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Effect of reflex bleeding of a predatory ladybird beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), as a means of avoiding intraguild predation and its cost

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Abstract

In aphidophagous ladybird beetle, larvae reflexively exude hemolymph, i.e. reflex bleeding, when disturbed. In the present study, to evaluate the effectiveness of hemolymph as a defense against intraguild predation and its bleeding cost, laboratory experiments were conducted using two species, *Coccinella septempunctata brucki* and *Harmonia axyridis*. In the first experiment, to evaluate the effect of *H. axyridis* hemolymph as a means of avoiding intraguild predation by *C. septempunctata brucki*, the incidence of cannibalism of *C. septempunctata brucki* eggs by fourth instars was determined in relation to the presence of *H. axyridis* hemolymph. In this experiment, 80% eggs of *C. septempunctata brucki* were cannibalized when no *H. axyridis* hemolymph was present; however, only 20% of eggs were cannibalized when *H. axyridis* hemolymph was present. In the second experiment, average adult body weight of *H. axyridis* at its emergence differed after individuals had experienced no versus daily reflex bleeding during the fourth instar. The body weight of adult *H. axyridis* averaged 32.8 ± 1.3 mg ($n=11$) and 35.3 ± 0.9 mg ($n=19$) for males and females, respectively, when they did not reflex bleed during the fourth instar; however, their body weight tended to be lower when they had reflex bled daily during the fourth instar.

Key words: Avoidance of intraguild predation; chemical protection; hemolymph; predatory ladybird beetles; reflex bleeding

INTRODUCTION

The general theory of intraguild predation predicts that communities including intraguild predators are unlikely to persist (Polis et al., 1989; Holt and Polis, 1997); however, intraguild prey species often persist despite the presence of intraguild predators (Diehl, 1993). This suggests that avoiding intraguild predation is likely important for the persistence of community structure among predator species. If this is generally true, then it is necessary to study the means of avoiding intraguild predation to better understand the ecology of predators, especially their interactions with other species in the same predator guild.

Aphidophagous ladybird beetles forming guilds are thought to avoid intraguild predation. As larvae are adversely affected by consuming poor quality food, such as intraguild prey (Agarwala and Dixon, 1992; Yasuda and Ohnuma, 1999; Dixon, 2000; Hemptinne et al., 2000a; Sato and Dixon, 2004), larvae should not consume other species of ladybird beetles when their primary prey, aphids, is available. In fact, chemicals present on the body surfaces of ladybird beetles, i.e., surface chemicals (Hemptinne et al., 2000b), enable larvae to recognize other species of ladybird beetles and to avoid consuming them; that is, in the aphidophagous ladybird beetle guild, larvae are likely to be protected from intraguild predation to some extent when aphid availability is unlimited.

As starvation readily results in death (Dimetry, 1976; Kawauchi, 1979), however, starving larvae consume any prey, including intraguild prey (Hemptinne et al., 2000a), regardless of quality; that is, it is suggested that surface chemical-based protection is an ineffective means for larvae to avoid intraguild predation when aphid availability

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is limited. In the field, larvae often face limited aphid availability (Osawa, 1989; Yasuda and Shinya, 1997; Jansen and Hautier, 2008). In this circumstance, one possible way of avoiding intraguild predation more effectively is by reflex bleeding-based protection (Komai, 1956; Holloway et al., 1991, 1993).

Ladybird beetles exude when agitated and this reflex bleeding is thought to be a defense against predators (Komai, 1956; Majerus and Kearns, 1989; Holloway et al., 1991, 1993). Thus, reflex bleeding may also provide protection against intraguild predators, whereas its actual efficiency as a mean of avoiding intraguild predation is unknown. In addition, the chemical components of reflex exudates are similar to those of the hemolymph (Hodek and Honek, 1996; Dixon, 2000), suggesting that reflex bleeding may result in considerable nutritional loss. In the present study, to determine the actual efficiency of larval hemolymph as a means of avoiding intraguild predation and its cost, a series of experiments was conducted in the laboratory using two species of aphidophagous ladybird beetles, *Coccinella septempunctata brucki* (Mulsant) and *Harmonia axyridis* (Pallas), which co-occur on hibiscus trees and form an aphidophagous ladybird beetle guild in Yamagata, Japan (Yasuda and Shinya, 1997).

MATERIALS AND METHODS

Ladybird beetles used in the experiments. Pairs of new adults of *C. septempunctata brucki* and *H. axyridis* were obtained from ladybird beetle cultures at Yamagata University, Tsuruoka, Japan. Pairs were kept individually in small Petri dishes (3 cm in diameter) and fed an excess of pea aphid, *Acyrthosiphon pisum* (Harris) daily. All pairs were checked daily for oviposition, and egg clusters were removed and kept individually in another Petri dish (9 cm in diameter) until egg hatching. Hatchling larvae were also fed an excess of pea aphids daily until used in the following two experiments. All fourth instars of *C. septempunctata brucki* used in the experiments were starved for 24 h prior to the start of the experiment.

Experiment 1: Efficiency of hemolymph. Twenty fourth-instar *C. septempunctata brucki* were placed individually into Petri dishes (3 cm in diameter), and were each supplied five eggs of their own species, which were painted with either distilled water or hemolymph of *H. axyridis*. In this experiment, the number of eggs of *C. septempunctata brucki* that remained was recorded 30 min after the start of the experiment. Hemolymph was collected immediately before it was painted on the experimental eggs.

Experiment 2: Cost of bleeding hemolymph. Sixty fourth-instar *H. axyridis*, which had molted within 12 h prior to the experiments, were placed individually in Petri dishes (3 cm in diameter) and fed an excess of pea aphids in a controlled cabinet (20°C, 16L8D). Half of these fourth instars were gently stimulated once a day using a paint brush for hemolymph bleeding, and the hemolymph was removed at each bleeding event. These procedures were continued until all fourth instars had pupated. All pupae were checked every 12 h until adult emergence, and then emerged adults were weighed and sexed. In this experiment, no fourth instars died before pupating. All data were analyzed using the Mann-Whitney *U* test.

RESULTS

Experiment 1: Efficiency of hemolymph

The average number of eggs of *C. septempunctata brucki* cannibalized by a fourth instar is shown in Fig. 1. Although over 80% of eggs were cannibalized when water painted, this fell to 20% when

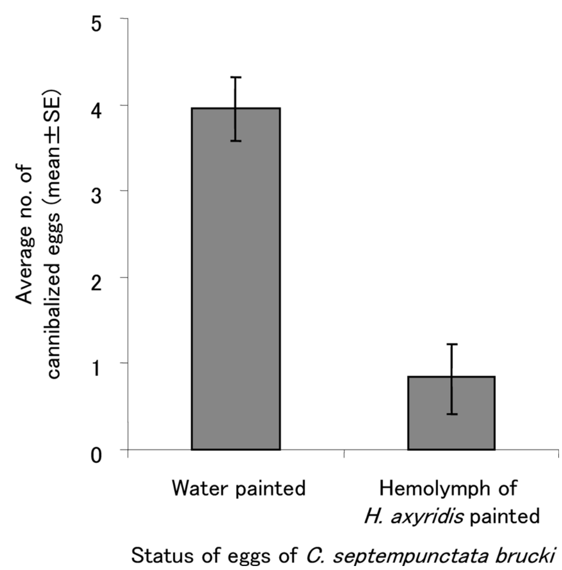

Fig. 1. Average number of *C. septempunctata brucki* eggs cannibalized by fourth instars in relation to the presence of *H. axyridis* hemolymph.

Fig. 2. Average adult body weight of *H. axyridis* when they had experienced no and daily reflex bleeding as fourth instars.

H. axyridis hemolymph was present on the eggs (Mann-Whitney $U=53$, $n=20$, $p<0.0001$); that is, *H. axyridis* hemolymph markedly increased the degree of protection of *C. septempunctata brucki* eggs from cannibalism by fourth instars.

Experiment 2: Cost of bleeding hemolymph

In males, the body weight of adult *H. axyridis* averaged 32.8 ± 1.3 mg ($n=11$) when they did not reflex bleed during the fourth instar (Fig. 2), and this significantly decreased by 15% to 27.6 ± 1.1 mg (Mann-Whitney $U=22$, $n=16$, $p=0.015$) with reflex bleeding. Also in females, their average body weight when they did not reflex bleed during the fourth instar, 35.3 ± 0.9 mg ($n=19$), tended to fall, although the difference was not significant (Mann-Whitney $U=102$, $n=14$, $p=0.259$).

DISCUSSION

In general, the physical vulnerability of ladybird beetles to cannibalism or intraguild predation depends on their relative mobility and size as compared with these attributes of their cannibal or intraguild predator (Dixon, 2000). Thus, the eggs used in the present study were particularly vulnerable to such predation. Accordingly, it is likely that any reduction in the percentage of eggs of *C. septempunctata brucki* that were cannibalized by fourth instars when painted with *H. axyridis* hemolymph purely reflects the effect of the hemolymph. In the present study, fourth-instar *C. septempunctata brucki* were extremely reluctant to cannibalize eggs painted with *H. axyridis* hemolymph, suggesting that *H. axyridis* hemolymph strongly deters attack by fourth-instar *C. septempunctata brucki*. If so, bleeding hemolymph when attacked (Komai, 1956; Majerus and Kearns, 1989; Holloway et al., 1991, 1993) may efficiently enable larvae of *H. axyridis* to avoid intraguild predation by intraguild predators such as *C. septempunctata brucki*.

Although bleeding hemolymph may be a very efficient way of avoiding intraguild predation by other species, there is likely to be a cost. As shown in the present study, reflex bleeding by larvae adversely affected their weight as adults (see also Grill and Moore, 1998); however, the unlimited availability of aphids may sometimes allow larvae to offset the cost of reflex bleeding, especially in females. In some species, females are more voracious than males (Yasuda and Dixon, 2002). If this is generally true, it is likely that a different rate of prey consumption between males and females can account for our results.

Furthermore, the adverse effect of reflex bleeding is especially likely to be strong in the field, where the availability of aphids is often limited (Yasuda and Shinya, 1997; Sato, 2001). In addition, the use of reflex bleeding is likely to be limited in some way; for example, larvae exude up to 20% of their body weight in a single reflex bleeding event (Dejong et al., 1991; Holloway et al., 1991), suggesting that reflex bleeding is limited in its frequency of use; however, this was not shown in the present study.

In conclusion, the present study suggests that reflex bleeding can provide important protection against intraguild predation in aphidophagous ladybird beetles, whereas this adversely affects the performance of larvae however, the fact that larvae of some species do reflex bleed when attacked by their intraguild predator (Kushibuchi, personal observation) suggests that this behavior is adaptive, especially for *H. axyridis*. In the present study, as the actual efficiency of reflex bleeding is still unknown, this should be confirmed by more realistic experiments.

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REFERENCES

Agarwala, B. K. and A. F. G. Dixon (1992) Laboratory study of cannibalism and interspecific predation in ladybirds. *Ecol. Entomol.* 17: 303–309.

Dejong, P. W., G. J. Holloway, P. M. Brakefield and H. de Vos (1991) Chemical defense in ladybird beetles (Coccinellidae). II. Amount of reflex fluid, the alkaloid adaline and individual variation in defence in 2-spot ladybirds (*Adalia bipunctata*). *Chemoecology* 2: 15–19.

Diehl, S. (1993) Relative consumer sizes and the strengths of direct and indirect interactions in omnivorous feeding relationships. *Oikos* 68: 151–157.

Dimetry, N. Z. (1976) The role of predator and prey density as factors affecting behavioural and biological aspects of *Adalia bipunctata* (L.) larvae. *Zeit. Ange. Entomol.* 81: 386–392.

Dixon, A. F. G. (2000) *Insect Predator-Prey Dynamics: Ladybird Beetles and Biological Control*. Cambridge University Press, London. 268 pp.

Grill, C. P. and A. J. Moore (1998) Effects of a larval antipredator response and larval diet on adult phenotype in an aposematic ladybird beetle. *Oecologia* 114: 274–282.

Hemptinne, J. L., A. F. G. Dixon and C. Gauthier (2000a) Nutritive cost of intraguild predation on eggs of *Coccinella septempunctata* and *Adalia bipunctata* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 97: 559–562.

Hemptinne, J. L., G. Lognay, C. Gauthier and A. F. G. Dixon (2000b) Role of surface chemical signals in egg cannibalism and intraguild predation in ladybirds (Coleoptera: Coccinellidae). *Chemoecology* 10: 123–128.

Hodek, I. and A. Honek (1996) *Ecology of Coccinellidae*. Kluwer Academic Publishers, Dordrecht. 464 pp.

Holloway, G. J., P. W. Dejong, P. M. Brakefield and H. de Vos (1991) Chemical defence in ladybird beetles (Coccinellidae). I. Distribution of coccinelline and individual variation in defence in 7-spot ladybirds (*Coccinella septempunctata*). *Chemoecology* 2: 7–14.

Holloway, G. J., P. W. Dejong and M. Ottenheim (1993) The genetics and cost of chemical defense in the 2-spot ladybird (*Adalia bipunctata* L). *Evolution* 47: 1229–1239.

Holt, R. D. and G. A. Polis (1997) A theoretical framework for intraguild predation. *Am. Natur.* 149: 745–764.

Jansen, J. P. and L. Hautier (2008) Ladybird population dynamics in potato: comparison of native species with an invasive species, *Harmonia axyridis*. *Biocontrol* 53: 223–233.

Kawauchi, S. (1979) Effects of prey density on the rate of prey consumption development, and survival of *Propylea japonica* Thunberg. *Kontyu* 47: 204–212.

Komai, T. (1956) Genetics of ladybeetles. *Adv. Genet.* 8: 155–188.

Majerus, M. and P. Kearns (1989) *Ladybirds, Naturalist Handbooks 10*. Richmond Publishing, Slough. 103 pp.

Osawa, N. (1989) Sibling and non-sibling cannibalism by larvae of a lady beetle *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae) in the field. *Res. Popul. Ecol.* 31: 153–160.

Polis, G. A., C. A. Myers and R. D. Holt (1989) The ecology and evolution of intraguild predation-potential competitors that eat each other. *Annu. Rev. Ecol. Syst.* 20: 297–330.

Sato, S. (2001) Ecology of ladybirds—factors influencing their survival. PhD thesis, University of East Anglia, Norwich, UK. 120 pp.

Sato, S. and A. F. G. Dixon (2004) Effect of intraguild predation on the survival and development of three species of aphidophagous ladybirds: consequences for invasive species. *J. Agric. Forest Entomol.* 6: 21–24.

Yasuda, H. and A. F. G. Dixon (2002) Sexual size dimorphism in the two spot ladybird beetle *Adalia bipunctata*: developmental mechanism and its consequences for mating. *Ecol. Entomol.* 27: 493–498.

Yasuda, H. and N. Ohnuma (1999) Effect of cannibalism and predation on the larval performance of two ladybird beetles. *Entomol. Exp. Appl.* 93: 63–67.

Yasuda, H. and S. Shinya (1997) Cannibalism and interspecific predation in two predatory ladybirds in relation to prey abundance in the field. *Entomophaga* 42: 153–163.