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Behavioral mechanisms underlie an ant-plant mutualism

Abstract Predators can reduce herbivory by consuming herbivores (a consumptive effect) and by altering herbivore behavior, life history, physiology or distribution (non-consumptive effects). The non-consumptive, or trait-mediated, effects of predators on prey may have important functions in the dynamics of communities. In a facultative ant-plant mutualism, we investigated whether these non-consumptive effects influenced the host plants of prey. Here, predaceous ants (*Forelius pruinosus*) consume and disturb a dominant lepidopteran folivore (*Bucculatrix thurberiella*) of wild cotton plants (*Gossypium thurberi*). Season-long ant exclusion experiments revealed that ants had a larger proportional effect on damage by *B. thurberiella* than on caterpillar abundance, a result that suggests ants have a strong non-consumptive effect. Behavioral experiments conducted in two populations over 2 years demonstrated that *B. thurberiella* caterpillars were substantially less likely to damage wild cotton leaves in the presence of ants due to ant-induced changes in caterpillar behavior. In the absence of ants caterpillars spent more time stationary (potential feeding time) and less time dropping from leaves by a thread of silk than when ants were present. Furthermore, ants altered the spatial distribution of both caterpillars and damage; caterpillars spent relatively more time on the upper surfaces of leaves and caused damage further from the leaf margin in ant exclusion treatments. Both direct encounters with ants and information conveyed when ants walked onto leaves were key events leading to the anti-predator behaviors of caterpillars. This study contributes to a small body of evidence from terrestrial systems demonstrating that the trait-mediated effects of predators can cascade to the host plants of prey.

Keywords *Forelius* · *Gossypium* · Trait-mediated predation · Tri-trophic interactions

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Introduction

The effects of predators can cascade through communities of organisms (reviews: Polis 1999; Polis et al. 2000; Schmitz 2000; Schmitz et al. 2000; Halaj and Wise 2001). While predators can reduce prey in at least two ways – by consuming them (a consumptive or density-mediated effect) or by altering their behavior, morphology, physiology, distribution, or life history (a non-consumptive or trait-mediated effect) (Abrams 1995; Lima and Dill 1990; Lima 1998), the relative importance of these two mechanisms in the dynamics of communities is not well resolved. Recent models have shown that incorporating the non-consumptive effects of predators can dramatically alter the conclusions drawn from classic models of predation (Abrams 1995; Schmitz and Booth 1997; Abrams and Schmitz 1999; Luttbeg and Schmitz 2000). In addition, a number of empirical studies in aquatic communities have found important non-consumptive effects of predators (e.g., Power 1987; Werner 1992; Persson 1999; Bernot and Turner 2001; Peacor and Werner 2001). However, in terrestrial habitats, such effects have been explored in only four systems (e.g., Messina 1981; Beckerman et al. 1997; Schmitz 1998; Gastreich 1999; Pusenius and Ostfeld 2000; Schmitz and Suttle 2001), all of which suggest that non-consumptive effects can influence other community members, namely plants. The non-consumptive effects of predators may be particularly important when there is a strong link between

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predators and plant fitness for example, in mutualistic systems such as ant-plant associations. Despite an extensive literature documenting that ants benefit plants by reducing herbivory (reviewed in Koptur 1992; Bronstein 1998; Agrawal and Rutter 1998), few studies have examined potential behavioral mechanisms underlying these interactions (but see Lees and Smith 1991; Freitas and Oliveira 1994). Whether these benefits to plants result from ants' consumptive or non-consumptive effects (or both) remains unclear.

Using an ant-plant association from the Sonoran Desert, we investigated the effects of a predator on both the density and behavior of an herbivore. We then explored the consequences for a third community member, the herbivore's host plant. By conducting trials in the field with prey that had prior exposure to predators, we avoid important shortcomings of many previous experiments (identified by Sih et al. 2000), which have isolated prey in the laboratory for extended time periods and subjected them to only short bouts of predation risk. Our experiments were conducted in the wild cotton system (*Gossypium thurberi*), in which mutualistic ants visit extrafloral nectaries and enhance seed production by reducing herbivory from a specialist lepidopteran (*Bucculatrix thurberiella*) as well as other folivores (Rudgers 2002). Specifically, we addressed the following questions: (1) What are the relative effects of ant exclusion on *B. thurberiella* caterpillar numbers versus *B. thurberiella* caterpillar damage? (2) Do ants modify the spatial distribution of caterpillar damage? (3) Does the presence of ants influence the amount of time caterpillars engage in foraging and anti-predator behaviors? (4) Are caterpillars more likely to damage leaves when ants are absent? (5) Do ants alter caterpillar location within a plant? (6) What information do caterpillars use to assess the risk of predation?

Materials and methods

Natural history of the study species

Gossypium thurberi (Malvaceae, wild cotton) is a perennial shrub native to canyons of the Sonoran Desert of Arizona, USA and Sonora, Mexico (Hanson 1923). Our experiments occurred in two populations in the Santa Rita Mts., Pima Co., Ariz.: Florida Canyon in the north (31°45.664'N, 110°50.763'W, elevation 1,426 m) and Agua Caliente Canyon in the south (31°41.539'N, 110°57.378'W, elevation 1,225 m). Following the monsoon rains beginning in July, *G. thurberi* plants produce a main flush of leaves. Flowering begins in early September, and plants typically yield mature fruits and drop their leaves by mid-December. Many *Gossypium* species (including *G. thurberi*) bear extrafloral nectaries that attract arthropods, such as predaceous ants (e.g., Henneberry et al. 1977; Rudgers 2002). In *G. thurberi*, extrafloral nectaries occur singly on the undersides of leaves and also beneath the bracts (Fryxell 1979).

Bucculatrix thurberiella (Lepidoptera: Lyonetiidae, cotton leaf perforator) is a common folivore of *G. thurberi* (Karban 1993a, 1993b; Karban and Adler 1996). A specialist on *Gossypium*, *B. thurberiella* spends the first three instars as a leaf miner, then emerges and feeds externally. Emerged caterpillars are small (3–5 mm) (Rejesus 1968) and seldom move between plants. Whereas a single leaf mine rarely occupies more than 1% of a leaf (J.A.

Rudgers, personal observations), a newly eclosed fourth instar caterpillar will consume approximately 10–20% of one leaf before pupating (Karban 1993b). *Bucculatrix thurberiella* caterpillars cause a unique pattern of leaf damage by leaving a layer of leaf epidermis and avoiding gossypol glands (J.A. Rudgers, personal observations), pigmented cavities that harbor plant secondary metabolites such as terpenoid aldehydes (Bell et al. 1978). Caterpillar damage has been shown to reduce the growth and reproduction of *G. thurberi* (Rudgers 2002). Adult moths are nocturnal, consume nectar, including *G. thurberi* extrafloral nectar, and lay eggs on wild cotton leaves (Rejesus 1968, J.A. Rudgers, personal observations).

Forelius pruinosus (Hymenoptera: Formicidae) dominates the ant assemblage on *G. thurberi* in both Florida and Agua Caliente canyons; in 2000, approximately 93% ±27.2 SE and 98% ±13.4 SE of all ant visitors were *F. pruinosus*, respectively. *Forelius pruinosus* is an aggressive, generalist species that kills free-feeding *B. thurberiella* caterpillars (both fourth and fifth instars). In addition, encounters with *F. pruinosus* frequently cause caterpillars to drop from leaves and hang from a thread of silk (silking). *Forelius pruinosus* is a diurnal visitor.

Ant exclusion experiment

Ants were excluded from naturally occurring, randomly chosen plants as part of a larger experiment that also manipulated extrafloral nectar (Rudgers 2002). Data discussed here include only the subset of plants that had ambient levels of extrafloral nectar because these plants represented the natural conditions in which ants and caterpillars interact. We used a sticky barrier (Tanglefoot, Tanglefoot Co., Grand Rapids, Mich.) to reduce ant visitation to *G. thurberi* for entire growing seasons (July–December). At Florida Canyon plants received treatments from 1998 to 2000; at Agua Caliente Canyon treatments lasted from 1999 to 2000. Plant stems were protected with masking tape (1998–1999) or Tangleguard (2000, Tanglefoot Co.), and Tanglefoot was reapplied as necessary (approx. monthly). Samples sizes were unequal (Agua Caliente: Ants=28, No Ants=25; Florida: Ants=31, No Ants=15). At peak ant abundances, exclusion barriers on *G. thurberi* substantially reduced ant visitation by 98% at Agua Caliente Canyon and by 54% at Florida Canyon (Rudgers 2002). Our experiments were conducted in the context of a diverse interaction web, including more than 100 arthropod species that visit *G. thurberi* plants (J.A. Rudgers, unpublished data). However, exclusion barriers had no significant effects on other common predators (spiders in Dictynidae, Oxyopidae, Thomisidae) (Rudgers 2002).

We assessed *B. thurberiella* numbers and damage in both populations during 2000. Caterpillars were counted once every 2 weeks, and damage was estimated approximately monthly between July and December. The peaks of caterpillar density and damage were used in our analysis and occurred between 25 October and 15 November. Damage was assessed for 15 randomly selected leaves per plant. Leaf area was measured with a transparent grid comprising 1.0 cm^2 cells by counting the number of grid squares containing >50% of leaf tissue; missing leaf area was visually estimated. Leaf damage was estimated to the nearest 0.25 cm^2 using the grid. Because ant exclusion reduced plant size (Rudgers 2002), caterpillar density was adjusted by dividing by the number of late-season leaves per plant (25 October). Ant exclusion had no significant effect on leaf area ($F=0.32$, $P=0.57$). Both the number of caterpillars per leaf and proportional leaf damage were angular-transformed. Populations were examined separately using ANOVA because experiments ran for different lengths of time (SAS 1999). For each population, we determined the proportional effect of ant exclusion [(1−least squares (LS) mean for Ants/LS mean for No Ants)]. To test the null hypothesis that the effect of ant exclusion on caterpillar damage was equivalent to the effect on caterpillar number, we bootstrapped the difference in proportional effects (proportional effect on damage−proportional effect on number) for each population with the SAS Jackboot macro (SAS 1995). We

employed the bias-corrected bootstrap method to approximate 95% confidence intervals for the difference, although basic, accelerated, and percentile bootstraps gave similar results (Dixon 2001). If the confidence limits for the difference do not include zero, then the effect of ants on damage significantly differs ($P<0.05$) from the effect of ants on caterpillar number.

Spatial patterns of caterpillar damage

We predicted that ants would alter the spatial distribution of damage within leaves by causing *B. thurberiella* caterpillars to feed near the leaf margin. Caterpillars may prefer leaf margins to facilitate abandoning the leaf and because they typically return to the margin when recovering from silking. During 25–30 October 2000, five randomly selected leaves were collected from ant exclusion plants and controls in each population ($n=17$ plants treatment^{-1} population^{-1}). To describe the proximity of damage to the leaf margin, we measured the shortest distance from the margin to the nearest *B. thurberiella* perforation (to the nearest 0.01 mm) with digital calipers. We also assessed the total number of perforations per leaf and the proportion of perforations that extended to the leaf margin such that one side of the perforation was the edge of the leaf.

To evaluate the effects of ants on the distribution of damage, we used mixed model MANOVA (SAS 1999). We report Pillai's Trace values following Scheiner (2001). The distance of perforations from the leaf margin, the number of perforations per leaf, and the proportion of perforations on the margin were reciprocal-, log- and angular-transformed for normality, respectively. Fixed effects in the model included population, ant treatment, and population × ant treatment. Population was considered fixed because sites were selected non-randomly to represent high and low densities of ants. The fixed effects were tested explicitly using the random effect of plant (nested within population × ant treatment) as the error term because a Satterthwaite approximation of the denominator degrees of freedom could not be used for the multivariate test. The error term was no more than 1% larger than the expected value for the quasi-F statistic, which included residual error; therefore, this test was conservative (N. Willits, Department of Statistics, University of California, Davis, Calif., personal communication). F statistics were reported for univariate analyses only when factors were significant in the MANOVA.

Behavior of experimentally introduced caterpillars

In 1999, *B. thurberiella* caterpillars were introduced to plants on which ants were experimentally manipulated. For each population we selected plants with *F. pruinosus* ant visitors (Agua Caliente $n=30$, Florida $n=27$). Two similar branches (same number of fruits, flowers, and buds) were chosen per plant and assigned randomly to ant access or ant exclusion. The ant accessible treatment had unrestricted ant visitation. For ant exclusion we brushed ants from the branch and prevented visitation using a Tanglefoot barrier. Observers were assigned randomly to treatments with two observers per plant. Each observer simultaneously placed a caterpillar in the center lobe of the topmost, fully expanded leaf of a branch using a paintbrush. Caterpillars were introduced immediately after ants were removed, and no acclimation period was permitted. Observers recorded the foraging and anti-predator behaviors of caterpillars (see below) continuously for 10 min using a hand-held tape recorder. Observations occurred on 16–24 October 1999. During this time, unmanipulated plants in the two populations had similar mean numbers of ants (Agua Caliente 4.8±1.69 SE, $n=30$; Florida 4.1±3.70 SE, $n=34$).

B. thurberiella caterpillars used in the experiment originated from two sources. At Florida Canyon we used caterpillars naturally occurring on cultivated cotton plants (*G. hirsutum*) grown outdoors approximately 0.8 km away. At Agua Caliente Canyon caterpillars were collected haphazardly from naturally occurring *G. thurberi*. Caterpillars from both sources were very likely to have had prior exposure to *F. pruinosus*. We collected caterpillars on the day of experimentation and assigned them randomly to treatments.

Behavior of naturally occurring caterpillars

To observe behavior under less artificial conditions, we used naturally occurring *B. thurberiella* in 2000. In each population we selected 60 plants that had both *F. pruinosus* visitors and at least one caterpillar. Plants were randomly assigned to either an ant access (not manipulated) or ant exclusion treatment. Ants were excluded with an application of Tanglefoot to the base of a branch, and ants were gently brushed from the branch without disturbing the caterpillar. As in 1999, caterpillar behavior was recorded for 10 min, and observations began immediately after ants were removed. Caterpillars were observed on 15–19 October 2000 when unmanipulated plants in the two populations had different mean densities of ants (Agua Caliente 31.6±8.46 SE, $n=28$; Florida 5.8±2.51 SE, $n=31$).

Behavior experiments: response variables

We characterized a number of foraging and anti-predator behaviors of caterpillars. In both years we determined the total time caterpillars engaged in the following activities: stationary (not moving), walking, and silking (hanging by a thread of silk from the leaf). Due to the small size of *B. thurberiella*, we could not visually assess the amount of time caterpillars consumed leaf tissue without disturbing them; however, caterpillars only appear damage leaves when they are stationary (J.A. Rudgers, personal observation). We documented the following locations of caterpillars during each behavior: the upper surface or underside of the leaf, the stem (and petiole), or off the plant (while silking). The number of leaves visited by each caterpillar was also recorded. At the end of each trial, leaves were examined for new perforations caused by focal caterpillars.

Behavior experiments: statistical analyses

Foraging and anti-predator behaviors were examined with separate MANOVA models for each experiment (SAS 1999). Because the response variables were proportions that summed to one (angular-transformed), one behavior was chosen at random (walking) and excluded from the multivariate analysis (e.g., Cisneros and Rosenheim 1998). For this behavior, a separate univariate test was conducted. In all models we evaluated the fixed effects of population, ant treatment, and population × ant treatment. ANOVA was used to decompose effects for each response, and F statistics are given only for factors significant in the MANOVA.

Analysis of the propensity for caterpillars to damage leaves required different statistical methods for each year. Because a caterpillar typically caused a single, small perforation (<5 mm^2) during a trial, the binary response variable in both experiments was whether or not a caterpillar initiated damage. To accommodate the paired nature of the introduced caterpillar experiment (1999), the effect of population was examined first with a chi-square test (SAS 1999). Because the population effect was not significant at $P<0.05$, we conducted a sign test to evaluate the effect of ant treatment on the pooled data from both populations. For naturally occurring caterpillars (2000), we used logistic regression with the fixed effects of population, ant treatment, and population × ant treatment (SAS 1999), and we present likelihood ratio tests with chi-square (χ^2) statistics (Stokes et al. 1995).

We evaluated the influence of ants and population on the location of caterpillars with MANOVA on (1) the proportion of time caterpillars spent on the underside of leaves divided by the proportion of time caterpillars spent on the upper side of leaves, (2) the proportion of time caterpillars spent on the stem divided by the proportion of time caterpillars spent on leaves, and (3) the mean number of leaves visited during trials. Using relative proportions

allowed us to explore location responses without confounding these data with the absolute proportion of time caterpillars devoted to different behaviors. Because of extreme non-normality, location responses were rank-transformed to make the analysis non-parametric (Conover and Iman 1981). *F* statistics from univariate analyses were reported for significant effects in the MANOVA.

Proximate information for risk assessment

To evaluate the importance of information used in risk assessment, we recorded each time a caterpillar was touched by an ant ("direct contact") and each time that an ant walked on the same leaf as the caterpillar without touching it ("indirect interaction") during the behavioral trials. Ant movement on leaves may influence caterpillars via leaf vibrations (e.g., Castellanos et al. 2000) or through other information such as chemical signals (e.g., Kats and Dill 1998; Grostal and Dicke 1999). From these data, we calculated the percentage of direct contacts (*n*=16 trials; range 1–4 contacts trial^{-1}) and the percentage of indirect interactions per trial (*n*=27 trials; range 1–6 interactions trial^{-1}) that resulted immediately in silking by caterpillars. We compare these values qualitatively to assess the relevance of each event for inducing anti-predator behaviors.

Results

## What are the relative effects of ant exclusion on caterpillar numbers versus caterpillar damage?

Ant exclusion did not significantly increase the peak number of caterpillars per leaf at Agua Caliente Canyon (LS mean ±SE, Ants=0.69±0.061, No Ants 0.84±0.065; *F*=2.86, *P*=0.0967) or at Florida Canyon (Ants= 0.38±0.029, No Ants 0.45±0.043; *F*=1.79, *P*=0.1875), although at both sites the trend was for more caterpillars on plants without ants (see also Rudgers 2002). In contrast, ant exclusion strongly increased damage by caterpillars at both sites (Agua Caliente LS mean ±SE, Ants=0.18±0.021, No Ants 0.30±0.022; *F*=15.33, *P*=0.0003; Florida Ants=0.14±0.013, No Ants 0.19± 0.020; *F*=5.26, *P*=0.0268). For both populations the proportional effect of ant exclusion was substantially greater for damage than for caterpillar number. The effect of ants on caterpillar damage was 54% greater than the effect on caterpillar number at Agua Caliente Canyon, and the difference was significant at *P*<0.05 [Boot-strapped difference (95% CI)=0.22 (0.04–0.40); Fig. 1]. At Florida Canyon, the effect of ants on damage was 43% greater than the effect on number, but the difference was not statistically significantly different from zero [0.13 (−0.10–0.36); Fig. 1]. These results suggest a strong non-consumptive effect of ants.

Do ants modify the spatial distribution of caterpillar damage?

Ant exclusion during an entire growing season significantly altered the distribution of damage within leaves. As predicted, when plants had ants, caterpillars were more likely to cause damage near the leaf margin than when
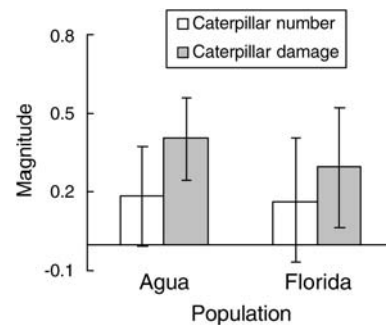
Fig. 1 The magnitude of the effect of season-long ant exclusion (1–LS mean for ant treatment/ LS mean for no ant treatment) ±95% CI for caterpillar number and caterpillar damage at Agua Caliente and Florida canyons
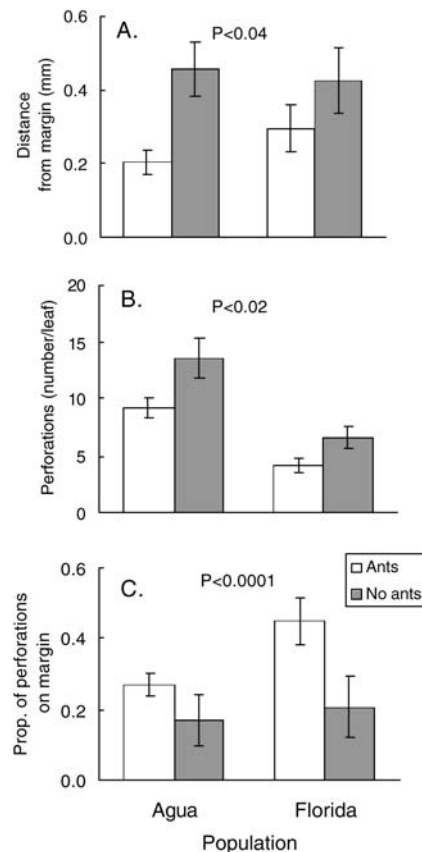
Fig. 2A–C Effects of season-long ant exclusion on the spatial distribution of damage. Means±SE for **A** the distance from the leaf margin to the nearest *Bucculatrix thurberiella* perforation, **B** the total number of perforations per leaf, and **C** the proportion of perforations occurring directly on the leaf margin by ant treatment and population. *P* values represent a significant effect of the ant treatment

ants were excluded (Fig. 2a, Table 1). However, the difference in distance to margin between ant access and ant exclusion treatments represented only 5% of the length of a caterpillar and 0.1% the mean width of a leaf lobe. Ant exclusion also resulted in more perforations per

Table 1 Statistical results from M/ANCOVA assessing the effects of population and ant treatment on the spatial distribution of *Bucculatrix thurberiella* caterpillar damage, including the distance from the leaf margin to the nearest perforation, total perforation number per leaf, and the proportion of perforations occurring on the leaf margin

	MANOVA				ANOVA	Distance from margin		Perforation number		Proportion of perforations	
	df	Pillai	F	P	df	F	P	F	P	F	P
Population	3,62	0.44	16.14	<0.0001	1,66	0.35	0.5584	38.53	<0.0001	6.95	0.0104
Ant	3,62	0.33	10.25	<0.0001	1,66	4.60	0.0356	5.89	0.0179	28.87	<0.0001
Population× Ant	3,62	0.10	2.21	0.0955							
Plant	192,720	0.79	1.35	0.0038	64,246	1.22	0.1450	1.74	0.0014	1.22	0.1480

Table 2 Results from analysis of variance examining the effects of population and ant exclusion on the proportion of time *B. thurberiella* caterpillars spent walking, stationary, or silking. Experimentally introduced caterpillars (1999) and naturally occurring caterpillars (2000)

|	MANOVA				ANOVA	Walking		Stationary		Silking	
	df	Pillai	F	P	df	F	P	F	P	F	P
1999											
Population	2,109	0.06	3.26	0.0421	1,110	4.13	0.0445	6.57	0.0117	0.16	0.6875
Ant	2,109	0.17	11.14	<0.0001	1,110	2.08	0.1518	1.68	0.1981	21.89	<0.0001
Population×Ant	2,109	0.01	0.81	0.4487							
2000											
Population	2,117	0.03	1.91	0.1533							
Ant	2,117	0.16	10.74	<0.0001	1,118	3.85	0.0520	11.81	0.0008	21.53	<0.0001
Population×Ant	2,117	0.02	1.01	0.3663							

leaf compared to plants with ants (Fig. 2b, Table 1), which corresponded with the result that ant exclusion increased leaf damage on whole plants (Fig. 1). Finally, plants without ants had a significantly smaller proportion of perforations occurring directly on the leaf margin (Fig. 2c, Table 1). Thus, ant exclusion altered the spatial distribution of damage such that damage was concentrated toward the center of leaves when ants were absent. While the populations differed in patterns of damage (at Florida Canyon leaves had fewer perforations and a greater fraction of perforations on the margin than at Agua Caliente Canyon, Fig. 2), the effect of ant exclusion was consistent across populations (Table 1).

Does the presence of ants influence the amount of time caterpillars engage in foraging and anti-predator behaviors?

The presence of ants significantly altered the behavior of *Bucculatrix thurberiella* caterpillars. For experimentally introduced caterpillars, this effect was largely attributable to caterpillars spending approximately 28-fold more time silking in the presence of ants (Fig. 3a, b; Table 2). Naturally occurring caterpillars not only invested 10-fold more time silking but also spent 2.3 times more of their activity budget walking and 13% less time stationary in the presence of ants (Fig. 3c, d; Table 2). While the effects of ants were similar for both experiments, averaged over treatments and populations, naturally
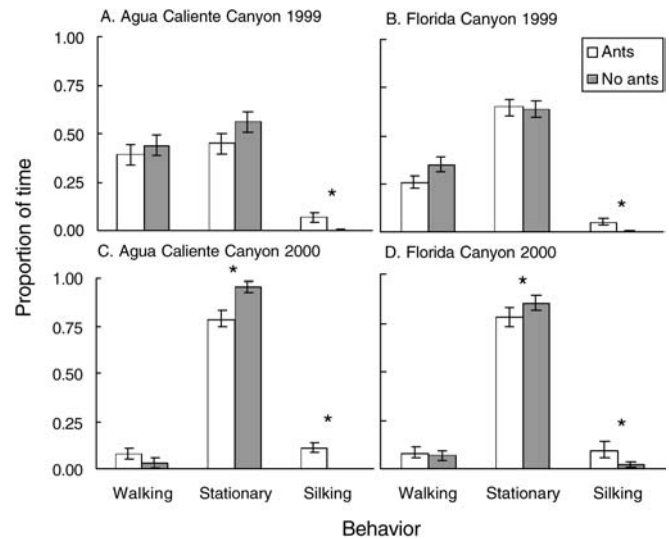

Fig. 3A–D Means±SE for the proportion of time caterpillars engaged in different behaviors [walking, stationary (not moving), or silking] by ant treatment and population. Introduced caterpillars (1999) in **A** Agua Caliente Canyon and **B** Florida Canyon, and naturally-occurring caterpillars (2000) in **C** Agua Caliente Canyon and **D** Florida Canyon. *Asterisks* indicate significant univariate tests for the effect of ants, averaged across populations ($P<0.05$)

occurring caterpillars spent 32% more time stationary than introduced caterpillars (Fig. 3). The effects of ants were also consistent across populations (Table 2), although across treatments populations differed in the
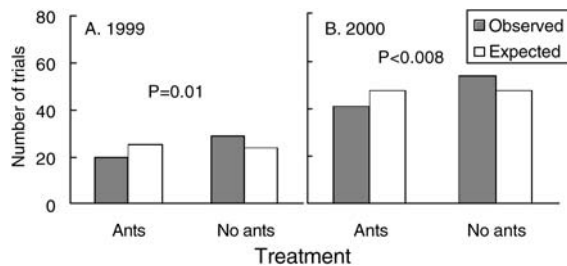
Fig. 4 Observed and expected frequencies for the number of trials in which caterpillars damaged leaves in the Ants and No ants treatments of the behavioral experiments, averaged over populations with **A** introduced caterpillars (1999) and **B** naturally occurring caterpillars (2000)

0.01 ± 0.007 SE; $F_{(1,118)}=10.49$, $P<0.002$). There were no differences between treatments in the initial location of caterpillars on leaves in 2000 ($\chi^2=0.70$, $df=1$, $P=0.40$). In contrast, ants had no effect on the proportion of time caterpillars spent on leaves versus stems ($F_{(1,118)}=1.34$, $P=0.25$), and caterpillars visited the same number of leaves, regardless of ant treatment ($F_{(1,118)}=0.35$, $P=0.56$). Population did not affect caterpillar location in either experiment (1999 Pillai's Trace=0.002, $F_{(3,107)}=0.09$, $P=0.96$; 2000 Pillai's Trace=0.02, $F_{(3,116)}=0.94$, $P=0.42$), nor were there ant treatment by population interactions (1999: Pillai's Trace=0.004, $F_{(3,107)}=0.13$, $P=0.94$; 2000: Pillai's Trace=0.03, $F_{(3,116)}=1.22$, $P=0.31$).

What information do caterpillars use to assess the risk of predation?

Both direct contact with ants and the indirect interaction of ants walking on the same leaf were key events leading to silking behavior in *B. thurberiella*. In 2000, we observed 43 trials during which direct contact or indirect interactions occurred at least once. In the ant-accessible treatment, caterpillars silked on average $98\pm2.0\%$ of times immediately after an ant touched them ($n=27$ trials, means ±SE), and they silked on average $41\pm9.6\%$ of times after an ant walked onto the same leaf ($n=16$ trials). Of the 59 instances of silking behavior that occurred in the ant-accessible treatment, 64% were preceded by one of these two cues. In the remaining silking events, the cues were unclear. Thus, physical contact with ants was a more reliable trigger for caterpillar silking behavior than ants walking onto leaves, but both cues provided information that provoked anti-predator behaviors in *B. thurberiella*.

responses of introduced caterpillars, which spent more time stationary and less time walking at Florida Canyon than at Agua Caliente Canyon (Fig. 3a, b; Table 2).

Predation by *Forelius pruinosus* occurred only when caterpillars were experimentally introduced. Because we observed only four predation events, statistical analyses were not performed. Dividing the number of predation events by the total amount of observation time, we determined a predation rate of 4.8 caterpillars plant^{-1}day^{-1} at Florida Canyon and 14.4 caterpillars plant^{-1} day^{-1} at Agua Caliente Canyon. This rate assumes that predation is constant over time.

Are caterpillars more likely to damage leaves when ants are absent?

The absence of ants increased the probability that caterpillars damaged *Gossypium thurberi* leaves. With ants excluded, caterpillars damaged leaves 22% more often than expected by chance when they were introduced [sign test, Pr($X=24$) ~ Bin($P=0.5$, $n=35$), $P=0.01$; Fig. 4a] and 14% more often than expected by chance when they occurred naturally ($\chi^2=7.14$, $df=1$, $P<0.008$; Fig. 4b). Population did not affect the propensity for caterpillars to damage leaves in either year (1999 $\chi^2=0.09$, $df=1$, $P=0.77$; 2000 $\chi^2=0.40$, $df=1$, $P=0.53$), and the effects of ants were similar across populations (2000 only, population × ant $\chi^2=0.09$, $df=1$, $P=0.76$).

Do ants alter caterpillar location within a plant?

Whether ants influenced caterpillar location within a plant depended on the type of behavioral experiment. For introduced caterpillars, ant treatment did not modify the location of caterpillars within plants or the number of leaves visited (Pillai's Trace=0.02, $F_{(3,107)}=0.61$, $P=0.61$). However, for naturally occurring caterpillars, ants altered location (Pillai's Trace=0.09, $F_{(3,116)}=3.70$, $P=0.01$). Caterpillars on plants with ants spent a greater proportion of time on the undersides of leaves relative to the upper surfaces (Ants: 0.40 ± 0.295 SE, No Ants:

Discussion

Behavioral mechanisms in ant-wild cotton mutualisms

Prior work has documented a mutualism between wild cotton and ants, wherein ants reduced damage by herbivores (including *Bucculatrix thurberiella*) and ultimately enhanced seed production (Rudgers 2002). Our work here shows that non-consumptive effects of ants underlie this mutualism. While ants had some effects on the abundance of caterpillars during season-long ant exclusion (Rudgers 2002), these experiments demonstrated that ants had a greater influence on damage caused by *B. thurberiella* caterpillars than on caterpillar densities. Thus, although ants do consume *B. thurberiella* caterpillars, their non-consumptive effects appear to play a large role in reducing herbivore damage and may ultimately have important fitness consequences for wild cotton plants.

Ants induced a number of behavioral changes in *B. thurberiella* that provide mechanisms to explain their

non-consumptive effects. First, caterpillars spent less time remaining stationary in the presence of ants than when ants were excluded. Because caterpillars typically cause damage when stationary on leaves, ant exclusion lengthened the potential amount of time spent feeding. Second, ants also caused caterpillars to spend more time silking. Silking may ultimately reduce *B. thurberiella* fitness (e.g., via less time spent feeding or via the cost of silk, Berenbaum et al. 1993), perhaps by decreasing development time and causing caterpillars to pupate at a smaller size. Silking may also increase emigration of caterpillars from plants, an additional non-consumptive effect. Third, ant exclusion significantly increased the probability that caterpillars initiated damage to leaves, providing a direct link between the behavioral modifications induced by ants and the results of season-long ant exclusion. Lastly, ants altered the distribution of prey within plants. Caterpillars spent more time on the underside of leaves compared to the upper surface when ants were present. This effect was likely related to recovery from silking, as caterpillars often return to the undersides of leaves after dropping (J.A. Rudgers, personal observations). Ants' modification of the location of prey may influence the foraging behaviors of parasitoids and other predators (e.g., Godfray 1994; Whelan 2001) and thereby affect additional interactions in the community.

Season-long ant exclusion also significantly altered the spatial distribution of damage by *B. thurberiella*. Most important in this respect was that the proportion of perforations occurring directly on the leaf margin was on average twice as great in the presence of ants than in the absence of ants. These within leaf effects may have resulted from increased caterpillar silking behavior in the presence of ants (as a by-product of recovery from silking) or from preferences for margins as quick escape routes. In other species, the degree and pattern of herbivore perforations within leaves can alter photosynthetic rates and water-use efficiency (Oleksyn et al. 1998; Zangerl et al. 2002). It is unlikely, however, that the small effects of ants on the distribution of caterpillar damage within leaves had important effects on *Gossypium thurberi* fitness. Nevertheless, ants may alter the distribution of caterpillars within plants because silking may cause caterpillars to be concentrated on lower leaves. While this effect has not yet been investigated in *G. thurberi*, changes in the spatial distribution of damage within whole plants can affect reproduction in other plant species (Marquis 1988, 1992, 1996; Mauricio et al. 1993).

Trait-mediated predator-prey interactions
in natural interaction webs

Many prior studies have been criticized for assessing the non-consumptive effects of predators only under controlled laboratory conditions (Sih et al. 2002). Our work supports the hypothesis that small differences in experimental design can have large effects on animal behavior and suggests a need for more natural experiments. In wild cotton, introducing caterpillars to plants appears to have modified both ant and caterpillar behaviors relative to naturally occurring animals. For example, we observed predation events only when caterpillars were introduced to plants; this result suggests that the consumptive effect of ants was not as strong under natural conditions as when caterpillars were experimentally added. Furthermore, naturally occurring caterpillars spent more time stationary on leaves (potential feeding time) than did introduced caterpillars. More finely tuned experiments in this system and others could help elucidate the contribution of experimental design to the outcome of predator and prey manipulations.

Assessing the risk of predation

Understanding the information content of cues can be crucial for modeling the dynamics of predator-prey interactions. For example, introducing a time lag in which prey use cues to assess the risk of predation destabilized predator-prey population dynamics in one model (Luttbeg and Schmitz 2000). In our experiments, *B. thurberiella* caterpillars responded to two distinct types of events: direct physical encounters with ants and indirect interactions caused by ants walking onto the same leaf. In combination, these two events explained the majority of caterpillars' silking behaviors, with silking occurring more frequently after direct contact than after indirect interactions. These results demonstrate that anti-predator behaviors in *B. thurberiella* (silking) can be triggered by simple cues from predaceous ants.

Non-consumptive effects of predators
in terrestrial communities

The non-consumptive effects of predators may be widespread in ant-plant interactions as well as in other terrestrial systems. An obvious example occurs in some myrmecophytic plants in which ants expel herbivores and depend on specialized plant resources for food (e.g., acacias, Janzen 1966). A few additional ant-plant studies also suggest a role for non-consumptive effects (Messina 1981; De la Fuente and Marquis 1999). Stronger evidence comes from spider studies, where spiders' presence alone altered the host-use of grasshoppers (Schmitz 1998) and unoccupied webs discouraged ant visitors (Gasterich 1999). Only one study found a contrasting pattern: vole herbivory was greater when the risk of predation was higher (Pusenius and Ostfeld 2000). Research in other systems would help resolve how widespread and important are the non-consumptive effects of predation in terrestrial habitats. Results from wild cotton show that ants alter herbivore foraging and anti-predator behaviors, with effects that cascade to host plants; this work contributes to the small body of evidence from terrestrial systems that documents the non-consumptive effects of predators on communities.

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References

Abrams PA (1995) Implications of dynamically variable traits for identifying, classifying and measuring direct and indirect effects in ecological communities. Am Nat 146:112–134

Abrams PA, Schmitz OJ (1999) The effect of risk of mortality on the foraging behaviour of animals faced with time and digestive capacity constraints. Evol Ecol Res 1:285–301

Agrawal AA, Rutter MT (1998) Dynamic anti-herbivore defense in ant-plants: the role of induced responses. Oikos 83:227–236

Beckerman AP, Uriarte M, Schmitz OJ (1997) Experimental evidence for a behavior-mediated trophic cascade in a terrestrial food chain. Proc Natl Acad Sci USA 94:10735–10738

Bell AA, Stipanovic RD, O'Brien DH, Fryxell PA (1978) Sesquiterpenoid aldehyde quinones and derivatives in pigment glands of Gossypium. Phytochemistry 17:1297–1305

Berenbaum MR, Green ES, Zangerl AR (1993) Web costs and web defense in the parsnip webworm (Lepidoptera: Oeocophoridae). Environ Entomol 22:791–795

Bernot RJ, Turner AM (2001) Predator identity and trait-mediated indirect effects in a littoral food web. Oecologia 129:139–146

Bronstein JL (1998) The contribution of ant-plant protection studies to our understanding of mutualism. Biotropica 30:150–161

Castellanos I, Kraljevik A, Barbosa P (2000) Costs of anti-predator behavior in a forest caterpillar. Bull Ecol Soc Am 80:69

Cisneros JJ, Rosenheim JA (1998) Changes in the foraging behavior, within-plant vertical distribution, and microhabitat selection of a generalist insect predator: an age analysis. Environ Entomol 27:949–957

Conover WJ, Iman RL (1981) Rank transformations as a bridge between parametric and nonparametric statistics. Am Stat 35:124–133

De la Fuente MAS, Marquis RJ (1999) The role of ant-tended extrafloral nectaries in the protection and benefit of a Neotropical rainforest tree. Oecologia 118:192–202

Dixon PM (2001) The bootstrap and the jackknife: Describing the precision of ecological indices. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments, 2nd edn. Oxford University Press, Oxford, pp 267–288

Freitas AVL, Oliveira PS (1994) Biology and behavior of the neotropical butterfly Eunica bechina (Nymphalidae) with special reference to larval defence against ant predation. J Res Lepid 31:1–11

Fryxell PA (1979) The natural history of the cotton tribe. Texas A&M, College Station, Tex.

Gastreich KR (1999) Trait-mediated indirect effects of a theridiid spider on an ant-plant mutualism. Ecology 80:1066–1070

Godfray HCJ (1994) Parasitoids: behavioral and evolutionary ecology. Princeton University Press, Princeton, N.J.

Grostal P, Dicke M (1999) Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. Behav Ecol 10:422–427

Halaj J, Wise DH (2001) Terrestrial trophic cascades: how much do they trickle? Am Nat 157:262–281

Hanson HC (1923) Distribution of Arizona wild cotton. Ariz Agric Exp Stn Tech Bull 3:48–59

Henneberry TJ, Bariola LA, Kittock DL (1977) Nectariles cotton: effect on cotton leaf perforator and other cotton insects in Arizona. J Econ Entomol 70:797–799

Janzen DH (1966) Coevolution of mutualism between ants and acacias in Central America. Evolution 20:249–275

Karban R (1993a) Costs and benefits of induced resistance and plant density for a native shrub, Gossypium thurberi. Ecology 74:9–19

Karban R (1993b) Induced resistance and plant density of a native shrub, Gossypium thurberi, affect its herbivores. Ecology 74:1–8

Karban R, Adler FR (1996) Induced resistance to herbivores and the information content of early season attack. Oecologia 107:379–385

Kats LB, Dill LM (1998) The scent of death: Chemosensory assessment of predation risk by prey animals. Ecoscience 5:361–394

Koptur S (1992) Extrafloral nectary-mediated interactions between insects and plants. In: Bernays E (ed) Insect-plant interactions. IV. CRC, Boca Raton, Fla., pp 81–129

Lees DC, Smith NG (1991) Foodplant associations of the Uraniinae (Uraniidae) and their systematic evolutionary, and ecological significance. J Lepid Soc 45:296–347

Lima SL (1998) Nonlethal effects in the ecology of predator-prey interactions. BioScience 48:25–34

Lima SL, Dill LM (1990) Behavioral decisions made under the risk of predation a review and prospectus. Can J Zool 68:619–640

Luttbeg B, Schmitz OJ (2000) Predator and prey models with flexible individual behavior and imperfect information. Am Nat 155:669–683

Marquis RJ (1988) Intra-crown variation in leaf herbivory and seed production in striped maple, Acer pensylvanicum L. (Aceraceae). Oecologia 77:51–55

Marquis RJ (1992) A bite is a bite is a bite? Constraints on response to folivory in Piper arietinum (Piperaceae). Ecology 73:143–152

Marquis RJ (1996) Plant architecture, sectoriality and plant tolerance to herbivores. Vegetatio 127:85–97

Mauricio R, Bowers MD, Bazzaz FA (1993) Pattern of leaf damage affects fitness of the annual plant Raphanus sativus (Brassicaceae). Ecology 74:2066–2071

Messina FJ (1981) Plant protection as a consequence of an ant-membracid mutualism: interactions on goldenrod (Solidago sp.). Ecology 62:1433–1440

Oleksyn J, Karolewski P, Giertych MJ, Zytkowiak R, Reich PB, Tjoelker MG (1998) Primary and secondary host plants differ in leaf-level photosynthetic response to herbivory: evidence from Alnus and Betula grazed by the alder beetle, Agelastica alni. New Phytol 140:239–249

Peacor SD, Werner EE (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. Proc Natl Acad Sci USA 98:3904–3908

Persson L (1999) Trophic cascades: abiding heterogeneity and the trophic level concept at the end of the road. Oikos 85:385–397

Polis GA (1999) Why are parts of the world green? Multiple factors control productivity and the distribution of biomass. Oikos 86:3–15

Polis GA, Sears ALW, Huxel GR, Strong DR, Maron J (2000) When is a trophic cascade a trophic cascade? Trends Ecol Evol 15:473–475

Power ME (1987) Predator avoidance by grazing fishes in temperate and tropical streams: importance of stream depth and prey size. In: Kerfoot WC, Sih A (eds) Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, N.H., pp 333–351

Pusenius J, Ostfeld RS (2000) Effects of stoat's presence and auditory cues indicating its presence on tree seedling predation by meadow voles. Oikos 91:123–130

Rejesus RS (1968) Bio-ecological studies on the cotton leaf perforator, Bucculatrix thurberiella Busck (Lepidoptera: Lyonetiidae). PhD Thesis, University of California, Riverside, Calif.

Rudgers JA (2002) Evolutionary ecology of ant-wild cotton associations. PhD Dissertation, University of California, Davis, Calif.

SAS (1995) Jackboot macro. SAS Institute, Cary, N.C.

SAS (1999) SAS version 8.00. SAS Institute, Cary, N.C.

Scheiner SM (2001) MANOVA: Multiple response variables and multispecies interactions. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments, 2nd edn. Oxford University Press, Oxford, pp 99–115

Schmitz OJ (1998) Direct and indirect effects of predation and predation risk in old-field interaction webs. Am Nat 151:327–342

Schmitz OJ (2000) Combining field experiments and individual-based modeling to identify the dynamically relevant organizational scale in a field system. Oikos 89:471–484

Schmitz OJ, Booth G (1997) Modelling food web complexity: The consequences of individual-based, spatially explicit behavioural ecology on trophic interactions. Evol Ecol 11:379–398

Schmitz OJ, Suttle KB (2001) Effects of top predator species on direct and indirect interactions in a food web. Ecology 82:2072–2081

Schmitz OJ, Hamback PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: a review of the effects of carnivore removals on plants. Am Nat 155:141–153

Sih A, Ziemba R, Harding KC (2000) New insights on how temporal variation in predation risk shapes prey behavior. Trends Ecol Evol 15:3–4

Stokes ME, Davis CS, Koch GG (1995) Categorical data analysis using the SAS system. SAS Institute, Cary, N.C.

Werner EE (1992) Individual behavior and higher-order species interactions. Am Nat 140:S5-S32

Whelan CJ (2001) Foliage structure influences foraging of insectivorous forest birds: an experimental study. Ecology 82:219–231

Zangerl AR, Hamilton JG, Miller TJ, Crofts AR, Oxborough K, Berenbaum MR, de Lucia EH (2002) Impact of folivory on photosynthesis is greater than the sum of its holes. Proc Natl Acad Sci USA 99:1088–1091