

A selection mosaic in the facultative mutualism between ants and wild cotton

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In protection mutualisms, one mutualist defends its partner against a natural enemy in exchange for a reward, usually food or shelter. For both partners, the costs and benefits of these interactions often vary considerably in space because the outcome (positive, negative or neutral) depends on the local abundance of at least three species: the protector, the beneficiary of protection and the beneficiary's natural enemy. In *Gossypium thurberi* (wild cotton), ants benefit nutritionally from the plant's extrafloral nectaries and guard plants from herbivores. Experimentally altering the availability of both ants and extrafloral nectar in three populations demonstrated that the mutualism is facultative, depending, in part, on the abundance of ants and the level of herbivore damage. The species composition of ants and a parasitic alga that clogs extrafloral nectaries were also implicated in altering the outcome of plant–ant interactions. Furthermore, experimental treatments that excluded ants (the putative selective agents) in combination with phenotypic selection analyses revealed that selection on extrafloral nectary traits was mediated by ants and, importantly, varied across populations. This work is some of the first to manipulate interactions experimentally across multiple sites and thereby document that geographically variable selection, mediated by a mutualist, can shape the evolution of plant traits.

Keywords: ant–plant; coevolution; *Forelius*; geographical mosaic; *Gossypium*; indirect defence

1. INTRODUCTION

The process of coevolution is fundamental to the evolution of species traits (Thompson 1994, 1999). Recent work on coevolution has embraced a commonly observed biological reality: that the outcomes of species interactions (positive, negative or neutral for each species) vary in space. Predicting how traits are shaped by coevolutionary interactions should thus require an understanding of spatially variable patterns of selection, metapopulation dynamics, gene flow among populations and genetic drift within populations (the geographical mosaic theory of coevolution (Thompson 1999)). Empirical research has focused on demonstrating that the *outcomes* of species interactions vary across populations. For example, Thompson & Cunningham (2002) showed that interactions between *Greya* moths and *Lithophragma* plants vary geographically, with the moths providing pollination services in some populations and parasitizing *Lithophragma* seeds in others. However, in this well-studied association, it remains unclear which traits (either of the plants or the moths) are affected by the variable outcomes of these interactions. What is missing from most prior research is evidence that variation in the *outcomes* of coevolutionary interactions actually translates into variation in *selection on traits* that mediate these interactions (see Strauss *et al.* 2004). The evidence we do have comes mainly from comparative, rather than experimental, studies (e.g. Brodie *et al.* 2002; Zangerl & Berenbaum 2003; Siepielski & Benkman 2004; but see Stinchcombe & Rausher 2002). We take an experimental approach to exploring spatially structured coevolution by manipulating

agents of selection as well as species traits in three geographically distinct locations.

Protection mutualisms, with their pervasive and well-documented variability, provide excellent models for understanding the geographical context of coevolution. In protection mutualisms, one mutualist guards its partner against a natural enemy, often in return for nutrition or shelter. These interactions occur commonly in nature (Bronstein & Barbosa 2002). For example, fungal endophytes defend grasses against herbivores (Clay 1990), noxious algae protect decorator crabs from predators (Stachowicz & Hay 1999), oxpeckers reduce parasites on cattle (Weeks 1999), and spiders protect plants from seed predators (Whitney 2004). Additionally, ants guard plants against herbivores and pathogens (Huxley & Cutler 1991), while plants reward ants with food (e.g. extrafloral nectar; Koptur 1992) or refuge (e.g. swollen thorns; Janzen 1966).

For both partners, the costs and benefits of engaging in protection mutualisms are markedly variable in space and time because the interactions depend on the local abundance of at least three species—the protector, the beneficiary of protection, and the beneficiary's natural enemy (Bronstein 1998). For example, in protection mutualisms between ants and plants, variation can arise from changes in the abundance of partners (Di Giusto *et al.* 2001), the identity of partners (Horvitz & Schemske 1984), the availability of alternative resources for partners (Gaume *et al.* 1998) or the identity and abundance of plant consumers (Barton 1986). Therefore, the net outcome of these interactions often shifts along a continuum from mutualism to parasitism.

Using a protection mutualism between ants and wild cotton, we took an experimental approach to investigate how geographical variation in the nature of interactions

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between ants, herbivores and plants influences the evolution of plant traits. Wild cotton (*Gossypium thurberi*) possesses extrafloral nectaries attractive to ants that consume its herbivores. First, we asked: are ant–wild cotton interactions geographically variable? Geographical variation in the outcome of the interaction should depend on the local abundance of several interacting groups in this protection mutualism (the plant, its herbivores, ants and a parasitic alga that clogs extrafloral nectaries). In three populations, we experimentally reduced ants and extrafloral nectar to test the predictions that extrafloral nectar increases wild cotton fitness when ants are abundant and that extrafloral nectar is costly when ants are absent. Second, we addressed the question: do ants mediate geographically variable selection on extrafloral nectary traits? In three populations, we experimentally released plants from selection imposed by ants to determine whether variation in the level of protection conferred by ants creates a geographical mosaic of selection on wild cotton traits.

2. MATERIAL AND METHODS

(a) *Study organisms*

Wild cotton, *Gossypium thurberi* Todaro (Malvaceae), is a deciduous, perennial shrub native to canyons of the Sonoran Desert, USA and Mexico (see Hanson (1923) for distribution map). Wild cotton bears three extrafloral nectaries below the bracts surrounding reproductive tissues, and single nectaries are located on the undersides of leaves along the midvein (Fryxell 1979). Unlike bract nectaries, which are ubiquitous, foliar nectaries are not expressed on all leaves, and prior work has demonstrated a heritable basis for the proportion of leaves bearing nectaries and for extrafloral nectary size (Rudgers 2004).

Both folivores and predators of reproductive tissue attack wild cotton. Of the folivores, the moth *Bucculatrix thurberiella* Busck (Lyonetiidae) inflicts the greatest damage (Karban 1993). A specialist on *Gossypium*, *B. thurberiella* occurs throughout *G. thurberi*'s range (Smith & Flint 1977). Adult *B. thurberiella* consume nectar, including the extrafloral nectar of wild cotton (personal observation).

Forelius pruinosus Roger (Formicidae) dominates the ant assemblage in some populations, although other ant species are also observed at extrafloral nectaries (see § 3a(v)). *Forelius pruinosus* has a broader geographical range than wild cotton (Mackay & Mackay 2002). Coccinellids, parasitoids and predaceous wasps also occasionally visit extrafloral nectaries; however, these enemies did not respond to experimental manipulations of extrafloral nectar (Rudgers 2002, 2004).

Populations of wild cotton were located in southern Arizona, USA. Agua Caliente Canyon (31°41'32" N, 10°57'23" W, elevation 1225 m) and Florida Canyon (31°45'40" N, 10°50'46" W, elevation 1426 m, 13 km northeast of Agua) are in the Santa Rita Mountains and Molino Basin (32°20'22" N, 110°41'14" W, elevation 1341 m) is in the Santa Catalina Mountains ca. 70 km to the north of Florida Canyon. Populations were chosen based on ease of accessibility. Results from experiments conducted at Agua Caliente Canyon are presented in greater detail elsewhere (Rudgers 2004) and are included here to illustrate differences among populations.

(b) *Are ant–wild cotton interactions geographically variable?*

In three populations of wild cotton, we employed a 2 × 2 design with an ant treatment (ant access or ant exclusion) and an extra-

floral nectar treatment (ambient extrafloral nectar or extrafloral nectar reduction). First, if ants benefit wild cotton in a given population, then plants with ants should have higher fitness than plants with ants excluded. Second, if extrafloral nectar mediates the benefit of ants, then a significant ant × extrafloral nectar interaction should occur, with extrafloral nectar enhancing plant fitness only in the presence of ants. The manipulation of extrafloral nectar is crucial to determining the importance of this plant trait in mediating the interaction with ants; few other ant–plant studies include such manipulations (reviewed by Rudgers 2004).

(i) *Experimental treatments*

Randomly selected, naturally occurring plants were assigned at random to treatments using a random number table. 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).

Nectar reduction was achieved by covering all extrafloral nectaries with a drop of non-toxic, waterproof glue (Aleene's Co., Buellton, CA, USA). Control plants had the same amount of glue placed less than 1 cm from the nectary. All plants received glue throughout the season as new nectaries were produced.

Ants were deterred from plants with a sticky barrier applied over protective tape (Tanglefoot Co., Grand Rapids, MI, USA). Tape was applied in a triple-layered, 10 cm wide band around the woody trunk of the plant, ca. 10 cm above the ground. The sticky barrier was painted in a 2.5 cm wide band in the middle of the tape. During 1998–1999, control plants received tape only. In 2000, all plants had both tape and the sticky barrier, and for control plants we added a short section of stem cut from a non-manipulated wild cotton plant that allowed ants to circumvent the sticky barrier. Minor differences among years in treatments did not affect the results because plant, herbivore and ant responses were consistent across years (see § 3a(i–iv)). All plants for which the sticky barrier accidentally contacted the bark were excluded from the analysis, producing unequal sample sizes in this factorial design (ant access + ambient nectar, ant access + reduced nectar, ant exclusion + ambient nectar, ant exclusion + reduced nectar; Agua $n = 28, 30, 25, 27$; Florida $n = 31, 31, 15, 9$; Molino $n = 27, 25, 30, 26$).

(ii) *Response variables*

Ant visitation, herbivory, and seed production were assessed. The number of ants per plant was counted once every two weeks from approximately 1 July to 30 November. Ants were identified to species in the field and were not removed from plants. Percentage leaf damage was estimated non-destructively (transparent grid with 1 cm² cells) on 15 randomly selected leaves per plant during monthly censuses (August–November 1998, July–November 2000). In 1999, leaf damage was not measured. We focused on leaf damage rather than herbivore number because ants can reduce herbivore feeding through behaviourally mediated indirect effects, without affecting herbivore density (Rudgers *et al.* 2003). Plant fitness was estimated by the number of undamaged seeds per plant. Plant growth was not strongly affected by the treatments, and flower number showed the same patterns as seed number (Rudgers 2002).

(iii) *Statistical analyses*

Repeated-measures analysis of covariance (proc mixed; SAS Institute 2000) was conducted using data from all populations (1999–2000). Three response variables were examined: the mean number of ants per plant (square-root transformed) calculated

across all census dates for each year, cumulative percentage leaf damage (angular-transformed), which was the maximum amount of damage observed per plant across the five herbivory censuses, and total seed production per plant per year (log-transformed). Population was a random effect, and the ant and extrafloral nectar treatments were fixed effects. Because naturally occurring plants were used, pre-treatment plant size (height (cm) + basal stem diameter (mm) + leaf number) was used as a covariate. Year (1999 or 2000) was the repeated factor (von Ende 2001). All interactions were included in the model with the exception of interactions involving the covariate, which were non-significant. When a significant population \times ant \times extrafloral nectar interaction was observed, differences among treatments within a population were tested using *a posteriori* Tukey HSD tests. No analyses deviated significantly from the assumptions of normality and homogeneity of variances following transformations of the response variables.

(iv) Variation in community composition

Variation among populations in the abundance and composition of the ant assemblage may affect the benefits afforded to wild cotton. Using control plants (ant access + ambient nectar) from the experiment, we determined the mean number of ants per plant (averaged over all censuses from the year 2000) and the Simpson's diversity index per plant (Simpson 1949), using the mean numbers of each ant species per plant during 2000. ANCOVA analyses included the random effect of population, pre-treatment plant size as a covariate, and *a posteriori* Tukey HSD tests for pairwise comparisons among populations (proc glm; SAS Institute 2000).

A parasitic green alga (*Cephaleuros* sp., Trentepohliaceae) may influence the importance of extrafloral nectar to ants. *Cephaleuros* spreads across leaf surfaces and clogs extrafloral nectaries, preventing visible nectar secretion. We recorded whether plants had evidence of *Cephaleuros* every two weeks from approximately 1 July to 30 November 2000. For each plant, we determined the proportion of sampling dates (angular-transformed) that the parasite was observed. We tested for population differences with ANCOVA, as described above for ants. Prior analysis showed that ant and extrafloral nectar treatments did not affect *Cephaleuros* prevalence (Rudgers 2002).

(c) Do ants mediate geographically variable selection on extrafloral nectaries?

Phenotypic selection analyses, in which extrafloral nectary traits are regressed against relative fitness of individuals (Lande & Arnold 1983; Brodie *et al.* 1995), were used to assess whether populations experienced different selective pressures in the presence versus absence of ants. Although phenotypic selection analysis has important limitations (Rauscher 1992), by experimentally excluding ants (the putative agents of selection), we could determine whether ants affected current selection on extrafloral nectary traits (see Mauricio & Rauscher 1997).

(i) Extrafloral nectary traits

Phenotypic selection analyses were conducted for each population \times ant treatment combination, using only the subset of plants with ambient levels of extrafloral nectar (proc reg; SAS Institute 2000). Sample sizes are given above. Two extrafloral nectary traits, the proportion of leaves with nectaries (extrafloral nectary frequency) and mean extrafloral nectary length (mm), were assessed in the field. Because ants are likely to respond differently to the frequency versus the size of extrafloral nectaries (see § 2c(ii)

below), examining both traits gives a more complete representation of selection. Extrafloral nectary frequency was determined for all leaves per plant every six weeks from 15 July 2000 to 21 November 2000. Extrafloral nectary length (a correlate of nectar production (Rudgers 2004)) was measured on 15 randomly chosen leaves per plant once during 1–7 July and again during 1–7 October. The maximum length of each nectary was measured to the nearest 0.01 mm with digital calipers. Estimates of plant size were included in the selection models to account for environmental variation: we estimated leaf area with a transparent grid of 1 cm² cells for 15 leaves per plant and counted leaf number (15 July 2000). For all traits, mean values were calculated per plant. The ant treatment did not affect the plant traits examined in the selection analyses (Rudgers 2002).

(ii) Statistical analyses

Phenotypic selection analyses followed methods in Lande & Arnold (1983). The number of seeds in the year 2000 served as a proxy for plant fitness, and relative fitness (calculated within each population \times ant combination) was used in the analyses. Data did not deviate from assumptions of normality, independence and homoscedasticity with the exception of extrafloral nectary frequency, which was non-normally distributed. Angular transformation normalized this variable but did not qualitatively change the results; therefore, untransformed values were used for ease of interpretation. No multicollinearity problems were detected. Models to estimate nonlinear and trait interaction selection gradients lacked sufficient power given criteria in Lande & Arnold (1983); therefore, only directional selection was estimated. The directional selection gradients are the partial regression coefficients simultaneously fitted to all traits in multiple regression analysis. Confidence limits for the selection gradients were obtained using the bias-corrected accelerated bootstrap with 10 000 re-samples (Efron & Tibshirani 1993; jackboot (SAS Institute 1995)). In addition, ANCOVA compared selection gradients among populations and ant treatments following Mauricio & Rauscher (1997).

(iii) Ants' response to extrafloral nectary traits

We used the same multiple-regression analysis described above to compare the relative importance of extrafloral nectary length and frequency in attracting ants. Here, the total number of ants per plant (square-root transformed) was the response variable, rather than relative plant fitness. We focused our analysis on ants at Agua Caliente during early September 2000 (ants + ambient extrafloral nectar treatment, $n=28$), because ants were very abundant (Rudgers 2004) and because extrafloral nectaries were measured during that year. Predictor variables included the September estimate of nectary frequency, and July estimates of nectary length, leaf area and leaf number, as described previously.

3. RESULTS

(a) Are ant–wild cotton interactions geographically variable?

(i) Summary

The existence of an ant–wild cotton mutualism varied across populations (population \times ant \times extrafloral nectaries (EFN) interactions; table 1). Ants benefited wild cotton plants at both Agua Caliente and Florida Canyons, where plants with ants experienced less herbivory and produced more seeds than plants with ants excluded (figure 1). By contrast, ant exclusion at Molino Basin had no effect on leaf damage or plant fitness (figure 1). In addition, the

importance of extrafloral nectar in attracting ants varied among populations (population \times ant \times EFN interactions; table 1) and was important in attracting ants only in the Agua Caliente Canyon population (figure 1). The result that extrafloral nectar does not always mediate ants' benefits to plants appears to depend on ant abundance, the species composition of the ant community, the prevalence of the parasitic alga and overall levels of herbivory (figures 1 and 2).

(ii) *Effects of treatments on ants*

In all populations, the Tanglefoot barrier effectively reduced ant visitation (figure 1; ant $p < 0.0001$; table 1). However, reduction of extrafloral nectar only affected ant visitation in Agua Caliente Canyon (figure 1; population \times ant \times EFN, $p = 0.02$; table 1), where ant visitation (averaged over censuses and years) was *ca.* 50% lower for plants with reduced extrafloral nectar as compared with plants with ambient extrafloral nectar.

(iii) *Effects of treatments on herbivory*

The populations differed in the effects of both ants and extrafloral nectar on herbivory (population \times ant \times EFN, $p = 0.0122$; table 1). At Agua Caliente Canyon, both ant exclusion and extrafloral nectar reduction increased leaf damage (figure 1); for plants with ants, extrafloral nectar reduction resulted in more than 35% greater herbivory compared with plants with ambient nectar. At Florida Canyon in 1998, plants with ants excluded experienced more than 30% more leaf damage than controls, but there was no effect of nectar reduction on damage (figure 1). This result suggests that ants reduce leaf herbivory, but their effects are not mediated by the presence of extrafloral nectar. At Florida Canyon in 2000, ant-exclusion plants with ambient extrafloral nectar had significantly more leaf damage than plants in all other treatments (figure 1), demonstrating that extrafloral nectar can have ecological costs in the absence of ants by increasing leaf damage. Finally, at Molino Basin, neither ants nor extrafloral nectar affected leaf damage (figure 1).

(iv) *Effects of treatments on seed production*

Populations varied in the response of seed production to ant exclusion and extrafloral nectar reduction (population \times ant \times EFN, $p = 0.0413$; table 1). Seed number was reduced by both ant exclusion and extrafloral nectar reduction at Agua Caliente Canyon (figure 1). For plants with ants, mean seed production during the 2-year experiment was more than 30% lower for extrafloral nectar reduction than for ambient extrafloral nectar. At Florida Canyon, only ant exclusion reduced seed production; plants with ants excluded had *ca.* 70% fewer seeds than plants with ants. Consistent with the lack of an effect of the treatments on herbivory, there were no effects of ants or extrafloral nectar on seed production at Molino Basin.

(v) *Variation in community composition*

Both the density and composition of ant visitors varied among wild cotton populations. Agua Caliente Canyon, where plants responded most strongly to both ants and extrafloral nectar, had the highest ambient densities of ants per plant (mean number of ants per plant \pm s.e.; different letters show populations that significantly differed

Table 1. Statistical results from repeated-measures ANCOVA examining the effects of the population (Agua Caliente Canyon, Florida Canyon or Molino Basin), the ant treatment (access or exclusion), the extrafloral nectar (EFN) treatment (ambient or reduced) and the repeated factor of year (1999 and 2000) on (i) the mean number of ants per plant, (ii) the cumulative percentage leaf damage per plant (2000 only), and (iii) the total seed production per plant. (Plant size is the covariate, pre-treatment plant size. p values of less than 0.05 are indicated in bold.)

effect	mean number of ants			percentage leaf damage			number of seeds		
	d.f.	<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>
ant	1,614	160.05	< 0.0001	1,280	0.48	0.5602	1,688	35.60	< 0.0001
EFN	1,614	5.09	0.0245	1,280	0.75	0.4766	1,688	0.06	0.8142
ant \times EFN	1,614	4.31	0.0384	1,280	2.20	0.2743	1,688	0.77	0.3807
plant size	1,614	8.43	0.0038	1,280	0.88	0.3489	1,688	7.83	0.0053
population	2,614	74.12	< 0.0001	2,280	4.22	0.4151	2,688	17.46	< 0.0001
population \times ant	2,614	31.20	< 0.0001	2,280	1.25	0.4449	2,688	10.01	< 0.0001
population \times EFN	2,614	5.62	0.0038	2,280	0.72	0.5813	2,688	1.47	0.2318
population \times ant \times EFN	2,614	3.94	0.0200	2,280	4.48	0.0122	2,688	3.20	0.0413
year	1,614	19.21	< 0.0001	—	—	—	1,688	486.93	< 0.0001
year \times ant	1,614	3.22	0.0731	—	—	—	1,688	1.78	0.1829
year \times EFN	1,614	0.13	0.7166	—	—	—	1,688	0.00	0.9577
year \times ant \times EFN	1,614	0.22	0.6395	—	—	—	1,688	0.07	0.7883
year \times population	2,614	42.61	< 0.0001	—	—	—	2,688	45.19	< 0.0001
year \times population \times ant	2,614	9.59	< 0.0001	—	—	—	2,688	6.44	0.0017
year \times population \times EFN	2,614	0.33	0.7215	—	—	—	2,688	1.64	0.1940
year \times population \times ant \times EFN	2,614	0.40	0.6689	—	—	—	2,688	0.19	0.8261

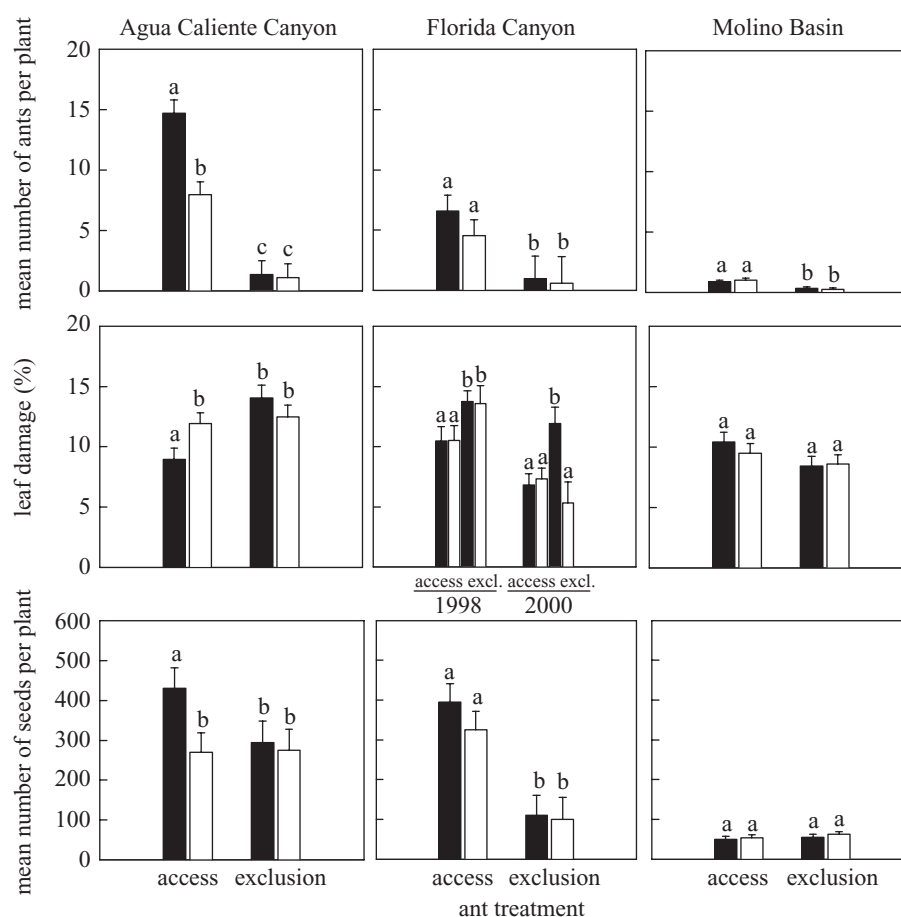


Figure 1. The effects of the ant treatment (access or exclusion) and the extrafloral nectar treatment (ambient EFN, filled bars; or reduced EFN, open bars) on the mean number of ants per plant (averaged over all censuses taken during 1999–2000), the cumulative percentage leaf damage (data available for only 1998 and 2000), and the number of seeds per plant (averaged over the years 1999–2000). Data are shown for three wild cotton populations, Agua Caliente Canyon, Florida Canyon and Molino Basin. Bars are means \pm 1 s.e. For a given response variable, significant differences among the four treatments within a population are indicated by different letters, as determined by *a posteriori* Tukey HSD tests.

via Tukey HSD; Agua $14.7 \pm 1.1a$, Florida $6.6 \pm 1.3b$, Molino $0.9 \pm 0.1c$, population $F_{2,168} = 46.1$, $p < 0.0001$). Furthermore, plants in the two populations that benefited from ants (Agua Caliente and Florida Canyons) were dominated by a single, aggressive species, *Forelius pruinosus*, unlike at Molino Basin where *F. pruinosus* was a minor component of a more diverse ant assemblage (figure 2).

Populations also strongly differed in *Cephaluros* attack. Agua Caliente Canyon, the only population in which ants responded to extrafloral nectar, had a much lower prevalence of *Cephaluros* (mean percentage of censuses on which individuals had *Cephaluros* \pm s.e.; populations that significantly differed via Tukey HSD tests have different letters; Agua $3.7\% \pm 0.8a$, $n = 111$) than did Florida Canyon ($50.8\% \pm 3.3b$, $n = 83$) or Molino Basin ($60.2\% \pm 1.9b$, $n = 108$; population $F_{2,298} = 254.1$, $p < 0.0001$). The prevalence of *Cephaluros* was not correlated with plant fitness within or across populations (J. A. Rudgers, unpublished data).

(b) Do ants mediate geographically variable selection on extrafloral nectaries?

Populations strongly differed in the importance of ants as agents of selection on extrafloral nectary traits, as demonstrated by significant population \times ant \times EFN frequency

($F_{4,110} = 2.44$, $p < 0.05$) and population \times ant \times EFN length effects ($F_{4,110} = 4.32$, $p = 0.003$) in ANCOVA including the relative fitness of plants in all populations. Patterns of selection where extrafloral nectar strongly attracted ants (Agua Caliente) were in the opposite direction from patterns in the sites where extrafloral nectar did not attract ants (Florida Canyon, Molino Basin; figure 3).

(i) Frequency

For plants with ants, phenotypic selection analyses suggest that increased extrafloral nectary frequency should be favoured at Agua Caliente (positive β) and not affected at Florida Canyon (n.s. β), and that decreased extrafloral nectary frequency should be marginally favoured at Molino Basin (marginally significantly negative β ; figure 3), to the extent that this trait is heritable in each population (known to be heritable in Agua Caliente Canyon (Rudgers 2004)). Importantly, in support of the hypothesis that ants are a primary selective agent on this trait, selection gradients on extrafloral nectary frequency were never significant in ant-excluded plants from any population (figure 3).

(ii) Length

In the presence of ants, selection analyses suggest that decreased extrafloral nectary length should be favoured in

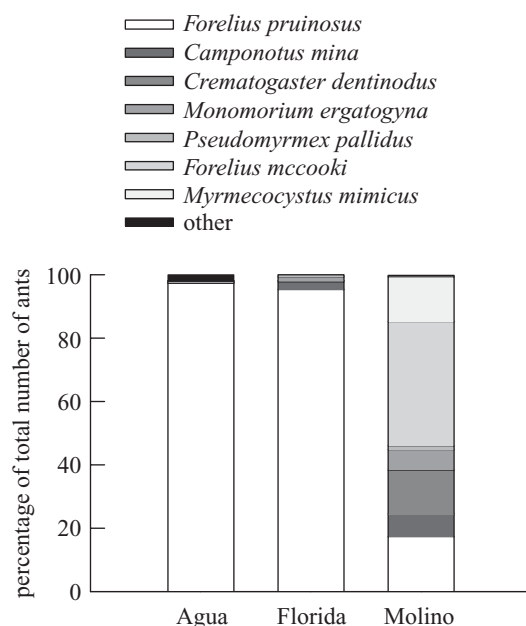


Figure 2. Variation among populations in the composition of the ant assemblage. Stacked bars show the percentage each ant species contributes to the total number of ants per plant. Simpson's diversity indices for ant composition \pm s.e.: Agua Caliente Canyon, $0.03 \pm 0.06a$; Florida Canyon, $0.26 \pm 0.05b$; Molino Basin, $0.44 \pm 0.06c$. Populations that differed significantly according to *a posteriori* Tukey HSD tests have different letters (population $F_{2,80} = 12.2$, $p < 0.0001$).

Agua Caliente Canyon (negative β) and increased length should be favoured in Florida Canyon and Molino Basin (positive β ; figure 3), to the extent that length is heritable (heritable in Agua Caliente Canyon (Rudgers 2004)). Extrafloral nectary frequency and length were negatively correlated (Agua: $r = -0.39$, $p < 0.05$; Florida: $r = -0.45$, $p < 0.02$; Molino: $r = -0.45$, $p < 0.05$).

(iii) *Ants' response to extrafloral nectary traits*

At Agua Caliente Canyon, the number of ants per plant (September 2000) was significantly greater on plants with a greater frequency of extrafloral nectaries, as demonstrated by multiple regression analysis (β (95% confidence interval (CI)), 9.03 (2.54 – 22.12)). By contrast, ant abundance was negatively related to the length of extrafloral nectaries, although not significantly (β (95% CI), -2.27 (-4.86 – 0.90)). These patterns indicate that extrafloral nectary frequency was more important in attracting ants than extrafloral nectary size.

4. DISCUSSION

To our knowledge, this is the first experimental study to demonstrate that spatial variation in the outcome of a protection mutualism results in spatially variable selection on species traits. Current selection on allocation to extrafloral nectar differed among wild cotton populations in the presence of ants, but not in the absence of ants. Specifically, we found strong positive selection for an increased frequency of extrafloral nectaries in just one of the three populations (Agua Caliente Canyon) and only in the presence of ants. Supporting this pattern, we also found that more ants visited plants with a greater frequency of extrafloral nectaries in this population. The lack of positive selection on

the frequency of extrafloral nectaries in the other two populations and in the absence of ants in all populations suggests that ants are responsible for geographical variation in selection on extrafloral nectary traits.

Across the three populations, the outcome of the interaction between ants and plants changed sign (from positive to neutral). Ants protected plants from herbivory and increased fitness in two populations (Agua Caliente and Florida Canyons) but did not affect herbivory or fitness in a third site (Molino Basin). What factors might influence the importance of ants as mutualists? As in other facultative associations involving ants (Bronstein 1998), benefits to wild cotton are likely to depend on both the density and the identity of the mutualists, with more ants conferring larger benefits, and more aggressive species providing greater protection. In the population where ants did not benefit plants (Molino Basin), the density of ants on plants was less than 10% of ant densities at Agua Caliente or Florida Canyons. Furthermore, the aggressive ant, *Forelius pruinosus*, which dominates the other wild cotton populations, was infrequently observed. The lack of abundant, aggressive mutualists at Molino Basin provides a plausible explanation for why ant exclusion did not affect herbivory or plant success in this population. However, differences in water availability (which could affect rates of nectar production or ant distributions (Kaspari & Valone 2002)), the abundance of other plant species bearing extrafloral nectaries (which may affect the abundance and distribution of ants (Rico-Gray *et al.* 1998; Rudgers & Gardener 2004)), the level of phenotypic plasticity in extrafloral nectar production (Wäckers *et al.* 2001) and the composition of the herbivore assemblage are alternative factors that may also contribute to the outcome of interactions between ants and wild cotton.

In addition, populations varied in the importance of extrafloral nectar to attracting ants. Although experimental reduction of extrafloral nectar strongly reduced ant visitation at Agua Caliente Canyon, extrafloral nectar did not affect ant visitation at Florida Canyon and Molino Basin. One potential mechanism underlying this variation could be the prevalence of the parasitic green alga, *Cephaleuros*. *Cephaleuros* clogs extrafloral nectaries and may have reduced the difference between ambient nectar and experimentally reduced nectar. *Cephaleuros* was more prevalent at Florida Canyon and Molino Basin (present in 50–60% of surveys) than at Agua Caliente Canyon (4%). Thus, the lack of an effect of the extrafloral nectar treatment on ants may not result from a lack of ants' response to nectar, but rather from interference from *Cephaleuros* that masks variation among plants in nectar availability. If supported, such a pattern would reinforce the idea that geographically variable selection can be influenced by numerous players, requiring a deep understanding of the natural history of the system to decipher the mechanisms underlying spatial patterns (Thompson 1994). Experimental manipulations of *Cephaleuros* would be necessary to determine the extent to which this parasite may affect selection on extrafloral nectary traits. Our current data do not show an effect of *Cephaleuros* on plant fitness, nor does inclusion of its prevalence result in altered patterns of selection on nectary traits in our analyses. However, the prevalence data collected were admittedly coarse (number of census dates with alga) and may thus not be detailed enough to detect such effects.

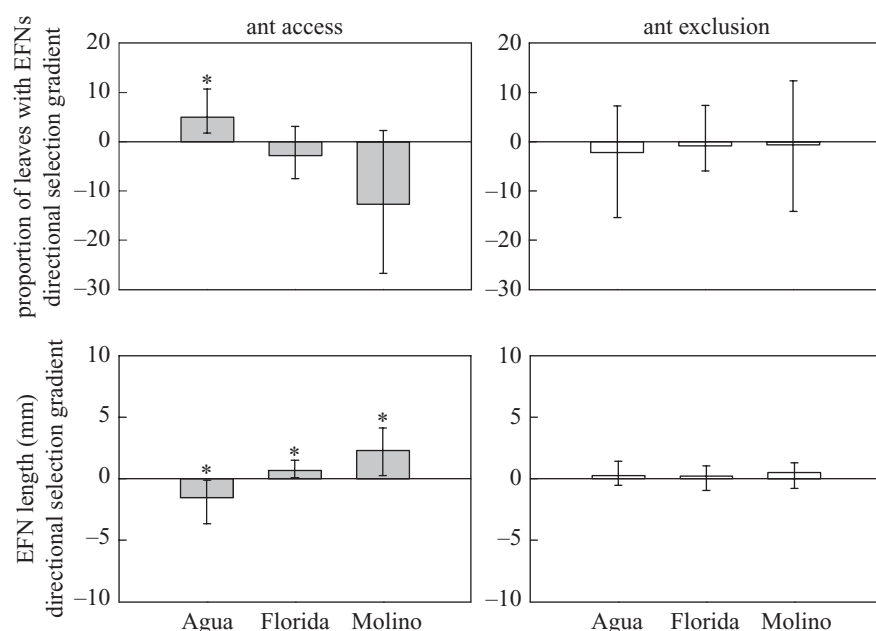


Figure 3. Directional selection gradients (in units of measurement) for the three populations (Agua Caliente Canyon, Florida Canyon and Molino Basin) and the two ant treatments (ant access or ant exclusion). Bars are directional selection gradients (partial regression coefficients from a multiple regression analysis) \pm 95% CI. At Molino Basin, the estimate of the selection gradient was not significant at $p < 0.05$, but 90% confidence limits bounded the estimate away from zero ($\beta = -12.66$, 90% CI = -23.94 to -0.52). * $p < 0.05$.

Alternative hypotheses to explain population differences include that ants may be attracted to prey, rather than nectar, and that other nectar sources may be more important to ants than wild cotton extrafloral nectar at Florida Canyon and Molino Basin.

Finally, experimental evidence suggests that extrafloral nectar can have an ecological cost by enhancing herbivory in the absence of ants (Florida Canyon in 2000; see also Rudgers (2004)). Similarly, in agricultural fields with few ants, cultivated cotton varieties bearing extrafloral nectaries often attract more insect herbivores and herbivory as compared with nectary-free varieties (e.g. Henneberry *et al.* 1977; Adjei-Mafo *et al.* 1983). Despite the increased herbivory on wild cotton plants with extrafloral nectar in the absence of ants, this ecological cost did not affect plant fitness during the years of this study.

Geographical mosaics of selection are likely to be a common feature of protection mutualisms, which depend on the abundances and identities of at least three, and often more, interacting species. In wild cotton, we found geographical variation in the importance of ants to plant defence and in the importance of extrafloral nectar to attracting ants. This variation in the ecological outcomes of interactions between ants and wild cotton produced variation in evolutionary selective pressures on wild cotton extrafloral nectary traits.

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REFERENCES

- Adjei-Mafo, I. K., Wilson, L. T., Thomson, N. J. & Blood, P. R. B. 1983 Effect of pest damage intensity on the growth, maturation, and yield of nectaried and nectariless cotton. *Environ. Entomol.* **12**, 353–358.
- Barton, A. M. 1986 Spatial variation in the effect of ants on an extrafloral nectary plant. *Ecology* **67**, 495–504.
- Brodie III, E. D., Moore, A. J. & Janzen, F. J. 1995 Visualizing and quantifying natural selection. *Trends Ecol. Evol.* **10**, 313–318.
- Brodie, E. D., Ridenhour, B. J. & Brodie, E. D. 2002 The evolutionary response of predators to dangerous prey: hot-spots and coldspots in the geographic mosaic of coevolution between garter snakes and newts. *Evolution* **56**, 2067–2082.
- Bronstein, J. L. 1998 The contribution of ant-plant protection studies to our understanding of mutualism. *Biotropica* **30**, 150–161.
- Bronstein, J. L. & Barbosa, P. 2002 Multitrophic/multispecies mutualistic interactions: the role of non-mutualists in shaping and mediating mutualisms. In *Multitrophic interactions* (ed. T. Tscharntke & B. A. Hawkins), pp. 44–66. Cambridge University Press.
- Clay, K. 1990 Fungal endophytes of grasses. *A. Rev. Ecol. Syst.* **21**, 275–297.
- Di Giusto, B., Anstett, M., Dounias, E. & McKey, D. B. 2001 Variation in the effectiveness of biotic defense: the case of an opportunistic ant-plant protection mutualism. *Oecologia* **129**, 367–375.
- Efron, B. & Tibshirani, R. 1993 *An introduction to the bootstrap*. Monographs on statistics and applied probability no. 57. New York: Chapman & Hall.
- Fryxell, P. A. 1979 *The natural history of the cotton tribe*. College Station, TX: Texas A&M University Press.

- Gaume, L., McKey, D. & Terrin, S. 1998 Ant-plant-homopteran mutualism: how the third partner affects the interaction between a plant-specialist ant and its myrmecophyte host. *Proc. R. Soc. Lond. B* **265**, 569–575. (doi:10.1098/rspb.1998.0332)
- Hanson, H. C. 1923 Distribution of Arizona wild cotton. *Arizona Exp. Station Tech. Bull.* **3**, 48–59.
- Henneberry, T. J., Bariola, L. A. & Kittock, D. L. 1977 Nectariless cotton: effect on cotton leafperforator and other cotton insects in Arizona. *J. Econ. Entomol.* **70**, 797–799.
- Horvitz, C. C. & Schemske, D. W. 1984 Effects of ants and an ant-tended herbivore on seed production of a neotropical herb. *Ecology* **65**, 1369–1378.
- Huxley, C. R. & Cutler, D. F. (eds) 1991 *Ant-plant interactions*. Oxford University Press.
- Janzen, D. H. 1966 Coevolution of mutualism between ants and acacias in Central America. *Evolution* **20**, 249–275.
- Karban, R. 1993 Costs and benefits of induced resistance and plant density for a native shrub *Gossypium thurberi*. *Ecology* **74**, 9–19.
- Kaspari, M. & Valone, T. J. 2002 On ectotherm abundance in a seasonal environment—studies of a desert ant assemblage. *Ecology* **83**, 2991–2996.
- Koptur, S. 1992 Extrafloral nectary-mediated interactions between insects and plants. *Insect-plant interactions IV* (ed. E. Bernays), pp. 81–129. Boca Raton, FL: CRC Press.
- Lande, R. & Arnold, S. J. 1983 The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226.
- Mackay, W. P. & Mackay, E. E. 2002 The ants of North America: key to *Forelius* Centennial Museum, Laboratory for Environmental Biology, The University of Texas, El Paso, Texas, USA. See <http://www.utep.edu/leb/ants/Forelius.doc>.
- Mauricio, R. & Rausher, M. D. 1997 Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**, 1435–1444.
- Rausher, M. D. 1992 The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* **46**, 616–626.
- Rico-Gray, V., Garcia-Franco, J. G., Palacios-Rios, M., Diaz-Castelazo, C., Parra-Tabla, V. & Navarro, J. A. 1998 Geographical and seasonal variation in the richness of ant-plant interactions in Mexico. *Biotropica* **30**, 190–200.
- Rudgers, J. A. 2002 Evolutionary ecology of ant-wild cotton associations. Dissertation, University of California at Davis, CA.
- Rudgers, J. A. 2004 Enemies of herbivores can shape plant traits: selection in a facultative ant-plant mutualism. *Ecology* **85**, 192–205.
- Rudgers, J. A. & Gardener, M. C. 2004 Extrafloral nectar as a resource mediating multispecies interactions. *Ecology* **85**, 1495–1502.
- Rudgers, J. A., Hodgen, J. G. & White, J. W. 2003 Behavioral mechanisms underlie an ant-plant mutualism. *Oecologia* **135**, 51–59.
- SAS Institute 1995 *Jackboot macro*. Cary, NC: SAS Institute.
- SAS Institute 2000 *SAS version 8.1*. Cary, NC: SAS Institute.
- Siepielski, A. M. & Benkman, C. W. 2004 Interactions among moths, crossbills, squirrels, and lodgepole pine in a geographic selection mosaic. *Evolution* **58**, 95–101.
- Simpson, E. H. 1949 Measurement of diversity. *Nature* **163**, 688.
- Smith, R. L. & Flint, H. M. 1977 A bibliography of the cotton leafperforator, *Bucculatrix thurberiella*, and a related species, *Bucculatrix gossypiella*, that also feeds on cotton (Lepidoptera: Lyonetiidae). *Bull. Entomol. Soc. Am.* **23**, 195–198.
- Stachowicz, J. J. & Hay, M. E. 1999 Mutualism and coral persistence: the role of herbivore resistance to algal chemical defense. *Ecology* **80**, 2085–2101.
- Stinchcombe, J. R. & Rausher, M. D. 2002 The evolution of tolerance to deer herbivory: modifications caused by the abundance of insect herbivores. *Proc. R. Soc. Lond. B* **269**, 1241–1246. (doi:10.1098/rspb.2002.2015)
- Strauss, S. Y., Connor, J. K. & Sahli, H. 2004 Toward a more trait-centered view of diffuse (co)evolution. *New Phytol.* (In the press.)
- Thompson, J. N. 1994 *The coevolutionary process*. University of Chicago Press.
- Thompson, J. N. 1999 Specific hypotheses on the geographic mosaic of coevolution. *Am. Nat.* **153**, S1–S14.
- Thompson, J. N. & Cunningham, B. M. 2002 Geographic structure and dynamics of coevolutionary selection. *Nature* **417**, 735–738.
- von Ende, C. N. 2001 Repeated measures analysis: growth and other time dependent measures. In *Design and analysis of ecological experiments* (ed. S. M. Scheiner & J. Gurevitch), pp. 134–157. New York: Oxford University Press.
- Wackers, F. L., Zuber, D., Wunderlin, R. & Keller, F. 2001 The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Ann. Bot.* **87**, 365–370.
- Weeks, P. 1999 Interactions between red-billed oxpeckers, *Buphagus erythrorhynchus*, and domestic cattle, *Bos taurus*, in Zimbabwe. *Anim. Behav.* **58**, 1253–1259.
- Whitney, K. D. 2004 Experimental evidence that both parties benefit in a facultative plant-spider mutualism. *Ecology* **85**, 1642–1650.
- Zangerl, A. R. & Berenbaum, M. R. 2003 Phenotype matching in wild parsnip and parsnip webworms: causes and consequences. *Evolution* **57**, 806–815.

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