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Geographic variation in a facultative mutualism: consequences for local arthropod composition and diversity

Jennifer A. Rudgers · Amy M. Savage · Megan A. Rúa

Abstract Geographic variation in the outcome of inter-specific interactions may influence not only the evolutionary trajectories of species but also the structure of local communities. We investigated this community consequence of geographic variation for a facultative mutualism between ants and wild cotton (*Gossypium thurberi*). Ants consume wild cotton extrafloral nectar and can protect plants from herbivores. We chose three sites that differed in interaction outcome, including a mutualism (ants provided the greatest benefits to plant fitness and responded to manipulations of extrafloral nectar), a potential commensalism (ants increased plant fitness but were unresponsive to extrafloral nectar), and a neutral interaction (ants neither affected plant fitness nor responded to extrafloral nectar). At all sites, we manipulated ants and extrafloral nectar in a factorial design and monitored the abundance, diversity, and composition of other arthropods occurring on wild cotton plants. We predicted that the effects of ants and extrafloral nectar on arthropods would be largest in the location with the mutualism and weakest where the interaction was neutral. A non-metric multidimensional scaling analysis revealed that the presence of ants altered arthropod composition, but only at the two sites in which ants increased plant fitness. At the site with the mutualism, ants also suppressed detritivore/scavenger abundance and increased aphids. The presence of extrafloral nectar increased arthropod abundance where mutual benefits were the strongest, whereas both arthropod abundance and morphospecies richness declined with extrafloral nectar availability at the site with the weakest ant–plant interaction. Some responses were geographically invariable: total arthropod richness and evenness declined by approximately 20% on plants with ants, and extrafloral nectar reduced carnivore abundance when ants were excluded from plants. These results demonstrate that a facultative ant–plant mutualism can alter the composition of arthropod assemblages on plants and that these community-level consequences vary across the landscape.

Keywords Ant–plant · Community structure · Conditionality · Geographic mosaic · *Gossypium*

J. A. Rudgers (✉) · A. M. Savage
Department of Ecology and Evolutionary Biology,
Rice University, 6100 Main Street, Houston,
Texas 77005, USA
e-mail: jrudgers@rice.edu

Present Address:
M. A. Rúa
Curriculum in Ecology, University of North Carolina-Chapel Hill,
Chapel Hill, NC 27599, USA

Introduction

Geographic variation in interaction outcome appears to be a common feature of mutualisms (Peterson 1995; Bronstein 1998; Thompson and Cunningham 2002; Cheney and Cote 2005; Bronstein et al. 2006; Gove and Rico-Gray 2006; Ness 2006; Rey and Manzaneda 2007; Thrall et al. 2007). In particular, protection mutalisms, in which one species guards another species against a natural enemy, can be highly variable, with the net outcome shifting along a continuum from mutualism to parasitism depending on the local biotic or abiotic context (Rico-Gray et al. 1998; de la Fuente and Marquis 1999; Despres et al. 2007). For example,

in protection interactions between ants and plants, variation can arise from changes in the abundance of the partners (Di Giusto et al. 2001), the identity of the partners (Horvitz and Schemske 1984), the availability of alternative resources for the partners (Gaume et al. 1998), the identity and abundance of the plant consumers (Barton 1986), and the abiotic context (Kersch and Fonseca 2005).

Recent research efforts have sought to understand how this geographic variation in species interactions alters selection on species traits and ultimately influences coevolutionary dynamics (Bronstein et al. 2003; Thompson 2005). However, geographic variation in the outcome of species interactions may also influence the diversity and composition of co-occurring organisms, generating ecological consequences at the community level. For instance, within a field of cultivated cotton, areas with higher densities of ant-tended aphids also had larger negative impacts of ants on the surrounding arthropod community (Kaplan and Eubanks 2005). Thus, regions with stronger pair-wise interactions (e.g., between aphids and ants) should show stronger community-level impacts of the interaction. The degree to which geographic variation in the outcome of a species interaction causes changes at the community-level remains unclear. Understanding this variation would increase the ability to predict when and where species interactions will alter community structure.

Geographic variation in the outcome of mutualisms may be an important factor to consider for predicting spatial variation in community composition. However, even for single locations or populations, experimental tests for community-level impacts are lacking for most types of mutualisms, particularly in terrestrial habitats (Stachowicz 2001; Styrsky and Eubanks 2007; Rudgers and Clay 2008). Although mutualisms are common and abundant (Bronstein 1994), they have often been assumed to be less important than antagonisms with respect to their effects on community structure (Bruno et al. 2003; Hay et al. 2004). Despite this assumption, studies conducted thus far suggest that mutualism may rival competition and predation in the magnitude of its influence on the community (van der Heijden et al. 1998; Mooney 2007; Elias et al. 2008; Bastolla et al. 2009). For example, the presence of an ant–aphid mutualism reduced arthropod species richness and abundance on cottonwood trees compared to conspecific trees lacking the mutualism (Wimp and Whitham 2001). Similarly, the protection mutualism between a fungal endophyte and host grass reduced plant and arthropod diversity in a grassland ecosystem (Rudgers et al. 2007; Rudgers and Clay 2008).

We used field experiments to test whether geographic variation in the outcome of a facultative mutualism between ants and wild cotton (*Gossypium thurberi*) was associated with the effects of the mutualism on local arthropod composition. Wild cotton plants possess extrafloral nectaries attractive to ants, which in turn can deter insect herbivores (Rudgers 2004). Prior work has revealed geographic variation in the outcome of the ant–wild cotton interaction, as quantified in the currency of plant fitness (Rudgers 2004; Rudgers and Strauss 2004). The presence of ants has been found to protect plants from damage by the specialist caterpillar, *Bucculatrix thurberiella*, and to increase plant fitness at two sites (Agua Caliente and Florida canyons), but not to affect herbivory or fitness at a third site (Molino Basin) at which ant visitation was relatively low (Rudgers and Strauss 2004). In addition, sites varied in the attractiveness of extrafloral nectar to ants: blocking extrafloral nectar production significantly reduced ant visitation and plant fitness only at one location (Agua Caliente Canyon) (Rudgers and Strauss 2004). Ant abundance and plant investment in extrafloral nectar corresponded with this geographic variation. At the site where ants were most beneficial to plants (Agua Caliente Canyon), ants were 15-fold more abundant on plants (Rudgers and Strauss 2004), and plants produced two- to four-fold more extrafloral nectar than at the site where plants received no benefits from ants (Molino Basin) (Rudgers and Gardener 2004).

Here, we report our study of whether this previously documented variation in interaction outcome is associated with variation in the community response to the ant–wild cotton interaction. We experimentally altered the interaction in two ways—by excluding ants from plants and by eliminating the reward of extrafloral nectar—and we monitored the response of arthropods that were less directly involved in the facultative mutualism. Specifically, we asked (1) does ant presence or extrafloral nectar availability affect the composition, abundance, or diversity of arthropods on plants? While extrafloral nectar may be readily consumed by many arthropod species, it is often monopolized by ants (Rudgers and Gardener 2004; Blüthgen and Stork 2007). Thus, we predicted that the influence of extrafloral nectar would depend on ant presence. In the presence of ants, extrafloral nectar should increase ant visitation, and more ants should reduce the abundance of other arthropods. In the absence of ants, however, extrafloral nectar may attract non-ant arthropods by providing a carbohydrate-rich resource.

Given previously documented geographic variation in the ant–wild cotton interaction, we additionally investigated (2) do the effects of the ant–wild cotton interaction on arthropods vary geographically? We expected that disrupting the interaction would have the largest effect on plant-associated arthropods where the mutualism was strongest (Agua Caliente Canyon) and the weakest effect where the interaction was neutral (Molino Basin). While an ideal test would also directly manipulate interaction outcome to determine causality, this would be challenging for any ant–plant system in the field. Thus, we evaluated whether interaction

outcome across sites was associated with the effects of our treatments on the composition of the local arthropod community.

Materials and methods

Study system

Wild cotton, *Gossypium thurberi* Todaro (Malvaceae), is a deciduous, perennial shrub native to canyons of the Sonoran Desert, USA and Mexico (Hanson 1923). Plants bear three extrafloral nectaries below the bracts surrounding the reproductive tissues, and single nectaries are located on the undersides of leaves along the midvein (Fryxell 1979). Prior work has demonstrated heritability for the proportion of leaves bearing nectaries and for extrafloral nectary size (Rudgers 2004).

Field sites were located in southern Arizona, USA. Agua Caliente Canyon (31°41′32″N, 110°57′23″W, elevation 1,225 m a.s.l.) and Florida Canyon (31°45′40″N, 110°50′46″W, elevation 1,426 m a.s.l., 13 km northeast of Agua) are in the Santa Rita Mountains. Molino Basin (32°20′22″N, 110°41′14″W, elevation 1,341 m a.s.l.) is in the Santa Catalina Mountains approximately 70 km to the north of Florida Canyon.

Both the density and composition of ant visitors varied among the wild cotton populations. Agua Caliente Canyon, where plants accrued the most benefits from ants, had the highest ambient densities of ants per plant (Rudgers and Strauss 2004). In addition, in both populations that benefited from ants (Agua Caliente and Florida Canyons), plants were dominated by a single, aggressive species, *Forelius pruinosus*, whereas *F. pruinosus* was a minor component of a more diverse assemblage of ants at Molino Basin (Rudgers and Strauss 2004). Ant species less commonly observed on wild cotton include *Camponotus mina*, *Crematogaster dentinodus*, *Monomorium ergatogyna*, *Pseudomyrmex pallidus*, *Forelius mccooki*, and *Myrmecocystus mimicus*.

Of the herbivores on wild cotton in these sites, the moth, *Bucculatrix thurberiella* Busck (Lyonetiidae), inflicted the greatest damage during this study (Rudgers 2004; Rudgers and Strauss 2004). A specialist on *Gossypium*, *B. thurberiella* occurs throughout *G. thurberi*'s range (Smith and Flint 1977). Prior work showed that ants reduced *B. thurberiella* abundance and damage at both Agua Caliente and Florida canyons, but not at Molino Basin (Rudgers et al. 2003; Rudgers 2004; Rudgers and Strauss 2004).

Experimental treatments

In each of three sites, we employed a 2×2 design with an ant treatment (ant access or ant exclusion) and an extrafloral nectar treatment (ambient extrafloral nectar or extrafloral nectar reduction). Randomly selected, naturally occurring plants were assigned at random to treatments (additional details can be found in Rudgers 2004; Rudgers and Strauss 2004). Treatments began during the first leaf flush following monsoon rains in July. The same individuals received the same treatments for 2–3 consecutive years (Florida Canyon 1998–2000, Agua Caliente, and Molino Basin 1999–2000). In the final year (2000), we assessed arthropod responses.

The manipulation of extrafloral nectar is crucial to determining the importance of this plant trait in mediating the community consequences of interactions with ants, although few ant–plant studies have included such manipulations (Rudgers 2004; Rudgers and Gardener 2004). Nectar reduction was achieved by covering all extrafloral nectaries with a drop of non-toxic, waterproof glue (Aleene's Co., Buellton, CA). Control plants had the same amount of glue placed on the midrib <1 cm from the nectary. All plants received glue throughout the season as new nectaries were produced. Treatments ended in late November, corresponding with leaf senescence.

Ants were deterred from plants with a sticky barrier applied over the protective tape (Tanglefoot and Tangleguard; Tanglefoot Co., Grand Rapids, MI). We wrapped the tape in a 10-cm-wide band around the woody trunk (approx. 10 cm above the ground) and painted the sticky barrier in a 2.5-cm-wide layer in the middle of the tape. In prior years, ant-accessible plants received tape only. In the year of this study, all plants received both tape and the sticky barrier. For ant-accessible plants, we added a short section of stem cut from a non-manipulated wild cotton plant that allowed ants to circumvent the barrier. The barrier effectively deterred ants at all three sites (Rudgers and Strauss 2004). Plants that died before the end of the growing season were excluded, producing unequal sample sizes (ant access + ambient nectar, ant access + reduced nectar, ant exclusion + ambient nectar, ant exclusion + reduced nectar: Agua $n = 26, 25, 23, 28$; Florida $n = 31, 29, 14, 8$; Molino $n = 23, 25, 22, 23$, respectively).

Response variables

In this study, we focused on the response of *G. thurberi*-associated arthropods other than ants or *B. thurberiella*. In wild cotton, leaves flush with the monsoon rains in early July and are typically shed in November. Thus, beginning on 14 July 2000, and continuing once every two weeks until early-November (eight census dates), we recorded all arthropod visitors to each plant during a 5–10-min census interval. Arthropods were censused non-destructively. Voucher specimens of each morphospecies were collected and identified to order and family and are permanently

stored at Rice University. During the season, we recorded >5,900 individuals, spanning 13 orders and 67 families.

We evaluated treatment effects and site differences for arthropod composition (abundance of each morphospecies), the total abundance of arthropods, and both richness (number of taxa per plant per census) and evenness [Shannon evenness index (J')] (Begon et al. 2006) at the levels of morphospecies, family, and order. Based on family- and species-level identifications, we also grouped arthropods into one of the following categories: carnivores (i.e., predators and parasitoids, excluding ants), herbivores (excluding the specialist *B. thurberiella* and aphids), or detritivores/ scavengers. Detritivores/scavengers consisted primarily of adult Dermestid beetles, which are scavengers. Too few pollinators were observed to permit robust statistical analysis, and wild cotton is self-compatible. All response variables excluded ants and *Bucculatrix thurberiella* because these results were reported in previous publications (Rudgers 2004; Rudgers and Strauss 2004) and because our focus here was on organisms other than the central participants in the facultative mutualism. In addition, because aphids (the primary honeydew-excreting insects at our sites) can benefit from protection by tending ants, we analyzed their response separately; we expected aphids would respond to our treatments oppositely from other plant-visiting arthropods.

Statistical analyses

We predicted that manipulating the ant–plant interaction would have divergent effects on arthropods across sites. Statistically, this pattern would be evident from significant site × ant or site × extrafloral nectar interactions. When these interactions were significant ($P < 0.05$), we then tested for effects of the treatments within each site because we had an a priori expectation that the sites would differ.

We used repeated measures general linear models to evaluate responses in arthropod diversity (separated into richness and evenness at the levels of morphospecies, family, and order) (von Ende 2001; SAS Institute 2004). The repeated effect was census date, and the model included the fixed factors of site, ant treatment, extrafloral nectar treatment, and all interactions. Site was considered fixed because sites were chosen to test hypotheses about known geographic variation in interaction outcome. We included initial plant size as a covariate because we applied treatments to naturally occurring plants that varied in size (see Rudgers 2004; Rudgers and Strauss 2004).

For abundance responses (total, carnivore, herbivore, aphid, and detritivore/scavenger), we calculated cumulative arthropod number per plant over the course of the growing season and applied general linear models with the fixed factors of site, ant treatment, extrafloral nectar treatment, all interactions, and the covariate initial plant size. All response variables were log-transformed to satisfy the assumptions of normality of residuals and homogeneity of variances. In addition, we omitted four observations on single census dates (out of 2,224 total) because these generated outliers in the cumulative abundances that caused non-normal distributions of the residuals.

We examined the composition of the arthropod assemblage using nonmetric multidimensional scaling analysis (NMS) with a Bray–Curtis distance measure and 9,999 iterations (Clarke and Gorley 2007). To test for treatment and site effects, we used analysis of similarities (ANOSIM) (Clarke and Gorley 2007). ANOSIM detects differences in species assemblages between two or more groups (Clarke et al. 2006). The initial ordination showed unacceptably high stress [that is, distance in the three-dimensional (3D) ordination space did not adequately reflect dissimilarity among plants in the multi-dimensional species space] (McCune and Grace 2002). We resolved this issue by creating plant size class categories, which reduced variability and the number of zeros in the data matrix. At each site, plants were sorted by initial, pre-treatment plant size (see Rudgers 2004; Rudgers and Strauss 2004), and each plant was assigned a rank. We used these rankings to create three plant size classes within each site, with each size class containing a similar number of plants within each treatment combination. We then used the matrix of mean morphospecies abundances for each size class by treatment combination for the NMS and ANOSIM analyses. To test for treatment by site interactions, we conducted two-way crossed ANOSIM (9,999 permutations) including the independent factors of site (Agua Caliente, Florida Canyon, or Molino Basin) and either ant treatment (access/exclusion), nectar treatment (ambient/reduced), or the composite factor of treatment combination (four levels). Following a significant site × treatment (ant, nectar or composite) interaction, we tested treatment effects within each site. We also transformed morphospecies abundances into a presence/absence matrix and repeated the analyses, which generated qualitatively similar results. For simplicity, we only present findings for the abundance data. Finally, to identify the morphospecies contributing most to differences among treatments and sites, we used SIMPER analysis, which ranks individual species by their contribution to the partitioning of treatments (or sites) in the ordination (Clarke and Gorley 2007).

Finally, to compare arthropod communities among sites, we tested the effect of site and covariate of initial plant size for the subset of plants with ambient levels of extrafloral nectar and ants (the control treatment). We used the post-hoc Tukey HSD test to determine how the three sites differed in terms of the abundance, richness, and evenness of total arthropods and in the abundances of carnivores, herbivores, aphids, and detritivores/scavengers.

Results

Does ant presence or extrafloral nectar availability affect the composition, abundance, or diversity of arthropods on plants?

Some arthropod responses were consistent across sites. At all sites, the presence of ants reduced total arthropod richness on plants by 8–19% at the taxonomic levels of morphospecies [mean ± standard error (SE); ant access $1.7 ± 0.07$, ant exclusion $2.1 ± 0.11$; $F_{1,264} = 4.1$, $P = 0.044$), family (ant access $1.3 ± 0.06$, ant exclusion $1.5 ± 0.08$; $F_{1,264} = 5.9$, $P = 0.016$), and order (ant access $1.2 ± 0.05$, ant exclusion $1.3 ± 0.06$; $F_{1,264} = 6.5$, $P = 0.011$) [complete statistical results are given in the Electronic Supplementary Material (ESM) Table S1]. Arthropod evenness declined by 14% in the presence of ants; these effects were significant at the level of family (ant access $0.31 ± 0.02$, ant exclusion $0.36 ± 0.02$; $F_{1,264} = 4.3$, $P = 0.039$) and order (ant access $0.38 ± 0.02$, ant exclusion $0.44 ± 0.02$; $F_{1,264} = 4.8$, $P = 0.029$), but not morphospecies ($F_{1,264} = 2.8$, $P = 0.095$). The ant treatment had no consistent influence on total arthropod abundance across sites ($F_{1,264} = 2.4$, $P = 0.122$).

We predicted that the response of non-ant arthropods to extrafloral nectar would depend on ant presence, with stronger positive responses to nectar in the absence of ants. However, only carnivore abundance varied with both the ant and nectar treatments, and the effects were in the opposite direction of our prediction. Across sites, carnivores were most abundant on plants that lacked both ants and extrafloral nectar (EFN) (Fig. 1; ant × EFN, $F_{1,264} = 4.2$, $P = 0.041$). The manipulation of extrafloral nectar did not have a consistent influence across sites on any other measure of arthropod community structure (all $P > 0.08$; complete statistical results are given in the ESM, Tables S1, S2).

Do effects of the ant-wild cotton interaction on arthropods vary geographically?

Several arthropod responses to the mutualism varied among the three sites, as evidenced by significant interactions between site and the experimental treatments (ESM, Tables S1, S2). Most notably, in the two sites where ants increased plant fitness, the ordination of arthropod composition (reflecting the abundances of each morphospecies) diverged between the ant access and ant exclusion treatments (Fig. 2; site × ant, ANOSIM $R = 0.173$, $P = 0.008$, NMS 3D stress = 0.08). In contrast, at Molino Basin, where the ant–wild cotton interaction was neutral for plant fitness (Rudgers and Strauss 2004), arthropod composition did not differ between the ant treatments (Fig. 2).
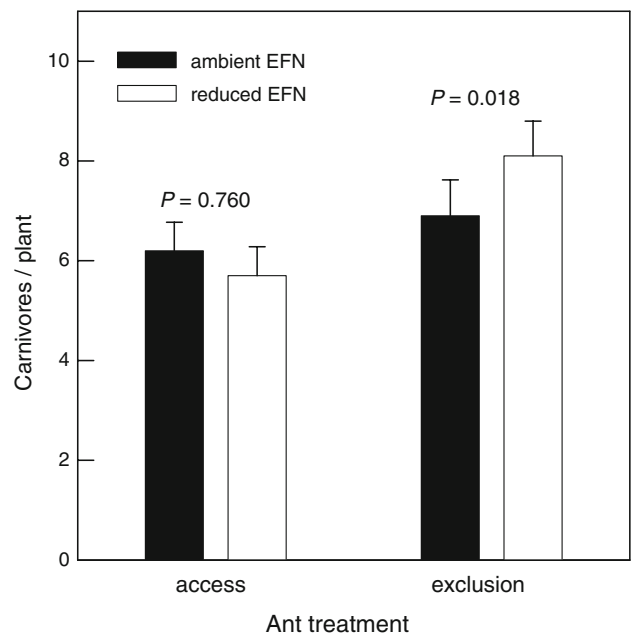

Fig. 1 Cumulative abundance of (non-ant) carnivores in response to experimental 664 manipulations of ants (access or exclusion) and extrafloral nectar (*EFN*) availability (ambient or reduced) on wild cotton plants. *Bars* Means + SE for cumulative data spanning eight census dates and averaged across sites. *P* values are shown for significant extrafloral nectar treatment effects within each ant treatment

Agua Caliente Canyon and Florida Canyon differed in terms of the identities of arthropod morphospecies that contributed to the dissimilarity between the ant treatments. At the Agua Caliente Canyon, flies in the family Ulidiidae and spiders both declined in the presence of ants. SIMPER analysis revealed that a single ulidiid morphospecies contributed to 18% of the dissimilarity between ant treatments and was 160% lower in the presence of ants. A green lynx spider (*Peucetia* sp., Oxyopidae) and one crab spider (Thomisidae) explained 13 and 7% of the dissimilarity between the treatments and were 870 and 10% less abundant in the presence of ants, respectively. In contrast, at Florida Canyon, a dictynid spider was 210% more abundant on plants with ants (contributing 14% of the dissimilarity between treatments), a soft-winged flower beetle (Melyridae) was 80% more abundant on plants with ants (8% of the dissimilarity), and a thomisid spider was 180% more abundant on plants with ants (6% of the dissimilarity). At both sites, all other morphospecies each contributed ≤5% to the dissimilarity between the ant treatments. Despite a strong partitioning of the arthropod communities due to the ant treatment, the reduction of extrafloral nectar did not affect the NMS ordination of arthropod composition (site × EFN: ANOSIM $R = -0.003$, $P = 0.474$; site × treatment combination (four levels): $R = 0.071$, $P = 0.197$).
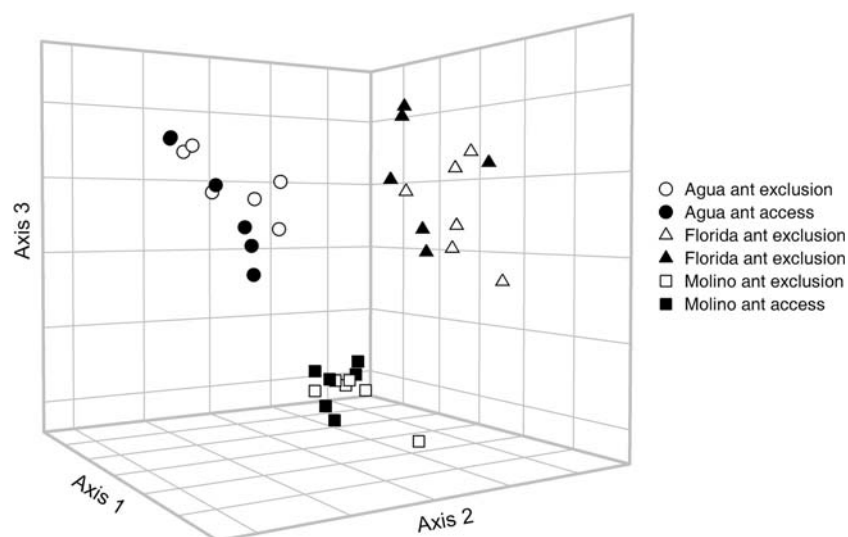
Fig. 2 Non-metric multidimensional scaling analysis showing site differences in arthropod species composition in response to the ant treatment. *Filled symbols* Ant access, *open symbols* ant exclusion. *Points* represent average responses for each plant size class within each site. Three-dimensional stress of the ordination (including all sites) = 0.08, indicating a low risk for drawing false inferences (McCune and Grace 2002). Agua Caliente Canyon (*circles*) within-site analysis of similarities (ANOSIM): ant treatment $R = 0.231$, $P = 0.037$, stress = 0.02. Florida Canyon (*triangles*) within-site ANOSIM: ant treatment $R = 0.393$, $P = 0.004$, stress = 0.05. Molino Basin (*squares*) within-site ANOSIM: ant treatment $R = -0.106$, $P = 0.993$, stress = 0.04

We expected that ant exclusion would have the largest effects on arthropods at Agua Caliente Canyon because wild cotton at this site showed the strongest increases in fitness due to ants (Rudgers and Strauss 2004). As predicted, at Agua Caliente Canyon, ant presence reduced detritivore/scavenger abundance by 60% (Fig. 3a) and carnivore abundance by 45% (mean ± SE: ant access 4.8 ± 0.39, ant exclusion 8.8 ± 0.85). In contrast, carnivores declined by only 11% in the presence of ants at both Florida Canyon (ant access 8.0 ± 0.96, ant exclusion 8.7 ± 1.49) and Molino Basin (ant access 5.0 ± 0.57, ant exclusion 5.6 ± 0.49), and detritivores were affected only at Molino Basin, showing a 47% decline due to ants (Fig. 3d). At Agua Caliente Canyon, carnivores in the families Lycosidae (Araneae), Cleridae (Coleoptera), Leucospidae (Hymenoptera), and Chrysopidae (Neuroptera) were only observed on plants in which ants were excluded. Further, it was only at Agua Caliente Canyon that the ant treatment affected aphids, with a cumulative aphid abundance that was ten fold higher in the presence of ants (Fig. 3b). Despite these changes in the abundance of particular trophic groups, there was no significant change in total arthropod abundance (Fig. 3a) or in the abundance of generalist herbivore taxa (Fig. 3c) due to ant presence (ESM, Table S2). Treatments also had no influence when internally feeding herbivores and externally feeding herbivores were analyzed separately (data not shown).
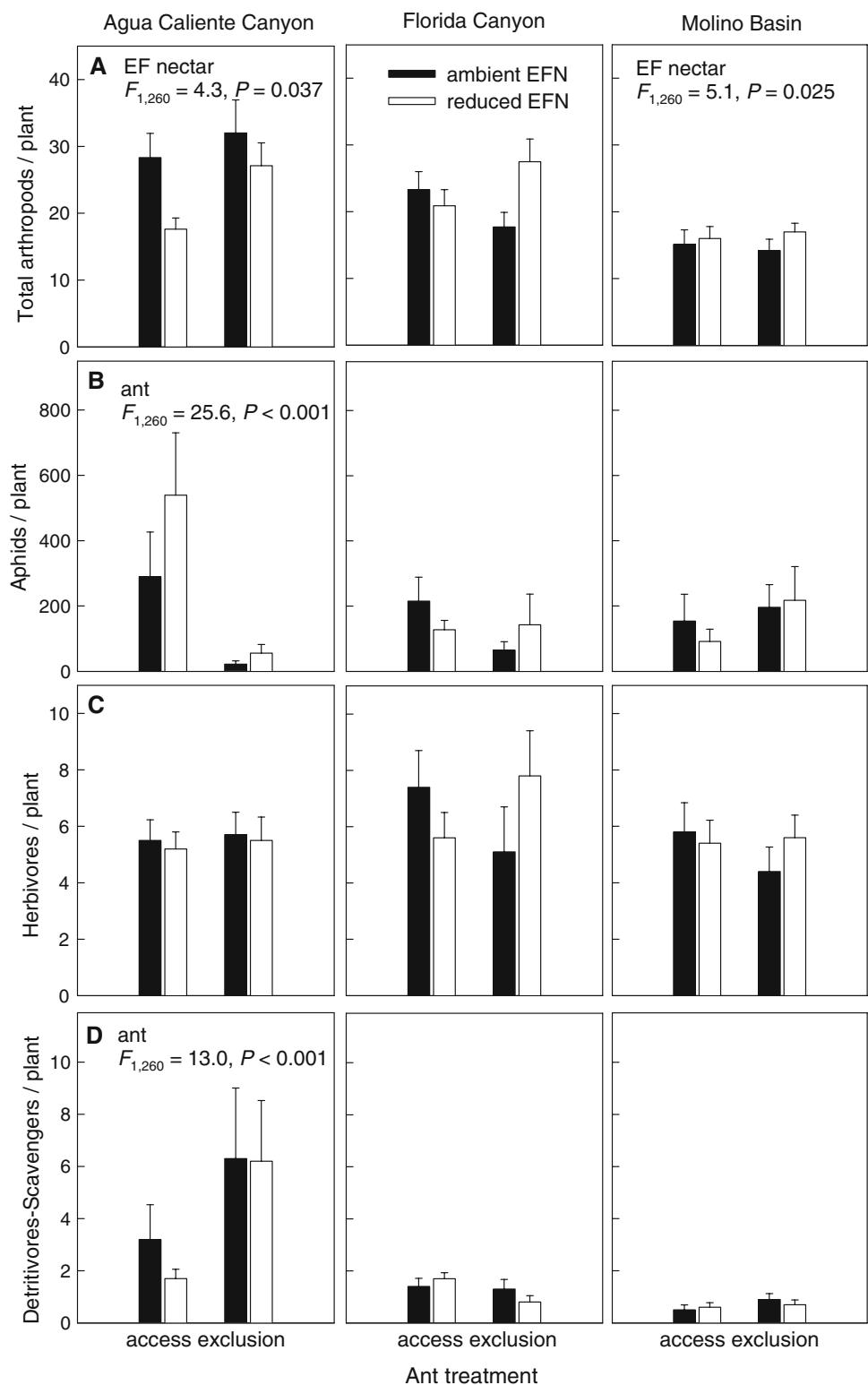
Because prior experiments demonstrated that ants were most attracted to extrafloral nectar at Agua Caliente Canyon and not significantly affected by nectar at Florida Canyon or Molino Basin (Rudgers and Strauss 2004), we expected that greater extrafloral nectar availability would intensify the negative impacts of ants on arthropods only at Agua Caliente Canyon. However, our data did not support this hypothesis (site × ant × EFN, all $P > 0.2$, complete statistical results in ESM, Tables S1, S2). The consequences of reducing extrafloral nectar varied among sites, but the effects of nectar were independent of the ant treatment. At Agua Caliente Canyon, plants with ambient levels of extrafloral nectar attracted 33% more total arthropods than plants with reduced nectar (Fig. 3a). In contrast, at Molino Basin, where extrafloral nectar did not influence ants (Rudgers and Strauss 2004), plants with ambient extrafloral nectar attracted 12% fewer arthropods than plants with reduced extrafloral nectar (Fig. 3a) and had 13% fewer morphospecies per plant (mean ± SE; ambient EFN 1.49 ± 0.14, reduced EFN 1.69 ± 0.10, Molino: ambient vs. reduced EFN contrast $F_{1,264} = 4.2$, $P = 0.040$; time × ambient vs. reduced EFN contrast $F_{7,258} = 1.4$, $P = 0.209$). While extrafloral nectar had opposite effects at Agua Caliente Canyon versus Molino Basin, there were no effects of the extrafloral nectar treatment at Florida Canyon (Fig. 3a, Tables S1, S2).

Site differences

Wild cotton sites diverged in arthropod composition (Fig. 2; ANOSIM site $R = 0.765$, $P < 0.001$; Agua vs. Florida

Fig. 3 Cumulative abundances of total arthropod (**a**), aphids (**b**), generalist herbivores (**c**), and detritivores/scavengers (**d**) aphids in response to experimental manipulations of ants (access or exclusion) on wild cotton plants at three sites: Agua Caliente Canyon, Florida Canyon, and Molino Basin. *Bars* Means + SE for cumulative data across eight census dates. *P* values are given for significant treatment effects within a site

$R = 0.685$, $P < 0.001$; Agua vs. Molino $R = 0.768$, $P < 0.001$; Florida vs. Molino $R = 0.850$, $P < 0.001$) and in the abundance, richness, and evenness of arthropods visiting plants (Table 1). On wild cotton plants with ambient levels of ants and extrafloral nectar, arthropod abundance and diversity were generally lowest at the Molino Basin (Table 1). Wild cotton plants at Agua Caliente and Florida canyons supported 170% more total arthropods and had 110% higher morphospecies richness and evenness than plants at Molino Basin (Table 1). Plants at Florida Canyon

Table 1 Comparison of arthropod community composition across three wild cotton sites: Agua Caliente Canyon, Florida Canyon, and Molino Basin

Community variable (per plant)	Natural site differences					
	Agua Caliente		Florida Canyon		Molino Basin	
	Mean	SE	Mean	SE	Mean	SE
Cumulative abundance	27.4 a	2.94	27.7 a	3.22	10.2 b	3.70
Morphospecies richness	2.0 a	0.16	2.2 a	0.18	1.0 b	0.21
Morphospecies evenness	0.47 a	0.032	0.44 a	0.035	0.22 b	0.041
Cumulative carnivores	5.3 a	0.90	9.7 b	1.17	3.9 a	1.13
Cumulative herbivores	5.3 a	0.98	9.6 b	1.28	3.9 a	1.23
Cumulative aphids	290.8 a	103.5	236.9 a	134.6	165.4 a	129.4
Cumulative detritivores/scavengers	0.7 a	0.1	0.3 a	0.07	0.6 a	0.09
Mean spA census^{-1} (Thomisidae)	0.02 a	0.04	0.09 a	0.04	0.19 b	0.04
Mean spB census^{-1} (Ulidiidae)	0.3 a	0.09	0.2 a	0.11	0.0 b	0.10
Mean spC census^{-1} (Dictynidae)	0.0 a	0.00	0.4 b	0.08	0.0 a	0.00
Mean spD census^{-1} (Tortricidae)	0.01 a	0.03	0.08 ab	0.03	0.19 b	0.03

Least squares means are presented with SE

Values followed by different lower-case letters are statistically significantly different among sites ($P < 0.05$) according to a Tukey HSD post-hoc test

had the greatest numbers of both carnivores and herbivores, with 110% more than at Agua Caliente or Molino Basin (Table 1). Aphid abundances on plants did not significantly differ among sites (Table 1). Four morphospecies contributed a maximum of 6–14% to the dissimilarity between sites, including an ulidiid fly, dictynid and thomisid spiders, and a leaf rolling caterpillar (Tortricidae) (Table 1).

Discussion

The facultative mutualism altered arthropod composition and diversity

Overall, the patterns supported our prediction that the facultative mutualism between ants and wild cotton would negatively affect other arthropods visiting plants. Across all sites, the presence of ants depressed arthropod richness, evenness, and the abundance of non-ant carnivores relative to the ant exclusion treatment. Because effects on evenness were only significant at the family and order levels, it may be important to examine diversity at multiple scales when assessing arthropod community responses. In addition, the probability of detecting new taxa declines when fewer individuals are observed (Gotelli and Colwell 2001). However, the effects of ants on arthropod richness and evenness were not likely driven by a reduction in arthropod abundance because the ant treatment had no consistent influence on total arthropod abundance across sites. Declines in arthropod richness and evenness in the ant access treatment were opposite to the increases expected if the sticky barrier used

to deter ants also excluded other arthropods. Thus, results support a causal role for ants in structuring the arthropod assemblage on wild cotton. Negative effects of the mutualism on arthropods could result from direct aggression by ants or could occur indirectly, for example, via ant-mediated reductions in the dominant herbivore (*Bucculatrix thurberiella*) (Rudgers et al. 2003; Rudgers and Strauss 2004).

In general, these findings agree with other recent studies of ant–plant protective mutualisms showing that ant presence can reduce the numbers of individual predator and parasitoid species visiting plants (e.g. Fernandes et al. 1999; Mody and Linsenmair 2004; Renault et al. 2005). Surprisingly, however, ants did not consistently reduce the numbers of casual herbivores of wild cotton (i.e., herbivores other than the specialist *B. thurberiella* or aphids) and instead had stronger impacts on carnivores and detritivores/scavengers. Thus, despite their presumed protective value to plants, ants probably have more variable influences on plants than is commonly presumed. For example, Cuautle and Rico-Gray (2003) found that extrafloral nectar attracted both ants and parasitic wasps to *Turnera ulmifolia* but that wasps actually provided stronger benefits to plants than ants. The presence of an aggressive ant partner could lead to negative fitness consequences for extrafloral nectar-bearing plants, for instance, if the non-ant carnivores deterred are important for controlling herbivory (see also Ness 2006).

We predicted that the influence of extrafloral nectar would depend on ant presence: in the presence of ants, extrafloral nectar should increase ant visitation, and thereby strengthen the negative effect of ants on other arthropods.

For example, Kaplan and Eubanks (2005) found negative effects of fire ants (*Solenopsis invicta*) on both herbivores and predators on cultivated cotton plants, but only when ants had access to honeydew-excreting aphids. In the absence of ants, we predicted that extrafloral nectar would increase the abundance of other arthropods by providing a carbohydrate-rich resource (Stapel 1997; Rudgers and Gardener 2004; Rose et al. 2006; Taylor and Pfannenstiel 2008). In our study, only non-ant carnivores responded non-additively to the ant and nectar treatments across sites, and our findings do not support the initial prediction that extrafloral nectar resources would strengthen the negative effects of ants. In fact, carnivores were most abundant in the absence of both ants and extrafloral nectar resources. Ants significantly reduced herbivory and the abundance of the specialist herbivore *Bucculatrix thurberiella* at both the Agua Caliente and Florida Canyons (Rudgers and Strauss 2004). Thus, higher abundances of the specialist *B. thurberiella* or reduced ant aggression may explain why carnivores were more abundant on plants that lacked ants, but the specific ecological mechanisms driving carnivore responses to extrafloral nectar remain unclear.

Although relatively few studies have experimentally tested whether the presence of mutualism affects community composition (e.g. Mooney 2007; Savage and Peterson 2007), our findings are largely consistent with results from other systems in showing declines in the diversity in the presence of the mutualism. Across studies, results thus far suggest that mutualisms could be as important as competition or predation to the structure of ecological communities (Stachowicz 2001; Rudgers and Clay 2008). Furthermore, protection mutualisms, such as those between ants and plants, may commonly have negative impacts on the diversity of associated species. Studies that experimentally manipulate other types of mutualisms will help to elucidate the generality of these patterns.

Effects of the facultative mutualism on arthropods varied geographically

We found site-dependent effects of both ants and extrafloral nectar, demonstrating that community-level consequences of the facultative ant-wild cotton mutualism vary across the landscape. Although several recent studies have revealed geographic variation in interspecific interaction outcome or strength (Brodie and Ridenhour 2002; Berenbaum and Zangerl 2006; Thompson and Cunningham 2002; Edelaar and Benkman 2006; Thompson and Fernandez 2006), our study is one of the first attempts to test whether geographic variation in the outcome of a mutualism is associated with variable effects of the mutualism on community structure.

Most notably, ant exclusion treatments had the strongest effects on arthropods at the site with the strongest mutualism (Agua Caliente Canyon) and few significant effects where the interaction was neutral (Molino Basin). Ants altered overall arthropod composition (NMS ordination) at both the Agua Caliente and Florida Canyons, but had no influence at Molino Basin. The absence of an ant effect at Molino Basin was likely related to the low abundance of ants visiting wild cotton plants at this site (Rudgers and Strauss 2004). However, it was not due to a low overall abundance of ants at the Molino Basin site because ground foraging ant densities were high (J. A. Rudgers, unpublished data).

In addition to geographic variation in the effects of ants, the influence of extrafloral nectar varied among sites. Although we initially predicted that extrafloral nectar effects would depend on the presence of ants, ants and nectar had additive effects on all groups except carnivores. The strongest patterns occurred at Agua Caliente Canyon, where ambient levels of extrafloral nectar increased total arthropod visitation to plants by 33% relative to plants with reduced nectar. In contrast, arthropods responded to extrafloral nectar in the opposite direction at Molino Basin. Here, plants with ambient nectar attracted 12–13% fewer total arthropods and morphospecies than plants with reduced extrafloral nectar. Agua Caliente Canyon had a two- to fourfold greater extrafloral nectar production than Molino Basin (Rudgers and Gardener 2004), which may explain why the arthropod response was strong and positive at this site. However, it remains unclear which mechanisms may underlie the negative response of arthropods to extrafloral nectar at Molino Basin. In both sites, arthropods appear to respond to ant presence and extrafloral nectar availability independently.

Ants and extrafloral nectar largely affected different components of arthropod composition. For example, at Agua Caliente Canyon where treatment effects were the strongest, ant presence reduced arthropod diversity, altered the NMS ordination of morphospecies composition, and suppressed abundances of carnivores and detritivores/scavengers. In contrast, extrafloral nectar altered total arthropod abundance. Overall, manipulations of ants had stronger magnitudes of effects on arthropods (up to 60%) than manipulations of extrafloral nectar (33%), suggesting that the negative effects of ants outweigh the attractiveness of extrafloral nectar. Similarly, Lach (2008) showed that ants strongly influenced the arthropod community associated with flowers of *Leucospermum conocarpodendron*, while extrafloral nectar had no effect. However, in this system, there was strong temporal variation in the amount of available extrafloral nectar resources, and plants were producing little extrafloral nectar when flowers were blooming. Mathews et al. (2009) recently documented the effects of ants and extrafloral nectar on arthropods visiting peach plants (*Prunus persica*). While extrafloral nectar initially

reduced arthropod community diversity, in the second year of the study, plants bearing extrafloral nectaries supported higher levels of arthropod diversity than nectar-free plants. Similarly, studies comparing commercial cotton plants (*Gossypium hirsutum*) that lacked extrafloral nectaries to isogenic, nectaried lines have also reported positive effects of extrafloral nectaries on plant-visiting arthropods (Lukefahr 1960; Schuster et al. 1976; Rose et al. 2006). Further studies spanning a broader diversity of systems should help to resolve when ants versus extrafloral nectar resources are more likely to influence arthropod composition.

What factors may influence the importance of the mutualism to arthropod composition?

The outcome of mutualism is likely to depend on both the density and identity of the mutualist partners, for example, with more ants and more aggressive ant species conferring the largest benefits (Bronstein 1998). In our study, the site at which plants received the greatest benefits from ants also demonstrated the strongest community-level impacts of the mutualism. However, to test the hypothesis that interaction outcome caused these patterns would additionally require experimental manipulation of the outcome of the mutualism, a task that would be logistically difficult for any ant–plant system. Other differences among sites could also contribute to the magnitude of treatment effects on arthropods. For example, across treatments, there were more arthropods and a greater diversity of taxa at Agua Caliente Canyon (Table 1); this may have increased the ability to detect treatment effects. In addition, at Molino Basin where treatments had few effects on arthropods, not only were ant densities on plants low, but the aggressive ant, *Forelius pruinosus*, was effectively absent from plants (Rudgers and Strauss 2004). The presence of a parasitic green alga that clogs extrafloral nectaries was much higher at both Florida Canyon and Molino Basin than at Agua Caliente Canyon (Rudgers and Strauss 2004), which could also influence the responses of ants and other arthropods to extrafloral nectar. Finally, we manipulated the mutualism at the plant level in this study. Since most arthropods (including ants) are highly mobile, plot- or site-level manipulations of both mutualist partners may provide a more realistic test of the influence of mutualisms on community structure. Elucidating the mechanisms driving geographic variation in the community response to ants and extrafloral nectar will require further study.

Conclusion

Geographic variation in interaction outcome may be a common feature of protection mutualisms, which depend on the abundances of at least three, and often more, interacting species. Across the landscape of the Sonoran Desert, wild cotton sites differed in terms of the importance of ants to plant defense and the importance of extrafloral nectar to attracting ants. Here, we have shown that responses of associated arthropods to the mutualism also varied geographically, corresponding to differences among sites in the outcome of the ant-plant mutualism. Our findings underscore the importance of incorporating geographic variation into investigations of the community-level influences of mutualisms, as the influence of the ant–wild cotton interaction on other community members varied considerably among sites.

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