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EXTRAFLORAL NECTAR AS A RESOURCE MEDIATING MULTISPECIES INTERACTIONS

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Abstract. Extrafloral (EF) nectar resources can affect the dynamics of species interactions at the community scale. Furthermore, selection acting on EF nectary traits may extend beyond simple mutualisms between plants and the enemies of herbivores to involve other community members that use EF nectar. We examine how EF nectaries influence and are influenced by interactions with multiple species, highlighting our review with original data from the association between ants and wild cotton (*Gossypium thurberi*). Our survey of the literature suggested that EF nectar attracts a diverse assemblage of arthropods and may enhance the diversity and abundance of arthropod assemblages. However, experimental evidence on the importance of EF nectar to terrestrial food webs was equivocal. Exploring potential avenues for selection, we uncovered several costs and benefits of EF nectary traits that have received little empirical attention. These include a constraint faced by plants when attracting both pollinators and protectors via nectar, as well as an ecological cost of nectar when herbivores consume EF nectar as adults. Finally, we discuss how geographic variation in multispecies interactions may affect selection on EF nectary traits. In wild cotton, variation among populations in EF nectar composition was consistent with the benefits afforded by attracting ants; however, non-ant species may also mediate spatially variable selection on EF nectaries. Our synthesis underscores a need for studies that manipulate EF resources at the community level and investigate selection on EF nectaries in a multispecies and geographic context.

Key words: extrafloral nectar; geographic variation; Gossypium thurberi; *indirect effects; multispecies interactions; nectar chemistry; species diversity; wild cotton.*

INTRODUCTION

Extrafloral (EF) nectaries occur in more than 90 plant families and 330 genera (Koptur 1992*a*). These secretory structures are thought to function primarily in protection mutualisms, most commonly involving ants that reduce herbivory in exchange for nectar (Bentley 1977, Koptur 1992*a*). However, carbohydrate-rich, EF nectar rewards are also consumed by insect herbivores, parasitoids, spiders, and even birds (Adjei-Maafo and Wilson 1983, Hespenheide 1985, Vanstone and Paton 1988, Taylor and Foster 1996, Cuautle and Rico-Gray 2003). Although few studies have directly investigated the effects of EF nectar on communities, current evidence suggests that EF nectar may influence species composition, abundance, and interactions at the community scale (Keeler 1978, Rico-Gray et al. 1998).

In turn, the evolution of EF nectary traits should be affected by a broad array of community members. Tra-

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ditionally, ants have been viewed as the most important agents of selection on EF nectary traits; however, several other taxa may influence the evolution of EF nectaries, including pollinators and herbivores. Selective regimes will depend on a balance between the costs and benefits of EF nectar. This balance is likely to shift with the local ecological context because interactions involving EF nectar are characteristically variable in space and time (Bronstein 1998).

We explore multispecies interactions mediated by EF nectar from both ecological and evolutionary perspectives. First, we ask, what are the community-level effects of EF nectar resources? We evaluate the potential for EF nectar to affect entire assemblages of arthropods, examine methods to assess the contribution of EF nectar to terrestrial food webs, and suggest a role for EF nectar in mediating plant–plant interactions. Second, given the potential for many species to interact with EF nectar, we ask how multispecies interactions might shape the evolution of EF nectary traits. To this end, we examine costs as well as nondefensive benefits of EF nectar. Finally, we ask, how does geographic variation in multispecies interactions affect the evolution of EF nectaries? We include examples from the current literature and emphasize our published and new work on ants and the EF nectary-bearing wild cotton (*Gossypium thurberi*). Throughout the paper, our goal

is to use these examples to highlight areas that we feel are important to future research on EF nectar-mediated interactions.

WHAT ARE THE COMMUNITY-LEVEL EFFECTS OF EF NECTAR RESOURCES?

The prevalence of EF nectary-bearing plants provides some indication of the importance of EF nectar in provisioning nectar-feeding animals. As the percentage of plant species bearing EF nectaries can reach nearly 30% in some regions (Oliveira and Oliveira-Filho 1991, Schupp and Feener 1991, Blüthgen et al. 2000), EF nectar has the potential to provide a considerable community-level resource. We summarize the evidence assessing whether EF nectar rewards are substantial enough to affect the diversity or abundance of arthropod assemblages. We also present new data that address this hypothesis. We then suggest novel ways to explore the relative contribution of EF nectar vs. other resources to terrestrial food webs. Finally, we propose that EF nectaries may mediate species interactions beyond the simple tri-trophic food chain of plants–herbivores–enemies, specifically by facilitating interactions among plants that may share or compete for nectary visitors.

Effects of EF nectar on arthropod diversity and abundance

EF nectar can attract a diverse assemblage of visitors to plants. We conducted a survey of published records and determined a mean of 11.7 ± 6.5 (1 SD) ant species per EF nectary-bearing plant species (range 1–27 ant species, $n = 35$ plant species; Appendix). This result suggests that EF nectar often hosts diverse, rather than homogeneous, assemblages of ants (see also Rico-Gray 1993). Although current records are insufficient to allow a similar survey of non-ant arthropods, diverse visitors have been documented, including herbivore, parasite, and pollinator taxa in at least 10 arthropod orders (Koptur 1992a).

By providing carbohydrates, plants with EF nectar may support a greater diversity or abundance of arthropods than nectary-less plants. An analogous example comes from gall-forming aphids (Pemphigus betae), which provide honeydew and thereby increase the species richness and abundance of arthropods on cottonwood compared to conspecifics without aphids (Dickson and Whitham 1996). Evidence for similar community-level effects of EF nectar is limited. However, in cultivated cotton (Gossypium hirsutum), greater abundances of herbivores, parasitoids, and non-ant predators were found on plants with EF nectaries compared to near-isogenic (or related) nectary-less lines (Henneberry et al. 1977, Adjei-Maafo and Wilson 1983).

In contrast, new data from a wild cotton species (Gossypium thurberi, Malvaceae), native to the Sonoran Desert of Arizona and Mexico, indicate that EF nectar may not always promote arthropod abundance or diversity. During 8–10 September 2000, all arthropods were counted on wild cotton plants in Agua Caliente Canyon, Arizona, USA ($31°41'32''$ N, $110°57'23''$ W). Plants were exposed to one of four treatment combinations: ant access + ambient EF nectar, ant access + reduced EF nectar, ant exclusion + ambient EF nectar, ant exclusion + reduced EF nectar ($n = 28$, 30, 26, 28 plants per treatment, respectively). Beginning in July 2000, EF nectar was reduced by covering nectaries with nontoxic glue (Aleene's, Buellton, California, USA); controls had glue placed near, but not covering, nectaries. Ants were excluded with a sticky barrier (Tanglefoot, Grand Rapids, Michigan, USA) spread over a band of Tangleguard (Tanglefoot), and controls had the barrier plus ant bridges (for additional experimental details, see Rudgers 2004). Although EF nectar occlusion significantly reduced visitation by ants (Rudgers 2004), EF nectar did not affect the Shannon-Weiner diversity index for non-ant arthropods (ANOVA: EF nectar, $F_{1, 108} = 1.54$, $P = 0.22$; EF nectar × ant, $F_{1, 108} = 0.06$, $P = 0.81$). In addition, plants with access to ants supported a lower diversity of non-ant arthropods than plants with ants excluded (ant, $F_{1, 108} = 12.33$, $P = 0.0007$; plants with ant access had a Shannon-Weiner diversity index of 1.26 ± 0.08 [mean $\pm$ 1 SE]; plants with ant exclusion had a diversity index of 1.61 ± 0.06). In a similar example, honeydew-producing, ant-tended aphids (Chaitophorus populicola) reduced arthropod species richness and abundance on cottonwood trees compared to conspecific trees without the mutualism (Wimp and Whitham 2001). These contrasting results in cultivated vs. native cotton and among aphids on cottonwood highlight a need for additional studies that assess the role of EF nectar in structuring arthropod assemblages. We predict that arthropod diversity will increase with the availability of nectar resources when these arthropods can resist competitive exclusion by ants or when ant abundances are low.

Compared to single plant species, assemblages of EF nectary-bearing plants have received even less attention with respect to effects on arthropods. However, observations indicate that habitats supplying EF nectar may create islands of high arthropod density and diversity. For example, parasitism of gypsy moths (Lymantria dispar) increased marginally with the abundance of plants with EF nectaries across forest sites in South Korea (Pemberton and Lee 1996); more parasitoids may occur in areas with greater EF nectar, or parasitoids may alter their behavior (e.g., attack more moths) in sites with more EF nectar. Similarly, ant species richness was positively correlated with the percentage of vegetation composed of EF nectary-bearing plants in Nebraska (Keeler 1980) and in Florida, USA (Koptur 1992b, but see Keeler 1979). Conceivably, the spatial distribution of EF nectar in communities could prevent a single arthropod from monopolizing the re-

source and thus promote species coexistence (Blüthgen et al. 2000).

Experiments manipulating EF nectar resources at the community level have not been performed outside of agricultural systems (e.g., EF nectary and nectary-less cotton cultivars). Plantings of nectary-bearing plants with crops lacking nectaries have successfully recruited biological control agents to agroecosystems (Atsatt and O'Dowd 1976), but are EF nectaries as attractive to arthropods in more complex, natural food webs? Manipulations of EF nectar (independently of plant species) could determine whether this resource affects the diversity or abundance of arthropods. If strong effects are found, a next step is to examine how variation in EF nectary *traits* (volume, composition, volatiles) affects arthropods. How these traits mediate interactions is not well resolved, even for the most common species of ants (Lanza et al. 1993, Heil et al. 2001), because most studies exclude ants but do not manipulate EF nectar (see Rudgers 2004).

Contribution of EF nectar to food web dynamics

In communities rich with EF nectary-bearing plants, nectar may be a vital resource in food webs. Currently, few data are available to address this hypothesis. Fisher et al. (1990) used stable-isotope analyses to determine that the contribution of EF nectar to the diets of 11 orchid-associated ant species ranged from 11% to 48%. Although ants were confined to plants in that study (potentially inflating the dietary contribution of nectar), stable-isotope analyses of natural diets could provide a useful metric for assessing the benefits of nectar to arthropods. Such analyses would be especially informative in seasonal systems in which EF nectar is ephemeral relative to arthropod activity (see Rico-Gray and Sternberg 1991). Future work that evaluates the relative contribution of EF nectar vs. other resources (insect prey, floral nectar, homopteran honeydew) will help to discern the importance of EF nectar in subsidizing food webs (Rico-Gray 1993, Rico-Gray et al. 1998).

Effects of EF nectar on plant–plant interactions

Just as Homoptera can compete for the defense conferred by tending ants (Fischer et al. 2001), plant species may compete for the protection provided by ants and other arthropods (e.g., Apple and Feener 2001). Alternatively, co-occurring plant species may benefit from associational resistance gained by sharing herbivores' enemies (Leius 1967, Atsatt and O'Dowd 1976). Experiments are needed that determine the conditions under which plants compete for mutualists or profit from sharing them.

HOW DO MULTISPECIES INTERACTIONS SHAPE THE EVOLUTION OF EF NECTARIES?

The diversity of EF nectar consumers just described suggests that multiple species may exert selection on

EF nectaries. Identifying and quantifying the costs and benefits of EF nectary traits will enhance our understanding of selection on these characters; however, few studies have examined EF nectary traits in a cost–benefit framework. Costs of EF nectar may be expressed physiologically (direct costs) or ecologically via interactions involving other species (ecological costs) (Strauss et al. 2002). For example, EF nectaries that attract ants to deter generalist herbivores may also lure specialized, ant-tended herbivores, exacting an ecological cost when specialized herbivores are abundant (Buckley 1983). Benefits of EF nectaries may include both the commonly studied protective benefits as well as other, nondefensive benefits, such as the attraction of pollinators or seed dispersers.

Costs

Physiological costs.—EF nectar typically contains carbohydrates with dilute concentrations of amino acids (Baker et al. 1978). Although EF nectar production is presumed to be inexpensive, this assumption has rarely been tested. The best estimate of which we are aware indicates that EF nectar is relatively cheap, equal to ~1% of the total energy invested per leaf (O'Dowd 1979). Comparing the fitness of plants for which nectar is repeatedly removed to controls without removals could estimate the physiological cost of EF nectar production (see Pyke 1991). Furthermore, the cost of nectary structures might be determined by growing isogenic lines of nectaried and nectary-less plants in the absence of arthropods. Because physiological costs (if they occur) may vary depending on local resources, experiments altering nutrient and water availability may also be informative.

Phenotypic plasticity.—Following leaf damage, several species, including cultivated cotton, increase production of EF nectar (Agrawal and Rutter 1998, Heil et al. 2001, Wäckers et al. 2001, Ness 2003). This induced response to damage may indicate that constitutively high levels of nectar are costly to maintain. Similarly, EF nectar flow stops or slows in some plants when nectar is allowed to accumulate (Heil et al. 2000, but see Torres-Hernández et al. 2000), although nectaries may simply become clogged, rather than being regulated. These data suggest that plants may moderate the physiological costs of EF nectar via plasticity in nectar production; however, direct evidence for the genetic basis and the adaptive significance of this plasticity is lacking.

Allocation trade-offs and pleiotropy.—As plants possess finite resources, allocation to one plant function (e.g., indirect defense via EF nectar production) may diminish the availability of resources for other plant functions, a physiologically-based allocation trade-off (Mole 1994). For example, plants that invest in EF nectar-mediated defense may express reduced investment in other resistance traits (e.g., toxic secondary compounds; Heil et al. 2002; Rudgers et al. 2004). In

TABLE 1. Ant–herbivore contexts for three southern Arizona wild cotton populations with corresponding variation in traits (mean ± 1 SE) for two extrafloral nectary types, sub-bracteal and foliar.

Nectary type by population[†]	Accumulated volume per nectary (μL/24 h)	Conc. of total carbohydrates (μg/μL)	Proportion of sugars	
			Fructose	Sucrose
Sub-bracteal				
Agua Caliente	0.201[a] ± 0.025	716.1[a] ± 29.9	0.458[a] ± 0.005	0.159[a] ± 0.006
Florida Canyon	0.285[a] ± 0.093	632.1[ab] ± 60.9	0.460[a] ± 0.011	0.117[ab] ± 0.009
Molino Basin	0.059[b] ± 0.013	444.6[b] ± 208.3	0.477[a] ± 0.052	0.029[b] ± 0.017
Foliar				
Agua Caliente	0.036[a] ± 0.004	845.8[a] ± 137.4	0.436[a] ± 0.024	0.178[a] ± 0.022
Florida Canyon	0.021[ab] ± 0.006	522.0[ab] ± 82.0	0.412[ab] ± 0.017	0.127[ab] ± 0.053
Molino Basin	0.015[b] ± 0.005	344.0[b] ± 78.1	0.569[b] ± 0.035	0.030[b] ± 0.017

Notes: ANOVA results are as follows: for accumulated volume/nectary, Population $F_{2,29} = 7.29$, $P < 0.01$; Nectary $F_{1,29} = 46.28$, $P < 0.001$, Population × Nectary $F_{2,29} = 0.35$, $P > 0.05$; for concentration of total carbohydrates, Population $F_{2,46} = 8.91$, $P < 0.001$; Nectary $F_{1,46} = 0.19$, $P > 0.05$, Population × Nectary $F_{2,46} = 1.54$, $P > 0.05$; and for total amino acids, Population $F_{2,35} = 0.66$, $P > 0.05$; Nectary $F_{1,35} = 27.97$, $P < 0.001$, Population × Nectary $F_{2,35} = 0.58$, $P > 0.05$. Protected ANOVAs were preceded by MANOVA, which showed a significant effect of population. The ant exclusion treatment led to increased damage and reduced seed production at both Agua Caliente and Florida Canyon, but had no effect at Molino Basin. Nectar reduction in the presence of ants led to increased damage and reduced seed production at Agua Caliente, but had no effect at Florida Canyon or Molino Basin.

† Sub-bracteal nectaries occur below the bracts surrounding reproductive tissues. Foliar nectaries occur along the midvein on the undersides of leaves. For each nectary type separately, within a column, different letters indicate significant differences between the three populations at $P < 0.01$, as determined by post hoc Tukey's hsd tests. An alpha level of 0.01 was chosen to reduce Type I error.

a similar manner, investment in floral nectar to attract pollinators may limit resources available to attract protective arthropods via EF nectar. For example, preliminary data from wild cotton showed a negative phenotypic correlation between the volume of floral nectar and the volume of EF nectar (M. C. Gardener and J. A. Rudgers, *unpublished data*). Alternatively, if genes underlying floral and extrafloral traits interact through pleiotropy or are closely linked, then positive correlations among reward traits may also occur (e.g., similarities in the composition of floral and EF nectar). To our knowledge, constraints derived from supporting multiple mutualists have not been investigated in other systems.

Ecological costs.—In contrast to physiological costs, the ecological costs of EF nectar have received more attention. In cotton fields, where the abundance of ants is kept low by pesticides and tilling, cultivars with EF nectaries commonly attract more herbivores than related nectary-less lines; this cost results because some herbivores feed on nectar as adults (Henneberry et al. 1977, Adjei-Maafo and Wilson 1983). At least one of these herbivores exhibits preferences among nectar sugars (Ramaswamy 1987), and therefore could influence selection on EF nectar composition. Similarly, ant-free wild cotton plants at one site experienced greater leaf damage when they had EF nectar than when EF nectar was experimentally reduced (J. A. Rudgers, *unpublished manuscript*). As in cultivated cotton, adult lepidopteran herbivores consume wild cotton EF nectar. In addition to herbivores, other plant consumers (e.g., fungi) may also utilize EF nectar in the absence of ants (Torres-Hernández et al. 2000). In sum, these examples suggest that some types of plant consumers may select

against investment in EF nectar, particularly when the abundances of ants or other enemies are low.

Ecological costs may also arise through other pathways. Because EF nectaries commonly occur on exposed leaf surfaces, EF nectar thievery may be widespread, with nonprotective arthropods taking EF nectar without benefiting the plant. Such thievery could reduce the attractiveness of plants to beneficial arthropods in the same way that nectar robbers can reduce the attractiveness of floral displays. Although many visitors that do not confer protection to plants have been documented at EF nectaries (Keeler 1978, Hespenheide 1985), an assessment of EF nectar thievery has not yet been undertaken. In addition to thievery, ecological costs may arise when species that are attracted to EF nectaries, such as ants, deter other plant mutualists (e.g., seed dispersers, Altshuler 1999). Interestingly, some plants release a chemical signal that repels ants from flowers (Ghazoul 2001) and may protect pollinators from harassment by ants.

Benefits

Conventional wisdom holds that EF nectaries benefit plants by attracting arthropods that reduce herbivores, pathogens, or parasites (Bentley 1977, Koptur 1992a). EF nectaries can also lure arthropods that deter floral nectar robbers (e.g., O'Dowd 1979), although robbing has received less attention than herbivory and pathogen attack. Most studies to date have excluded ants to assess the fitness benefits of protection (but see Cuautle and Rico-Gray 2003), and selective exclusions of other potentially beneficial arthropods (e.g., predaceous wasps) are much needed. In addition, several studies

TABLE 1.   Extended.

Proportion of sugars		Total amino acid conc. (pmol/μL)	Aspargine proportion
Glucose	Inositol		
$0.363^a \pm 0.007$	$0.007^a \pm 0.002$	$6017^a \pm 641$	$0.026^a \pm 0.006$
$0.387^a \pm 0.010$	$0.017^{ab} \pm 0.012$	$5775^a \pm 800$	$0.010^a \pm 0.004$
$0.339^a \pm 0.063$	$0.151^b \pm 0.120$	$4697^a \pm 1598$	$0.027^a \pm 0.015$
$0.316^a \pm 0.019$	$0.059^a \pm 0.015$	$17287^a \pm 1912$	$0.061^a \pm 0.013$
$0.329^a \pm 0.024$	$0.132^{ab} \pm 0.054$	$15730^a \pm 3067$	$0.045^{ab} \pm 0.021$
$0.232^a \pm 0.025$	$0.169^b \pm 0.032$	$18969^a \pm 2078$	$0.129^b \pm 0.021$

indicate that EF nectaries benefit plants through alternative pathways.

Alteration of ant behavior.—Some of these benefits originate by averting ant behaviors that are harmful to plants. For example, EF nectar may create a diversion from flowers where ants could disturb pollinators, steal nectar, or reduce pollen germination via their antimicrobial integuments (Beattie et al. 1984, Wagner and Kay 2002). In a similar manner, EF nectaries may distract ants from tending Homoptera that reduce plant fitness (Becerra and Venable 1989). However, evidence thus far is equivocal; EF nectar reduces tending behavior in some cases (Offenberg 2001) and encourages tending ants in others (Buckley 1983, Del-Claro and Oliveira 1993). The net benefit of EF nectar to plants may depend on the local abundance of both ants and tended herbivores, in addition to other nectar visitors.

Attraction of alternative mutualists.—EF nectaries may also attract alternative mutualists that could select for nectary traits. For example, EF nectaries in some acacias (located near the nectarless inflorescences) attract avian pollinators (Vanstone and Paton 1988); pollen is distributed when the bird's plumage contacts the flowers. Honeybees are common visitors to EF nectaries on cotton (Eiskowitch and Loper 1984), although abundant EF nectar could reduce floral visitation by distracting these pollinators from flowers. EF nectar may also attract seed dispersers, as in *Acacia ligulata*, where EF nectar feeds ants that disperse the plants' arillate seeds (Whitney 2002; K. D. Whitney, *personal communication*). Finally, because ant colonies accumulate nutrient-rich debris, plants may also derive resource benefits if ants preferentially nest near (or inside of) plants that exude copious nectar (Wagner 1997).

How Does Geographic Variation Affect the Evolution of EF Nectaries?

When selection on EF nectary traits is influenced by multiple species, the evolution of EF nectar traits may strongly depend on local ecological conditions. Studies often demonstrate that the net outcome of ecological interactions between ants and plants (positive, negative, or neutral) varies in space or time (Bronstein

1998). If the outcomes of interactions vary among populations, then species in local populations are likely to experience different patterns of selection, with reciprocal selection between ants and plants occurring only in some locations ("Geographic mosaic theory of coevolution"; Thompson 1999). Geographically structured coevolution is expected to result in differences among populations in the traits shaped by coevolutionary interactions (Thompson 1999). For example, we may expect populations that vary in the benefits gained from ants to differ in investment in EF nectar. This prediction has seldom been tested, because most studies do not manipulate or measure EF nectary traits in multiple populations (but see Inouye and Inouye 1980, Daehler et al. 1999). In addition, gene flow, genetic drift, and metapopulation dynamics may also influence the EF nectary evolution (Thompson 1999), yet these factors have not been considered in prior studies. Little is currently known about how variation in the outcomes of multispecies interactions affects the evolution of EF nectary traits.

We explored the spatial component of variable selection on EF nectaries by examining multiple populations of wild cotton. Prior work with wild cotton has revealed significant spatial variation in the strength and outcome of interactions with ants (Rudgers 2002, 2004; J. A. Rudgers, *unpublished manuscript*). Specifically, experiments reducing ants (via sticky barriers) and EF nectar (via gluing) revealed variation among populations in both the importance of EF nectar in attracting ants and the importance of ants to plant fitness (Table 1). We used these results to predict how populations might differ in EF nectary traits. To do so, we assumed that ants (by reducing herbivory) were the primary agents of selection and that interactions varied more in space than in time. We based this assumption on the temporal consistency of ant–wild cotton interactions during 2–3 years of field experiments in three populations. Specifically, we predicted that where ants enhanced plant fitness *and* where ants responded to the availability of EF nectar (Agua Caliente Canyon, southern Santa Rita Mountains, Arizona, USA), plants would invest significantly in EF nectar. Where ants protected

and benefited wild cotton regardless of EF nectar availability (Florida Canyon, northern Santa Rita Mountains), we expected intermediate EF nectary traits. Finally, where ants were infrequent visitors that did not protect plants (Molino Basin, Santa Catalina Mountains, Arizona, USA), we anticipated minimal allocation to EF nectar. Here, we present new data documenting among-population variation in EF nectary traits in light of these predictions. It is important to keep in mind that if gene flow among populations is high or if selection is exceedingly variable over time, populations may be ecologically mismatched for their EF nectary traits and may fail to reflect our predictions (Thompson 1999).

We assessed variation in the production and composition of EF nectar among three wild cotton populations. Single branches were selected at random from within randomly selected plants ($n = 16–32$ plants/population), cut, and transferred immediately to water-filled tubes (2–5 September 2001). Nectaries lacked visible nectar at the time of collection, probably because of the arid climate and abundance of nectary visitors. Cuttings were maintained in the laboratory to permit nectar accumulation. Although cuttings may have altered physiology, we were interested only in relative differences among populations, and we treated collections identically. After 24 h, we assessed nectar volume with glass microcapillaries. Nectar samples were then analyzed via HPLC (high-pressure liquid chromatography) for amino acids (Gardener and Gillman 2001) and for sugars (by D. L. Hendrix following Hendrix and Wei 1994). Means were calculated per nectary per plant with 2–10 nectaries examined per plant. Statistical analysis employed general linear models (MANOVA and ANOVA) including the effects of population, EF nectary type (foliar or sub-bracteal, see Table 1), and plant, a random effect nested within population (SAS Institute 2000). The proportional contribution of each amino acid or sugar to the total concentration was calculated and was arcsine square-root transformed to improve the distribution and homoscedasticity of the residuals.

Population differences in the production and composition of EF nectar corresponded with geographic variation in the outcome of interactions between wild cotton and ants. In the Agua Caliente population, in which ants were most beneficial and most responsive to EF nectar, we found the highest concentration of carbohydrates as well as high nectar volume (Table 1). The proportion of carbohydrates comprised of sucrose was more than five times greater at Agua Caliente than at Molino Basin, where plants received no benefits from ants. However, proportions of fructose and inositol in foliar nectaries were highest at Molino Basin. The compositional variation leads to the prediction that ants in this system may prefer sucrose over other sugars. In addition, plants from Agua Caliente produced two times more foliar nectar and nearly four times more

sub-bracteal nectar than did plants from Molino Basin. Surprisingly, Agua Caliente and Florida canyons did not strongly differ in EF nectar investment, despite the fact that three years of experiments at Florida Canyon failed to demonstrate a fitness effect of reduced EF nectar production (J. A. Rudgers, *unpublished manuscript*). Whether gene flow from other populations swamps selection at Florida Canyon, high investment in EF nectar represents historical selection, or other ecological interactions are operating remains to be determined.

In spite of differences in volume and carbohydrates, amino acid concentration did not vary among populations (Table 1). In total, we found 21 amino acids in wild cotton EF nectar, although some were not found in all samples. This value is high compared to other EF nectars examined (Baker et al. 1978). The only significant difference in the profiles of individual amino acids was for the proportion of asparagine in foliar EF nectar (highest at Molino Basin, Table 1); asparagine is not a typical component of EF nectar (Baker et al. 1978).

We can conclude from these results that wild cotton populations vary in EF nectary traits in a manner consistent with geographically variable selection that is mediated by ants. We predict that among-population variation in EF nectary traits will be common in facultative mutualisms that are mediated by EF nectar. Thus, explorations in multiple populations may be critical to understanding selection on EF nectaries. In wild cotton, examining a single population would have given an inadequate view of the interactions with ants. Although we have focused here on what is assumed to be the primary agent of selection on EF nectaries (ants), we envision future studies that examine alternative costs and benefits of EF nectary traits. When we consider EF nectaries in a broader community context, predictions become more complex. In the wild cotton system, for example, (1) adult moths of a dominant herbivore are attracted to EF nectar, representing a potential ecological cost; (2) an algal parasite that covers leaf surfaces reduces EF nectar and may negate the benefits of EF nectar (Rudgers 2002); (3) some EF nectar-visiting ant species also tend aphids, possibly limiting the benefits of nectar; (4) investment in floral nectar may constrain selection on EF nectar and thereby reflect interactions with pollinators, and (5) benefits to ants are seasonal, because wild cotton typically produces leaves from July to December.

CONCLUSION

The evidence presented here suggests that EF nectar resources can affect the composition and dynamics of communities. In some systems, plants bearing EF nectaries contribute to a considerable fraction of the total flora and are visited by a large and diverse arthropod assemblage. Thus, attempts to understand the factors that influence arthropod diversity and abundance, as

well as analyses of terrestrial food web dynamics, could benefit from considering EF nectar resources. In addition, because of the myriad species that forage for EF nectar, natural selection on EF nectary traits is likely to reflect interactions beyond the protective services offered by herbivores' enemies, such as ants. Finally, the widespread occurrence of spatial and temporal variation in the outcomes of EF nectar-mediated interactions calls for an expansion of our investigations from those focused on local dynamics to those that encompass geographic mosaics of selection.

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APPENDIX

A table showing the total number of ant species recorded visiting plants with extrafloral nectaries (for plants that have facultative associations with ants) is available in ESA's Electronic Data Archive: *Ecological Archives* E085-040-A1.