Notes

A FOX IN SHEEP'S CLOTHING: FURTIVE PREDATORS BENEFIT FROM THE COMMUNAL DEFENSE OF THEIR PREY

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Abstract. Many animals live in temporary or permanent groups, either as gregarious or social species, to reduce predation risk. The solitary midge, *Aphidoletes aphidimyza,* preys specifically on aphids and spends its entire larval development within a prey colony where it is susceptible to intraguild predation. We hypothesized that midge larvae profit from a dilution effect produced by aphids which enhances their chances of survival. We examined the defensive behaviors of aphids in response to foraging midges, and investigated the effect of aphid density on the predation risk of *A. aphidimyza* by the lacewing *Chrysoperla rufilabris.* We found that a foraging midge displays furtive hunting behavior which triggers little defensive reaction by aphids, and does not stimulate significant disturbance of the gregarious prey. Within the aphid colony the midge benefits from a dilution effect which reduces the incidence of predation by lacewing larvae. However, the effectiveness of such a mechanism is determined by the level of disturbance caused by foraging intraguild predators. Large lacewing larvae tend to dislodge aphids from their feeding sites, thereby eliminating the dilution effect. We conclude that prey not only provide food to midge larvae, but also protection against natural enemies.

Key words: aphid; Aphidoletes aphidimyza; Chrysoperla rufilabris; *defensive mechanism; dilution effect; ectosymbiosis; furtive behavior; intraguild predation; predatory midge.*

INTRODUCTION

Potential benefits of living in groups include increased foraging efficiency, information transfer among individuals, mate location, thermoregulation, and defense against natural enemies (Pulliam and Caraco 1984, Vulinec 1990, Danchin and Wagner 1997). One form of communal defense is the dilution effect which decreases the individual's probability of being attacked once the prey patch has been detected by predators (Taylor 1977, Inman and Krebs 1987). The dilution effect has been observed in many different organisms, including zooplankton, spiders, insects, fish, birds, and mammals (see Inman and Krebs 1987, Uetz and Hieber 1994 and references therein). Mixed groups of different prey species may also experience the dilution effect, as benefits are independent of genetic relatedness among individuals (Treisman 1975). However, the intriguing case in which predators profit from the communal defense of their prey has never been reported.

Aphids are small, sedentary, plant-sucking insects that often form dense aggregations. They are patchily distributed in most terrestrial ecosystems, and parthenogenetic populations usually have a high potential rate of increase (Dixon 1998). Aphids are exploited by many specialist and generalist aphidophagous species and have evolved a variety of individual and group defenses. Individual aphids living in a group may benefit from the presence of sterile soldier castes (Aoki 1982), the spatial structure of the colony (Völkl and Stadler 1996), the release of alarm pheromones (Nault et al. 1973), and the dilution effect (Kidd 1982, Turchin and Kareiva 1989).

The predatory midge *Aphidoletes aphidimyza* Rondani (Diptera: Cecidomyiidae) is specific to aphids in its larval stages. Females lay eggs close to aphid colonies and, upon hatching, neonate larvae enter the colony (Lucas and Brodeur 1999). The larvae possess specialized mouthparts to pierce the aphid integument, inject a paralyzing toxin, and then suck the aphid's body fluids. Confined within aphid colonies, eggs, and larvae of *A. aphidimyza* are vulnerable to intraguild predation. However, the incidence of mortality decreases with increased aphid density (Lucas et al. 1998).

We hypothesized that midge larvae profit from a di-

lution effect produced by aphids which enhances the predators' chances of survival. In the laboratory, we first compared patterns of predation behavior of *A. aphidimyza* with that of the lacewing, *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae), a predator of aphids and other arthropods. We then examined how aphid density influences the predation risk of *A. aphidimyza* by *C. rufilabris*.

METHODS

Chrysoperla rufilabris was purchased from Groupe Biocontrôle (Sainte-Foy, Québec, Canada) and *A. aphidimyza* from Plant Products (Montréal, Québec, Canada). Predators were reared on the potato aphid, *Macrosiphum euphorbiae* Thomas (Homoptera: Aphididae). Midge larvae were selected based on size since there is a controversy over the number of *A. aphidimyza* larval stages (see Lucas et al. 1998). Only large larvae (>168 h old) were used in our experiments. Lacewing larvae were used 24 h after hatching (first instar) or 48 h after molting (second and third instar). To establish aphid colonies on potato plants, early second-instar aphids were introduced on the lower surface of a leaf 24 h prior to testing. Clip-cages were used to prevent aphids from dispersing and thus favor cohesion among individuals. All experiments were carried out at 23°C, 60–70% relative humidity, under a 16:8 h light:dark photoregime.

To examine defensive behaviors of potato aphids in response to attacks by predators displaying different foraging strategies, we used large *A. aphidimyza* larvae and first instar *C. rufilabris,* as these two organisms are of similar size (Lucas et al. 1998). Following clip-cage removal, tests were started by introducing a predator larva close (<1 cm) to a colony of 9–13 aphids. Each test lasted 45 min during which aphids were monitored continuously. As we observed each predator approach, we recorded the number of attempted attacks, the proportion of successful predation events, and the proportion of aphids displaying defensive behaviors. These were classified as: walking away from the feeding site, dropping off the plant, and swiveling, a typical behavior performed in response to the release of alarm pheromone and characterized by repeated up and down movements of the abdomen (Dixon 1998). Aphid colonies without a predator served as controls. There were twelve replicates per treatment. Data were analyzed using a one-way ANOVA. Proportion data (successful attack and defensive behavior) were arcsine transformed prior to the analysis.

To test whether or not *A. aphidimyza* larvae benefit from a dilution effect, we examined their susceptibility to predation by second and third instar *C. rufilabris* within aphid colonies of three different sizes: 4, 10, and 20 individuals/colony. Aphid colonies were formed

as previously described and 30 min prior to the test a larva of *A. aphidimyza* was gently introduced in the center of the aphid colony using a fine paintbrush. Tests started with the release of a *C. rufilabris* larva and ended when the lacewing either killed the midge or left the leaf. There were a minimum of 14 replicates per treatment. We first compared the lacewing capture success on aphids and midges, defined as the total number of successful captures divided by the total number of attacks, regardless of colony sizes. We then compared (1) midge mortality risk (the number of midges killed by lacewings divided by the total number of midges tested); (2) frequency of first attack on midges (the proportion of replicates where the midge was the first prey attacked); (3) time elapsed before midge death in replicates where midges died; and (4) number of aphids killed prior to midge capture, as a function of aphid colony size. Lacewing capture success on aphids and midges, midge mortality risk, and frequency of first attack on midges were compared using *G* tests (likelihood ratio). These tests were followed by post hoc multiple comparisons tests corresponding to the experimentwise error state (Scherrer 1984), which consists of recalculating the alpha significance level (original $\alpha = 0.05$) and comparing the group two by two with *G* tests. Time to midge death and number of aphids killed prior to midge capture were analyzed using the Kruskal-Wallis procedure. The Student-Newman-Keuls test was used to separate means (Snedecor and Cochran 1967).

RESULTS

Attempted attacks ($F_{1,22} = 0.044$, $P = 0.836$) and successful attacks ($F_{1,19} = 0.286$, $P = 0.599$) were similar for lacewing and midge larvae. Foraging lacewing larvae induced significant defensive responses of aphids ($F_{2,23} = 24.95$, $P < 0.0001$; Fig. 1). Most aphids were dislodged from their feeding sites, thereby disrupting the integrity of the colony. In contrast, midges did not elicit strong defensive reactions in the aphids, with the incidences of dropping (LSD, $P = 0.367$) and swiveling (LSD, $P = 0.235$) being similar to controls. However, the incidence of walking was higher in the midge treatment than in the control (LSD, $P = 0.014$). During bioassays, 95% of the *A. aphidimyza* larvae were found within the aphid colony.

Capture success of lacewing larvae was similar for aphids and midges (*G* test; second instar, $G_5 = 1.37$, $P > 0.900$; third instar, $G_5 = 4.73$, $P > 0.250$), suggesting that differences in prey vulnerability did not interfere with mortality patterns. Mortality of midges to second instar *C. rufilabris* decreased significantly with an increase in aphid density ($G_2 = 10.00$, $P = 0.009$; Fig. 2A), as did the occurrence of first attack on midges ($G_2 = 11.04$, $P = 0.004$; Fig. 2B). However,
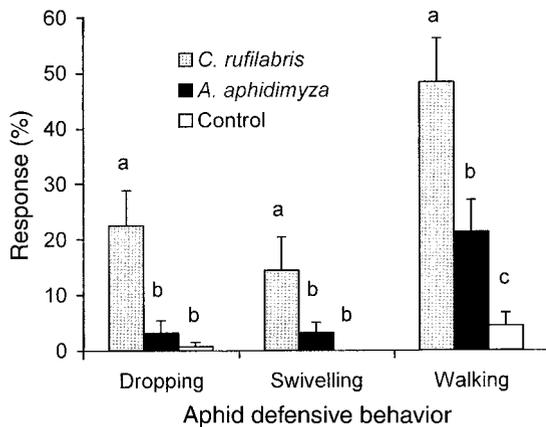
FIG. 1. Proportion of *Macrosiphum euphorbiae* that exhibited defensive behaviors in response to attack by *Aphidoletes aphidimyza* and *Chrysoperla rufilabris*. For each behavior, percentages followed by different letters are significantly different (ANOVA, $P < 0.05$, $N = 12$). Vertical lines represent $+1$ SE.

these parameters were not significantly affected by colony size when the lacewing was a third instar, (mortality, $G_2 = 0.66$, $P > 0.5$; Fig. 2A: occurrence of first attack, $G_2 = 3.12$, $P > 0.1$; Fig. 2B). The time to midge death (Kruskal-Wallis; second instar, $H = 7.54$, df = 2, $P = 0.023$; third instar, $H = 11.86$, df = 2, $P = 0.003$) and the number of aphids killed prior to midge capture (second instar, $H = 17.84$, df = 2, $P < 0.001$; third instar, $H = 12.68$, df = 2, $P = 0.002$) increased with an increase in aphid density for both predator larval instars (Fig. 2C, D).

DISCUSSION

A suite of behavioral traits characterized the atypical foraging strategy of *A. aphidimyza* larvae. During our experiments, most individuals were found within the aphid colony where they remained immobile in the absence of feeding. They also often covered themselves with aphid exuvia and dead or paralyzed aphids (E. Lucas, *personal observation*). This may serve as camouflage as shown for chrysopid larvae (Eisner et al. 1978, Milbrath et al. 1993). Hunting midges were slow-moving predators: they approached their victim by inconspicuous creeping movements and subdued aphids by injecting a paralyzing toxin, usually in the aphid's leg, thereby deactivating behavioral defenses of the prey (Klingauf 1967). They extracted the aphids' body contents on site without stimulating any significant increased in dropping behavior and alarm signaling of the remaining aphids in the colony. Although predatory midges caused a number of aphids to momentarily stop feeding and walk away, the overall cohesion among aphids was maintained in most colonies where *A. aphi-*

dimyza had been introduced. These tactics correspond to furtive predation, an unusual form of foraging behavior that allows predators to circumvent detection and defensive responses of their prey.

Aphid communities constitute a favorable template for intra- and interspecific encounters between natural enemies (Brodeur and Rosenheim 2000). In natural and managed ecosystems, intraguild predation occurs widely among aphidophagous species and may represent an important mortality factor that reduces substantially intraguild prey populations (Rosenheim et al. 1993, Müller et al. 1999). Lucas et al. (1998) showed that predatory midge larvae were highly vulnerable to intraguild predation because of their small size, low mobility, and feeding specificity. Consistent with our initial hypothesis, we found that foraging *A. aphidimyza* larvae may benefit from a dilution effect within the aphid colony that reduces intraguild predation. Thus, the prey not only serves as a food source, but may also provide protection against predators. However, the effectiveness of such a mechanism is influenced by the nature of intraguild predators. Most aphids were disturbed and eventually dislodged from their feeding sites by large predators, thereby eliminating the dilution effect. Third instar lacewing larvae are robust, highly mobile, and voracious active-searching predators. These characteristics contribute to magnify the impact a predator might have on both the structure of an aphid colony and the predation risk.

The benefits of living in groups may be negated by an increased number of attacks on the group (Turner and Pitcher 1986, Wrona and Dixon 1991, Uetz and Hieber 1994), as natural enemies tend to aggregate at large groups of prey (Taylor 1984). This question was critically explored for aphids by Turchin and Kareiva (1989). Under field conditions, they showed that aggregation of conspecific fireweed aphids, *Aphis varians* Patch, significantly reduced predation risk to individual aphids by *Hippodamia convergens* Guérin-Méneville, a common ladybird beetle. Although coccinellids exhibited both strong numerical and functional responses to aphid colonies, the aphid's probability of being killed was nevertheless reduced through a dilution effect that grew with colony size. Such an outcome may be true for *A. aphidimyza* threatened by similar aphidophagous guilds; field experiments are needed to determine the ecological conditions under which predatory midges may benefit from exploiting aphid defense.

Our findings are important in three respects. First, they provide a unique example of an adaptive process in which a predator usurps (sensu Brodeur and Vet 1994), for its own benefits, the communal defense of its prey. Aphids could, therefore, be viewed as a functional component of *A. aphidimyza* defensive strategy that contributes to protect developing larvae from in-
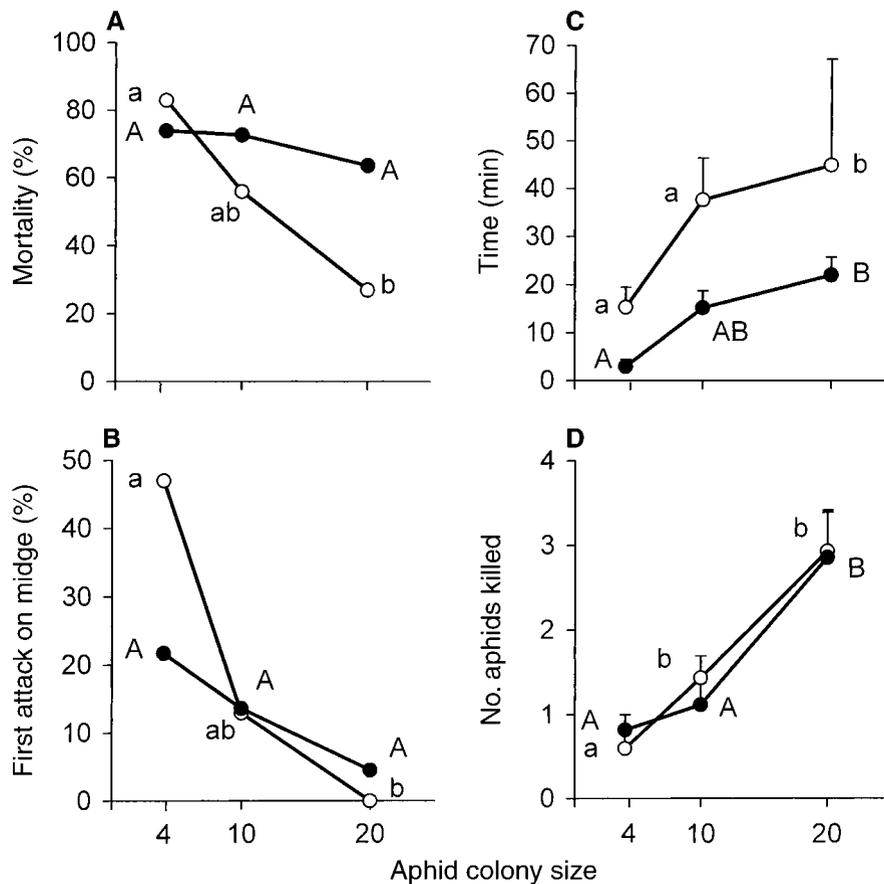
FIG. 2. Susceptibility of *Aphidoletes aphidimyza* larvae to second-instar (open circle) and third-instar (solid circle) larvae of *Chrysoperla rufilabris* as a function of aphid density: (A) percentage of experimental midge larvae that were killed by the predator, (B) percentage of replicates in which the first attack was directed toward the midge rather than an aphid, (C) time elapsed before midge death in replicates where midges died, and (D) number of aphids killed before midge death. For each predator developmental stage, values followed by different letters are significantly different (C and D, Kruskal-Wallis; A and B, *G* tests; $\propto = 0.05$; $N > 14$). Vertical error bars represent +1 SE.

traguild predators. Yet the effectiveness of the dilution effect would depend on the nature, number and foraging behavior of predators.

Second, selective pressures other than those to reduce predation risk are likely to have had an important role in shaping furtive behavior of *A. aphidimyza.* For example, furtive predation may constitute a significant mechanism for the evolution of prey specialization, or may reflect phylogenetic inertia (many Dipteran larvae are sluggish).

Third, the present findings may have significant implications for the evolution of ectosymbiosis. Predatory ectosymbionts of eusocial insects are associated with the host colony during part of their life cycle. They constitute a rich community of species (Kistner 1981) that share several biological attributes of furtive predators. Of significance, they live in the vicinity of the prey and have the capacity to deactivate prey defensive systems (Hölldobler and Wilson 1990). Furtive behavior might therefore constitute a preadaptation leading to predatory ectosymbiosis. Within the Cecidomyiidae, a family characterized by its life history diversity, species may be mycophagous, phytophagous, gall-making, predator, or parasitic (Gagné 1981). One *Termitomastus* species has been shown to develop as an ectosymbiont of eusocial termites (Silvestri 1920, cited in Wilson 1971) and, conceivably, may have originated from a furtive predatory ancestor. Efforts to examine the importance of furtive predation by species that exploit colonial or social prey will contribute to develop a more complete theory of predator–prey associations.

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