individuals used in this study were obtained from a laboratory population reared in an incubator (Sanyo, Japan) maintained at 25°C under a 16 h/8 h light/dark cycle (7.00 lights on, 23.00 lights off) with food (19 : 1 wholemeal flour : brewer's yeast). This beetle was originally collected from a wild population in Japan, in June 1957 (see [20] for details).

The predation experiment used female *H. adansoni* ($n = 10$) captured indoors as model predators. This experiment followed a previous study [16]. One starved female *H. adansoni* and one male *G. cornutus* were placed in an 80 mm diameter, 20 mm high Petri dish and observed for 15 min. The experiment was replicated using 10 different predator–prey pairs.

Virgin 14–21-day-old *G. cornutus* males ($n = 192$) were randomly collected from the laboratory population, and we measured prothorax width and mandible length (figure 1) as indicators of body size and weapon size, respectively [20]. Each trait was measured to the nearest 0.01 mm using a dissecting microscope (VM-60; Olympus, Japan). To evaluate the effects of relative weapon size, we also used the residual mandible length for the analysis after regressing mandible length on prothorax width.

We observed the frequency and duration of tonic immobility in the male *G. cornutus* according to the method of Miyatake *et al.* [16]. To avoid interference from other beetles, each male was placed in a 17 mm diameter, 20 mm high plastic container with food until the observations. Each beetle was moved gently onto a small plate, and tonic immobility was induced by touching the abdomen of the beetle with a thin stick. When the beetle showed tonic immobility, we recorded its duration using a stopwatch. The duration of tonic immobility was defined as the length of time between touching the beetle and detecting its first movement. If the beetle did not respond, the stimulus was repeated up to three times. When the beetle showed tonic immobility, the duration was recorded. If the beetle was unresponsive to these stimulations, it was recorded as a non-responsive individual (see electronic supplementary material). All observations were conducted from 12.00 to 18.00 in a room maintained at 25°C.

The frequency of tonic immobility was analysed using a generalized linear model (GLM) with a binomial distribution and log link function, using prothorax width, absolute mandible length and residual mandible length as explanatory variables. To analyse the duration of tonic immobility, we used a GLM with a gamma

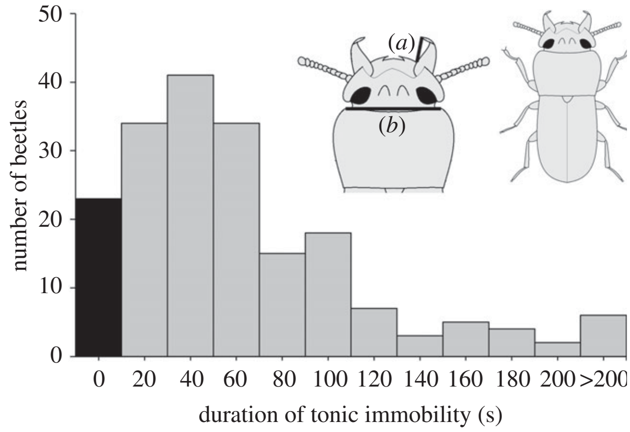

Figure 1. Distribution of the duration of tonic immobility in males of *Gnatocerus cornutus*. For example, bar '20' shows the number of beetles with a 1–20 s duration of tonic immobility. The black-filled bar shows beetles that did not exhibit tonic immobility. The straight lines in the beetle illustration indicate the mandible length (*a*) and prothorax width (*b*).

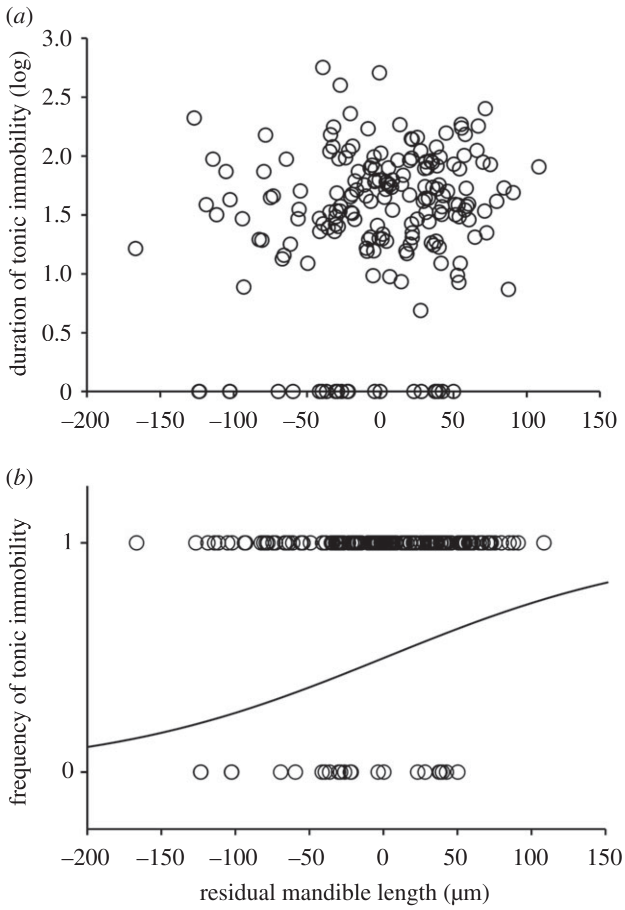

Figure 2. Relationships of mandible length with the duration (*a*) and frequency (*b*) of tonic immobility in *G. cornutus*. In (*b*), '1' indicates beetles with tonic immobility and '0' beetles without tonic immobility.

Table 1. Results of GLMs evaluating the effects of prothorax width, absolute mandible length, and residual mandible length on the frequency and duration of tonic immobility in *G. cornutus*. Values in bold are statistically significant ($p < 0.05$).

tonic immobility	factor	d.f.	χ^2	*p*-value
frequency	prothorax width	1	0.72	0.3977
	error	190		
	absolute mandible length	1	6.23	**0.0126**
	error	190		
	residual mandible length	1	6.10	**0.0136**
	error	190		
duration	prothorax width	1	0.57	0.4499
	error	190		
	absolute mandible length	1	0.00	0.9709
	error	190		
	residual mandible length	1	0.37	0.5406
	error	190		

distribution, using prothorax width, absolute mandible length and residual mandible length as explanatory variables. All analyses were conducted using R v. 3.4.3 [21].

3. Results

In all the 10 cases, the spider attacked the beetle when the beetle approached. The spider did not eat the beetle immediately, but observed its response for a while. If the prey showed tonic immobility after the spider attack, the predator lost interest after several seconds and the prey survived ($n = 8$ of 10; see electronic supplementary material, movie S1). If the beetle struggled with the spider or moved, the spider attacked it again, and beetle was eaten ($n = 2$ of 10; see electronic supplementary material, movie S2).

Figure 1 shows the distribution of tonic immobility duration in *G. cornutus*. Many beetles had shorter durations, while a few had longer durations (range 0–563.31 s, mean ± s.d. = 58.44 ± 73.27 s). Figure 2 shows the relationship between residual mandible length and the duration (figure 2*a*) or frequency (figure 2*b*) of tonic immobility. The GLMs did not show a significant correlation between the duration of tonic immobility and any explanatory variable, whereas the tonic immobility frequency was significantly correlated with mandible length and residual mandible length, but not prothorax width (table 1). Tonic immobility was more frequent in males with relatively long mandibles.

4. Discussion

When *G. cornutus* encountered *H. adansoni*, beetles that showed tonic immobility survived the attack by the predator. In *G. cornutus*, males with longer mandibles performed tonic immobility significantly more frequently than did males with shorter mandibles. This suggests that males with longer mandibles adopt tonic immobility as an anti-predator tactic. Tonic immobility does not necessitate movement. Studies of *G. cornutus* found that longer-mandible males were less mobile than shorter-mandible males [6,22]. Therefore, because mandible size is correlated with mobility in males, anti-predator tactics may differ between males with longer and shorter mandibles in this beetle. This is the first study to our knowledge to report a relationship between a male weapon trait and anti-predator behaviour in insects.

The development of mandible traits and fighting behaviour requires many resources [4,23–25]. Because tonic immobility does not consume energy compared with moving escape tactics [26], tonic immobility may be the optimal tactic for males with larger mandibles. Moreover, larger weapons may have higher physiological costs and consume more resources during development. Therefore, this correlation may also be an adaptive strategy for resource allocation.

The duration of tonic immobility was not correlated with mandible length. In many beetle studies, tonic immobility duration was considered an important anti-predator behaviour [16,27]. However, a longer duration of tonic immobility also increases physiological costs [28] and the risk of being discovered and carried off by ants [19]. Because we found that weapon size is correlated with the frequency but not duration of tonic immobility, the importance of the frequency of tonic immobility should be evaluated as an anti-predator strategy (see [29]).

Because male *G. cornutus* with shorter mandibles are more mobile [6,22], they may adopt an escape tactic rather than tonic immobility. Additional studies are needed to investigate anti-predator behaviour in shorter-mandible males. Although we expected that larger weapons lead to an increased predation risk, in some cases, the size or existence of weapons may have a positive relationship with predator defence [10]. Coevolution between weapon and predation avoidance may differ among species.

Ethics. The research conducted by the authors has been approved by the research ethics review of Kagawa University (AP0000353996) and Okayama University (no. 6433471). The beetle (*G. cornutus*) and the jumping spider (*H. adansoni*) used in this study are invertebrates and therefore not subject to animal ethics review. The study was conducted in a manner that avoided or minimized discomfort or distress to the laboratory animals, and efforts were made to ensure that the animals did not suffer unnecessarily at any stage of the experiment. We also used a sample size that minimizes the number of animals that experience discomfort or distress but is not statistically problematic in predation experiments. The *G. cornutus* beetle culture has been maintained in the laboratory of the National Food Research Institute, Japan

for 50 years. They have been maintained at Okayama University for over 15 years. This population has been kept on wholemeal flour with yeast. We reared this population at 25°C, which is similar to the natural conditions for this insect. All animals in the study were handled carefully. The *H. adansoni* spiders used in the predation experiments were collected from Kagawa University and Okayama University premises using appropriate methods. After completion of the experiment, the animals were reared with food in an environment similar to the natural conditions for this spider.

Data accessibility. Movies of the predation experiment are provided as electronic supplementary material. Data are available in the Dryad Digital Repository: https://doi.org/10.5061/dryad.8gtht76mt [30].

Authors' contributions. K.M. conceived and designed the study. K.Y. performed the experiment. Y.F. performed analyses. All authors contributed to the interpretation of data. K.M. and T.M. drafted the manuscript. All authors critically revised the manuscript for important intellectual content. All authors approved the final version and agree to be accountable for the content therein.

Competing interests. We declare we have no competing interests.

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