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**Variation in counterattack effect against a phytoseiid predator between
two forms of the social spider mite, *Stigmaeopsis miscanthi***

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Counterattack

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Abstract

In Japan *Stigmaeopsis miscanthi* (Saito) occurs in two forms that are differentiated by the intensity of male-to-male aggression, i.e. there is a low aggression and a high aggression group (known as the LW and HG forms respectively). The effects of counterattack behavior against predators were experimentally compared between the two forms. Parental males and females of both forms could achieve significant counterattack success against *Typhlodromus bambusae*, a specific predator, and counterattack efficiency increased significantly with parental density. Furthermore, the HG form showed a stronger tendency than the LW form to kill predator larvae. Thus variation in counterattack success may exist between these two forms, and there is some correspondence between male-to-male belligerence and counterattack effectiveness against predators.

Introduction

Although prey species counterattacking against their natural enemies is a relatively common phenomenon in the animal kingdom (Brodie et al 2002; Wignall and Taylor 2009), instances of prey directly and regularly killing their predators are still relatively scarce. One rare example is when mature fish eat the predatory plankton that pose a threat to their young, a phenomenon known as an “inverse trophic relationship in the hydrosphere” (Garcia and Alejandre 1995).

Several species within the genus *Stigmaeopsis* (Tetranychidae) show sociality (Saito 2010). Saito (1986a,b) reported a biparental defense system in *Stigmaeopsis longus* (Saito) against *Typhlodromus bambusae* Ehara, a specific natural enemy of *Stigmaeopsis* species. The counterattack behavior of *S. longus* becomes increasingly effective if more than one adult inhabits the same nest, thus the group living guaranteed by large nests is considered to be adaptive (Mori and Saito 2004, 2005; Saito 2010). Following these studies, Zhang et al. (2001) discovered similar counterattack behavior in *Stigmaeopsis nanjingensis* (Ma et Yuan), and Mori and Saito (2004) observed weak counterattack behavior against *T. bambusae* in *Stigmaeopsis celarius* (Banks), both close relatives of *S. longus* (Saito et al. 2004, Sakagami et al. 2009).

Stigmaeopsis miscanthi has also been shown to possess a comparable sociality with biparental defense against *T. bambusae* (Saito 1990), but the effect(s) of its counterattack behavior have never been evaluated experimentally. In addition, this species consists of two forms that show different intensities of male-to-male aggression (hereafter, the low aggression form is referred to as the LW form and the high aggression form as the HG form, in accordance with Saito 1995; Saito and Sahara 1999; Sato et al. 2008). This difference may be caused by the effect of cooperative male defense (i.e. counterattack) and the relatedness of the confronting males (Saito 2010). However, whether there is a difference in counterattack efficiency between the two forms of *S. miscanthi* remains undetermined. Here we evaluated the counterattack efficiency of *S. miscanthi* against *T. bambusae* under experimental conditions similar to Saito (1986b).

Materials and methods

Mites

The *S. miscanthi* populations used in the present experiments were collected on June 15, 2003 from *Miscanthus sinensis* in the Tobuko (HG form) and Mt. Unzen (LW form) regions of Nagasaki Prefecture, Japan.

The phytoseiid mite *T. bambusae* (known to be an important predator of *S. miscanthi* in China, Tsuji et al. 2010) used in this study was collected on May 10, 2003 from *S. longus* nests on *Sasa senanensis* (Franch. et Sav.) in Sapporo, Japan. *T. bambusae* was reared on *M. sinensis* leaves using *S. miscanthi* as prey under conditions of $25\pm 2^{\circ}\text{C}$, 40-70% r.h. and a photoperiod of 15L-9D.

Both the LW and HG forms were reared for at least four generations (ca. a half year) on detached *M. sinensis* leaves (as described in Saito 1990) prior to the experiments under the same conditions as *T. bambusae*. The two *S. miscanthi* forms (LW and HG) were distinguished by the criterion of relative leg length (I leg length/ III leg length) in accordance with Sato et al. (2008).

The *M. sinensis* host plants used for rearing and experiments were originally collected in Nagasaki Prefecture. The plants were cultivated in plastic containers

(40cm x 50cm x 30cm) in a glasshouse and supplied with only water and ammonium sulfate fertilizer.

Experiments

To evaluate how adult density affects counterattack success against *T. bambusae* in the two *S. miscanthi* forms, experiments were carried out using the undersides of detached *M. sinensis* leaves (facing upward) that included the midrib. Arenas were prepared by surrounding 2 cm x 1 cm leaf sections with water-soaked cotton strips. Both forms of *S. miscanthi* were introduced onto each experimental arena in the combinations shown in Table 1 and kept at 25±1°C, 50 -70% r.h. and a photoperiod of 15L-9D. After the adult females had constructed their nests and laid ca. 10 eggs in them (4-5 days after introduction), the numbers of prey adults were arranged as listed in Table 1 by removing all surplus individuals other than eggs. Then a single predator larva (at most one-day old after hatching) was introduced onto each experimental arena and the interaction between prey and predator was observed for 2 days. Any predators that

disappeared from the experimental arenas, usually due to drowning in the water-soaked cotton, were omitted from the data analyses.

Statistics

Actual counterattack success (driving away + killing) was analyzed as follows: A logistic model (GLM with binomial distribution) was constructed, in which the response variable was counterattack success, and explanatory variables were parental attendance (0 or 1 mite parent, nominal variable), parental form (HG or LW, nominal variable), and parental sex (female or male, nominal variable). Another logistic model using parental density (1, 2 or 4 mite parents, continuous variable) as one of the explanatory variables instead of parental attendance was also constructed. In the model using parental attendance, the data set from the control and the treatments of one mite per nest were used (Lf1, Lfc, Lm1, Lmc, Hf1, Hfc, Hm1 and Hmc; Table 1). In the model using parental density, the data sets from the treatments of one, two and four LW and HG form female mites per nest, and one and two LW form male mites per nest were used (Lf1, Lf2, Lf4, Hf1, Hf2, Hf4, Lm1 and

Lm2; Table 1). The effect of each explanatory variable was tested by the likelihood ratio test to compare the model with the explanatory variable to the model without it (LRT, Faraway 2006).

Killing success was analyzed as follows. Logistic models (GLM with binomial distribution) were constructed in which the response variable was the killing success, and the explanatory variables were the same as in the models used to analyze counterattack success. Since killing behavior wasn't observed in any of the LW form female treatments or one LW form males treatments, the data set from the control and the treatments of the one HG form male and female mite per nest were adopted in the model using parental attendance (Hf1, Hfc, Hm1 and Hmc; Table 1). Since we couldn't prepare any multiple HG males per nest treatments (because of the relentless tendency of HG males to kill each other), the data set from the treatments of one, two and four HG form female mites per nest were adopted in the model using female density (Hf1, Hf2 and Hf4; Table 1). The effect of each explanatory variable was tested in the same way as mentioned previously. These analyses were performed with the statistical package R ver. 2.11 (R Development Team 2010).

In addition to the above, Fisher's exact probability test (two-tailed, abbreviated hereafter as FEP) with sequential Bonferroni adjustments for multiple comparisons (abbreviated as BA) was used to test for differences between treatments when GLMs was difficult to be applied.

Results

Actual counterattack success (driving away + killing)

Actual counterattack success was observed in both forms and both sexes of *S. miscanthi* (Figs. 1 and 2). The effect of parental attendance on the actual rate of counterattack was significant regardless of the sex or form of the parent, and the counterattack success rate was significantly higher in the HG form than in LW form (Figs. 1, 2 and Table 2 (a), parental attendance: $P < 0.001$; form: $P < 0.05$; parental sex: $P = 0.343$).

As shown in Figs. 1 and 2, in both the LW and HG forms, the rate of actual counterattack success significantly increased with parental density regardless of parental

form or sex (Table 2 (b), parental density: $P < 0.01$; form: $P = 0.311$; sex: $P = 0.351$),

suggesting that the actual counterattack success rate is parental density dependent.

Killing success

Killing behavior was not observed in either the LW form male or female in the one mite per nest treatment, but was observed in both the HG form sexes (Figs. 1 and 2). The effect of parental attendance on the killing success rate was significant in the HG form, with males enjoying a significantly higher success rate than females (parental attendance: $P < 0.001$; sex: $P < 0.001$ in Table 3 (a)). The effect of parental density on the killing success rate was significant for the HG form females ($P < 0.05$ in Table 3 (b)).

The effect of cohabiting male and female(s) on the success of nest defense

Saito (1986b) reported a synergetic effect in counterattack success (killing predators) between male and female *S. longus* parents. Whether there is such an effect in either form of *S. miscanthi* was thus observed.

There were no significant differences in killing success between Lf1 and Lf1m1, between Lm1 and Lf1m1, between Lf2 and Lf2m1, between Lm1 and Lf2m1, and between Lf1m1 and Lf2m1 (by FEP with BA, at least $P > 0.3$), suggesting that the addition of extra males or females had no effect on the counterattack success (Fig. 3 and Table 4).

On the other hand, when one HG form male was paired with either one or two females, the actual success rates increased over 80% (Fig. 3). There were significant differences in killing success when single males were added to the nests defended by one or two females (Fig. 3 and Table 4, Hf1 vs. Hf1m1: $P = 0.025$; Hf2 vs. Hf2m1: $P = 0.0124$ by FEP with BA), though no significant differences when single females were added to the nests defended by single males ($P > 0.99$ between Hm1 vs. Hf1m1 and Hm1 vs. Hf2m1 by FEP with BA, Table 4).

Discussion

From the present study, it was apparent that both forms of *S. miscanthi* have the ability to counterattack against phytoseiid predators. In *S. longus*, another social spider mite species that occurs on *Sasa* dwarf bamboo, both males and females enjoyed considerable counterattack success against *T. bambusae* (specifically, 46% of the predator larvae were killed in nests defended by single males, Saito 1986b). In the *S. miscanthi* HG form the successful kill rate (Fig. 2) was similar to that of *S. longus*, whereas in the LW form it tended to be lower than both *S. longus* and the HG form (female and male). Ultimately we could ascertain that four species (one of which is comprised of 2 forms) within the genus *Stigmaeopsis* display effective biparental defense against a specific phytoseiid natural enemy.

On the other hand, we could detect a positive effect of parental density on counterattack success rate in both forms, i.e. the effect of parental attendance on the actual rate of counterattack was significant regardless of the sex or form of the parent. Saito and Takada (2009) provided a new game model that can explain the variation of male aggressiveness as a compromise (ESS) between individual (intra-sexual) and kin

selections from three parameters: the effect of cooperation on nest defense, the relatedness between males and the risk of male-to-male aggression. Among these three parameters, the male defensive success rate must be related to the cooperation effect and the risk of male-to-male aggression in their model. If the males always attempt to exclude each other as in the HG form of *S. miscanthi*, the counterattack effect does not increase with male density, but rather decreases. In this study we revealed that counterattack success rates positively increase with male as well as female density. These facts may provide consistency to the Saito and Takada (2009) game theory model about compromise between male-aggressiveness and male-cooperation, because the existence of male cooperation (including no interference) is one of the necessary conditions for their model.

As male and female(s) cohabitation is an ordinary situation for *S. miscanthi* in the field, understanding the counterattack effect of biparental attendance must be important. Saito (1986b) pointed out that cooperation between the male and female(s) quite possibly enhances nest defense efficiency in *S. longus*. In either form of *S. miscanthi*, female cohabitation showed no such strengthening from the viewpoint of males,

although there were no apparent negative effects of cohabitation either. On the other hand, HG form females must very much welcome male cohabitation in order to increase their offspring fitness, because it significantly enhances nest defense (Table 4). However, there is a conflict that male density can never increase in the HG form, because of the aggressive male-to-male nature. Such a contradiction in desire between the two sexes in nest defense has to be considered to understand the sociality of *S. miscanthi*.

Lastly, the question remains as to why HG form adults display stronger counterattack abilities than LW adults. For example, a single HG form male could kill *T. bambusae* larvae at 48% probability, but LW males never killed any larvae during the experiments ($P < 0.0001$ by FEP, Fig. 2), a consistent trend throughout our experimental results (Figs. 1-3). Saito (2010) discussed how the counterattack effect against predators does not always correspond to the intensity of male-to-male aggression, because *S. longus* males have a very strong counterattack ability against predators yet show no aggressive behavior towards conspecific males (Saito 1990). Saito (2010) went on to stress the importance of distinguishing male attacks against predators from male aggression towards conspecific males. The present results, namely the HG form's strong

male-to-male aggression and high ability to counterattack against predators, appear to contradict his conclusion however. Although we still have no definitive explanation for these phenomena, a recent report that *S. miscanthi* evolved from a common ancestor of *S. longus* through a host plant shift from bamboo to Chinese silvergrass (Sakagami et al. 2009) hints at a possible solution to this question: The ability to counterattack against predators first evolved in a common ancestor of *S. longus* and *S. miscanthi*, prior to the latter developing male-to-male combat tendencies after its host plant shift to Chinese silvergrass. Even so, precisely why the counterattack ability varies between species and forms is still awaiting a definitive explanation.

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Appl Acarol Spec Pub 9: 11-21

Figure legends:

Fig. 1. Counterattack success of female(s) of *S. miscanthi* LW and HG forms against *T. bambusae* larvae. D, driving away from nest; K, killing. Other explanations appear in Table 1.

Fig. 2. Counterattack success of male(s) of *S. miscanthi* LW and HG forms against *T. bambusae* larvae. D, driving away from nest; K, killing. Other explanations appear in Table 1.

Fig. 3. Counterattack success when male(s) and female(s) of *S. miscanthi* LW and HG forms defended nests against *T. bambusae* larvae. D, driving away from nest; K, killing. Other explanations appear in Table 1.

Table 1. Experimental design for evaluating density effect of two forms of *Stigmaeopsis miscanthi* against *Typhlodromus bambusae*.

Exp. No.	No. tests	No. of prey introduced initially		Prey adult combination tested		Note
		Male ¹	Female ¹	Male	Female	
LW form						
Lf1	25	1	4	0	1	
Lf2	25	1	4	0	2	
Lf4	25	1	4	0	4	
Lfc	25	1	4	0	0	Control
Lm1	25	2	4	1	0	
Lm2	25	2	4	2	0	
Lmc	25	2	4	0	0	Control
Lf1m1	25	1	4	1	1	
Lf2m1	25	1	4	1	2	
Lfmc	25	1	4	0	0	Control
HG form						
Hf1	25	1	4	0	1	
Hf2	25	1	4	0	2	
Hf4	25	1	4	0	4	
Hfc	25	1	4	0	0	Control
Hm1 ²	25	1	4	1	0	
Hmc	25	1	4	0	0	Control
Hf1m1	25	1	4	1	1	
Hf2m1	25	1	4	1	2	
Hfmc	25	1	4	0	0	Control

1. Virgin adults were introduced. 2. 2 males experiment (Hm2) could not conducted because of high male-to-male aggressive nature.

Table 2. Results of likelihood ratio test in actual counterattack success.					
(a) the model including parent attendance					
	<i>df</i>	Deviance	AIC	LRT	<i>P</i> -value
<none>		2.882	25.312		
Form	1	8.504	28.934	5.621	<0.05
Parent attendance	1	78.047	98.477	75.164	<0.001
Parent sex	1	3.782	24.213	0.901	0.343
(b) the model including parent density					
	<i>df</i>	Deviance	AIC	LRT	<i>P</i> -value
<none>		1.45	38.319		
Form	1	2.472	37.342	1.023	0.312
Parent density	1	2.319	43.807	7.488	<0.01
Parent sex	1	8.938	37.189	0.870	0.351

Table 3. Results of likelihood ratio test in killing success.					
(a) the model including parent attendance					
	<i>df</i>	Deviance	AIC	LRT	<i>P</i> -value
<none>		0	12.229		
Parent attendance	1	23.113	33.342	23.113	<0.001
Parent sex	1	12.172	22.401	12.172	<0.001
(b) the model including parental density					
	<i>df</i>	Deviance	AIC	LRT	<i>P</i> -value
<none>		0.047	13.282		
Parent density	1	6.468	17.702	6.4206	<0.05

Table 4. Effects of biparental denfense (killing success) tested by Fisher's exact probability test. Results of LW form were neglected because killing success was zero in almost all experiments.

HG form			
	Hf1	Hm1	Hf2
Hf1m1	$P = 0.017$	$P > 0.99$	-
Hf2m1	-	$P > 0.99$	$P = 0.0093$
The sequential Bonferroni adjustment for posterior multiple comparisons (4 combinations) was made for P .			

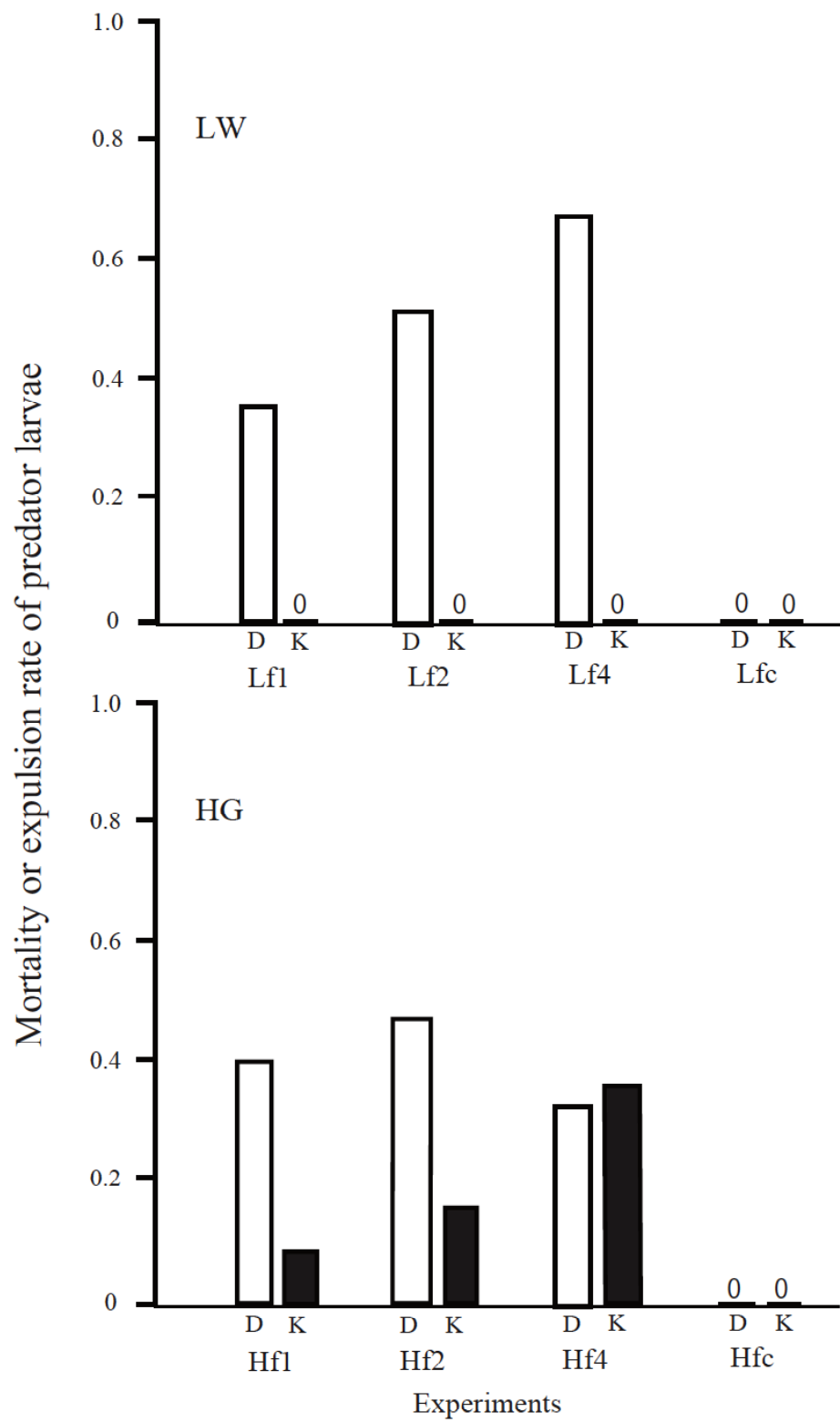


Fig. 1

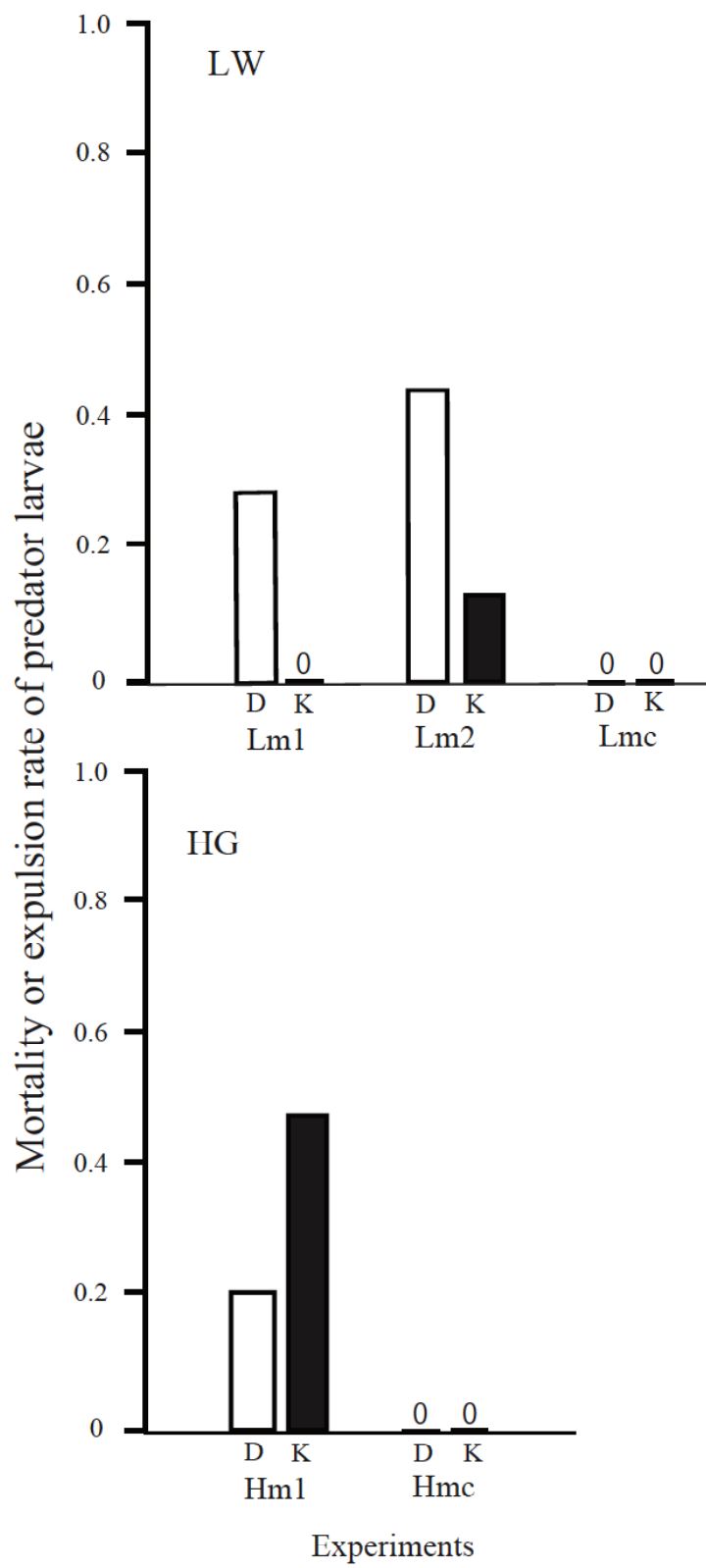


Fig. 2

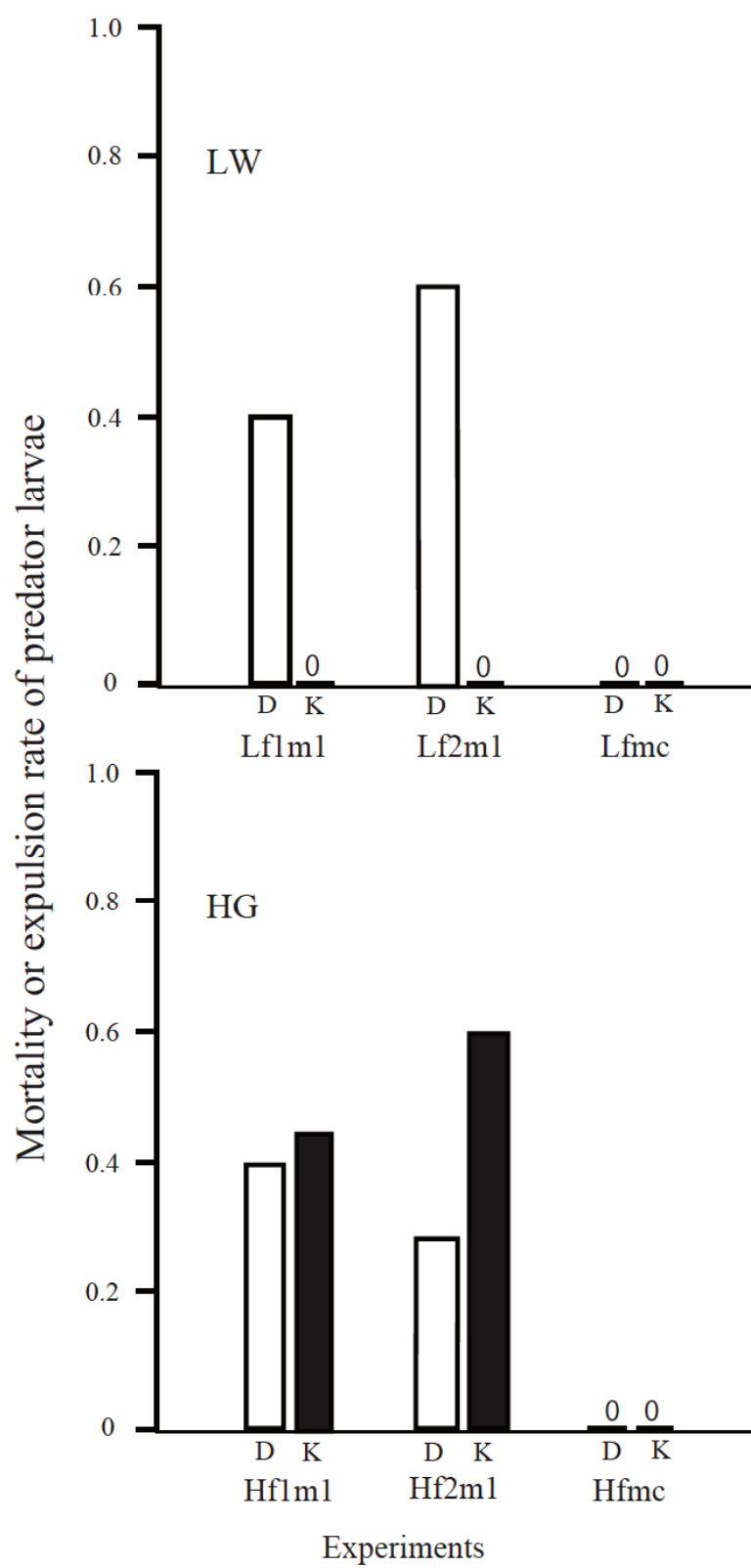


Fig 3