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How do plants balance multiple mutualists? Correlations among traits for attracting protective bodyguards and pollinators in cotton (*Gossypium*)

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Abstract   Many species, both plants and animals, are simultaneously engaged in inter-actions with multiple mutualists. However, the extent to which separate traits that attract different mutualist guilds display negative or positive relationships remains largely unstudied. We asked whether correlations exist among extrafloral nectary traits to attract arthropod bodyguards and floral traits to attract pollinator mutualists. For 37 species in the cotton genus (*Gossypium*), we evaluated correlations among six extrafloral nectary traits and four floral traits in a common greenhouse environment, with and without correction for phylogenetic non-independence. Across *Gossypium* species, greater investment in extrafloral nectary traits was positively correlated with greater investment in floral traits. Positive correlations remained after accounting for the evolutionary history of the clade. Our results demonstrate that traits to maintain multiple mutualist guilds can be positively correlated across related species and build a more general understanding of the constraints on trait evolution in plants.

Keywords   Ant · Extrafloral nectar · Evolutionary constraint · Floral nectar · Mutualism · Trade-off

Introduction

Mutualisms are ubiquitous in natural systems, and many species engage with more than one mutualist species simultaneously (Bronstein 1994; Mack and Rudgers 2008). Inter-actions involving a shared partner and more than one type of mutualist (i.e., interguild mutualisms) are common in nature, but have been little studied relative to pairwise and intraguild mutualisms (but see Stachowicz and Whitlatch 2005; Morris et al. 2007;

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Whitney and Rudgers 2009). For example, most angiosperms interact simultaneously with pollinators and mycorrhizal fungi, both of which acquire carbon from the plant (Gange and Smith 2005; Cahill et al. 2008).

Understanding how organisms allocate resources among guilds of mutualists is important for predicting constraints on the evolution of plant traits. Within species, the costs of rewarding mutualists could lead to trade-offs among investment in traits that affect different mutualist guilds. For example, in grasses that support both nutritional mutualists (mycorrhizal fungi) and protection mutualists (fungal endophytes), the presence of endophytes in leaves reduced the rate of colonization by mycorrhizal fungi in roots (Omacini et al. 2006; Mack and Rudgers 2008). Through evolutionary time, such constraints could produce species that are specialized to reward a single mutualist guild. Alternatively, simultaneous interactions with two or more guilds of mutualists could lead to positive correlations among traits of the rewarding species, where investment in one mutualist guild is associated with increased investment in the other mutualist guild. Despite advances in our understanding of the correlated evolution of traits involved in antagonistic species interactions (Agrawal and Fishbein 2006; Agrawal 2011), we know little about the correlated evolution of traits that attract multiple mutualists. Whether traits are positively or negatively correlated has important implications for macroevolution (Agrawal 2011). For example, positively correlated evolution of plant defense traits is associated with adaptive radiation in *Asclepias* L. (Agrawal et al. 2009b). In addition, trait correlations can have ecological effects. For example, a trade-off between plant defense and plant growth among milkweed species (*Asclepias*) affected densities of insect herbivores (Mooney et al. 2010). As most species interact with multiple mutualists, correlations among traits to attract mutualists are likely to influence trait evolution.

Many plant species interact with both pollinating mutualists and protection mutualists. These plants invest in the production of nectar, pollen, and floral displays to attract insects and birds that move plant gametes (Klinkhamer et al. 1989; Waser et al. 1996; Brody and Mitchell 1997), while also producing nectar outside of the corolla (extrafloral nectar) to attract bodyguards, particularly ants, as a means of indirect defense against plant herbivores (Heil and McKey 2003). Plants with extrafloral nectaries are not as common as those with floral nectaries, but worldwide, there are 113 angiosperm plant families with extrafloral nectaries, including approximately 4,000 plant species (K. Keeler, personal communication). There is much evidence that pollinators exert selection on floral traits (e.g., Benitez-Vieyra et al. 2006; Gómez et al. 2008; Young 2008), and while the body of evidence is smaller, ant bodyguards can also act as agents of selection on extrafloral nectar traits (Rudgers 2004; Heil et al. 2005; Rutter and Rausher 2007). Although pollinating and protection mutualists are generally associated with floral and extrafloral nectaries, respectively, this is not always true, potentially complicating correlations among these traits. For example, ant protective bodyguards that are most commonly associated with extrafloral nectaries can also feed on floral nectar or anthers. Prior work suggests that ants generally reduce plant fitness as a result of visiting flowers (Fritz and Morse 1981; Ness 2006; Galen and Geib 2007; Lach 2007; but see Schatz et al. 2006). This indirect cost of ants could result in reduced investment in floral traits for plants that are dependent on bodyguards, as a mechanism to limit ant visitation to flowers. What remains unclear is whether one set of traits could constrain plant investment in the alternate set.

Here, we took a comparative approach using 37 species of *Gossypium* L. (cotton; Malvaceae) grown in a greenhouse to ask whether there are trade-offs or positive correlations among plant traits used to attract bodyguard mutualists versus pollinating mutualists. Specifically, we addressed the following questions: (1) Across *Gossypium* species,

do extrafloral nectary and floral traits display negative correlations, suggesting trade-offs, or positive correlations? (2) Do *Gossypium* traits to reward mutualists vary among clades or geographic regions of origin?

Materials and methods

Gossypium extrafloral and floral nectaries and their visitors

All *Gossypium* L. species except *G. tomentosum* Nuttall ex Seemann produce extrafloral nectaries on the veins of the abaxial leaf surface (foliar) as well as either subtending the bracts (calyx) or above the bracts (involucellar), and on the adaxial surface of sepals of buds, flowers, and fruits (Dejoode and Wendel 1992). Hereafter, we refer to calyx and involucellar nectaries collectively as bracteal nectaries. The number of foliar nectaries on a single leaf generally ranges from one to five. Both nectary types can vary in size, shape, and the volume of nectar produced. Visitors to extrafloral nectaries are largely ants, but may also include wasps, flies, lepidopterans, and other insects (see also Rudgers et al. 2010).

All *Gossypium* L. species produce floral nectar (Fryxell 1979). *Gossypium* L. flowers are either solitary or arranged in elongated sympodia, and each flower has five obovate, white to rose colored petals, often with a dark spot in the petal claw (Fryxell 1979). Flowers are hermaphroditic, and the androecium is composed of numerous stamens fused into a staminal column that surrounds the gynoecium (Fryxell 1979). The stigma is 3–5 lobed, and sometimes exceeds the anthers (Fryxell 1979; Kubitzki 2003); greater stigma exsertion is often correlated with greater outcrossing (Motten and Stone 2000). *Gossypium* L. flowers generally produce nectar diurnally, except for two species: *G. longicalyx* J.B. Hutchinson & Lee and *G. tomentosum* Nuttall ex Seemann (Fryxell 1979). Nocturnal flowers of these species are likely visited by lepidopterans, rather than bees, which are considered the primary pollinators of *Gossypium* L. with diurnal flowers (Fryxell 1979).

The genus *Gossypium* L. includes eight monophyletic diploid genome groups (hereafter 'clades'; A, B, C, D, E, F, G and K) and one tetraploid genome group (AD, including all the cultivated species; Wendel and Cronn 2003; Wendel et al. 2010). Clades correspond roughly with geographic distribution: Africa (B, E, and F); the Americas (D and AD); Asia (A, E); and Australia (C, G, and K). *Gossypium* L. is thought to have undergone rapid, global radiation early on in the genus, with closely spaced divergence events in time facilitated by long-distance trans-oceanic dispersal (Wendel and Cronn 2003).

Study location and organisms

We conducted studies on 37 *Gossypium* L. species, with geographic distributions spanning five continents and all eight phylogenetic clades (Appendix Table A1—Electronic Supplementary Material). Traits were measured in greenhouses at the United States Department of Agriculture (USDA) Southern Plains Agricultural Research Center in College Station, Texas, USA (30°37′3″N, 96°21′38″W) between November 2008 and August 2009. The advantage of a common greenhouse setting was that differences in trait investment among species were not confounded with particular environmental conditions unique to each species' habitat (see also Cavender-Bares et al. 2004; Agrawal et al. 2009a). Plants ranged in age from 1 to 5 years ($\bar{X} \pm 1\,SE = 2.9 \pm 0.2$), had produced significant woody biomass, and had reached reproductive maturity. Temperatures in the greenhouses ranged from 15.6

to 29.4°C ($\bar{X} = 21.1$°C), humidity was ambient (range $\sim$60 to 90%), and no supplemental lighting was used. Plants were grown from seed and represent a permanent germplasm collection maintained by the USDA.

Extrafloral and floral nectary traits

We obtained trait measurements on 1–3 individuals per plant species, effectively treating species as the unit of replication. This has been a common approach in studies across species (e.g., $n = 5$ in Agrawal and Fishbein 2006). Sample sizes per species were limited by the availability of plants in the permanent collection, and the large size at reproduction of some species required significant greenhouse space. The small number of individuals per species limits the precision of the estimate of the mean trait values for each species, but does not affect corrections for phylogenetic non-independence, because these methods analyze only the mean, not variance, within species. Methods are available for including variation among individuals within a species in independent contrasts (Ives et al. 2007; Felsenstein 2008), but we have only one individual for roughly half of our 37 species. More precise estimates of mean trait values would reduce variation in the data and increase the ability to detect pattern; thus, our methods make this study conservative if correlations among traits are detected, but cannot rule out the possibility that non-significant correlations could become significant with larger sample sizes.

We quantified six extrafloral nectary traits: the proportion of leaves with active extrafloral nectaries (number of leaves with active nectaries/total number of leaves), foliar extrafloral nectar volume, bracteal extrafloral nectar volume, foliar extrafloral nectary area, bracteal extrafloral nectary area, and foliar extrafloral nectar sugar concentration. All leaf traits were measured on randomly selected fully expanded leaves, to capture the range of variation present within a plant (Heil et al. 2000). Two traits, the proportion of leaves with extrafloral nectaries and extrafloral nectary area, have shown significant heritability and were under significant directional selection in one cotton species, *G. thurberi* Todaro (Rudgers 2004; Rudgers and Strauss 2004). We determined the proportion of leaves with extrafloral nectaries for 30 leaves per plant. We measured standing stocks of extrafloral nectar (all accumulated nectar produced on plants that were untouched) on three leaves per plant. Nectar was removed using glass microcapillary tubes (40, 2, or 1 µl) (Drummond Scientific Company, Broomall, PA, USA), and care was taken not to puncture plant tissue when removing nectar. We assessed nectar volume by measuring the length of fluid in the capillary to the nearest 0.01 mm using digital calipers. For a subset of 28 plant species, we also measured the rate of foliar nectar production 24 h^{-1} on the same three leaves. Foliar extrafloral nectar production rate 24 h^{-1} was positively correlated with standing stock (Pearson correlation, $r = 0.67$, $P = 0.0001$, $n = 28$ species; Appendix Fig. B1—Electronic Supplementary Material), suggesting that standing stock measurements provided a useful estimate of the species level trait of foliar extrafloral nectar production. After measurements were made for each plant, we removed leaves at the base of the petiole, transported them in a cooler, and stored them at -20°C following methods in Rudgers et al. (2004). We defrosted leaves, scanned them using an HP Scanjet 5590 digital scanner, and converted to TIFF images, at either 600 or 2,400 dpi, for measurements of leaf area and extrafloral nectary area, respectively. We determined the area of leaves and extrafloral nectaries to the nearest 0.001 mm with image analysis software (Scion Image, Scion, Fredrick, Maryland, USA). Standing stock nectar was positively correlated with extrafloral nectary size ($r = 0.51$, $P = 0.001$, $n = 28$) across species. We stored nectar in vials at -20°C until sugar concentration was quantified. Extrafloral and nectar samples were thawed and diluted with

deionized water to measure sugar concentration (sucrose equivalents) using temperature-corrected hand-held refractometers (Eclipse Series, Regular and Low-volume 0–50° BRIX, Bellingham & Stanley Inc., Lawrenceville, GA, USA).

We measured four floral traits: floral nectar volume, flower size, stigma exertion, and floral sugar concentration. We measured standing stocks of floral nectar on up to two flowers per plant between 12:00 and 15:00. We assumed that standing stocks of floral nectar adequately reflected differences among species because flowers persist for only a few hours to 1 day. Maximum petal length, maximum petal width, and stigma exertion (distance from tip of stigma to uppermost anther) were measured to the nearest 0.001 mm using digital calipers without removing flowers from the plants. Finally, we also measured plant size as basal stem diameter (mm) to assess whether traits were size-dependent (see "Trait correlations"; see also Rudgers 2004).

Trait correlations

We calculated Pearson correlation coefficients for all pairwise combinations of extrafloral nectary and floral traits using the *cor. test* function in R v.2.10.1 (R Development Core Team 2008). For all analyses, we averaged trait values when more than one individual per species was sampled, such that species was the unit of replication. The proportion active foliar extrafloral nectaries was arcsine square-root transformed, and foliar extrafloral nectar volume, foliar extrafloral nectar sugar concentration, foliar extrafloral nectary area, bracteal extrafloral nectar volume, bracteal extrafloral nectary size, floral nectar volume, floral nectar sugar concentration, and flower size were $\log_{10}$ transformed prior to analysis to meet assumptions of normality. We performed corrections for multiple tests using sequential Bonferroni correction (Holm 1979) separately for each of four extrafloral nectary-floral trait combinations. Although species traits often scale with organism size, we found no significant correlations between plant size (basal stem diameter) and any of the 10 extrafloral nectar or floral traits (Pearson product-moment correlation, range of $r = -0.41$ to 0.34, all $P \geq 0.07$). Furthermore, using the residuals of each trait regressed on plant size did not result in qualitatively different results from traits not regressed on plant size. For simplicity, we present data that has not been corrected for plant size.

To incorporate phylogenetic relatedness, we calculated standardized phylogenetically independent contrasts (PICs) for all 10 traits using the *Gossypium* L. phylogeny (Fig. 1; see *Phylogeny Reconstruction* methods in Appendix C—Electronic Supplementary Material), then analyzed the 24 pairwise extrafloral-floral trait correlations. We calculated Pearson product-moment correlation coefficients among trait contrasts, and we applied sequential Bonferroni corrections to reduce Type I error.

Ordination

To capture overall differences in trait investment among species, we conducted non-metric multidimensional scaling analysis in Primer v.6.1.10 (NMDS; Clark and Gorley 2007). We used five extrafloral nectary traits (proportion active foliar extrafloral nectaries, foliar extrafloral nectar volume [standing stock], foliar extrafloral nectary area, bracteal extrafloral nectar volume, and bracteal extrafloral nectary size) for the extrafloral nectary traits ordination, and three floral traits (floral nectar volume, flower size [maximum petal length × maximum petal width], and stigma exertion) for the floral traits ordination. Due to two negative values, we made stigma exertion values positive by adding the absolute value of the most negative stigma exertion value to all species. Foliar extrafloral nectar volume,
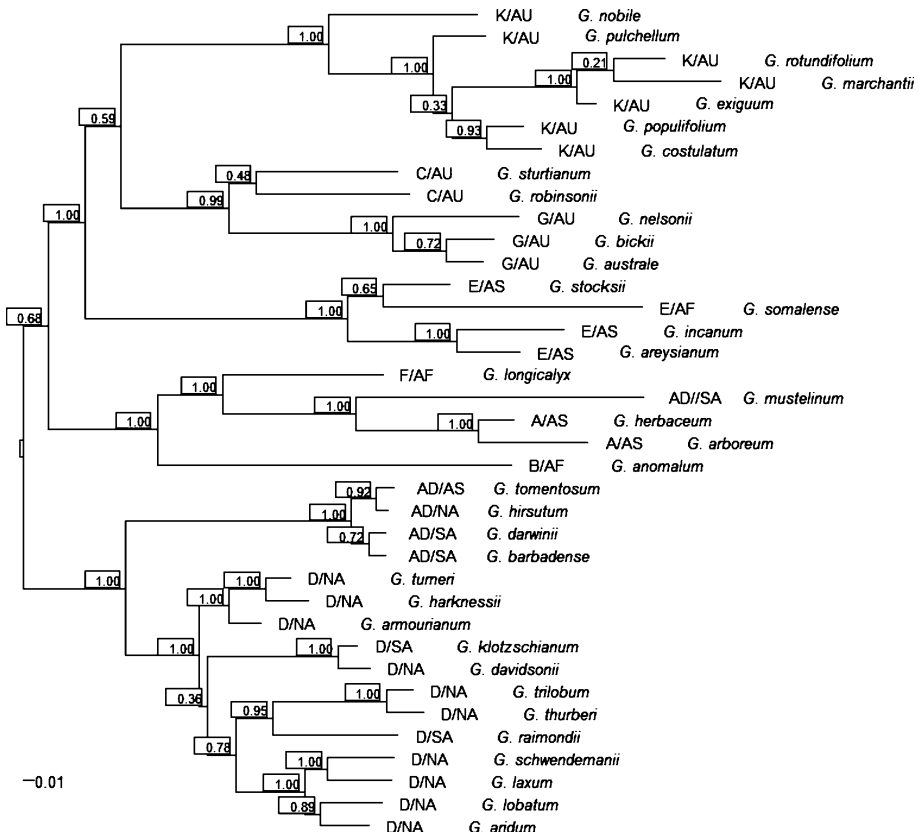
Fig. 1 Bayesian phylogeny of the 37 *Gossypium* species (the outgroup *Kokia drynarioides* is not shown to simplify the graphic). Supports on branches are Bayesian posterior probabilities; all posteriors are shown, regardless of magnitude. *Bar* in *bottom left* represents the inferred number of substitutions per site. Clades and geographic regions are indicated at tips of the phylogeny prior to species names: *Clades* are *A, B, C, D, E, F, G, K*, and *AD*. Geographic regions are AF (*Africa*), AS (*Asia*), AU (*Australia*), NA (*N. America*), SA (*S. America*)

bracteal extrafloral nectar volume, bracteal extrafloral nectary size, floral nectar volume, flower size, and stigma exertion were $\log_{10} + 1$ transformed prior to analysis to improve ordination fit. Proportion active foliar extrafloral nectaries and foliar extrafloral nectary area were arcsine square-root transformed. We only used *Gossypium* L. species for which we had all available data for the eight traits. We excluded sugar concentration data due to low sample sizes of species with these traits. We also excluded three extreme outlier species that inflated the stress of the ordination and prevented a satisfactory NMDS solution: (1) *G. tomentosum* Nuttall ex Seemann lacked extrafloral nectaries (i.e., zeros for all extrafloral nectary traits; and was also removed from the floral traits ordination for consistency), (2) *G. costulatum* Todaro had very large bracteal extrafloral nectary size (4,120% higher than the next largest species), and (3) *G. laxum* Phillips presented a low, outlying stigma exertion value. We standardized each trait to each trait's maximum. The final extrafloral nectary and floral traits ordinations were both run with 28 species (9,999 iterations, Bray-Curtis distance metric). We calculated Pearson's *r* for correlations between the traits and the NMDS axes to assess the influence of each trait on each axis.

Clustering traits within *Gossypium* clades and geographic regions of origin

We examined whether traits were clustered among the *Gossypium* L. clades in the NMDS using analysis of similarities (ANOSIM; Clark and Gorley 2007), which tested whether trait variation within each *Gossypium* L. clade designation was significantly smaller than variation between clades. We also tested for a geographic signal in extrafloral and floral traits ($n = 3$ groups: Asia, Australia, and the Americas). Statistical significance was assessed by permuting the grouping vector to obtain the distribution of the test statistic, *R* (a metric for the difference between the variation between groups and the variation within groups), under a null-model (Clark and Gorley 2007). We used a Bray-Curtis distance metric, with traits transformed as described in *Ordination* above. ANOSIM was run separately for extrafloral nectary and floral traits (9,999 permutations), with 28 species for each analysis. B and F clades were excluded from the test for clade effects, as each had only one representative species. In the case of a significant *R* value, we then examined pairwise tests of difference among individual clades or continents. If groups significantly differed, we identified the traits contributing most to differences among groups using SIMPER analysis, which ranks individual traits by their contribution to the partitioning of groups (Clark and Gorley 2007). Qualitatively similar results for these analyses were detected with PERMANOVA through the *adonis* procedure in R v.2.10.1 (R Development Core Team 2008).

Results

Across *Gossypium* species, are there significant correlations between floral traits and extrafloral nectary traits?

There were no significantly ($P < 0.05$) negative correlations among extrafloral nectary and floral traits, rejecting the hypothesis of trade-offs among investments in pollinators versus bodyguards. Furthermore, four of the 24 pairwise correlations and seven of the phylogenetically independent contrast (PIC) correlations among traits were significantly positive (Table 1). After sequential Bonferroni correction, three and four correlation coefficients remained significantly positive in the raw data and in the PICs, respectively (Table 1). Although accounting for evolutionary history did not strongly alter insights into relationships among extrafloral nectary and floral traits, four floral-extrafloral nectar trait correlations did change. Specifically, four pairwise trait combinations, floral nectar volume and proportion active foliar extrafloral nectaries, floral sugar concentration and proportion active foliar extrafloral nectaries, floral nectar volume and foliar extrafloral nectary area, and floral sugar concentration and bracteal extrafloral nectary area, changed from non-significant in the raw data analysis to significantly positive in the PICs analysis. In contrast, one pairwise trait combination, flower size and bracteal extrafloral nectary area, changed from significantly positive in the raw data analysis to non-significant in the PICs analysis.

Non-metric multidimensional scaling (NMDS) resulted in 2-dimensional solutions for both sets of mutualist-attracting traits (Fig. 2). In the extrafloral nectar trait NMDS, bracteal extrafloral nectary volume ($r = 0.83$) was most strongly positively correlated with Axis 1, while foliar extrafloral nectary volume was most strongly positively correlated with Axis 2 ($r = 0.82$; Appendix Table D1—Electronic Supplementary Material). There was no indication that species investing in foliar extrafloral nectar invested less in bracteal extrafloral nectar. In the floral trait NMDS, flower nectar volume was most strongly

Table 1 Pairwise Pearson correlation coefficients for all extrafloral nectary (EF)-floral trait combinations

	Proportion active foliar EF		Foliar EF volume		Foliar EF sugar		Foliar EF area		Bracteal EF volume		Bracteal EF area	
	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs	Raw	PICs
Floral nectar volume	0.34* (31)	0.48** (29)	**0.58***** (31)	**0.51**** (29)	0.14 (16)	−0.05 (14)	0.07 (30)	**0.59***** (28)	0.05 (30)	−0.24 (28)	0.31 (28)	0.01 (27)
Flower sugar	0.06 (17)	0.73*** (16)	0.28 (17)	0.19 (16)	0.30 (10)	0.33 (8)	**0.63***** (17)	0.68*** (16)	0.00 (17)	0.34 (15)	0.14 (17)	**0.80***** (15)
Flower size	0.07 (30)	0.37* (29)	**0.48***** (30)	**0.62***** (29)	0.35 (15)	0.02 (13)	0.35* (29)	−0.05 (28)	0.08 (29)	0.05 (28)	0.38** (28)	0.05 (27)
Stigma exertion	0.15 (30)	0.04 (28)	0.12 (30)	0.11 (29)	−0.12 (15)	−0.26 (14)	0.14 (29)	−0.14 (28)	−0.22 (29)	−0.39* (28)	0.28 (28)	0.19 (27)

Correlation coefficients (r) are given for raw data and for phylogenetically independent contrasts. Sample sizes (no. of species) are given parenthetically beside correlation coefficients. Asterisks indicate P values prior to correction for multiple tests. Those correlation coefficients remaining significant ($P < 0.05$) after sequential Bonferroni correction (Holm 1979) are shown in bold face type

* $P < 0.06$; ** $P < 0.05$; *** $P < 0.01$

Fig. 2 Two-dimensional NMDS ordination of *Gossypium* species defined by five extrafloral nectary traits (**a**) or three floral traits (**b**). Clark recommends that stress values between 5 and 10 represent good ordinations with no real risk of drawing false inferences (Clarke 1993). Stress for the final NMDS solutions were 11 and 7 for extrafloral nectary and floral traits ordinations, respectively. See Appendix D—Electronic Supplementary Material for correlations of traits with NMDS axes
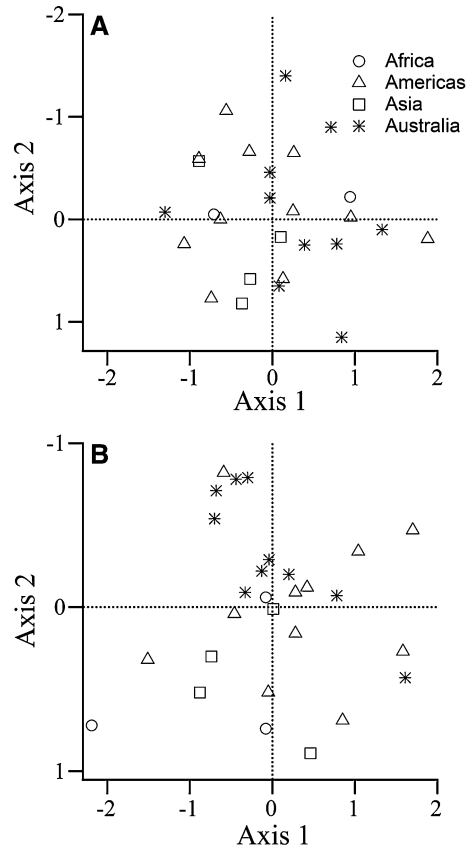
positively correlated with Axis 1 ($r = 0.92$), while stigma exertion ($r = 0.85$) and flower size ($r = 0.64$) were positively correlated with Axis 2 (Appendix Table D1—Electronic Supplementary Material). Thus, cotton species that produced more floral nectar also had larger flowers and greater stigma exertion (upper right quadrant Fig. 2b), possibly indicative of a suite of traits to enhance outcrossing.

Consistent with the positive correlations detected in the analysis of pairwise trait combinations, Axis 2 of the extrafloral nectary traits NMDS was significantly positively correlated with Axis 1 of the floral traits NMDS (Pearson's $r = 0.60$, $P = 0.0009$). The other NMDS axes were not significantly correlated (floral Axis 1-extrafloral Axis 1: $r = -0.05$, $P = 0.798$; floral Axis 2-extrafloral Axis 1: $r = 0.06$, $P = 0.771$; floral Axis 2-extrafloral Axis 2: $r = 0.02$, $P = 0.920$). These results reinforce the conclusion that foliar extrafloral nectary volume (as detected in pairwise analysis) was the key defensive mutualist trait that was positively associated with plants' investment in floral nectar.

Do *Gossypium* traits vary among clades or geographic regions?

In pairwise tests there was a difference between Australia and Asia (ANOSIM; $R = 0.34$, $P = 0.039$) in floral trait investment. A significant percentage of the difference between Australia and Asia was attributable to greater floral nectar volume (48%), larger flower size

(27%), and greater stigma exertion (25%) for species from Australia, as detected by SIMPER analysis. There was no significant difference among *Gossypium* clades in the extrafloral nectary traits ordination (ANOSIM; $R = -0.025$, $P = 0.570$) or in the floral traits ordination when all clades were included in the analysis (ANOSIM; $R = 0.09$, $P = 0.197$). Additionally, there was no significant difference among geographic regions in the extrafloral nectary traits ordination (ANOSIM; $R = 0.02$, $P = 0.373$; Fig. 2a) or in the floral traits ordination (ANOSIM; $R = 0.20$, $P = 0.014$; Fig. 2b).

Discussion

Our work is one of a few studies to examine correlations among traits that attract multiple mutualist guilds for any taxon (see also Whitney and Rudgers 2009), and the only study, to our knowledge, to investigate relationships among traits mediating pollination and protection mutualisms. Specifically, our results showed positive interspecific correlations between traits to attract ant bodyguards and traits to attract pollinators in the genus *Gossypium*.

Positive correlations remained when we accounted for the phylogenetic relatedness of *Gossypium* species, demonstrating that the associations between plant rewards to pollinator and protection guilds are not driven by phylogenetic non-independence in the data. The positive correlations we observed for extrafloral nectary and floral traits are consistent with the results of Whitney and Rudgers (2009), who found positive interspecific correlations between plant investment in fruit traits (seed dispersal) versus floral traits (pollination). In a recent meta-analysis, Morris et al. (2007) showed that the interactive effects of multiple mutualists on plant performance were, on average, positive. Although Morris et al. (2007) did not include species traits in their analysis, plant performance is often correlated with trait investment (e.g., Poorter et al. 2008), supporting the results we found here. In a similar study, albeit on traits involved in antagonisms, Agrawal and Fishbein (2006) demonstrated positive correlations between physical trichome defenses and chemical latex defense traits across 24 species of milkweed (*Asclepias*). Altogether, these results suggest that plants may experience fewer investment trade-offs among different functional traits than previously assumed.

There are several potential mechanisms that may underlie the positive correlations in this study. Positive correlations among extrafloral nectary and floral traits could be shaped by pleiotropy or tight linkage of genes underlying these traits. However, there is no evidence to date that genes for extrafloral nectary and floral traits are closely linked in any plant species (M. Heil, J. Wendel, personal communication), and further, we found little evidence for phylogenetic signal in either class of plant traits (see *Phylogenetic Signal* in Appendix C—Electronic Supplementary Material). Sequencing of a *Gossypium* genome is underway (Chen et al. 2007) and should help to inform whether genes for extrafloral nectary and floral traits are linked. Positive correlations among extrafloral nectary and floral traits suggest that the evolution of extrafloral nectaries and flowers may also be linked. For example, if selection were stronger on the traits of flowers than on extrafloral nectary traits, linked extrafloral nectary traits could be 'dragged along' with the evolution of flowers. Traits that are physiologically linked (e.g., extrafloral and floral nectar) may be more strongly correlated than traits that are not physiologically linked (e.g., extrafloral nectary size and flower size; Wright et al. 2007). Some evidence from our study supports this assertion. For example, of all the extrafloral nectary traits, foliar extrafloral nectar volume was most strongly correlated with floral nectar volume (Table 1). However, floral

nectar volume was only weakly correlated with nectar volume in bracteal nectaries, which are located spatially closer to flowers than foliar extrafloral nectaries. Lastly, it may be that both sets of traits are evolving independently, through different selection pressures.

A few caveats deserve consideration. First, we measured extrafloral nectary and floral traits in a greenhouse. Although this method controlled for environmental variation, species were removed from their ecological contexts, where trait expression may be environmentally plastic. Second, despite correlations among traits, we have no evidence that the floral or extrafloral traits have important ecological consequences for the majority of wild cotton species. However, the ecological context of extrafloral nectar traits has been well documented in *G. thurberi*: ant visits to plants were mediated by extrafloral nectar; plant benefits (reduced herbivory) increased with higher extrafloral nectar; plants with more extrafloral nectaries had higher fitness; and variation in plant fitness among populations corresponded to variation in ant abundance and community species composition (Rudgers and Gardener 2004; Rudgers and Strauss 2004). Third, although our statistical power is correctly based on species as replicates, we assumed that trait measurements on one to three individuals per species were adequate to represent each species. We argue that this effect makes any significant results conservative, provided that variation in species level traits is greater than variation within species, which appears to be the case for cotton (Eckstein et al. 1999; Rudgers et al. 2004; Hulshof and Swenson 2010). In addition, foliar extrafloral nectar volume was very similar between plants measured in the field (0.04 ± 0.01 SE μl, $n = 9$ plants) in Arizona (J.A.R., unpublished data) and in the greenhouse (0.05 μl, $n = 1$ plant). Last, if there is a trade-off between quantity and quality of extrafloral nectar, such that cotton species that produce greater quantity of extrafloral nectar have lesser quality extrafloral nectar, there may be a negative correlation between extrafloral nectar quality and floral traits. This possibility does not, however, negate the importance of the positive correlations between extrafloral nectar and floral nectar traits found here. The extent to which trait correlations drive fitness differences among species depends on ant bodyguard and pollinator preference for and recruitment rates to extrafloral nectar and floral nectar traits.

Conclusion

We have documented positive correlations among plant traits for attracting multiple mutualist guilds (ant bodyguards and pollinators) in the cotton genus (*Gossypium*). Specifically, we have shown that extrafloral nectar traits to attract ant bodyguards and floral traits to attract pollinators are often significantly positively correlated, and never significantly negatively correlated. In addition, positive correlations between extrafloral nectar and floral nectar traits remained when accounting for phylogenetic non-independence among species. Our work builds a more general understanding of how multi-species mutualisms can shape the evolution of plant traits.

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