

TRADE-OFFS AMONG ANTI-HERBIVORE RESISTANCE TRAITS: INSIGHTS FROM GOSSYPIDAE (MALVACEAE)¹

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Plant defense theories commonly predict negative correlations among anti-herbivore resistance traits. Although this prediction has been widely accepted, the majority of empirical studies have failed to account for similarities among species due to common ancestry, thus risking pseudoreplication. Wild cotton plants possess traits conferring both direct resistance (toxic leaf glands and trichomes) and indirect resistance (extrafloral nectaries that reward enemies of herbivores). The evidence for negative phenotypic correlations among these resistance traits was examined at two levels: within *Gossypium thurberi* (wild cotton) and across species in the cotton clade (Gossypidae). A phylogenetic analysis controlled for shared ancestry among species. Across the Gossypidae, a strong negative correlation emerged between the direct resistance traits, leaf gland and trichomes. This correlation may reflect costs of these traits, a negative genetic correlation, or redundancy in their actions against herbivores. In contrast, the direct resistance traits (glands and trichomes) were not correlated with the indirect resistance trait of extrafloral nectar, either within or across species. The robust lack of correlation suggests that these direct and indirect resistance mechanisms evolve independently over evolutionary time scales. This conclusion conflicts with both predictions of plant defense theory and the majority of prior comparisons of direct and indirect resistance traits and may reflect the facultative nature of indirect resistance in Gossypidae.

Key words: cost of resistance; defense; extrafloral nectar; *Gossypium*; herbivory; Malvaceae.

Herbivores often reduce plant growth or reproduction (Marquis, 1992); therefore, plant traits that reduce damage (resistance traits) are expected to enhance fitness and experience positive directional selection. Resistance traits can be classified as either direct resistance, in which the plant invests directly in the defense (e.g., secondary compounds in plant tissues or leaf trichomes) or as indirect resistance, in which reduction of herbivory occurs through a plant's investment in interactions with other species (Price et al., 1980), such as extrafloral nectar to attract predators (Koptur, 1992) or resources for endophytic fungi that are toxic to herbivores (Clay, 1996). Current theories on the evolution of plant resistance predict negative correlations among resistance traits when these traits are costly (including costs arising from competition with other plant functions for limited plant resources), negatively related due to pleiotropy, or redundant in ecological function (McKey, 1988; Steward and Keeler, 1988; Davidson and Fisher, 1991; Zangerl and Bazzaz, 1992; Mole, 1994).

A number of studies have sought empirical evidence for negative correlations (often called trade-offs) among anti-herbivore resistance traits, both within and across species. Few empirical studies have found strong negative relationships between direct resistance traits (Zangerl and Berenbaum, 1990; Brody and Karban, 1992; Mauricio and Rausher, 1997; Thaler and Karban, 1997; Underwood et al., 2000; Ruuhola et al.,

2001; Ward and Young, 2002; but see Björkman and Anderson, 1990; Silvertown and Dodd, 1996; Traw, 2002). In contrast, many comparisons between direct and indirect resistance, typically examined in systems with obligate ant–plant mutualisms, have detected the predicted negative correlations (Koptur, 1985; Turner, 1995; Heil et al., 1999, 2000; Dyer et al., 2001; Eck et al., 2001; Agrawal et al., 2002; but see Steward and Keeler, 1988; Letourneau and Barbosa, 1999; Heil et al., 2002). These empirical results suggest that certain resistance traits may be more likely to exhibit negative correlations, although mechanisms to explain these patterns are lacking.

We explored relationships among resistance traits in the cotton clade, Gossypidae (Malvaceae), most members of which invest in facultative mutualisms with ants or other predators. In Gossypidae, plants lack the large, specialized domatia and food bodies that characterize the obligate ant–plant mutualisms that have been the focus of many prior studies on indirect resistance. Gossypidae do, however, possess extrafloral nectaries (Fryxell, 1979) that can attract a diverse assemblage of predators and parasitoids of herbivores (Schuster et al., 1976), thereby reducing damage to plants and providing indirect resistance (e.g., Rudgers, 2004). In addition to extrafloral nectaries, species in the Gossypidae also share direct resistance traits, including trichomes on the leaf surface and gossypol glands, cavities in leaves that contain toxic secondary metabolites (reviewed by Schuster, 1979; Bell, 1986; Matthews, 1989; Smith, 1992).

We addressed the question: Are there negative phenotypic correlations among different mechanisms of resistance to herbivory? Importantly, we directly quantified investment in both direct and indirect resistance traits. Prior studies have typically compared plants with and without ants to examine indirect resistance (but see Steward and Keeler, 1988); thus, our work is some of the first to assess plants' investment in the traits conferring resistance (e.g., extrafloral nectaries). We explored investment in traits intraspecifically by examining phenotypic

¹ Manuscript received 29 August 2003; revision accepted 10 February 2004.

The authors thank A. Agrawal, R. Karban, W. Morris, M. Stanton, J. Rosenheim, T. Young, the Stachowicz Monte Carlo group, and the Clay lab for improving this manuscript. We are also indebted to R. Karban for introducing us to the *G. thurberi* system. M. Moua and D. Hogan assisted with image analyses, and D. Ackerly, C. Nunn, and T. Price provided thoughtful discussions on independent contrasts. K. Whitney gave help of many kinds. Research was supported by an EPA STAR fellowship to J. Rudgers, NSF grant DEB-98-07083 to S. Strauss, and by the Center for Population Biology of the University of California, Davis, California.

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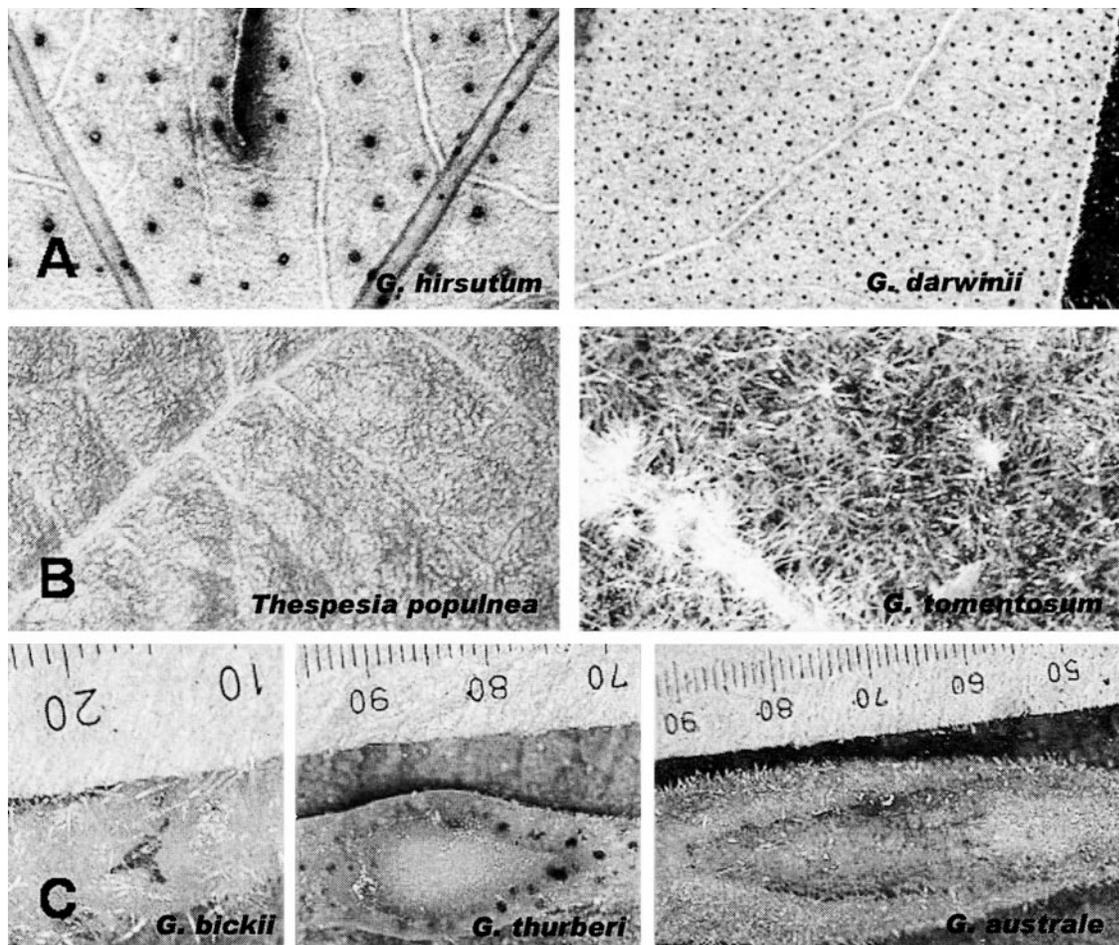


Fig. 1. Representative variation in resistance traits for Gossypieae, presented in rows. (A) Gossypol gland size and density, adaxial leaf surface (images were photographed at the same magnification). (B) Trichome cover, adaxial leaf surface (same magnification). (C) Extrafloral nectary size, abaxial leaf surface (scale is micrometers).

variation among individuals in three populations of *Gossypium thurberi* Todar (wild cotton) that differ in the degree to which ants provide effective indirect defense (Rudgers, 2002, 2004). We also considered relationships among the same traits more broadly by comparing investment in resistance across species in the cotton clade. Because comparisons made across species risk pseudoreplication if phylogeny is ignored (Felsenstein, 1985; Harvey and Pagel, 1991; Martins, 2000), we used the well-supported phylogeny of Gossypieae to evaluate phylogenetically independent correlations among resistance traits. To our knowledge, only two studies have evaluated correlations among resistance traits in a phylogenetic context (Silvertown and Dodd, 1996; Thaler and Karban, 1997); both examined only direct resistance traits.

Natural history of the Gossypieae—Gossypieae includes perennial shrubs, most of which are native to arid tropical and subtropical regions (Fryxell, 1979; Percival et al., 1999). *Gossypium*, the clade containing cultivated cotton, includes 45 species. Gossypieae species share several direct and indirect resistance traits, yet vary considerably in the degree to which they invest in these traits.

Direct resistance traits—Gossypol glands are lysigenous cavities in the leaves that contain terpenoid aldehydes and vol-

atile sesquiterpenes (Bell et al., 1975, 1978; Bell, 1986). These glands are known to be involved in plant defense in cultivated cotton, and the presence and density of gossypol glands are negatively associated with abundances, damage, and/or performance of several herbivores including insects, nematodes, rodents, and birds (Matthews, 1989; Summy and King, 1992). In addition, both the cotton leaf perforator (*Bucculatrix thurberiella*, Rudgers et al., 2003) and first instars of the tobacco bollworm (*Heliothis virescens*, Parrott, 1990) avoid glands by eating around them. Thus, assessing gland size and number allows quantification of an important component of direct resistance. In some species of *Gossypium*, herbivory can induce increased gland production (McAuslane et al., 1997; Agrawal and Karban, 2000), which may indicate that glands are costly and produced in large numbers only when needed. Glands are not universal deterrents, however, and they appear to have little effect on some insects, including boll weevils, whiteflies, and mites (Wilson and Lee, 1971; Meisner et al., 1977a, b, 1978; Matthews, 1989; Parrott et al., 1989; Parrott, 1990; Smith, 1992; Summy and King, 1992; McAuslane and Albourn, 1998; Agrawal and Karban, 2000).

Gland traits vary considerably among the Gossypieae (Fryxell, 1979; see Fig. 1A). In *G. hirsutum* (Mexico), two genes regulate the presence/absence of glands (McMichael, 1960),

and gland density is also genetically based (Bell, 1986). Terpenoid aldehydes and volatile sesquiterpenes are absent or much reduced in glandless isogenic lines of cotton crops (Bell et al., 1978; Bell, 1986; McAuslane and Alborn, 1998).

Trichomes also function in direct resistance in Gossypieae (Matthews, 1989; Butler et al., 1991; Smith, 1992). Cultivated varieties with dense trichomes have lower damage, abundance, or performance of several insect herbivores, including boll weevils, leafhoppers, and plant bugs, as compared to varieties with fewer leaf hairs; however, trichomes have either minor or positive effects on some lepidopterans, whiteflies, and mites (Wilson and George, 1986; Matthews, 1989; Butler et al., 1991; Smith, 1992; Summy and King, 1992).

The trichomes of cotton are typically unicellular and elongated appendages of the epidermis with needle-like, two-armed, or stellate morphology (Bondada and Oosterhuis, 2000). Trichome density ranges from zero to >20 trichomes per centimeter across species (Lee, 1985, and references therein; see Fig. 1B) and has a documented genetic basis in *G. hirsutum* (Wright et al., 1999). We are unaware of any work on Gossypieae that has shown trichome cover to be plastic in response to herbivore damage.

Indirect resistance traits—In cotton crops, indirect resistance includes extrafloral nectaries that can attract ants, other predatory insects, and spiders (Schuster et al., 1976; Way and Khoo, 1992). In *G. thurberi*, extrafloral nectaries reward ants that reduce damage by cotton leaf perforators and other herbivores (Rudgers, 2004). Extrafloral nectar production can increase in response to damage by herbivores in both cultivated and wild cotton (Mound, 1962; Wäckers et al., 2001; Rudgers and Gardener, 2004), providing further indication that extrafloral nectar functions in indirect resistance.

Extrafloral nectary traits vary both within and across species (Fig. 1C; Belcher et al., 1984; Rudgers, 2004). Simple nectaries comprised of aggregations of secretory cells with almost no protective tissue occur in *Lebronnecia* and *Thespesia* (Fryxell, 1979). More complex structures are more common throughout the clade, with papillate secretory cells surrounded by parenchyma cells and forming a pit (or Grubbenektarien) nectary structure (Fryxell, 1979; Elias, 1983). These nectaries, like the more simple forms, are nonvascularized, with vascular tissue absent in the subglandular tissues (Elias, 1983). Lack of nectaries has been identified as arising from homozygosity for recessive mutations at two duplicate loci in *G. tomentosum* (Meyer and Meyer, 1961). Extrafloral nectaries are also absent from *Kokia*; both taxa are endemic to Hawaii, where there are no native ants. Some *Cienfuegosia* species also lack extrafloral nectaries, although the presence of extrafloral nectaries is basal in *Gossypium*.

MATERIALS AND METHODS

Intraspecific investment in resistance traits—For *G. thurberi*, we assessed the resistance traits of naturally occurring individuals chosen at random from three populations: Florida Canyon (31°45'39.8" N, 110°50'45.8" W, $n = 43$) and Agua Caliente Canyon (31°41'32.4" N, 110°57'22.7" W, $n = 52$), both in the Santa Rita Mountains, Arizona, USA, and Molino Basin (32°20'21.5" N, 110°41'13.6" W, $n = 46$) in the Santa Catalina Mountains, Arizona. Prior experiments showed that ants conferred indirect defense in Florida and Agua Caliente canyon populations. In contrast, plants at Molino Basin experienced low visitation by ants and received no protective benefit (Rudgers, 2002, 2004).

Indirect resistance traits included the frequency and size of extrafloral nec-

taries. In *G. thurberi*, these traits did not respond to experimental manipulations of herbivory in the field (i.e., were not inducible, Rudgers, 2002). We determined the proportion of leaves with nectaries every 6 wk during the growing season (July–December 2000). Twice, we measured the maximum length of foliar nectaries to the nearest 0.01 mm with digital calipers (1 July, 1 October) on 15 randomly selected, fully expanded leaves per plant. Nectary size was positively correlated with nectar production in the greenhouse, and the availability of nectar increased visitation by ants in the field (Rudgers, 2004).

Direct resistance traits included the density and size of gossypol glands. In *G. thurberi*, neither character appeared to be induced by herbivory in the field (Rudgers, 2002). Trichomes were not examined because leaf pubescence was sparse and varied minimally. During 20–31 October 2000, five leaves per plant were collected at random and measured with a leaf area meter (LI 3000A, LI-COR, Lincoln, Nebraska, USA). Glands were counted under a microscope within a circular region (area = 2.4 mm²) for three sections of the leaf: adjacent to the midrib, halfway between the midrib and leaf margin, and adjacent to the margin. For three leaves per plant, we measured the maximum diameters of three glands per leaf to the nearest 0.001 mm with image analysis (Scion Image, Scion, Frederick, Maryland, USA). Gland volume was calculated assuming that each gland was spherical.

The mean values of resistance traits were determined across all leaf samples per plant and combined into indices of investment in gossypol glands (mean gland density × mean volume per gland) and extrafloral nectaries (proportion of leaves with nectaries × mean number of nectaries per leaf × mean size per nectary). The indices were particularly useful for the cross-species comparisons (see next) because nectary and gland traits varied markedly in size and number across species.

Within each population, we determined phenotypic correlations between the gossypol gland and extrafloral nectar indices. Phenotypic correlations can be reliable indicators of underlying genetic correlations (Roff, 1995). However, environmental, rather than genetic, covariation can underlie phenotypic relationships and is a limitation of this approach (Mitchell-Olds and Shaw, 1987). We used multiple regression analyses to reduce the influence of environmental covariation as indicated by leaf size and leaf number. The extrafloral nectary index was the dependent variable and the gland index, leaf area, and initial leaf number were independent variables. Gland and nectar indices as well as leaf area were log transformed, and leaf number was square-root transformed to achieve normality and homoscedasticity of residuals. No significant multicollinearity was detected (Proc REG collinoint, SAS Institute, 1999). Standard partial regression coefficients (b') (Sokal and Rohlf, 1995) were used to assess relationships among traits.

Interspecific investment in resistance traits—We examined 31 species of *Gossypium* and related taxa in the Gossypieae that vary considerably in both direct and indirect resistance. On 27 March and 3 April 2001, a minimum of five leaves was collected from plants (approximately 2–10 yr old) that were growing under standard glasshouse conditions (18–26°C, 12-h photoperiod, arthropod-free) at Iowa State University, Ames, Iowa, USA. Leaves were selected to maximize variation within a plant and included the oldest and the youngest fully expanded leaves on each individual. Because plants were grown in the absence of herbivory, our measures represent constitutive, rather than induced, levels of resistance. Whether these traits are inducible and the degree to which they can be induced is not known for the majority of Gossypieae. By using plants grown in a common environment, we controlled for genotype × environment interactions (Blomberg and Garland, 2002) and for unmeasured “third” traits associated with habitat (including herbivory and induced responses) that may correlate with resistance phenotypes (see Price, 1997). These controls confer an advantage over studies that use published data from multiple sources or samples collected from different environments.

In many cases (23 species), we used single individuals to represent a species, owing to a lack of other samples (some species are difficult to propagate). For the remaining species, 2–6 individuals were sampled (sample size = 3.0 ± 0.32 individuals [means ± 1 SE]). While we recognize that having a single individual to represent a species is less than ideal, standard programs designed to account for phylogeny (phylogenetically independent contrasts) do not in-

TABLE 1. Variances between Gossypieae species and within *Gossypium thurberi* for the log-transformed gland index, the log-transformed extrafloral nectary index, and the square-root transformed trichome index. For *G. thurberi*, variances are based on measurements in three different *G. thurberi* populations separated by over 80 km. *F* tests (two-tailed) tested the hypothesis that the variance between species differed from the variance within species.

Index	Between species		Within <i>G. thurberi</i>		<i>F</i>	<i>P</i>
	Variance	<i>n</i>	Variance	<i>n</i>		
Gossypol gland	0.138	31	0.049	141	2.79	<0.001
Extrafloral nectary	0.904	31	0.033	141	27.29	<0.001
Trichome	0.250	31	(not determined)			

corporate the sample size per species and assume that within-species variance is zero (see COMPARE 4.4, Martins, 2001 or Contrast 3.5c, Felsenstein, 1991). Thus, in using single individuals, we only reduced the precision of our estimate of the mean for those species. Phylogenetically independent contrast studies frequently sample single (or few) individuals per taxon (e.g., Vanhooydonck et al., 2000; Summers and Clough, 2001). These papers and ours assume that variation across species is greater than variation within species. Importantly, variation across Gossypieae species was significantly greater than variation within *G. thurberi*, for which we sampled 141 individuals across a range of ant-free and ant-tending environments and across varying levels of herbivory (Table 1; Rudgers, 2002).

We scored extrafloral nectary, gossypol gland, and trichome traits for each

species. The proportion of leaves with nectaries was determined in the glasshouse. All nectaries on each sampled leaf were counted and photographed under a microscope in the laboratory. We measured the area of nectar-producing cells with image analysis (Scion Image) and leaf area with a leaf area meter (LI-COR). To obtain gland density, we counted the number of glands within a circular region (area = 0.4 cm²) at the same location on each leaf, near the distal end of the primary lobe. Gland volume was determined as described earlier for a minimum of three glands per leaf and for five leaves per plant. Using a microscope, we scored trichome cover on a scale of zero (no trichomes) to 10 (trichomes so dense and long that the leaf surface was obscured). For each species, mean trait values were determined across all leaf samples, and indices of investment in gossypol glands and extrafloral nectaries were created as explained previously.

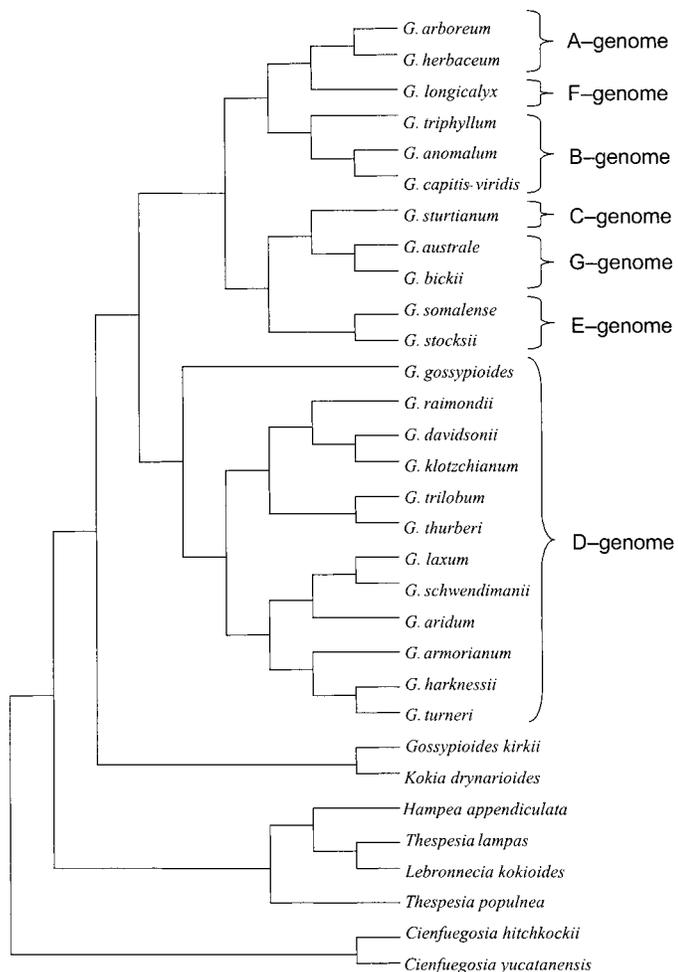


Fig. 2. Tree 1 is the best estimate of the phylogenetic relationships in the Gossypieae, from Seelanan et al. (1997), Liu et al. (2001), Cronn et al. (2002), and Wendel et al. (2002). Letters indicate monophyletic genome groups.

Phylogenetic trees—We used the well-documented phylogenetic hypotheses for the Gossypieae clade, which are based on phylogenetic analysis of molecular data sets derived from numerous chloroplast and nuclear gene sequences (Cronn et al., 2002, and references therein). Most branches of the synthesis shown in Fig. 2 have high internal support and/or are independently inferred from multiple, independent data sets (11 nuclear and four chloroplast loci in Cronn et al., 2002; see also Seelanan et al., 1997; Liu et al., 2001; Wendel et al., 2002). Not all taxa included in the work of Cronn et al. (2002) were used in the present study; thus, neither branch lengths nor internal support statistics are shown here. All phylogenetic analyses of *Gossypium* are congruent in showing each genome group to be monophyletic, although some areas of uncertainty remain, particularly regarding the placement of the B-genome clade (sister to either C + G or A + F) and the E-genome clade (sister to either C + G or the remainder of the African genome groups, A, F, B). Accordingly, we analyzed alternative trees that reflect this uncertainty: Tree 1 is our best estimate of the relationships among Gossypieae (Fig. 2). Tree 2 has the B-genome clade sister to C + G rather than to A + F, and Tree 3 has the E-genome clade sister to A + F + B instead of sister to C + G. Because it made the tree reticulate, we omitted a small, monophyletic clade of five allopolyploid species (*G. barbadense*, *G. hirsutum*, *G. darwinii*, *G. mustelinum*, *G. tomentosum*) that originated following hybridization between an A- and a D-genome diploid (Wendel, 1989).

Phylogenetically independent contrasts—The COMPARE program (Martins, 2001) was used to calculate standardized independent contrasts for the three alternative topologies, assuming a Brownian motion model of evolution. Because information was not available on branch lengths, all branch lengths were set equal to one. A simulation study found that the assumption of equal lengths produced lower inflations in error than did alternative methods (Ackery, 2000).

We used least squares regression to examine relationships among phylogenetically independent trait values (i.e., contrasts) (Proc REG, SAS Institute, 1999). Gland and nectary indices as well as leaf area were log transformed, and trichome cover was square-root transformed to achieve proper standardization of the contrasts (see Garland et al., 1992). We report standard regression coefficients (*b*) (Sokal and Rohlf, 1995) for regression lines through the origin (see Appendix 1 in Garland et al., 1992). For the extrafloral nectary index, multiple regression was used to control for variation in leaf area (a trait that was significantly correlated only with the extrafloral nectary index), and standard partial regression coefficients (*b'*) are reported. In addition, we

performed regressions for data that were not corrected for phylogeny. In all analyses, the data met assumptions of univariate normality, linearity, no multicollinearity, and homoscedasticity of residuals as required for regression techniques. Standard outlier detection methods resulted in the exclusion of two outliers from the phylogenetically independent correlation between gland index and trichome cover (Proc REG influence, SAS Institute, 1999). Confidence limits (95%) were determined for regression coefficients by bootstrapping (3000 iterations) using the bias-corrected bootstrap (Efron and Tibshirani, 1993; Jackboot macro, SAS Institute, 1999). Percentile and bias-corrected accelerated bootstraps gave similar results. If the assumption of equal branch lengths is incorrect, these confidence limits can lead to inflated type I error (Ackerly, 2000). Thus, we also present P values from a table of adjusted critical values from Ackerly (2000), which provides a correction for this error.

RESULTS

Phenotypic correlations in *G. thurberi*—Direct and indirect resistance traits were not correlated for any of the *G. thurberi* populations: Florida Canyon (Fig. 3A; standard partial regression coefficient $b' = -0.003$, $t = -0.02$, $P = 0.98$, $n = 43$), Agua Caliente Canyon (Fig. 3B; $b' = -0.046$, $t = -0.34$, $P = 0.74$, $n = 52$), Molino Basin (Fig. 3C; $b' = -0.095$, $t = -0.55$, $P = 0.59$, $n = 46$). The absolute values of the standard partial regression coefficients $|b'|$ needed to be greater than 0.33, 0.28, and 0.35, to achieve significance of a two-tailed t test at $P < 0.05$ in Florida, Agua Caliente, and Molino populations, respectively (following Zar, 1999). The lack of a relationship was consistent across populations, despite the fact that populations differed significantly in the levels of protection afforded by ants (Rudgers, 2002, 2004).

Correlations across the *Gossypieae*—In our examination of *Gossypieae*, correlations between direct and indirect resistance traits mirrored those documented within *G. thurberi*. Neither the gossypol gland index nor trichome cover was significantly negatively correlated with the extrafloral nectary index (Fig. 4A, B; Table 2). In fact, the standard partial regression coefficient for the relationship between extrafloral nectaries and glands was positive for all trees examined, although marginally significant only for Tree 3 (see 95% CL, Table 2). For Tree 1, to achieve significance at $P < 0.05$ using a two-tailed t test, the absolute value of the standard partial regression coefficient $|b'|$ must be greater than 0.371 for the extrafloral nectary index vs. the gland index and 0.369 for the extrafloral nectary index vs. the trichome index; the power of these tests was 0.65 and 0.52, respectively (following Zar, 1999).

In contrast, there was a strong negative correlation between investment in gossypol glands and investment in trichome cover across *Gossypieae* (Fig. 4C, Table 2, $P < 0.01$). The gossypol gland index explained approximately 34% of the variation in trichomes (Tree 1, $r^2 = 0.344$, $P < 0.01$; Tree 2, $r^2 = 0.338$, $P < 0.01$; Tree 3, $r^2 = 0.339$, $P < 0.01$).

Our cross-species results were not sensitive to uncertainty in the topology; patterns of association were similar for all three trees examined (Table 2). In addition, phylogenetic corrections did not significantly influence the correlations among resistance traits. The direction of the correlations and their significance levels were similar for both the phylogenetically independent contrasts and the species correlations (Table 2). In our case, accounting for phylogeny appeared to enhance the strength of the correlation between glands and trichomes, although not significantly (see 95% CL, Table 2).

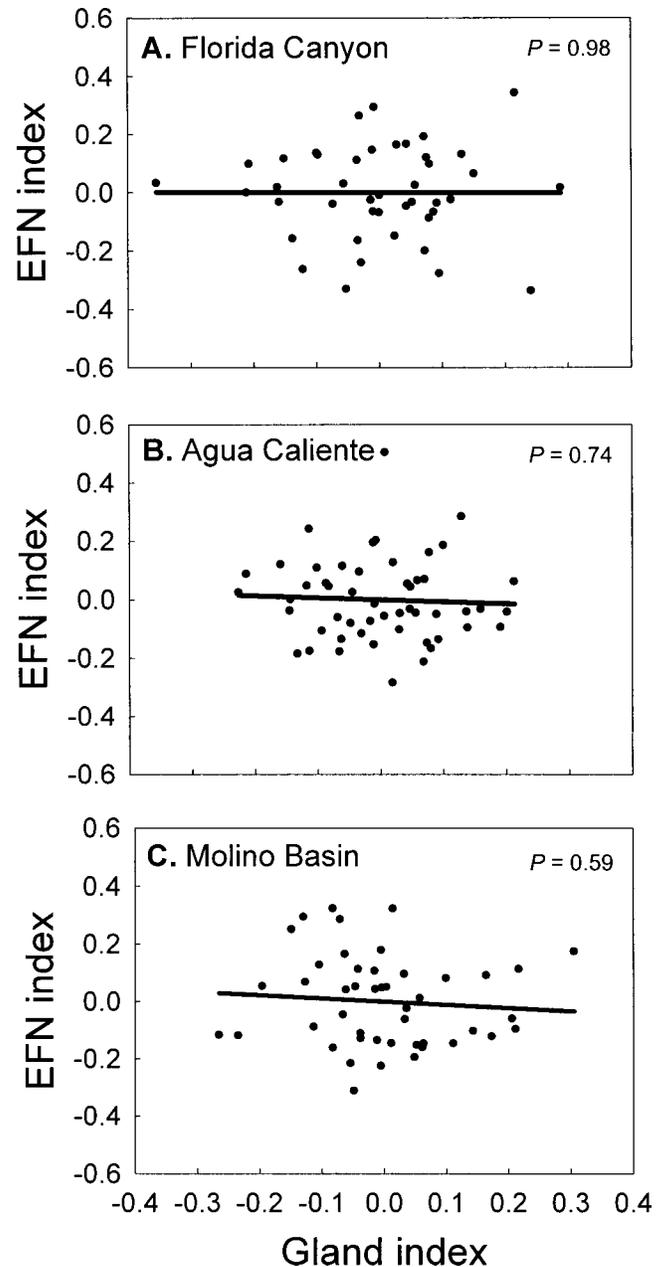


Fig. 3. Partial regression leverage plots for the gossypol gland index vs. the extrafloral nectary (EFN) index in each of three *Gossypium thurberi* populations: (A) Florida Canyon, (B) Agua Caliente Canyon, (C) Molino Basin. Plots show the residuals for the EFN index calculated with the gossypol gland index omitted from the regression analyses vs. the residuals for the gossypol gland index regressed on the remaining regressors (initial leaf number and leaf area) (SAS Institute, 1989, p. 1420). Slopes are given in the Results: Phenotypic correlations in *G. thurberi*.

DISCUSSION

Direct resistance—The direct resistance traits of leaf glands and trichomes were negatively correlated. Species with dense trichome cover had reduced investment in gossypol glands. This pattern was robust as it was maintained across three alternative trees that accounted for uncertainty in the phylogenetic hypotheses for *Gossypieae*. The negative correlation may reflect several underlying mechanisms. First, plants' invest-

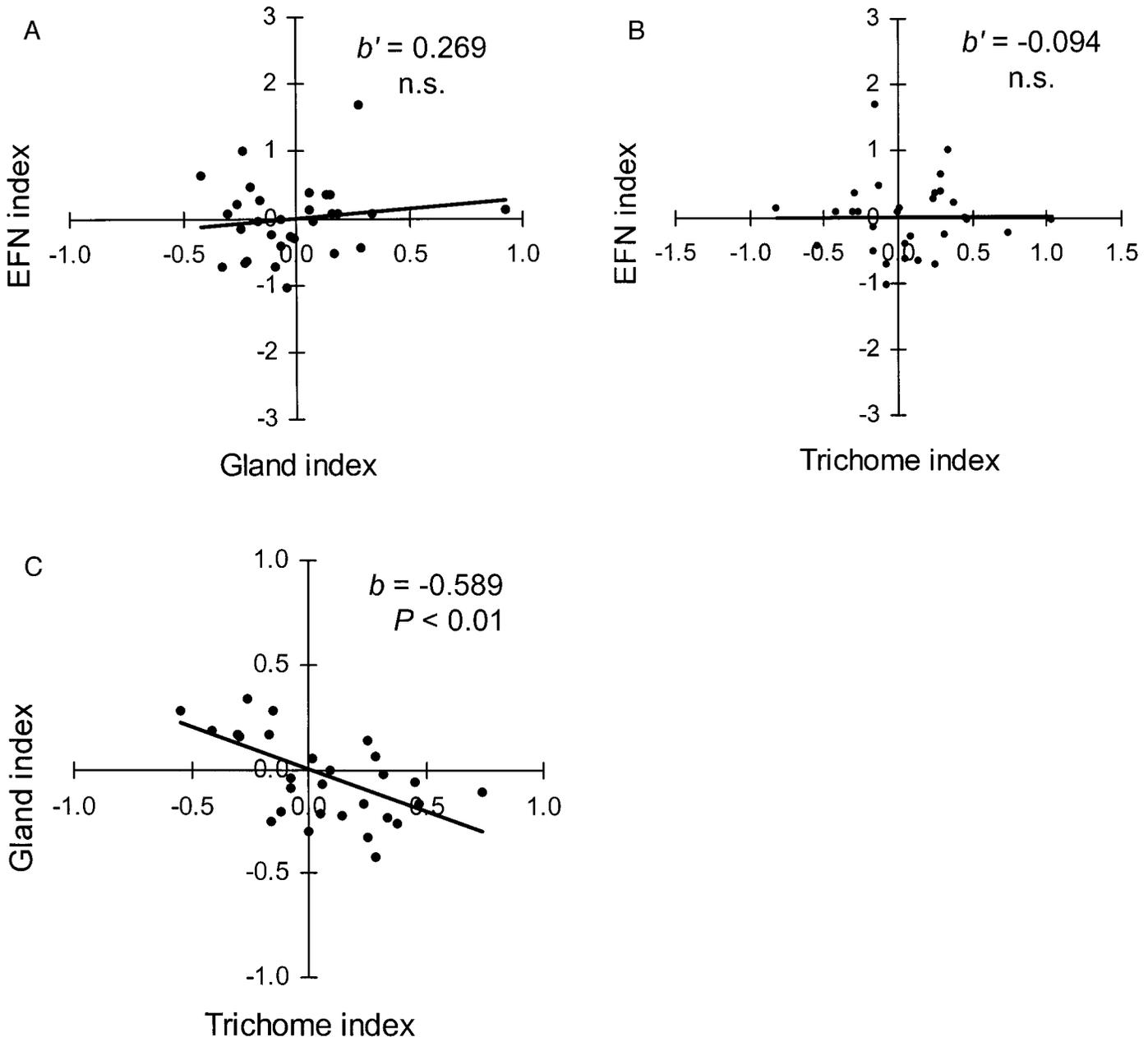


Fig. 4. Correlations between resistance indices showing standardized independent contrast values using the phylogenetic hypotheses in Tree 1: (A) the extrafloral nectary (EFN) index vs. the gossypol gland index, (B) the EFN index vs. the trichome index, and (C) the gossypol gland index vs. the trichome index. The trend line was restricted through the origin.

ment in both glands and trichomes may be constrained by costs. Costs may include both the structural investments (such as trichome cells or terpenoid aldehydes) as well as nondefensive functions of the traits (e.g., trichomes may reduce water loss [Ehleringer and Mooney, 1978], and glands may protect against pathogens [Bell, 1986]). Second, glands and trichomes may be negatively genetically correlated. Third, glands and trichomes may deter similar herbivores and therefore be redundant in ecological function. However, given that these traits deter different types of pests of cultivated cotton species (Jenkins et al., 1967; Bell, 1986; Matthews, 1989; Smith, 1992; Summy and King, 1992), the redundancy hypothesis seems unlikely. An examination of pleiotropy as well as an assess-

ment of fitness costs may help isolate the mechanisms underlying the negative correlation between glands and trichomes.

Direct resistance vs. indirect resistance—Both within and across species, indirect resistance (extrafloral nectar investment) was not correlated with either of the direct resistance traits (gossypol glands and trichomes). For each of three populations of *Gossypium thurberi*, there were no significantly negative phenotypic correlations between glands and extrafloral nectaries. The lack of significance is unlikely to be due to a lack of power because standard partial regression coefficients would only have to be larger than 0.35 to detect a significant relationship, given the sample sizes and variances for

TABLE 2. Standard regression coefficients for relationships among three resistance traits in the Gossypieae: the gossypol gland index (direct resistance), the index of trichome cover (direct resistance), and the extrafloral nectary (EFN) index (indirect resistance). Coefficients for EFN indices are standard partial regression coefficients (b') from multiple regressions that also included leaf area. Confidence limits that do not include zero indicate that the coefficient differed significantly from zero at $\alpha = 0.05$. Trees 1–3 represent alternative phylogenies of 31 diploid species. Species correlations included diploid species traits that were not corrected for phylogeny.

	Gossypol gland index vs. trichome index				Gossypol gland index vs. EFN index				Trichome index vs. EFN index			
	df	b'	95% CL	P^a	df	b'	95% CL	P^a	df	b'	95% CL	P^a
Tree 1	27	-0.589	-0.761, -0.359	<0.01	29	0.269	-0.071, 0.551	>0.1	29	-0.094	-0.346, 0.133	>0.1
Tree 2	27	-0.578	-0.821, -0.440	<0.01	29	0.164	-0.151, 0.509	>0.1	29	-0.020	-0.273, 0.210	>0.1
Tree 3	27	-0.583	-0.760, -0.349	<0.01	29	0.290	0.200, 0.647	>0.1	29	-0.154	-0.394, 0.082	>0.1
Species	29	-0.458	-0.714, -0.090	<0.01	29	0.247	-0.122, 0.590	>0.1	29	0.012	-0.286, 0.314	>0.1

^a For the independent contrasts (Trees 1–3), P values are from adjusted critical values in Ackerly (2000) that account for inflated Type I error due to the assumption of equal branch lengths. For species, P values are from t tests of the null hypothesis that the coefficient = 0 (SAS Institute, 1999). Bonferroni corrections for pair-wise correlations among three traits require a $P < 0.017$ for statistical significance.

each population. The evidence is consistent with the hypothesis that gland and extrafloral nectary traits evolve independently; however, phenotypic correlations are only a first step toward understanding correlated characters, and genetic studies are needed to determine the basis of these patterns.

Paralleling the results from *G. thurberi*, investment in direct resistance traits across the Gossypieae did not negatively correlate with allocation to indirect resistance for any phylogeny that we examined. The absence of a negative correlation between direct and indirect resistance traits stands in contrast to previous studies (see Introduction), which suggest that negative correlations occur more commonly between direct and indirect resistance traits than among types of direct resistance. Differences in methodology may explain the discrepancy between our results and those of previous studies, including the prior focus on obligate, rather than facultative, ant-plant mutualisms, the failure of previous studies to incorporate phylogeny in the analysis, or the prior use of ant exclusions in lieu of the quantification of plants' actual investment in indirect resistance traits. Phylogenetically corrected explorations of indirect resistance traits in both facultative and obligate mutualisms are needed to distinguish among these hypotheses. In addition, because correlations across species do not necessarily reflect patterns within species, individual species should be examined (as with *G. thurberi*) to determine whether patterns of investment hold within species.

When are negative correlations expected?—The lack of a negative relationship between direct and indirect resistance traits in Gossypieae may be explained by a number of non-mutually exclusive hypotheses. First, resistance traits may pose little cost to plants, although recent reviews of the fitness costs of resistance suggest otherwise (Koricheva, 2002; Strauss et al., 2002). Second, resistance traits may work in conjunction to reduce herbivory, and thus plants may require multiple traits to mount an effective defense (Futuyma, 2000). Some studies have found positive rather than negative correlations between resistance traits (e.g., Zangerl and Berenbaum, 1990; Ruuhola et al., 2001), a result that is consistent with the hypothesis that resistance traits may exhibit synergisms. For example, experimental evidence from *Acacia drepanolobium* showed that the combination of ants (indirect) and thorns (direct) synergistically reduced browsing by herbivores (Stapley, 1998). Similarly, a volatile sesquiterpene (caryophyllene oxide) in cotton that had no independent effect on herbivory, interacted synergistically with the terpenoid aldehyde, gossy-

pol, to reduce feeding by *Heliothis* caterpillars by 33% compared to gossypol alone (Gunasena et al., 1988).

Multiple mechanisms of resistance may be particularly crucial in Gossypieae species because extrafloral nectaries can directly attract some herbivore species. Extrafloral nectaries in cultivated cotton can enhance the abundance of or damage by several crop pests including lepidopterans and plant bugs (Trelease, 1879; Lukefahr and Rhyne, 1960; Lukefahr et al., 1960; Benschoter and Leal, 1974; Schuster et al., 1976; Wilson and Wilson, 1976; Henneberry et al., 1977; Adjei-Maafa et al., 1983; Beach et al., 1985; Smith, 1992; Summy and King, 1992), mainly because adults of these taxa consume extrafloral nectar. Other traits, such as gossypol glands and trichomes, may be needed to deter herbivores attracted to nectar. Therefore, in contrast to predictions made by current theories on plant defense, cotton species investing in extrafloral nectar may require alternative defenses due to the ecological cost of nectar in attracting some herbivore species. However, this ecological cost may be more apparent in cultivated cotton systems, where natural communities of predators are likely to be disrupted by routine tilling and pesticide application. Further studies in natural systems are needed.

The absence of a correlation between extrafloral nectary and gland/trichome traits does not preclude the possibility that correlations occur with other, as yet unmeasured, plant resistance characters. These traits include condensed tannins (Bell, 1986), leaf toughness (Coley, 1983), volatiles that function in indirect resistance by signaling predators and parasitoids of herbivores (Loughrin et al., 1995; Pare and Tumlinson, 1997), and induced forms of direct or indirect resistance to herbivory (Karban and Baldwin, 1997). An analysis encompassing additional resistance traits would provide an even broader picture of allocation to resistance in Gossypieae.

Finally, the robust lack of a correlation may reflect the facultative nature of indirect resistance in Gossypieae. Facultative mutualisms are prevalent in nature and highly conditional (Bronstein, 1998). Negative correlations with direct resistance traits may be most likely when indirect resistance is obligate, rather than facultative, in nature. For example, Turner (1995) showed that *Macaranga* plants engaged in obligate relationships with ants had lower levels of tannins than species with non-obligate associations. In addition, leaves from obligately ant-defended acacias often had lower concentrations of cyanogenic glycosides and less resistance to caterpillars than leaves from non-obligate acacias (Janzen, 1966; Rehr et al., 1973; Seigler and Ebinger, 1987). Exploring a range of mu-

tualisms from unspecialized and facultative to specialized and obligate may shed light on the conditions under which negative correlations among resistance traits occur. In such comparisons, controlling for phylogeny is essential, as plants in obligate associations may be more closely related to each other than to species in facultative associations (e.g., Davies et al., 2001).

In summary, explorations in the cotton tribe, Gossypieae, demonstrate patterns of investment in anti-herbivore resistance traits that differ from the majority of other studies on trade-offs among plant defenses. Negative correlations, as occurred between trichomes and gossypol glands in Gossypieae, may reflect costs or redundant functions of these constitutively expressed, direct resistance traits. In contrast, the lack of a negative correlation between these direct resistance traits and extrafloral nectar suggests that these traits evolve independently over evolutionary time scales. Finally, our results lead to the prediction that indirect defenses associated with unpredictable, facultative mutualisms (such as those in Gossypieae) will be less likely to be negatively correlated with other resistance traits than are indirect defenses that mediate obligate protective mutualisms.

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