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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Interactions between nectar robbers and seed predators mediated by a shared host plant, *Ipomopsis aggregata*

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Abstract Animals that consume plant parts or rewards but provide no services in return are likely to have significant impacts on the reproductive success of their host plants. The effects of multiple antagonists to plant reproduction may not be predictable from studying their individual effects in isolation. If consumer behaviors are contingent on each other, such interactions may limit the ability of the host to evolve in response to any one enemy. Here, we asked whether nectar robbing by a bumblebee (*Bombus occidentalis*) altered the likelihood of pre-dispersal seed predation by a fly (*Hylemya* sp.) on a shared host plant, *Ipomopsis aggregata* (Polemoniaceae). We estimated the fitness consequences of the combined interactions using experimental manipulations of nectar robbing within and among sites. Within sites, nectar robbing reduced the percentage of fruits destroyed by *Hylemya*. However, the negative effects of robbing on seed production outweighed any advantages associated with decreased seed predation in robbed plants. We found similar trends among sites when we manipulated robbing to all plants within a local population, although the results were not statistically significant. Taken together, our results suggest that seed predation is not independent of nectar robbing. Thus, accounting for the interactions among species is crucial to predicting their ecological effects and plant evolutionary response.

Keywords Scarlet gilia · Multispecies interactions · Nectar robbing · Plant–animal interactions · Pollination

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Introduction

Most flowering plants interact with a suite of mutualists and antagonists that may ultimately affect their reproductive success. However, the focus of many classical and contemporary studies in ecology and evolution has been on pairwise interactions between individuals and/or species (Miller and Travis 1996). The study of plant–pollinator mutualisms is a case in point, encompassing a literature dating back to Darwin (1862) and replete with studies that assess the impacts of pollinators on floral traits and plant fitness (for reviews, see Faegri and van der Pjil 1979; Proctor et al. 1996; Waser and Ollerton 2006). In addition to their interactions with pollinators, however, plants are often used simultaneously by herbivores, florivores, nectar robbers, and seed predators. These non-pollinating species can affect plant–pollinator interactions, plant fitness, and the evolution of floral traits (e.g., Schemske and Horvitz 1988; Lehtilä and Strauss 1997; Herrera 2000; Galen and Cuba 2001; Gómez 2003). Furthermore, the net effect of organisms that exploit mutualisms while providing no services in return may not be predictable from pairwise interactions (Bronstein et al. 2003), especially if one organism alters the

likelihood or intensity of an interaction with a second (Wootton 1993), or if the enemies have non-additive effects on plant fitness. The impacts of such third-party cheaters on host plant fitness can be dramatic, and may pose significant counter-selection on traits important to plants and their mutualist pollinators (Bronstein et al. 2006). For example, even evolutionarily tight linkages such as those between various species of *Yucca* and their coevolved moth pollinators can be exploited by parasites that effect no pollination while their offspring utilize the profits of mutualist–plant interaction—i.e., seeds (Pellmyr et al. 1996). In less specialized systems, a variety of other species may easily exploit the rewards offered to attract pollinators and/or consume flower parts or seeds (Galen 1999; Cariveau et al. 2004; Irwin 2006). For the vast majority of cases, two-party mutualisms are imbedded within a broader community that includes other mutualists and antagonists. Thus, understanding these myriad interactions is critical to predicting the long-term ecological and evolutionary trajectories of plants and their mutualist partners (Bronstein et al. 2003, 2006; Stanton 2003; Ferriere et al. 2007). Here, we examined interactions between two antagonists—nectar robbers and pre-dispersal seed predators—and their combined effects on host plant fitness that are, ultimately, dependent on the plant's interactions with mutualist pollinators.

Despite the commonness of nectar robbing (reviewed in Maloof and Inouye 2000; Irwin et al. 2001; Irwin and Maloof 2002), the importance of nectar robbers to other non-pollinating visitors, and the combined outcome of antagonists to pollination and plant fitness, has yet to be broadly considered. Although nectar robbers can significantly decrease plant fitness through direct consumption of reproductive parts (McDade and Kinsman 1980; Traveset et al. 1998; Galen 1999), the indirect effects of nectar robbers may be exacerbated or ameliorated by other species with which their host plants interact. For example, nectar robbing may significantly decrease pollination (Roubik 1982), and/or it might induce biotic or abiotic defense against flower- and seed-feeding insects (Newman and Thomson 2005).

Pre-dispersal seed predators can also have significant direct and indirect effects on pollination and plant fitness (Brody 1997; Brody and Mitchell 1997; Fenner et al. 2002). For cases in which seed predators do not also act as pollinators, pre-dispersal seed predators may significantly reduce the number of viable fruits and seeds that a plant produces and impose selection on floral and flowering traits (Brody 1992a; English-Loeb and Karban 1992; Pilson 2000; Fenner et al. 2002). In addition to their direct effects on plant fitness, pre-dispersal seed predators may affect the outcome of interactions between a plant and other floral enemies, such as nectar robbers. These interactions may be additive, or they may cancel each other out. For example, suppose nectar robbers reduce seed production by 50% (Irwin and Brody 1999). If seed predators consume an additional 50% of the remaining fruits, total female fitness will be additively reduced by 75%. Alternatively, if nectar-robbed flowers do not develop into fruits, the seed predators they harbor will perish because the survival of larvae of pre-dispersal seed predators is contingent upon the development of successful fruits. In that case, the effects of seed predation on robbed flowers would be negligible. Assessing the degree to which antagonists interact is one of the first steps toward understanding whether the traits important to one interaction evolve independently from traits involved in other interactions (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997).

We quantified the interactions between nectar-robbing bumblebees and a dipteran pre-dispersal seed predator utilizing a common sub-alpine host plant, scarlet gilia (*Ipomopsis aggregata*; Polemoniaceae), in the Rocky Mountains of Colorado, USA. We predicted that nectar robbing would both reduce plant attractiveness to pre-dispersal seed predators and decrease the effects of pre-dispersal seed predation on plant reproduction. Our hypotheses assume that adult seed predators can perceive robbed flowers and will avoid ovipositing on them, and/or larval survival of the seed predators is lowered on robbed versus unrobbed plants. Second, our hypotheses assume that because robbed flowers have a significantly lower probability of producing seeds (Irwin and Brody 1999), pre-dispersal seed predator larvae would more likely die on robbed flowers and thus their overall effects would be reduced. To examine these hypotheses, we asked:

1. Do pre-dispersal seed predators avoid ovipositing on flowers that are robbed and on plants with high levels of robbing?
2. Is the survivorship of seed-predator larvae (and thus destruction of fruits) lower on plants with high levels of robbing?
3. How do the combined effects of robbing and pre-dispersal seed predation affect estimates of host plant fitness?

Although the effects of nectar robbers and pre-dispersal seed predators on the reproduction of *I. aggregata* have been established, these studies have been conducted independently. In addition to linking the effects of multiple species on their host, we conducted experiments within and among sites as the outcomes of these interactions may vary across different spatial scales.

Materials and methods

Study system

We studied the montane perennial herb *I. aggregata* (scarlet gilia, Polemoniaceae) native to mountains of western

USA (Grant and Wilken 1986). Our study populations were near the Rocky Mountain Biological Laboratory (RMBL), located outside of Crested Butte, Colorado, at an elevation of 2,900 m. Near the RMBL, *I. aggregata* is monocarpic. Plants grow as vegetative rosettes for 3–7 years, flower once, and then die (Waser and Price 1989). Therefore, estimates of lifetime fitness can be obtained in a single flowering season. Plants begin flowering in mid-June and continue through mid-August. Each plant produces an inflorescence with numerous, red, trumpet-shaped flowers, and plants flower for approximately 6 weeks (Waser 1978). The hermaphroditic flowers are protandrous, and male- and female-phase flowers occur on the same plant at the same time. Corollas usually abscise after 3–5 days. The red, tubular flowers of *I. aggregata* are pollinated primarily by broad-tailed hummingbirds, *Selasphorus platycercus*, and rufous hummingbirds, *S. rufus*, near the RMBL (Waser 1978).

I. aggregata is nectar robbed by the bumblebee, *Bombus occidentalis* (Hymenoptera: Apidae). *B. occidentalis* uses its sharp, toothed mandibles to chew a hole through the side of the corolla. It inserts its proboscis into the hole and removes all available nectar without damaging the plant's reproductive structures (Irwin and Brody 1998). *B. occidentalis* does not, to our knowledge, pollinate *I. aggregata* and nectar robbing decreases visitation by hummingbird pollinators resulting in an approximate 50% reduction in both male and female plant-fitness estimates (Irwin and Brody 2000).

I. aggregata is also the host of a pre-dispersal seed predator, *Hylemya* sp. (Diptera: Anthomyiidae; Zimmerman 1980). *Hylemya* females oviposit under the sepals of buds and flowers, usually depositing a single egg at each oviposition event. The larva hatches and burrows into the ovary where it feeds on developing seeds. Most often, the larva on a given fruit destroys all of the seeds before it bores through the carpal wall, falls to the ground, and pupates in the soil. *Hylemya* often destroy up to 80% of fruits on individual *I. aggregata* plants (Brody 1991). Thus, these seed predators can greatly reduce total seed production (Juenger and Bergelson 1998). Unlike other plants which may abscise damaged flowers or selectively abort damaged fruits (Pellmyr and Huth 1994), *I. aggregata* does not abort fruits with *Hylemya* larvae and, in fact, the probability of fruit set is higher for flowers on which females oviposit than on other flowers of the same plant (see below, and Brody and Morita 2000).

Within sites, not all plants are likely to be robbed, and robbing levels (proportion of flowers robbed per plant) show a bimodal distribution with peaks at low and high robbing levels (Irwin and Brody 1998). Among sites, mean robbing levels vary almost 100-fold within the same flowering season (Irwin and Maloof 2002), and visitation by pollinators varies considerably within and among sites as well (Price et al. 2005). Given the demonstrated spatial variability in nectar robbing and pollination, we examined how robbing affected seed predation and subsequent plant reproduction both within and among sites. At the within-site spatial scale, we focused on how nectar robbing affected fruit destruction by *Hylemya* and subsequent plant reproduction. At the among-site spatial scale, we measured how nectar robbing affected *Hylemya* oviposition and survival, as well as how robbing affected fruit destruction by *Hylemya* and subsequent plant reproduction. We predicted that both within and among sites, high levels of nectar robbing would cancel out or reduce the effects of seed predation. Thus, we would predict that where nectar robbers are most active, they would be the primary agents interacting with pollinators to affect seed set (Irwin and Brody 2000), plant demography (Price et al. 2007), and floral evolution (Irwin 2006). Alternatively, where nectar robbers are rare or absent, we would predict that the interactions between pre-dispersal seed predators, pollinators and their hosts would be the primary agents of ecological and evolutionary effects.

Field methods

To examine how nectar robbing affected pre-dispersal seed predation and subsequent plant reproduction, we conducted experiments at two spatial scales. In the first experiment, we experimentally robbed individual plants within an *I. aggregata* population (hereafter referred to as a "site"). In the second experiment, we experimentally robbed plants within entire *I. aggregata* sites. We define an *I. aggregata* site as a population of *I. aggregata* that is separated from other populations by at least 100 m. We used these two spatial scales because robbing levels of *I. aggregata* can vary both within and among sites, and thus the interactions among robbers and pre-dispersal seed predators might vary within and among sites as well.

Effects of nectar robbing and seed predation within sites

Along the Copper Creek Trail, adjacent to the RMBL, we haphazardly chose 50 bolting *I. aggregata* in June 2005. We randomly assigned 25 plants each to either a robbing treatment (greater than 80% of flowers robbed) or a no-robbing (control) treatment. These levels represent high and low robbing levels commonly found in the field (Irwin and Maloof 2002). Plants assigned to the treatments did not differ in initial plant size (root stalk diameter, ANOVA, $F_{1,48} = 0.28$, $P = 0.60$). We artificially robbed flowers by cutting a hole, ca. 1 mm in diameter, in the side of the corolla using fine-tipped dissecting scissors. We then removed all available nectar from each flower using a microcapillary tube inserted in the hole. Our method

mimics natural robbing by *B. occidentalis* (Irwin and Brody 1998). We handled flowers on the control plants similarly, but did not make a hole in them. Natural robbing levels were low in the year of study and virtually none of the flowers on the control plants were robbed. We performed the robbing treatments 4 days per week throughout the blooming season.

At the end of the flowering season, we collected all fruits and scored them as having set seed or not (aborted), recorded if the fruits had been destroyed by *Hylemya*, and counted all seeds from intact fruits. *Hylemya* larvae destroy the seeds, leaving behind a mass of partially eaten seeds and frass and an exit hole in the carpal wall. Fruit destruction by *Hylemya* is distinctive and not easily mistaken for any other kind of damage (Brody 1992a).

Statistical analyses

To test the effects of nectar robbing on seed predation by *Hylemya*, we used an ANOVA with robbing treatment (robbing vs. control) as a fixed effect and the percent of fruits destroyed by *Hylemya* (arc-sine square-root transformed) as the response variable. The percent of destroyed fruits was calculated as the number of destroyed fruits divided by the total number of fruits produced per plant. To test the effects of nectar robbing and natural levels of seed predation on female plant reproduction, we used a multiple ANOVA (MANOVA) with robbing treatment as a fixed effect, and percent fruit set (number of expanded fruits divided by number of flowers produced per plant), seed set per undamaged fruit, and total seeds produced per plant as response variables. A MANOVA was used initially to control for multiple correlated dependent variables (Gotelli and Ellison 2004). A significant MANOVA was followed by univariate ANOVAs for each response variable.

Effects of nectar robbing and seed predation among sites

To examine how high levels of nectar robbing affected oviposition choices of *Hylemya*, and the combined effects of *Hylemya* and robbers, we chose three pairs of sites where *I. aggregata* was abundant in June 2000. Pairs of sites were chosen to be similar in slope, aspect, and the number of flowering *I. aggregata*. All sites were greater than 200 m apart. We randomly assigned one site of each pair to be robbed, and the other to remain as an unrobbed (control) site. We artificially robbed flowers and handled the control flowers, as described above. In the robbed sites, at least 80% of all flowers on each plant were robbed every other day from the beginning to the end of the flowering season, and approximately 80% of all plants at each site were robbed.

We chose 20 focal plants at each site and, at 5-day intervals, we examined all of the flowers of each plant for the presence of *Hylemya* eggs. If we found an egg, we left it undisturbed and marked the outside of the calyx with a dot of indelible ink. At the end of the season, we collected all fruits and scored them as having set seed or not (aborted) and recorded if the fruits had been destroyed by *Hylemya*, as described above. Finally, we counted all of the seeds produced per plant.

Statistical analyses

To test the effects of nectar robbing on *Hylemya* oviposition and offspring survival, we examined the percentage of flowers with eggs in robbed versus control sites. We averaged census data over the season to provide an overall mean percentage of flowers with eggs for each plant. We used percentage of fruits destroyed from flowers known to have a *Hylemya* egg (arcsine square-root transformed) to test if *Hylemya* survivorship was lower on plants from sites with high robbing.

To test the combined effects of nectar robbing and predispersal seed predation on plant reproduction, we examined the percent of flowers destroyed by *Hylemya* (arcsine square-root transformed), percent fruit set (arcsine square-root transformed), seeds per fruit (log transformed), and total seeds per plant (log transformed) averaged over all 20 focal plants in each site. We compared response variables between robbed and control sites using one-tailed, paired *t*-tests with the a priori hypothesis that seed predators would avoid ovipositing on robbed plants and flowers and that robbing would reduce levels of plant reproduction, assuming that the negative effects of robbing outweighed the benefits of deterring seed predators.

Because experimentally robbing all plants at a given site designated for robbing was time-consuming, we only had three site pairs per treatment and, thus, low statistical power to detect an effect of robbing on seed predation and plant reproduction (Hurlbert 2004). Therefore, in addition to reporting the significance values of the *t*-tests, we also calculated the effect size (Hedge's *d*) for each response variable for each site pair. The effect size measures the magnitude of the difference between robbed and control sites for each response variable in SD units (Gurevitch and Hedges 1993), and we calculated the cumulative effect size across the site pairs. The cumulative effect size measures the overall magnitude of the robbing treatment compared to the control treatment across site pairs. We calculated cumulative effect sizes using a random-effects model which assumes that, in addition to sampling error, there is random variation in effect sizes between site pairs (Gurevitch and Hedges 1993). In our analyses, a positive effect size means that plants in the control sites had a higher value for a given response variable than plants in the robbed sites, and vice versa for a negative effect size. An effect size of 0.2 is considered small, 0.5

medium, 0.8 large, and greater than 1.0 is considered very large (Cohen 1969). For all effect sizes, we calculated 95% confidence intervals (CIs) using bootstrapping (with 999 iterations) using MetaWin (Rosenberg et al. 2000). Effect sizes are considered statistically significant if they do not overlap zero (Gurevitch and Hedges 1993).

Last, we used a paired *t*-test across all sites to assess whether the presence versus absence of *Hylemya* eggs on flowers affected the probability that flowers set fruit.

Results

Effects of robbing and seed predation within sites

Within sites, both the number and the percent of fruits destroyed by *Hylemya* were significantly lower on robbed compared to control plants ($F_{1,47} = 5.09$, $P = 0.0287$, $F_{1,47} = 16.91$, $P = 0.0002$; Fig. 1a, b, respectively).

Although plants with high robbing experienced lower seed predation, they still had lower estimates of female reproductive success than control plants ($\lambda = 1.58$, $F_{3,43} = 22.58$, $P < 0.0001$). Percent fruit set was 47% lower in plants with high robbing compared to control plants ($F_{1,47} = 61.16$, $P < 0.0001$; Fig. 2a), and plants with high robbing produced 37% fewer seeds per plant than control plants ($F_{1,47} = 4.39$, $P = 0.04$; Fig. 2b). However, there was no difference in seeds produced per fruit in plants with high robbing (mean ± SE, 4.13 ± 0.30 seeds per fruit) versus control plants (mean ± SE, 4.35 ± 0.32 seeds per fruit; $F_{1,45} = 0.25$, $P = 0.62$).

Effects of nectar robbing and seed predation among sites

Hylemya females oviposited on 32% of all flowers on plants in robbed sites and on 35% of the flowers on plants in control sites ($t_2 = 2.30$, $P = 0.07$). The cumulative effect size between robbed and control sizes for *Hylemya* oviposition was small ($d = 0.25$), but the 95% CI did not overlap zero (95% CI, 0.11–0.50). Survivorship of *Hylemya* larvae on plants in the robbed sites was statistically indistinguishable from survivorship in the sites where plants were not

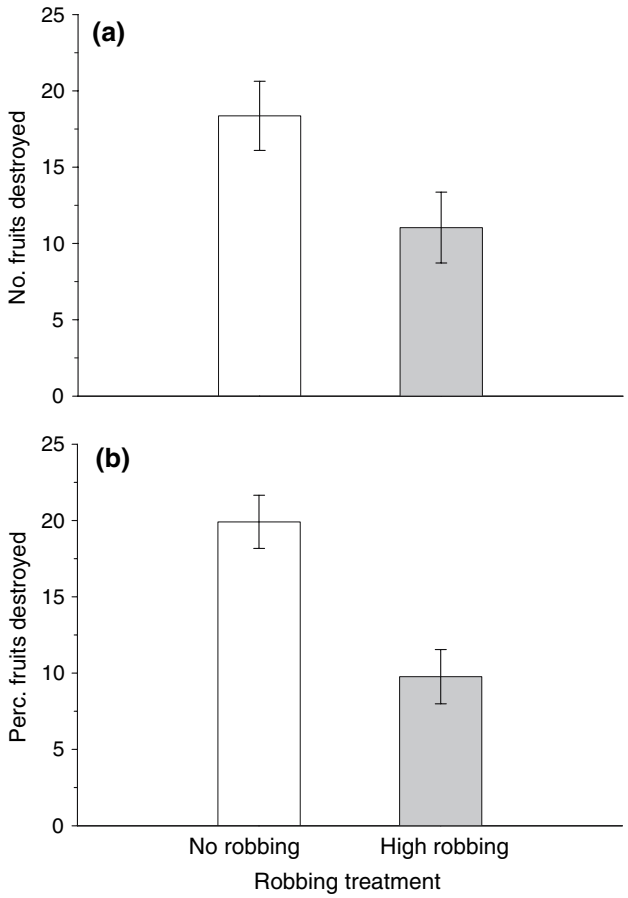

Fig. 1a, b Within-site effects of robbing on pre-dispersal seed predation. Plants had 80% of their flowers experimentally robbed or were assigned the no-robbing control

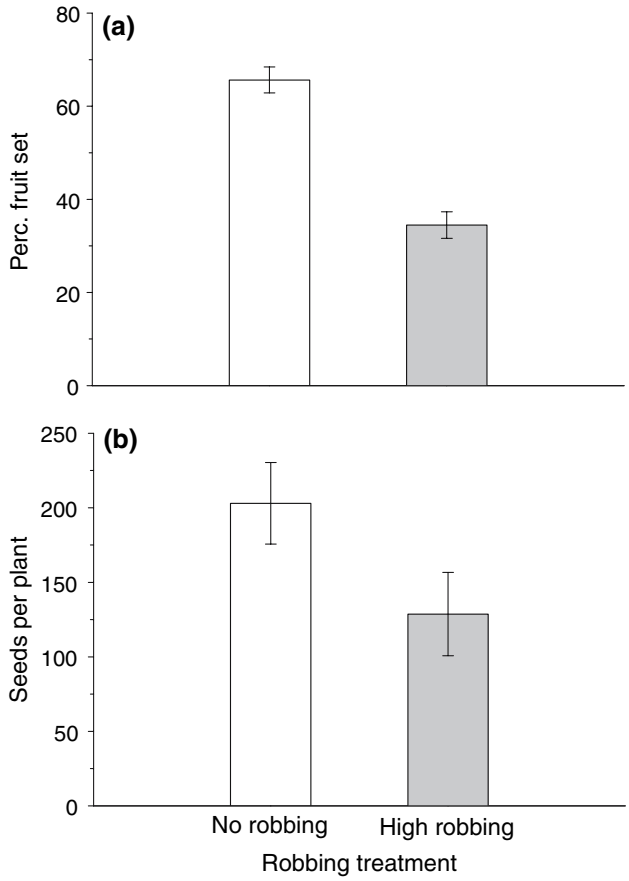

Fig. 2a, b Within-site effects of robbing and seed predation on estimates of female reproduction. Plants were robbed (80% of their flowers had all nectar removed) or were controls with no robbing imposed

robbed ($t_2 = 0.14$, $P = 0.55$). From the flowers that *Hylemya* oviposited on, 36% of the larvae survived in robbed sites and 37% of the larvae survived in the control sites. The effect size was small ($d = 0.02$), and the 95% CI overlapped zero (95% CI, −0.13 to 0.25).

Robbing had no statistically significant effect on levels of fruit destruction by seed predators or on estimates of plant reproductive success. We provide statistical values and effect sizes for each response variable to assess the biological range of effects. The percent of fruits destroyed by *Hylemya* was 27% lower on plants in robbed compared to control sites ($t_2 = 1.04$, $P = 0.20$; Fig. 3). The effect size was again moderate ($d = 0.53$), but the 95% CI overlapped zero (95% CI, −0.30 to 1.55). However, across all sites, flowers on which *Hylemya* oviposited were 32% more likely to set fruit than those the fly did not oviposit on (paired *t*-test within plants using plant as unit of replication, $t_{116} = 7.86$, $P < 0.0001$). Nonetheless, while *Hylemya* destroy fruits and seeds in addition to the losses attributable to robbing, robbing significantly lowered estimates of female plant reproduction, even though robbed plants had fewer fruits destroyed by *Hylemya*. Overall, robbing reduced percent fruit set by 22% ($t_2 = 1.42$, $P = 0.14$; Fig. 4a), seed set per fruit by 25% ($t_2 = 1.38$, $P = 0.15$; Fig. 4b), and total seeds produced per plant by 46% ($t_2 = 1.44$, $P = 0.14$; Fig. 4c) on plants in robbed compared to control sites. The cumulative effect sizes between robbed and control sites were large for percent fruit set ($d = 0.78$) and moderate for seed set per fruit ($d = 0.42$) and total seeds ($d = 0.60$); but in all cases the 95% CIs overlapped zero (95% CI, percent fruit set, −0.28 to 1.57; seed set per fruit, −0.27 to 0.80; total seeds, −0.13 to 1.27).

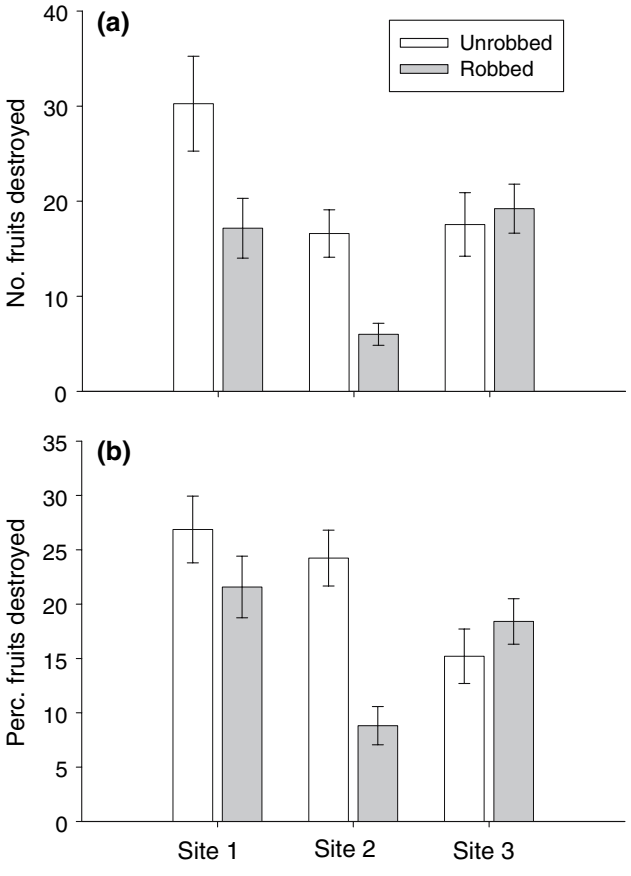

Fig. 3a, b Among-site effects of robbing on pre-dispersal seed predation. All plants were either robbed or not robbed. Robbing was imposed on at least 80% of the flowers on each plant over its lifetime. Unrobbed plants experienced no robbing

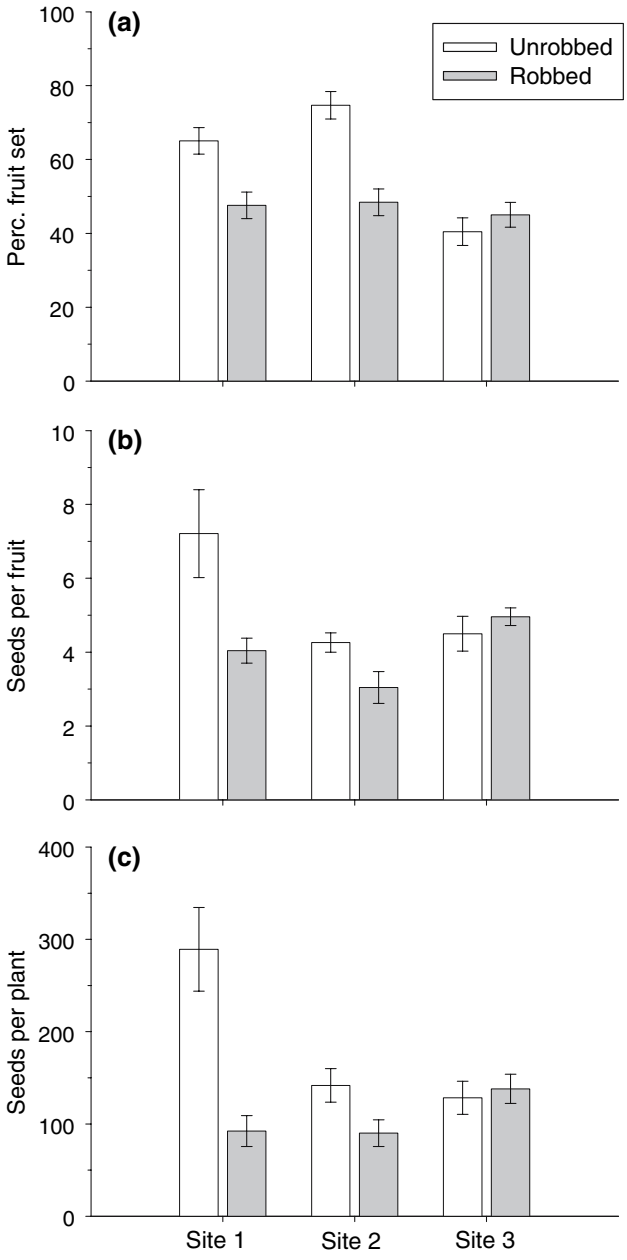

Fig. 4a–c Combined effects on female reproductive success among site pairs. Plants at robbed sites had 80% of their flowers robbed, unrobbed plants experienced no robbing

Discussion

Pairwise interactions, such as those between plants and pollinators, plants and nectar robbers, and plants and seed predators, do not often occur in isolation. Rather, a single host plant often interacts with a suite of organisms whose combined effects are not always predictable from tests of individual effects. The effects of multiple antagonists may not be independent—the effects of one antagonist might exacerbate or ameliorate the likelihood of interaction with another antagonist or the effects of another antagonist. Here, we show that pre-dispersal seed predation is not independent of nectar robbing either within or among populations of plants.

We expected that pre-dispersal seed predators would avoid individual plants and plant populations with high levels of robbing, or the effects of seed predation might cancel out the effects of nectar robbing. Indeed, we found that nectar-robbed plants had a lower percentage of fruits destroyed by seed predators. However, although robbed plants experienced lower seed predation, robbed plants still produced fewer seeds than control plants. These results suggest that the benefits of robbing in terms of reduced seed predation did not outweigh the costs of robbing for female plant reproduction. When we manipulated robbing between sites, the pattern was similar although not statistically significant. Our results suggest that patterns of host use by one community member (nectar robbers) can elicit changes in host use by subsequently interacting community members (seed predators) sharing the same host plant.

How might nectar robbing alter subsequent seed predation? At least two non-mutually exclusive mechanisms may be involved. First, because larval success of pre-dispersal seed predators depends on successful fruit production, seed predators should avoid ovipositing on robbed flowers. However, *Hylemya* females most often oviposit on flowers that are still in the elongated bud stage (Brody 1992b). Robbing does occur on elongated buds (R. E. Irwin, personal observation), but the probability of robbing increases as the flower ages. Even so, it is conceivable that *Hylemya* might avoid plants with high levels of robbing entirely. Second, seed predation may be lower on robbed plants and flowers if larval survivorship is reduced on robbed flowers. Do pre-dispersal seed predators avoid robbed flowers or plants with high levels of robbing? Perhaps. Robbing of all plants within sites resulted in a marginally significant reduction ($P = 0.07$) of oviposition by *Hylemya* relative to control sites. The cumulative effect sizes between robbed and control sites for *Hylemya* oviposition were small, but the 95% confidence intervals did not overlap zero. Thus, to the extent that they can find alternative hosts, *Hylemya* females may avoid sites with high levels of nectar robbing. Is larval survivorship reduced on robbed flowers or plants? Apparently not. The survivorship of *Hylemya* larvae on plants in the robbed sites was statistically indistinguishable from survivorship in the sites where plants were not robbed; 36% of the larvae survived in robbed sites and 37% of the larvae survived in control sites. In addition, we found no evidence for lower survivorship of larvae on robbed flowers. These lack of differences may be attributable to higher fruiting success of flowers on which *Hylemya* oviposit that we found here and previously as well.

Flowers upon which *Hylemya* oviposited had a higher probability of setting seed, regardless of whether or not they were robbed, compared to those flowers *Hylemya* females did not choose. The positive effect of *Hylemya* on the probability of a fruit setting seed has been previously documented and tested (Brody 1992b; Brody and Waser 1995; Brody and Morita 2000). Although the physiological mechanism remains a mystery, female *Hylemya*—by virtue of some subtle manipulation of the flowers on which they oviposit and not through choice of fertilized flowers—enhance fruit set of *I. aggregata* flowers (Brody and Morita 2000). Fruit and seed predation and herbivory on plants and flowers that are more likely to set fruit has been shown in other systems as well (Herrera 2000; Cariveau et al. 2004). For example, seed predation on *Castilleja linariaefolia* was up to 34% higher in plants that received supplemental hand pollination relative to open-pollinated plants (Cariveau et al. 2004). Here, for those unrobbed flowers on which *Hylemya* oviposits but the larvae perish, the net effect of oviposition could be positive.

The interactions of community members sharing host plants in different ways are increasingly being recognized and gaining attention (Strauss and Irwin 2004; Bronstein et al. 2006; Morris et al. 2007). For *I. aggregata*, not only does nectar robbing influence seed predation, early season herbivory by mule deer is likely to affect plant susceptibility to both robbers and seed predators. Early season herbivory delays flowering and lowers the per flower risk to *Hylemya* (Juenger and Bergelson 1998; Juenger et al. 2005). However, herbivory exacerbates the effects of nectar robbing because plants that bloom late in the season incur higher rates of robbing (Sharaf and Price 2004; Irwin 2006). In some years, but not all, nectar robbing increases as the season progresses (Irwin and Maloof 2002; Irwin 2006), and oviposition by *Hylemya* usually declines over the season (A. K. Brody, unpublished data). However, this is not always the case—2007 being a notable exception in robbing levels remaining consistently high over the entire flowering season, with a long period of overlap between robbers and seed predators (R. E. Irwin, personal observation).

In other systems, the ecological outcomes of multiple antagonists are complex as well. The flowering phenology of wild sunflower, *Helianthus annuus* (Asteraceae) is influenced by several flower- and seed-feeding herbivores. Plants that bloom late avoid heavy damage by head-clipping

weevils and moths that infest the buds and flower heads; however, such plants may incur heavier losses to seed flies than those that bloom later in the season (Pilson 2000). It is likely that early season florivores cancel the effects of those occurring late in the season, but to no benefit for their host. Similarly, herbivory from chrysomelid beetles negated the effects of browsing by deer on wild sumac. However, injury by cerambycids was magnified when it occurred in conjunction with attack by chrysomelids (Strauss 1991). Thus, the combined effects of multiple antagonists are not always additive and are often difficult to predict from pairwise studies (Wootton 1993).

In the 2 years of this study, fruit destruction by *Hylemya* was low (only 15–19%). By comparison, in previous years, up to 80% of fruits were destroyed (Brody 1991). In years when *Hylemya* is more abundant, the effects of nectar robbing may pale in comparison to the damage done by larval seed predators, and the benefits of low robbing may be outweighed by the costs of increased seed predation. Alternatively, in years when ovipositing females are abundant but, for whatever reason, larval survivorship is low (e.g., dry years with high levels of egg desiccation; Zimmerman and Brody 1998), *Hylemya* could exert positive effects on fruit set given that flowers that females oviposit on are more likely to set fruit. Ultimately, the spatial and temporal patterns of these interactions and gene flow among populations will drive the degree to which these species interactions affect long-term responses by their host plants (Thompson 1994).

Nectar robbing of *I. aggregata* varies both within and among sites, and thus we wanted to assess how robbing affected seed predation and subsequent plant performance at both spatial scales. The effects of nectar robbing on fruit destruction and subsequent seed production were statistically significant at the level of individual plants but not when we manipulated robbing at the level of sites, although both manipulations showed similar trends. Because we performed the manipulations in 2 years, the difference in the significance at the two spatial scales could be due to interannual differences in the effects of robbing on seed predation and plant reproduction, or simply due to the low statistical power we had to detect a difference in the effects of robbing at the among-site level (given that we only had three site pairs). The outcomes of interactions among species are inherently variable across space and time, and variation in the number and kind of floral visitors can have profound effects on estimates of plant fitness (e.g., Mayfield et al. 2001; Thompson 2001; Price et al. 2005). Variability in the strength and direction of interactions may help explain why genetic variation in traits thought to be under strong selection pressure is maintained. And, the presence of a variety of biotic selection pressures, such as those exerted by herbivores, florivores, nectar robbers, seed predators and pollinators, whose effects may oppose one another, provide the context for a suite of potential trade-offs faced by host plants. Although pollinators are clearly important to the evolution of flowering traits (reviewed in Lloyd and Barrett 1996), phenotypic variability and selection on floral traits may be attributable, at least in part, to interactions with other organisms (Armbruster 1997; Herrera et al. 2002; Gómez 2003; Irwin 2006; Morris et al. 2007).

The study of species interactions is increasingly moving toward comparative and interdisciplinary approaches. The past focus on the effects of single species on each other (e.g., a single pollinator, or herbivore) is being replaced by a broader focus on mutualism guilds (Stanton 2003), combined effects of mutualists and antagonists (Bronstein et al. 2003; Strauss and Irwin 2004), and the effects of multiple antagonists on their hosts, on each other, and on host–mutualist interactions (Iwao and Rausher 1997; Bronstein et al. 2003; Morris et al. 2007). The field is ripe for studies that both scale up to communities and ecosystems, and scale down to exploring the underlying mechanisms governing multi-species interactions. Knowledge of the extent to which interactions are consistent (or not) across the geographical range of species, coupled with knowledge of gene flow among populations, will allow for insights in reconstructing the past evolution of traits important to the interactions as well as provide predictive power regarding the stability of interactions in the future (Thompson 1994). In addition, knowledge of the underlying genetic basis and biochemical mechanisms that govern interactions among plants and their mutualist and antagonist partners will go far in advancing our understanding beyond descriptions of empirical effects. Ultimately, we need to explore the genetic basis for plant response to simultaneous attack by antagonists, and how that response affects traits important to mutualists, to understand how selection has and will respond to the full suite of species with which any given host plant interacts.

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References

Armbruster WS (1997) Exaptations link evolution of plant–herbivore and plant–pollinator interactions: a phylogenetic inquiry. Ecology 78:1661–1672

Brody AK (1991) Pre-dispersal seed predation by *Hylemya* (*Delia*) sp. (Diptera: Anthomyiidae): mechanisms and consequences of oviposition choice. University of California, Davis, Calif.

Brody AK (1992a) Oviposition choices by a predispersal seed predator (*Hylemya* sp.). 1. Correspondence with hummingbird pollinators, and the role of plant size, density and floral morphology. Oecologia 91:56–62

Brody AK (1992b) Oviposition choices by a predispersal seed predator (*Hylemya* sp.). 2. A positive association between female choice and fruit-set. Oecologia 91:63–67

Brody AK (1997) Effects of pollinators, herbivores, and seed predators on flowering phenology. Ecology 78:1624–1631

Brody AK, Mitchell RJ (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant, *Ipomopsis aggregata*. Oecologia 110:86–93

Brody AK, Morita S (2000) A positive association between oviposition and fruit set: female choice or manipulation? Oikos 124:418–425

Brody AK, Waser NM (1995) Oviposition patterns and larval success of a pre-dispersal seed predator attacking two confamilial host plants. Oikos 74:447–452

Bronstein JL, Wilson WG, Morris WE (2003) Ecological dynamics of mutualist/antagonist communities. Am Nat 162:S24–S39

Bronstein JL, Alarcon R, Geber M (2006) The evolution of plant–insect mutualisms. New Phytol 172:412–428

Cariveau D, Irwin RE, Brody AK, Sevillano LG-M, von der Ohe A (2004) Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. Oikos 104:15–26

Cohen J (1969) Statistical power analysis for the behavioral sciences. Academic Press, New York

Darwin CR (1862) On the various contrivances by which British and foreign orchids are fertilised by insects: and on the good effects of intercrossing. Murray, London

English-Loeb GM, Karban R (1992) Consequences of variation in flowering phenology for seed head herbivory and reproductive success in *Erigeron glaucus* (Compositae). Oecologia 89:588–595

Faegri K, van der Pjil P (1979) The principles of pollination ecology. Pergamon, Oxford

Fenner M, Cresswell JE, Hurley RA, Baldwin T (2002) Relationship between capitulum size and pre-dispersal seed predation by insect larvae in common *Asteraceae*. Oecologia 130:72–77

Ferriere R, Gauduchon M, Bronstein JL (2007) Evolution and persistence of obligate mutualists and exploiters: competition for partners and evolutionary immunization. Ecol Lett 10:115–126

Galen C (1999) Flowers and enemies: predation by nectar-thieving ants in relation to variation in floral form of an alpine wildflower, *Polemonium viscosum*. Oikos 85:426–434

Galen C, Cuba J (2001) Down the tube: pollinators, predators, and the evolution of flower shape in the alpine skypilot, *Polemonium viscosum*. Evolution 55:1963–1971

Gómez JM (2003) Herbivory reduces the strength of pollinator-mediated selection in the Mediterranean herb *Erysimum mediohispanicum*: consequences for plant specialization. Am Nat 162:242–256

Gotelli NJ, Ellison AM (2004) A primer of ecological statistics. Sinauer, Sunderland, Mass.

Grant V, Wilken DH (1986) Taxonomy of the *Ipomopsis aggregata* group (Polemoniaceae). Bot Gaz 147:359–371

Gurevitch J, Hedges LV (1993) Meta-analysis: combining the results of independent experiments. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman & Hall, New York, pp 378–398

Herrera CM (2000) Measuring the effects of pollinators and herbivores: evidence for non-additivity in a perennial herb. Ecology 81:2170–2176

Herrera CM, Medrano M, Rey PJ, Sánchez-Lafuente AM, García M, Guitián J, Manzaneda AJ (2002) Interaction of pollinators and herbivores on plant fitness suggests a pathway for correlated evolution of mutualism- and antagonism-related traits. Proc Natl Acad Sci USA 99:16823–16828

Hougen-Eitzman D, Rausher MD (1994) Interactions between herbivorous insects and plant−insect coevolution. Am Nat 143:677–697

Hurlbert SH (2004) On misinterpretations of pseudoreplication and related matters: a reply to Oksanen. Oikos 104:591–597

Irwin RE (2006) Consequences of direct versus indirect species interactions to selection on traits: pollination and nectar robbing in *Ipomopsis aggregata*. Am Nat 167:315–328

Irwin RE, Brody AK (1998) Nectar robbing in *Ipomopsis aggregata*: effects on pollinator behavior and plant fitness. Oecologia 116:519–527

Irwin RE, Brody AK (1999) Nectar-robbing bumblebees reduce the fitness of *Ipomopsis aggregata* (Polemoniaceae). Ecology 80:1703–1712

Irwin RE, Brody AK (2000) Consequences of nectar robbing for realized male function in a hummingbird-pollinated plant. Ecology 81:2637–2643

Irwin RE, Maloof JE (2002) Variation in nectar robbing over time, space, and species. Oecologia 133:525–533

Irwin RE, Brody AK, Waser NM (2001) The impact of floral larceny on individuals, populations, and communities. Oecologia 129:161–168

Iwao K, Rausher MD (1997) Evolution of plant resistance to multiple herbivores: quantifying diffuse coevolution. Am Nat 149:316–335

Juenger T, Bergelson J (1998) Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. Evolution 52:1583–1592

Juenger T, Morton TC, Miller RE, Bergelson J (2005) Scarlet gilia resistance to insect herbivory: the effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack. Evol Ecol 19:79–101

Lehtilä K, Strauss SY (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, *Raphanus raphanistrum*. Oecologia 111:396–403

Lloyd DG, Barrett SCH (1996) Floral biology: studies on floral evolution in animal-pollinated plants. Chapman & Hall, New York

Maloof JE, Inouye DW (2000) Are nectar robbers cheaters or mutualists? Ecology 81:2651–2661

Mayfield MM, Waser NM, Price MV (2001) Exploring the "most effective pollinator principle" with complex flowers: bumblebees and *Ipomopsis aggregata*. Ann Bot 88:591–596

McDade LA, Kinsman S (1980) The impact of floral parasitism in two neotropical hummingbird-pollinated plant species. Evolution 34:944–958

Miller TE, Travis J (1996) The evolutionary role of indirect effects in communities. Ecology 77:1329–1335

Morris W, Hufbauer R, Agrawal A, Bever J, Borowicz V, Gilbert G, Maron J, Mitchell C, Parker I, Power A, Torchin M, Vázquez D (2007) Direct and interactive effects of enemies and mutualists on plant performance: a meta-analysis. Ecology 88:1021–1029

Newman DA, Thomson JD (2005) Interactions among nectar robbing, floral herbivory, and ant protection in *Linaria vulgaris*. Oikos 110:497–506

Pellmyr O, Huth CJ (1994) Evolutionary stability of mutualism between yuccas and yucca moths. Nature 372:257–260

Pellmyr O, Leebens-Mack J, Huth CJ (1996) Non-mutualistic yucca moths and their evolutionary consequences. Nature 380:155–156

Pilson D (2000) Herbivory and natural selection on flowering phenology in wild sunflower, *Helianthus annuus*. Oecologia 122:72–82

Price MV, Waser NM, Irwin RE, Campbell DR, Brody AK (2005) Temporal and spatial variation in pollination of a montane herb: a seven year study. Ecology 86:2106–2116

Price MV, Campbell DR, Waser NM, Brody AK (2007) Bridging the generation gap in plants: pollination, parental fecundity, and offspring demography. Ecology (in review)

Proctor M, Yeo P, Lack A (1996) The natural history of pollination. Timber Press, Portland, Oreg.

Rosenberg MS, Adams DC, Gurevitch J (2000) *MetaWin*. Statistical software for conducting meta-analysis: fixed effect models, mixed effect models, and resampling tests. (Version 2.0). Sinauer, Sunderland, Mass.

Roubik DW (1982) The ecological impact of nectar-robbing bees and pollinating hummingbirds on a tropical shrub. Ecology 63:354–360

Schemske DW, Horvitz CC (1988) Plant–animal interactions and fruit production in a neotropical herb: a path analysis. Ecology 69:1128–1137

Sharaf KE, Price MV (2004) Does pollination limit tolerance to browsing in *Ipomopsis aggregata*? Oecologia 138:396–404

Stanton ML (2003) Interacting guilds: moving beyond the pairwise perspective on mutualisms. Am Nat 162:S10–S23

Strauss SY (1991) Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. Ecology 72:543–558

Strauss SY, Irwin RE (2004) Ecological and evolutionary consequences of multispecies plant–animal interactions. Annu Rev Ecol Evol Syst 35:435–466

Thompson JN (1994) The coevolutionary process. University of Chicago Press, Chicago, Ill.

Thomson JD (2001) How do visitation patterns vary among pollinators in relation to floral display and floral design in a generalist pollination system? Oecologia 126:386–394

Traveset A, Willson MF, Sabag C (1998) Effect of nectar-robbing birds on fruit set of *Fuchsia magellanica* in Tierra del Fuego: a disrupted mutualism. Funct Ecol 12:459–464

Waser NM (1978) Competition for hummingbird pollination and sequential flowering in Colorado wildflowers. Ecology 59:934–944

Waser NM, Ollerton J (2006) Plant–pollinator interactions: from specialization to generalization. University of Chicago Press, Chicago, Ill.

Waser NM, Price MV (1989) Optimal outcrossing in *Ipomopsis aggregata*: seed set and offspring fitness. Evolution 43:1097–1109

Wootton JT (1993) Indirect effects and habitat use in an intertidal community: interaction chains and interaction modifications. Am Nat 141:71–89

Zimmerman M (1980) Reproduction in *Polemonium*: pre-dispersal seed predation. Ecology 61:502–506

Zimmerman M, Brody AK (1998) Choices and consequences of oviposition by *Hylemya* (*Delia*) sp. (Diptera: Anthomyiidae). J Insect Behav 11:371–381