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Defensive functions of the trash-package of a green lacewing, *Mallada desjardinsi* (Neuroptera: Chrysopidae), against a ladybird, *Harmonia axyridis* (Coleoptera: Coccinellidae)

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Abstract

To elucidate the defensive functions of the trash-package of the green lacewing *Mallada desjardinsi* larva against the ladybird *Harmonia axyridis*, the contact frequency, attack rate, and capture rate of ladybirds were compared between the 'with trash' or 'naked' treatments of the green lacewing. The contact frequency until the ladybird captured the green lacewing was significantly more in the 'with trash' treatment (median: four times) than in the 'naked' treatment (median: one time), which indicates that the trash-package of the green lacewing offers protection from predation by ladybirds. The attack rate of the ladybirds on the 'with trash' green lacewing larvae (55%) was significantly lower than that on the 'naked' ones (90%). After the ladybirds first attacked, their capture rate of the 'with trash' green lacewing larvae (18%) was significantly lower than that of the 'naked' ones (83%). Thus, the trash-package of the green lacewing affords prevention against recognition (primary defense) and subjugation (secondary defense) from the ladybird.

Key words: Intra-guild predator; primary defense; secondary defense; defense strategy; camouflage

INTRODUCTION

Predator-prey interaction can be an important force driving natural selection, because almost all animals are at risk for predation by predators (Endler, 1991). Successful predation may characteristically involve five stages: detection, identification, approach, subjugation, and consumption (Endler, 1986). To interrupt this sequence, animals have evolved a variety of antipredator strategies such as cryptic coloration and camouflage (Campbell, 1993).

Larvae of some green lacewing species place extraneous materials (dead aphids and aphid's exuviae etc.) onto carrying structures that are located on the dorsal surface of the metathoracic and anterior abdominal tergites. This 'trash-package' of the green lacewing has been assumed to serve as an avoidance strategy against predation (New, 1969; Eisner et al., 1978, 2002; Milbrath et al., 1993; Tauber et al., 1995; Anderson et al., 2003). Defense strategies such as the 'trash-package' will affect the success of augmentative biological control programs, because heterospecific competitions between predators (e.g. intraguild predation) were found to reduce the efficacy of biological control (Hindayana et al., 2001). Therefore, a thorough understanding of the defense strategies of predators is important for appropriate selection of biological control agents.

The green lacewing *Mallada desjardinsi* (Navas), which places trash onto its back (Tsukaguchi, 1995), was considered to be a prospective biological control agent of aphids (Niijima, 1997). This green lacewing commonly co-occurs with the ladybird *Harmonia axyridis* Pallas on cherry and peach trees (unpublished data), and green lacewings and ladybirds are well known to have intra-guild predation interaction (e.g. Howell and Pienkowski, 1971; Wheeler, 1977). In this study, we elucidated the defensive functions of the trash-package of the green lacewing *M. desjardinsi* against the ladybird *H. axyridis*.

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MATERIALS AND METHODS

Insect culture. Laboratory cultures of the green lacewing *M. desjardinsi* were established from female adults collected in Nankoku City, Kochi, Japan. One to five individuals were maintained in a plastic cup (90 mm in diameter, 90 mm in depth). An artificial diet (1 : 1 dry yeast AY-65, Asahi Food & Healthcare Ltd., Tokyo, Japan, and honey) was provided as adult food with moistened cotton. The eggs laid were individually transferred to a plastic Petri dish (55 mm in diameter, 15 mm in depth). Newly hatched larvae were reared individually with *Ephestia kuehniella* Zeller eggs (Arysta LifeScience Corp. Entofood) in the same plastic Petri dish until adult emergence. This culture was maintained under a photoperiod of 16L : 8D and 25°C.

Effect of trash-package on predation. Newly hatched *M. desjardinsi* larvae, within 24 h of birth in the culture, were removed and individually reared with the aphid, *Myzus siegesbeckiae* Takahashi, in a plastic Petri dish (55 mm in diameter, 15 mm in depth). Second-instar larvae molting within 24 h to 48 h were used for the experiment. Second- or third-instar larvae of the ladybird *H. axyridis* were collected at Nankoku City, Kochi, Japan. They were transferred individually to a plastic Petri dish (55 mm in diameter, 15 mm in depth) and reared with the aphid *M. siegesbeckiae*. Fourth-instar ladybird larvae molting within 24 h to 48 h were used for the experiment. Larvae of both *M. desjardinsi* and *H. axyridis* were starved for 24 h before the experiments. All experimental preparations were maintained at a photoperiod of 16L : 8D and 20°C.

Forty green lacewing larvae were used for two treatments, 20 each for experimental groups 1 (with trash; Fig. 1A) and 2 (naked; Fig. 1B). The green lacewing larva of group 1 or 2 was put into a plastic Petri dish (55 mm in diameter, 15 mm in depth), and then a ladybird larva was added. Their behaviors were recorded via digital video camera to a DVD recorder system for 1 h under a fluorescent lamp (27 W) at room temperature of 24–27°C. Then, contact frequency until the ladybird captured the green lacewing was investigated. The possible sequences of ladybird behavior upon contact with the green lacewing are shown in Fig. 2. Attacking and capturing rates of the ladybirds were calculated by the following equations: Attack rate = Number of individuals attacked/Number of individuals contacted; Capture rate = Number of individuals captured/Number of individuals attacked. We used the observation results at first contact only for the calculation of attack and capture rates, because the trash on the green lacewings was sometimes removed by the attack of the ladybirds upon first contact.

Statistical analyses. The significance between

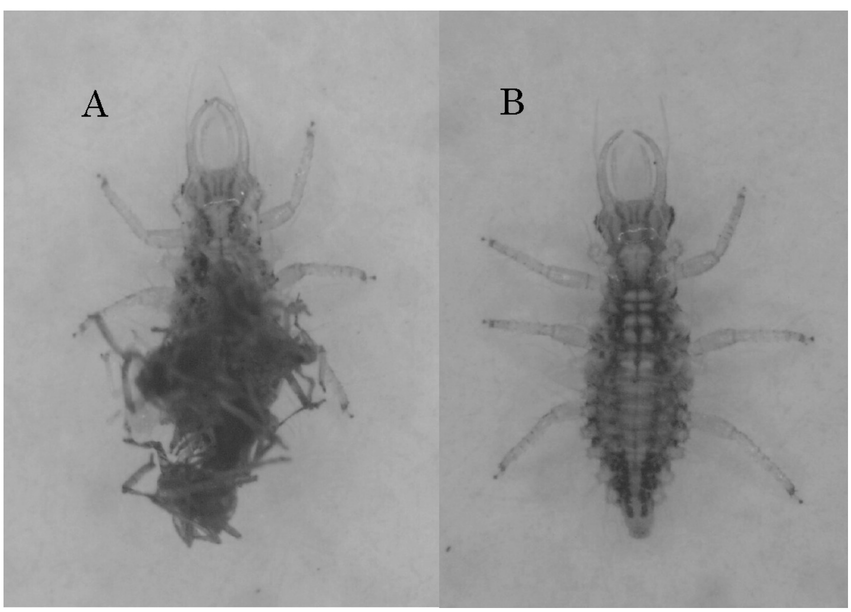

Fig. 1. Treatments of *M. desjardinsi* larvae, 'with trash' (A) and 'naked' (B).

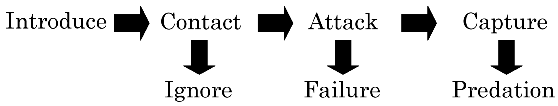

Fig. 2. Sequence of possible types of ladybird behavior from ladybirds coming into contact with prey.

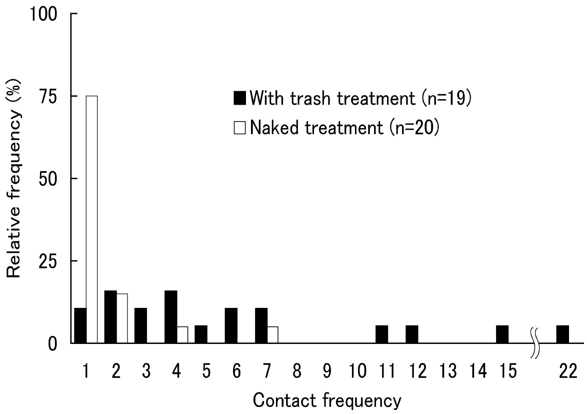

Fig. 3. Distribution of contact frequency until the *H. axyridis* capture of *M. desjardinsi* in the 'with trash' and 'naked' treatments.

'with trash' and 'naked' treatments was analyzed with the statistical software SPSS (SPSS, 2002). The contact frequency until the ladybird captured the lacewing was analyzed using the Mann-Whitney *U*-test. The attack and capture rates of the ladybirds at first contact were analyzed by Fisher's exact test.

RESULTS

Contact frequency until the ladybird captured the lacewing

The ladybird *H. axyridis*, larvae were not killed by the green lacewing *M. desjardinsi* larvae in any replications of either treatment. One lacewing in the 'with trash' treatment and none in the 'naked' treatment of the green lacewing had been captured by the end of the experiment. Once the ladybirds captured the green lacewings, all green lacewings were eaten. The contact frequency until the ladybird captured the green lacewing in the 'with trash' treatment (median: four times) was significantly more than that in the 'naked' treatment (median: one time) ($p<0.05$; Fig. 3).

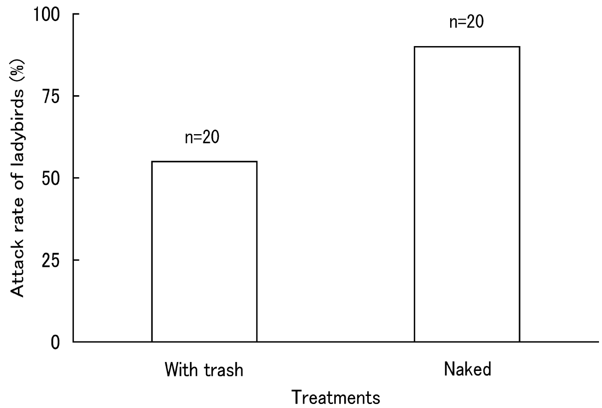

Fig. 4. Attack rate of *H. axyridis* in the 'with trash' and 'naked' treatments.

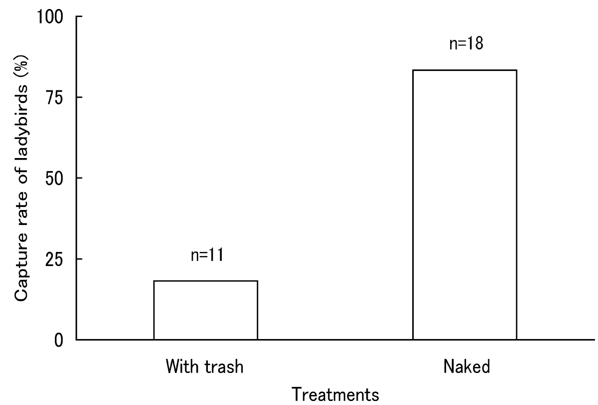

Fig. 5. Capture rate of *H. axyridis* in the 'with trash' and 'naked' treatments.

Attack and capture rates of the ladybird at first contact

The attack rate of the ladybirds on the 'with trash' green lacewing larvae (55%) was significantly lower than that on the 'naked' individuals (90%) ($p<0.05$; Fig. 4). After the ladybirds first attacked, their capture rate of the 'with trash' green lacewing larvae (18%) was significantly lower than that of the 'naked' individuals (83%) ($p<0.05$; Fig. 5).

DISCUSSION

Although ladybirds are known as predators of green lacewings (New, 1969; Ruzicka, 1997), there is no report on whether the trash-package of green lacewings offers protection against predation by ladybirds. In this study, more contact frequency until the ladybirds captured the green lacewings was observed in the 'with trash' treatment (Fig. 3). Therefore, we suggest that the trash-package of the green lacewing affords protection against predation by ladybirds. Similar results have been reported on the avoidance of predation from ants, green lacewings, anthocorises, and reduviids (New, 1969; Eisner et al., 1978, 2002; Anderson et al., 2003).

Masking (decoration of the body with foreign material) is common not only in the trash-package of the green lacewings, but also in a variety of animal groups such as spider crabs (Wicksten, 1993), marine gastropods (Portmann, 1956) and weevils (Gressitt and Samuelson, 1968). In these cases, the masking is reported to result in a cryptic resemblance to the respective animal's background and thus offers protection against 'visually oriented predators'. Ladybirds are generally 'not visually oriented predators', because they evidently do not perceive their prey until physical contact occurs (Fleschner, 1950; Robinson, 1952; Putman, 1955; Banks, 1957; Dixon, 1959; Kaddou, 1960; Kehat, 1968; Storch, 1976; Nakamuta and Saito, 1985). This contact seems to play a role in the prey 'recognition' of ladybirds. However, the ladybirds use volatile chemical cues in searching for prey (Jamal and Brown, 2001; Omkar et al., 2004), and the cues probably play a role in the prey 'detection' and/or 'recognition' of ladybirds. In this study, the ladybirds contacted the green lacewings in the 'with trash' and 'naked' treatments, which might be caused by the use of volatile chemical cues. A lower attack rate of the ladybirds was observed in the 'with trash' treatment (Fig. 4), which suggests that the trash-package of the green lacewing allows the prevention of 'recognition' by the ladybird. The trash-package of the green lacewing is not suitable prey for ladybirds, because trash-package components are sucked dry and quite old aphids. Consequently, the trash-package offers a camouflage effect which is caused by misrecognition as unsuitable prey by the ladybirds. The most effective antipredator adaptations of a potential prey are the avoidance of 'detection' or 'recognition', or both by its predators, because such prey individuals can eliminate the energetic costs of interacting with predators and the risks of injury or death (Lederhouse, 1990). These antipredator adaptations have been categorized as primary defense mechanisms (Robinson, 1969).

Secondary defense mechanisms come into play after the prey has been detected and recognized by a predator in its vicinity (Edmunds, 1974). The secondary defense of potential prey is also important because primary defense usually conflicts with other interests of the organism such as feeding, reproducing and dispersing (Evans, 1990). In this study, a lower capture rate by the ladybird was observed in the 'with trash' treatment (Fig. 5), which suggests that the trash-package of the green lacewing offered the prevention of 'subjugation' from the ladybird.

We suggest the trash-package of the green lacewing *M. desjardinsi* larvae offers defensive effectiveness against the ladybird *H. axyridis* through a combination of primary and secondary defense mechanisms.

The green lacewing *Chrysoperla nipponensis* (Okamoto) is also a prospective biological control agent of aphids in Japan (Niijima, 1997), and unlike *M. desjardinsi*, it is not a 'trash-package' species (Tsukaguchi, 1995). To select more useful green lacewings as biological control agents, the differences in the defense strategies and effectiveness between *M. desjardinsi* and *C. nipponensis* larvae should be investigated.

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