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Body-Size Dependent Difference in Death-Feigning Behavior of Adult *Callosobruchus chinensis*

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Size-dependent differences in death-feigning behavior, an anti-predator defense, was examined in the adult adzuki bean beetle, Callosobruchus chinensis (Coleoptera: Bruchidae). A positive correlation was observed between the duration of death-feigning and adult body weight in two replicated experiments. Moreover, under starved condition, beetles reared under high (=lighter beetles) larval density showed significantly shorter longevity and a shorter duration of death-feigning than those reared under low (=heavier beetles) larval density. The body-size-dependent difference in the duration of death-feigning is discussed from the viewpoint of the difference in survival strategies between small and large beetles. Because small beetles don't live as long, they have less time in which to reproduce and so any loss of that short time might be a greater proportion of their fitness lost. This is the first report on the body-size-dependent difference in death-feigning behavior in the adult holometabolic beetle.

KEY WORDS: anti-predatory behavior; body weight; larval density; thanatosis; tonic immobility.

INTRODUCTION

Predator evading responses in animals tend to be related to their physiological and environmental conditions (Lima and Dill, 1990; Evans and

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Schmidt, 1990). In some animals, anti-predator behavior is dependent on body size during growth; for example, lobsters exhibit an ontogenic shift in escape and defense behavior during growth (Lang *et al.*, 1977; Wahle, 1992), and stage-specific responses in defensive behavior have been reported in a grasshopper species (Danner and Joern, 2003). These studies describe stage-specific (=growth-dependent) differences in animal defense, whereas adults of some insect species cannot alter body size after eclosion. Therefore, the insect's fitness might be determined by the acquisition of nutrition during the larval stage if the body-size-dependent difference relates to their survival strategies. Body-size-dependent behavioral variation is frequently observed in adult insects during courtship and in male-male competition (e.g., Blum and Blum, 1979; Thornhill and Alcock, 1983; Simmons, 1986). To our knowledge, however, no studies have been conducted on the relation between body size at adult stage and anti-predatory behavior in holometabolic insect species, where adult body size is determined by the acquisition of nutrients during the larval stage, and no development is observed during the adult stage. We examined the effect of larval crowding, and hence adult body size on death-feigning (=an anti-predatory behavior) in a holometabolic beetle, the adzuki bean beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae).

Death-feigning (thanatosis), a state of tonic immobility assumed by many animals in response to external stimuli, is a defense mechanism against predators shared by diverse insect groups including beetles (Fabre,1900; Bleich, 1928; Frost, 1959; Chemsak and Linsley, 1970; Prohammer and Wade, 1981; Allen, 1990; Oliver, 1996; Acheampong and Mitchell, 1997; Miyatake, 2001a,b; Miyatake *et al.*, 2004), butterflies (Dudley, 1989; Larsen, 1991), moths (Blest, 1964; Tojo *et al.*, 1985), mantids (Edmunds, 1972), stick insects (Godden, 1972; Carlberg, 1986), a water-scorpion bug (Holmes, 1906), a stone fly (Moore and Williams, 1990) a cricket (Nishino and Sakai, 1996), a cicada (Villet, 1999) and a bee (van Veen *et al.*, 1999).

To date many descriptive studies have been conducted on individual variation in death-feigning behavior (DuPorte, 1916; Bleich, 1928). Some of its parameters, such as occurrence and duration of death-feigning, are affected by environmental factors including temperature (Holmes, 1906; Larsen, 1991) and light-dark conditions (Miyatake, 2001a), and physiological factors such as starvation (Acheampong and Mitchell, 1997; Miyatake, 2001b) and the behavior before a beetle was startled (Miyatake, 2001a). However, there is no study focused on the relation between body size and death-feigning behavior. This study is aimed at clarifying the effects of body weight on the duration of death-feigning (=recovery time) of adult *C. chinensis* in the laboratory.

MATERIALS AND METHODS

Culture and Insects

The beetle, *C. chinensis*, culture used in the present experiments is a laboratory population established by Utida (Utida, 1941a,b) and maintained for more than 60 years. It is referred to as the jC strain (Shimada, 1990). Before conducting our experiments, this population was maintained on adzuki beans *Vigna angularis*, under 25°C, 60% RH, and 14L10D conditions. All the experiments were conducted in a chamber (Sanyo, Tokyo) maintained at 25°C and 60% RH under a photoperiod of 14:10 (L:D) h (lights on at 0800, lights off at 2200).

Observation of Death-Feigning

Death-feigning behavior was induced by touching the abdomen of the beetle with the observer's forefinger. Death-feigning adults rolled over on their backs and lay immobile with the legs closely drawn up against the body. A trial consisted of provoking the behavior and recording its duration (the length of time between touching the beetle and detecting its first visible movement) with a stopwatch. If the beetle did not respond, the touch was repeated. No response after two stimuli was very infrequent (2.3%, n = 1492, N. Hozumi, unpublished data); therefore, we have not adopted the trait "frequency of death-feigning" described as another behavioral trait of feigning death at population level for another beetle (Miyatake, 2001a,b; Miyatake *et al.*, 2004). All the trials were conducted between 0830 and 1100 in the chamber mentioned above.

The Relation between Body Weight and the Duration of Death-Feigning

The relation between body weight and the duration of death-feigning was examined twice (replicates 1 and 2) in beetles reared under two different larval densities. Females collected randomly from the stock culture were allowed to lay 10–15 eggs and more than 25 eggs on an adzuki bean in replicates 1 and 2, respectively, to induce variation in the size of beetles. Each adzuki bean was transferred to one well of a 48-well tissue culture plate (Falcon, Becton Dickinson and Company, NJ, USA) before the adults emerged from the bean; virgin females and males were then collected. The adults were weighed individually to the nearest 0.01mg on an electronic balance (Genius ME215S, Sartorius, Goettingen, Germany) within 36 hours of emergence and observed for the death-feigning behavior on the second day after emergence. ANCOVA (SAS Institute, 1998) was used to analyze the relation between body weight and the duration of death-feigning with sex as covariate.

Effects of Rearing Density on Body Weight, Longevity, and Duration of Death-Feigning

The females were allowed to lay less than 5 eggs (low density) and more than 25 eggs (high density) on an adzuki bean to obtain heavier and lighter populations, respectively. Adults (40 males and 40 females) were randomly collected from each population, weighed individually as described above, and then observed for the death-feigning behavior on the second day after emergence. The beetles were reared without water or food, and survival was checked every day until death. All data for body weight, longevity under starvation, and duration of death-feigning were analyzed using ANOVA (SAS Institute, 1998), with rearing density as a random effect and sex a fixed effect.

RESULTS

The Relation between Body Weight and the Duration of Death-Feigning

The body weight of the beetles correlated positively with the duration of death-feigning in the two replications (Figs. 1 and 2 for the replicates 1 and 2, respectively). ANCOVA showed a significant effect of body weight on the duration of death-feigning in both replicates ($p < 0.0001$), but no relation was found between sex and the duration, and no interaction between the effect of body weight and sex in either replicates (Table I).

Effects of Rearing Density on Body Weight, Longevity, and Duration of Death-Feigning

Highly significant differences were observed in body weight between the two populations reared in the two different densities (Tables II and III). Heavier beetles (reared in the low larval density) demonstrated significantly longer longevity and duration of death-feigning than lighter populations reared in the high density, regardless of sex (Tables II and III). Significant associations were observed between sex and the three characteristics; females demonstrated heavier body weight, longer longevity, and longer duration of death-feigning than males (Tables II and III). Significant interaction effects were observed between rearing density

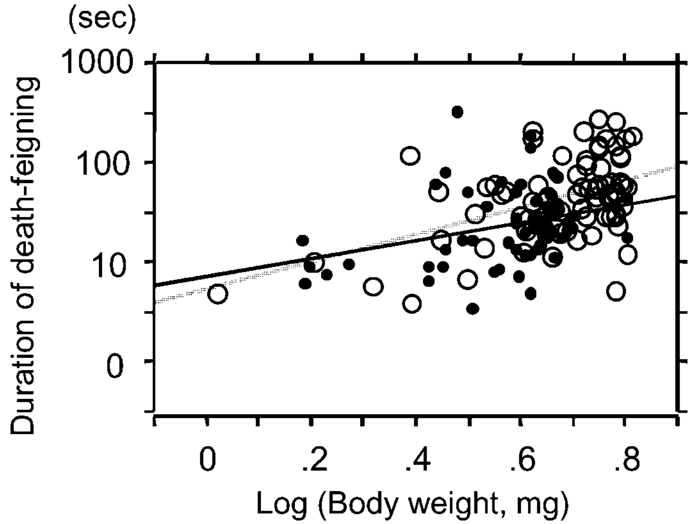

Fig. 1. Relation between adult body weight and duration of death-feigning in *Callosobruchus chinensis*; replicate 1. Closed and open circles show males and females, respectively. Solid and broken lines are fitted regression lines for males and females, respectively.

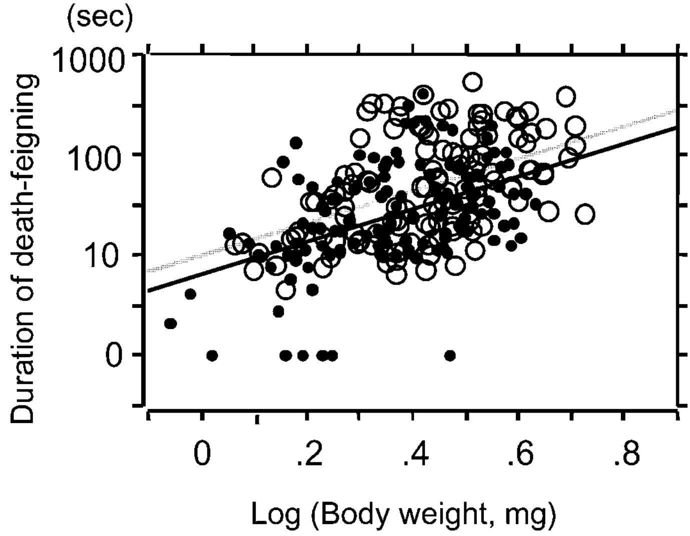

Fig. 2. Relation between adult body weight and duration of death-feigning in *Callosobruchus chinensis*; replicate 2. Closed and open circles show males and females, respectively. Solid and broken lines are fitted regression lines for males and females, respectively.

Table I. ANCOVA Test Results for Relation Among Body Weight, Sex, and the Duration of Death-Feigning

Experiment	Source	SS	*df*	*F*	*p*
1	Body weight	3.42	1	25.45	<0.0001
	Sex	0.03	1	0.20	0.6537
	Body weight × Sex	0.15	1	1.13	0.2896
	Error	18.26	136		
2	Body weight	14.60	1	69.20	<0.0001
	Sex	0.27	1	1.25	0.2640
	Body weight × Sex	0.00	1	0.00	0.9779
	Error	51.57	244		

and sex with regard to body weight and longevity, but not to the duration of death-feigning (Table III).

DISCUSSION

The results showed clear and positive correlations between the duration of death-feigning and body weight in *C. chinensis*, raising some questions about the adaptive significance of the death-feigning behavior. If the longer duration of death-feigning is effective in escaping from predators, a plausible reason has to be found as to why the duration of death-feigning is shorter in small beetles than in larger ones. It will be intriguing to discuss how the decrease in thanatosis duration may be adaptive in smaller beetles. Shorter longevity was found in smaller than larger beetles. Therefore, because small beetles don't live as long, they have less time in which to reproduce and so any loss of that short time might be a greater proportion of their fitness lost. In other words, the cost of long-duration thanatosis may increase with decreasing body size.

In insects, the difference in adult body size relates to different adaptive strategies and behavior during their life; for example, larval rearing density

Table II. Means (SDs) for Body Weight, Longevity, and the Duration of Death-Feigning in Male and Female Adults Reared in Low and High Larval Densities

Sex	Characteristics	Larval rearing density	
		Low	High
Males ($n = 40$)	Body weight (mg)	4.32 (0.27)	1.87 (0.39)
	Longevity (days)	13.25 (1.06)	7.05 (1.93)
	Duration of death-feigning (sec)	53.14 (39.50)	13.05 (10.89)
Females ($n = 40$)	Body weight (mg)	5.63 (0.40)	1.80 (0.30)
	Longevity (days)	14.80 (1.98)	7.33 (1.82)
	Duration of death-feigning (sec)	86.45 (52.94)	27.20 (23.80)

Table III. ANOVA Test Results for Body Weight, Longevity, and the Duration of Death-Feigning

Characteristic	Source	SS	*df*	*F*	*P*
Body weight	Rearing density (RD)	394.32	1	3328.17	< 0.0001
	Sex	15.34	1	129.46	< 0.0001
	RD × Sex	19.34	1	163.19	< 0.0001
	Error	18.48	156		
Longevity	Rearing density (RD)	1870.06	1	1870.06	< 0.0001
	Sex	33.31	1	11.04	0.0011
	RD × Sex	16.26	1	5.39	0.0216
	Error	470.58	156		
Duration of death-feigning	Rearing density (RD)	98703.23	1	78.21	< 0.0001
	Sex	22518.35	1	17.84	< 0.0001
	RD × Sex	3673.36	1	2.91	0.0900
	Error	193868.72	156		

influences adult dispersal (Dingle *et al.*, 1980; Sasaki *et al.*, 2002) and the volume of sperm ejaculated into females (Gage, 1995). Also, size-dependent variation is frequently observed in insect behavior such as courtship or male-male competition for resource or territory (e.g., Blum and Blum, 1979; Thornhill and Alcock, 1983). The present results suggest that larval rearing density influences anti-predator behavioral responses that may have a direct effect on fitness.

Another explanation for the body-size-dependent difference in the duration of death-feigning is related to physiological constraints attributable to body weight. Generally, animal body size influences physiological, behavioral, and reproductive traits (Peters, 1983). Analyzing the relation between energy demands and death-feigning behavior is worthy of future studies.

In *C. chinensis*, few beetles failed to respond to stimuli intended to induce death-feigning. Consequently, we were not able evaluate the effect of body weight on the occurrence of death-feigning in this beetle. The frequency rather than the duration of death-feigning might be more crucial from the viewpoint of the fitness of death-feigning beetles. Studies are needed on the effect of body weight in other beetles, such as the sweet potato weevil *Cylas formicarius* and the red flour beetle *Tribolium castaneum,* with large variations in the frequency of death-feigning at population level (Miyatake, 2001a,b; Miyatake *et al.*, 2004).

A shorter duration of death-feigning is observed in starved than in unstarved Colorado potato beetles *Leptinotarsa decemlineata* (Acheampong and Mitchell, 1997) and *Cylas formicarius* (Miyatake, 2001b). Starved beetles may have less resource-energy in their bodies. Probably, smaller beetles also have less resource-energy than larger ones; thus the lesser energy in the body might relate to the shorter duration of death-feigning. Nevertheless, the relation between death-feigning and energy consumption and that between death-feigning and the metabolic rate are not yet known. The present results also showed that under starved conditions the longevity of smaller beetles was shorter than that of larger ones (Table II), suggesting an association among starvation, longevity and the death-feigning behavior.

No significant effect of sex was found on the duration of death-feigning in the first experiment (Table I). In second experiment, however, significantly longer duration of death-feigning was detected in females than in males, evidencing a highly significant association between sex and the duration of death-feigning (Tables II and III). The reason for the different results, from the two experiments by different methods, could be due to its low sample size of very small beetles in the first experiment. Females also showed heavier body weight and longer longevity than males (Tables II and III). Also in the sweet potato weevil, *Cylas formicarius*, under starved conditions females show longer longevity than males, and the difference of sex has been observed in the relation between the duration of death-feigning and starvation (Miyatake, 2001b). Acheampong and Mitchell (1996), on the other hand, have shown no relation between the duration of death-feigning and sex in *Leptinotarsa decemlineata*. Further and more systematic studies are needed on the effect of sex on the death-feigning behavior.

In conclusion, the present study shows that the positive correlation between body weight and the duration of death-feigning in *C. chinensis* implies the necessity of including the effect of body size in future studies on death-feigning behavior. Investigation of positive correlations in other organisms are also needed to explore the generality of size-dependent-difference in the death-feigning behavior.

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