

## PLANT DEFENSE SYNDROMES

ANURAG A. AGRAWAL<sup>1,3</sup> AND MARK FISHBEIN<sup>2,4</sup>

<sup>1</sup>Department of Ecology and Evolutionary Biology and Department of Entomology, Cornell University, Ithaca, New York 14853 USA

<sup>2</sup>Department of Biological Sciences, Mississippi State University, Mississippi State, Mississippi 39762 USA

**Abstract.** Given that a plant's defensive strategy against herbivory is never likely to be a single trait, we develop the concept of plant defense syndromes, where association with specific ecological interactions can result in convergence on suites of covarying defensive traits. Defense syndromes can be studied within communities of diverse plant species as well as within clades of closely related species. In either case, theory predicts that plant defense traits can consistently covary across species, due to shared evolutionary ancestry or due to adaptive convergence.

We examined potential defense syndromes in 24 species of milkweeds (*Asclepias* spp.) in a field experiment. Employing phylogenetically independent contrasts, we found few correlations between seven defensive traits, no bivariate trade-offs, and notable positive correlations between trichome density and latex production, and between C:N ratio and leaf toughness. We then used a hierarchical cluster analysis to produce a phenogram of defense trait similarity among the 24 species. This analysis revealed three distinct clusters of species. The defense syndromes of these species clusters are associated with either low nutritional quality or a balance of higher nutritional quality coupled with physical or chemical defenses. The phenogram based on defense traits was not congruent, however, with a molecular phylogeny of the group, suggesting convergence on defense syndromes. Finally, we examined the performance of monarch butterfly caterpillars on the 24 milkweed species in the field; monarch growth and survival did not differ on plants in the three syndromes, although multiple regression revealed that leaf trichomes and toughness significantly reduced caterpillar growth. The discovery of convergent plant defense syndromes can be used as a framework to ask questions about how abiotic environments, communities of herbivores, and biogeography are associated with particular defense strategies of plants.

**Key words:** *Asclepias*; *cardenolides*; *chemical ecology*; *cluster analysis*; *coevolution*; *Danaus plexippus*; *herbivory*; *latex*; *milkweed*; *monarch butterfly*; *phylogenetically independent contrasts*; *phytochemistry*; *plant–insect interactions*.

### INTRODUCTION

Understanding the macroevolution of adaptive traits has inspired biologists for decades, yet has been challenging to study (Schluter 2000). The difficulty lies in (1) identifying the trait, or more commonly suites of traits, responsible for the adaptation of interest, (2) having adequate phylogenies to examine macroevolutionary patterns, (3) distinguishing apparent adaptation from “random” evolution along a diversifying phylogeny, and (4) matching the origins of adaptations to contemporaneous biotic and abiotic environmental factors that have likely driven adaptive changes. Despite these difficulties, the study of plant–herbivore interactions has contributed substantially to understanding the macroevolution of adaptive traits. Research on the macroevolution of plant defense has played a prominent

role in the development of ideas on both the ecology and evolution of plants and insect herbivores, two of the most diverse lineages of eukaryotes (Ehrlich and Raven 1964, Coley 1983, Farrell et al. 1991, Farrell and Mitter 1993, 1998, Becerra 1997, Berenbaum 2001). In this paper, we present a new synthesis of ideas on the macroevolution of plant defense traits, with an attempt to identify the relative roles of phylogenetic history and ecological variables in shaping the expression of suites of defense traits within species. We then test some of our proposed ideas and assumptions utilizing new data on the phylogeny and defense of milkweeds (*Asclepias* spp.).

Although it is convenient to consider plant defense as a single trait, plants typically utilize a broad arsenal of defensive traits against herbivores (Duffey and Stout 1996, Romeo et al. 1996). Even when a plant species is apparently defended by a single type or class of defense chemical, there are typically many specific forms of those compounds (Berenbaum et al. 1986, Malcolm 1991, Bennett and Wallsgrave 1994, Becerra 1997). Thus, it is more useful to think about plant defense as a suite of traits, which might include aspects of a plant's

Manuscript received 21 January 2005; revised 9 May 2005; accepted 21 June 2005; final version received 1 August 2005. Corresponding Editor (ad hoc): C. O. Webb. For reprints of this Special Issue, see footnote 1, p. S1.

<sup>3</sup> E-mail: aa337@cornell.edu

<sup>4</sup> Present address: Department of Biology, Portland State University, Portland, Oregon 97207 USA.

nutritional quality (e.g., proteins and antiproteins), physical characteristics (e.g., spines, trichomes, and leaf toughness), toxicity (e.g., cyanides and alkaloids), phenology, regrowth capacity (i.e., tolerance), and indirect defenses (e.g., volatiles and branching architecture). Synergistic interactions between multiple traits is particularly important in potentially providing a greater level of defense than would be possible if the traits were present independently (Broadway and Duffey 1988, Gunasena et al. 1988, Berenbaum et al. 1991, Stapley 1998).

Nonetheless, most attention historically has considered defenses as singleton strategies, with the typical prediction that there should be trade-offs among different antiherbivore strategies (because they could be costly and/or redundant) (Steward and Keeler 1988, Herms and Mattson 1992). We argue that this reasoning, in its simplest form, is inaccurate, because plants do simultaneously employ multiple defense traits. Of course, particular plant defenses might trade off against each other, but this should not be the a priori expectation for any two defense-related traits. If plant defenses, like most adaptations, are composed of multiple traits, they might be organized into coadapted complexes (Dobzhansky 1970).

The categorization of an organism's phenotype into suites of potentially covarying traits has a long tradition in biology, including the concepts of guilds and syndromes (Root 1967, Grime 1977, Fægri and van der Pijl 1979, Simberloff and Dayan 1991, Chapin et al. 1993, Cunningham et al. 1999, Fenster et al. 2004, Wilson et al. 2004). The syndrome concept has been rightly criticized as overly simplistic when applied uncritically (e.g., Waser et al. 1996); however, suites of covarying traits can be usefully employed in some cases to infer adaptation to specific selective contexts. For example, suites of traits associated with hummingbird-pollinated flowers (i.e., red color, tubular morphology, extended anther position, dilute nectar concentration, etc.) have repeatedly evolved from those of bee-pollinated ancestors in diverse lineages (McDade 1992, Beardsley et al. 2003, Castellanos et al. 2003, 2004, Fenster et al. 2004). Patterns of natural selection imposed by birds and bees implicate these pollinators as the agents favoring floral syndromes (e.g., Schemske and Bradshaw 1999). Although any two traits in a syndrome can be positively or negatively (or not at all) correlated across taxa, the plant defense syndrome hypothesis rejects the prediction that any two plant defenses are redundant, and thus should be negatively associated across species (Steward and Keeler 1988, Twigg and Socha 1996, Rudgers et al. 2004). Defense syndromes as whole, however, can trade off if they truly represent alternative adaptive strategies.

If a syndrome has independently evolved multiple times, it suggests that the selective forces driving the evolution of these convergent adaptations are common and widespread. If, however, closely related species

share a syndrome due to common ancestry, then common ancestry is sufficient to explain the association, and adaptive advantage need not necessarily be invoked. Like trade-offs among supposedly redundant traits, the correlated evolution of traits composing syndromes needs to be tested explicitly rather than assumed.

Although plant defense syndromes have received little attention in the past (but see Feeny 1976, Kursar and Coley 2003), we suggest that where distantly related plant species share a common assemblage of herbivores, they are likely to defend themselves with similar strategies. For example, plant species attacked primarily by vertebrate grazers should employ quite different strategies (e.g., spines, leaf toughness, and out-of-reach morphologies) than plants primarily attacked by caterpillars (e.g., trichomes, toxins, and parasitoid-attracting volatiles). Perhaps the original "plant defense syndrome" was the set of traits proposed to be associated with highly apparent plants (Feeny 1976). Feeny argued that apparent plants like oak trees were bound to be found, and thus defended with suites of traits that make them nutritionally poor: high concentrations of tannins, low water and nitrogen content, and tough leaves. We agree that such patterns are likely to have evolved repeatedly to converge on distinct syndromes of defensive traits, even in distantly related plant taxa. So why have most ecologists not explicitly considered plant defense syndromes? We believe that the traits of importance in defense against herbivores are too often unobservable by the naked eye of naturalists, and therefore have escaped attention.

#### *Plant defense syndromes in communities versus taxonomic groups*

The concept that plant species within a particular community may coexist due to trade-offs in fitness-enhancing traits is an old and central paradigm in plant ecology (reviewed by Tilman and Pacala [1993]). This logic was later applied to plant defense traits for coexisting species. Van der Meijden et al. (1988) proposed that suites of traits associated with resistance vs. regrowth (tolerance) might represent alternative strategies for coexisting temperate herbaceous plants. Spurred by Janzen's (1974) study of tropical blackwater rivers and emerging physiological plant defense theories (Bryant et al. 1983), Coley and colleagues hypothesized that plant species colonizing light gaps would have divergent (trading-off) suites of defense traits, compared to species colonizing the more resource-poor understory in tropical forests (Coley 1983, 1987, Coley et al. 1985). Such trade-offs explained divergent patterns of herbivory in different microenvironments and also likely contributed to the maintenance of diversity at larger spatial scales. Recent experimental work on phylogenetically paired species from white sand and clay soil habitats of Peru support the hypothesis (Fine et al. 2004, 2006).

Kursar and Coley (2003) have also expanded these ideas to explicitly consider the evolution of convergent defense syndromes in tropical trees. They argue that trees fall along an escape–defense continuum: extreme “escape” species are predicted to have few chemical defenses, but rapid synchronous leaf expansion, and low leaf nutritional quality during expansion; extreme “defense” species have high chemical defense, low nutritional quality, and asynchronous leaf expansion (Kursar and Coley 2003). Using phylogenetically independent contrasts, Silvertown and Dodd (1996) showed that herbaceous vs. woody plants had distinct types of chemical defenses (tannins vs. alkaloids), consistent with classic apparency theory (Feeny 1976). In each of these examples, the general finding has been that unrelated plant species within a particular community have converged on a suite of similar strategies that maximize fitness, given a particular set of ecological interactions. Thus within a regional community, plant species can converge on a few defense syndromes, yet the divergent strategies (across the syndromes) also can promote the coexistence of species.

The approach of examining syndromes within communities is an ecological question, first and foremost, and begins with identifying the traits of species that occur within a plant community irrespective of their evolutionary relationships. If patterns of defensive traits consistently occur among coexisting taxa, then the patterns of defense among species could be explained by either shared phylogenetic history or convergence due to similar selective agents causing repeated evolution of a defensive syndrome. We propose that phylogenetic history should provide the null hypothesis to explain the distribution of defense traits among species (Silvertown and Dodd 1996). The alternative hypothesis is that species’ defensive traits are evolutionarily labile, and species have repeatedly evolved homoplasious (i.e., independent, but similar) solutions to biotic and abiotic environmental challenges. For instance, in a temperate grassland community, patterns of plant defense could consistently fall into a few categories (e.g., toxic and ant-defended vs. tolerant and poor nutritional quality), but each of these categories might be phylogenetically homologous (e.g., evolving once each in legumes and grasses). Alternatively, unrelated taxa could converge on homoplasious suites of traits in response to the same or similar selective forces (Fine et al. 2004).

In community studies of syndromes, phylogenetic information need not be extremely detailed, and taxonomic information might be sufficient. If two species within a genus have different suites of defense traits and are ecologically divergent (i.e., have different types of species interactions, live in different microhabitats, etc), then selection is a reasonable hypothesis for the differences between the close relatives; if multiple, phylogenetically independent congeneric pairs show similar suites of defensive traits and divergent ecologies, convergence can be invoked (Conway-Morris 2003, Fine

et al. 2004). Of course, such an association does not prove adaptation, but, at minimum, implies an evolutionary association between traits and ecology.

The macroevolution of syndromes has also been studied within clades. Within the plant genus *Penstemon*, species have repeatedly evolved suites of traits associated with hummingbird pollination from Hymenoptera-pollinated ancestors (Thomson et al. 2000, Wilson et al. 2004). The repeated evolution of particular plant defense trait combinations within a taxonomic group (e.g., genus) is suggestive of “syndromes in clades.” Traits associated with induced resistance to herbivores evolved multiple times in the cotton genus, *Gossypium* (Thaler and Karban 1997). Work with ant-associated and nonant-associated *Acacia* spp. may reveal patterns of divergent defensive strategies in plants with altered species interactions (Rehr et al. 1973, Seigler and Ebinger 1987, Heil et al. 2000, 2002, 2004). Recent work by Becerra and colleagues (Becerra 1997, Becerra et al. 2001) demonstrates that there has been convergent evolution of terpene chemical defense and “squirt-gun” defense combinations in tropical *Bursera* spp. Species of specialist *Blepharida* beetles typically consume plants with similar defense traits, which are not necessarily the most closely related species or those with geographically similar ranges (Becerra 1997, Becerra and Venable 1999). Thus, traits can covary within a clade and are predicted to be associated with particular interactions (although convergent taxa do not necessarily coexist).

The approach of examining syndromes within a clade requires an accurate plant phylogeny and the existence of two or more syndromes (suites of traits). Thus, if suites of traits converge in different parts of the phylogeny, the question becomes this: Are there consistent ecological correlates that match these different syndromes? A key difference from the community approach is that syndromes within a closely related set of species in a clade can surface in geographically separated species that do not naturally coexist. The community approach specifically starts with species that coexist under relatively similar ecological conditions. Although similar in trying to disentangle the effects of phylogenetic history and ecology, the “community” and “clade” approaches start at the opposing ends of the phylogeny–ecology continuum.

#### *Defense syndromes in milkweeds (Asclepias)*

There are ~130 species of *Asclepias* native to North America, including Mesoamerica and the Caribbean (Woodson 1954; M. Fishbein et al., *unpublished manuscript*). Approximately six additional species are native to temperate South America (Bollwinkel 1969). The situation is more complicated in Africa, where up to 200 species have been or could potentially be included in the genus *Asclepias*, depending on the breadth of circumscription and phylogenetic relationships among African and American species (Fishbein 1996). However, preliminary analyses suggest that American and African

TABLE 1. Species sampled for defense-related traits, effects on herbivore performance, and phylogenetic relationships.

Species	Native Range
<i>Asclepias amplexicaulis</i> Sm.	Eastern USA
<i>Asclepias asperula</i> (Decne.) Woodson ssp. <i>asperula</i>	Southwestern USA and Mexico
<i>Asclepias californica</i> Greene	Western USA
<i>Asclepias cordifolia</i> (Benth.) Jeps.	Western USA
<i>Asclepias curassavica</i> L.	Neotropics
<i>Asclepias eriocarpa</i> Benth.	Western USA
<i>Asclepias exaltata</i> L.	Eastern USA
<i>Asclepias fascicularis</i> Decne.	Western USA and Mexico
<i>Asclepias hallii</i> A. Gray	Western USA
<i>Asclepias hirtella</i> (Pennell) Woodson	Central USA
<i>Asclepias incarnata</i> L. ssp. <i>incarnata</i>	Eastern USA
<i>Asclepias incarnata</i> L. ssp. <i>pulchra</i> (Ehrh. ex Willd.) Woodson	Eastern USA
<i>Asclepias oenotheroides</i> Schldt. & Cham.	Southwestern USA, Mexico, and Central America
<i>Asclepias perennis</i> Walter	Southeastern USA
<i>Asclepias purpurascens</i> L.	Eastern USA
<i>Asclepias speciosa</i> Torr.	Western USA
<i>Asclepias sullivantii</i> Engelm. ex A. Gray	Central USA
<i>Asclepias syriaca</i> L.	Eastern USA and Canada
<i>Asclepias tuberosa</i> L.	Eastern USA, southwestern USA, and Mexico
<i>Asclepias variegata</i> L.	Eastern USA
<i>Asclepias verticillata</i> L.	Eastern USA and Canada
<i>Asclepias viridis</i> Walter	Eastern USA
<i>Gomphocarpus cancellatus</i> (Burm. f.) Bruyns	Southern Africa
<i>Gomphocarpus fruticosus</i> (L.) W.T. Aiton	Africa

species belong to distinct sister clades (Rapini et al. 2003; M. Fishbein et al., *unpublished manuscript*), and thus *Asclepias* may be considered an exclusively American genus of ~150 species. In this study, we employ the results of the most intensively sampled study of *Asclepias* to date (M. Fishbein et al., *unpublished manuscript*), focusing on the American species as a framework for investigating macroevolution of defense traits. Of the species we studied, all are perennial, some are clonal, and genets are probably very long-lived (Table 1). In addition, most of the species are relatively rare in the landscape; those that are common now, such as *A. syriaca* in eastern North America, were probably much less abundant and widespread prior to the clearing of the eastern deciduous forest by colonists.

Plants in the genus *Asclepias* have been of major importance in the development of theories about plant–herbivore and tri-trophic interactions, and historically the focus has been primarily on cardenolides as a plant defense and the Monarch butterfly as the herbivore (Fig. 1) (Brower et al. 1967, 1972, Malcolm et al. 1989). However, the herbivore community of *Asclepias* spp. consists of tens of species that are principally host-specific insects (Weiss and Dickerson 1921, Rothschild et al. 1970, Wilbur 1976, Blakley and Dingle 1978, Price and Willson 1979, Morse 1985, Malcolm 1991, Fordyce and Malcolm 2000, Agrawal and Malcolm 2002). These herbivores fill almost every conceivable feeding guild: aphids feed on the phloem, beetles and caterpillars chew the leaves, flies mine the leaves, hemipterans eat the seeds, weevils bore through the stem and eat the pith, and beetle larvae bore through the roots.

For example, the common milkweed, *A. syriaca*, has 12 reported insect herbivores, including members of all

of the guilds. Some of the herbivores, such as Monarchs (*Danaus plexippus*) and Milkweed aphids (*Aphis nerii*), are broadly distributed and feed on many milkweeds. Other groups, such as the cerambycid beetles in the genus *Tetraopes* have radiated with the milkweed genus, and each of the 24 *Tetraopes* species is primarily associated with a single milkweed species (Farrell and Mitter 1998). Thus, each milkweed species is likely attacked by several herbivores, although the species composition and guilds attacking particular species vary widely. An important future goal in the study of plant defense macroevolution is to detail the particular insect communities that correspondingly attack plants with different defense syndromes.

The community of insects on milkweed thrives by either actively avoiding defenses in the plant or by consuming, sequestering, and advertising these same defenses. Probably the two most potent aspects of herbivore resistance in milkweeds are the production of cardenolides and latex. Cardenolides are bitter tasting steroids that occur in all milkweed tissues, including the latex, that act by disrupting the sodium and potassium flux in cells, and have toxic effects on most animals (Malcolm 1991). The sticky white latex is delivered via specialized canals (laticifers) to most plant parts, and can be copiously exuded upon tissue damage (Fig. 1). Latex of milkweed has been strongly implicated as a physical impediment to herbivory (Dussourd and Eisner 1987, Zalucki et al. 2001). Other potentially defensive and nutritional constituents that could influence herbivores include leaf toughness, trichome density, water content, nitrogen content, and specific leaf area (Mattson 1980, Coley 1983, Haddad and Hicks 2000, Lavoie and Oberhauser 2004). Most of these traits show plastic

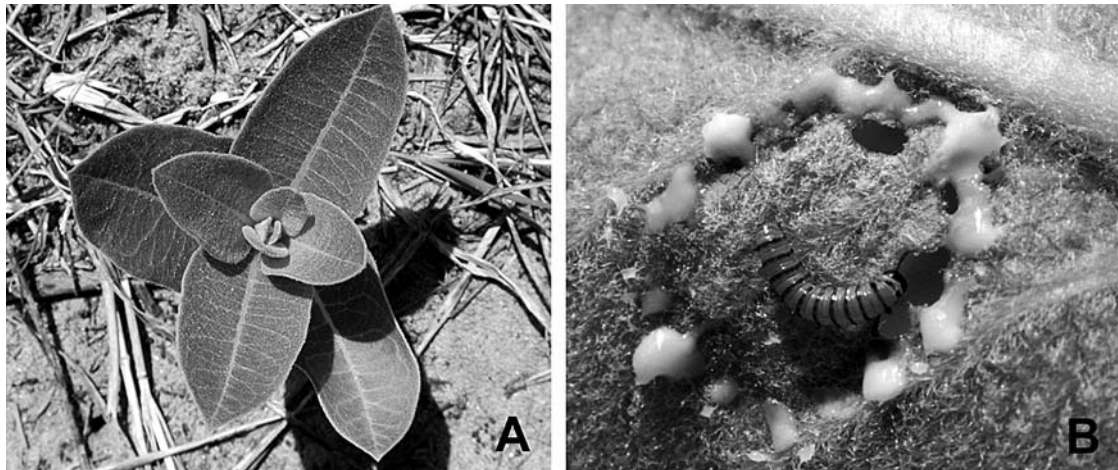


FIG. 1. (A) Common milkweed (*Asclepias syriaca*) viewed from above early in the growing season. (B) A newly hatched monarch caterpillar (*Danaus plexippus*). Before it can get its first meal, the caterpillar must graze the trichomes and avoid the droplets of latex. Exposure to latex is reduced by clipping laticifers in a circle and feeding on the tissue in the middle.

variation within species, are genetically variable in natural populations of single species, and are highly variable across species (Table 2).

Using 24 species of *Asclepias*, we begin to address the role of convergent evolution in giving rise to antiherbivore defense syndromes. We specifically addressed the following questions: (1) What are the pairwise associations of defense traits across taxa? Are defense traits typically negatively correlated as predicted by redundancy or trade-off theory, are they positively correlated as predicted by syndrome theory, or are they not correlated at all? (2) Do species cluster into syndromes of common patterns of defense trait expression? (3) Are phylogenetic relationships among *Asclepias* species inferred from DNA sequences congruent with patterns of defense trait similarity? If so, we can conclude that phylogenetic history is sufficient to explain plant defense trait associations; if not, there is a suggestion for convergence in defense syndromes. And (4) Are particular defense traits or syndromes associated with the performance of a common oligophagous herbivore of milkweeds (monarch caterpillars, *Danaus plexippus*)?

#### MATERIALS AND METHODS

##### *Measuring plant traits*

Seeds for 24 species of milkweeds (Table 1) were obtained from field collections, nurseries, and native plant seed suppliers. We germinated seeds by nicking the tips and placing the seeds on moist filter paper. Seedlings were grown in potting soil (~10-cm pots) in growth chambers for one month and out-planted in a completely randomized common garden in a plowed field at the Koffler Scientific Reserve at Jokers Hill, Southern Ontario (44°03' N, 79°29' W; information *available online*).<sup>5</sup> After

accounting for plant mortality, our common garden had approximately 12 replicate plants per species (mean  $\pm$  SE,  $11.8 \pm 0.7$ ). All measurements were taken from newly expanded, undamaged leaves of plants in the common garden.

We measured cardenolide concentrations as digitoxin equivalents (grams per gram dry tissue) extracted from 50 mg dry leaf tissue ( $n = 5$  replicates/species); we employed a spectrophotometric assay modified from Nelson (1993). We adapted the assay for sampling using a microplate reader (PowerWave X, Bio-Tek Instruments, Winooski, Vermont, USA). Field-collected leaf tissue was kept on ice, then frozen, freeze-dried, ground with a mortar and pestle, and weighed in 2-mL boil-proof tubes. To each tube, we added 1.9 mL of 95% ethanol, tubes were then vortexed, and floated in a sonicating water bath (65°C) for 10 min. We then centrifuged the tubes at 5000 rpm for five minutes at room temperature. Two 45- $\mu$ L aliquots of the supernatant from each tube were then pipetted into the wells of a 96-well plate, one above the other (active sample and blank, respectively). Each plate also contained six samples of digitoxin for the standard curve used to determine concentrations of cardenolides (0.125–3 mg/mL; Sigma Chemical, St. Louis, Missouri, USA). We then added 90  $\mu$ L of ethanol to the blanks and 90  $\mu$ L of 0.15% 2,2',4,4'-tetranitrodiphenyl (TNDP) in ethanol to the active samples. Finally, 70  $\mu$ L of 0.1 mol/L aqueous NaOH was added to all wells to make the solutions basic and to catalyze the colorimetric reaction. After 15 minutes, all wells in the plate were read at 620 nm using the microplate reader.

We measured latex from 5–10 replicates from each species by cutting the tip off (0.5 cm) an intact leaf in the field and collecting the exuding latex onto a 1 cm disc of filter paper. Latex stopped flowing after ~10 s, all latex was absorbed on the filter paper, and this disc was placed

<sup>5</sup> (<http://www.zoo.utoronto.ca/jokershill>)

TABLE 2. Seven putative defensive traits of milkweeds. Ranges for the actual trait values for treatment and for family and species means are presented, followed by percentage variation (in parentheses).

Putative defense trait	Plastic variation	Full-sib variation	Species level variation
Latex (mg)	1.05–1.35 (29%)	0.9–4.5 (400%)	0–4.8 (infinite)
Cardenolides (% dry mass)	2.7–4.0 (48%)	1.7–3.9 (129%)	2–16 (800%)
Trichomes (no. hairs/cm <sup>2</sup> )	NA	580–1090 (88%)	0–2019 (infinite)
Toughness (g)	NA	96–128 (33%)	58–177 (205%)
C:N	7.8–9.0 (15%)	11–14 (27%)	10–15 (50%)
Water content (%)	NA	NA	78–90 (15%)
Specific leaf area (mm <sup>2</sup> /mg)	NA	NA	8–23 (188%)

*Notes:* In experiments, we have characterized the level of variation in these traits among control *Asclepias syriaca* plants and those experimentally damaged by herbivores (plastic variation) (Van Zandt and Agrawal 2004a; A. A. Agrawal, *unpublished data*), 23 full-sibling families of *A. syriaca* (Agrawal 2004b, 2005), and 24 different species of *Asclepias* (Agrawal 2004a, this study). All data were collected from at least five individuals (per treatment, family, or species) typically growing in growth chambers (plastic variation) or independent field common gardens (full-sibling and species variation). There were significant differences between treatments, families, and species for all traits measured (ANOVA for all traits,  $P \ll 0.05$ ); families and species vary continuously between the extremes presented.

on top of another dry filter paper disc in a 24-well plate. The discs were dried at 60°C and then weighed to the microgram. This method has proven to be highly repeatable (Van Zandt and Agrawal 2004a, Agrawal 2005).

We assessed the trichome density of 5–10 replicate plants from each species by counting the tops and bottoms of leaf discs (28 mm<sup>2</sup>) under a dissection microscope. Leaf discs were taken from the tips of leaves. Water content was assessed by first weighing leaf discs wet and again after drying in an oven (60°C). Specific leaf area (SLA) was calculated as the area of the leaf disc divided by the dry mass. This measure can be thought of as an indicator of thickness or density; leaves with a higher SLA are typically thin and have greater levels of herbivory (Schädler et al. 2003).

We measured leaf toughness on 10 replicate plants from each species with a force gauge penetrometer (Type 516; Chatillon, Largo, Florida, USA) that measures the mass (in grams) needed to penetrate a surface. We sandwiched the leaf between two pieces of Plexiglas, each with a 0.5-cm hole, pushed the probe of the penetrometer through the leaf, and recorded the maximum force required for penetration. For each leaf, we measured toughness on each side of the mid-rib; these two measures were averaged and used as a single data point per plant.

Total leaf carbon (C) and nitrogen (N) concentration was measured from five replicates from each species by microcombustion, using 5 mg of dried ground leaf material in an Elemental Combustion System 4010, CHNS-O analyzer (Costech Analytical Technologies, Valencia, California, USA). We used the C:N ratio as indicator of plant nutritional quality.

Means and standard errors of the seven defense-related traits for each of the 24 milkweed species are reported in Appendix A.

#### *Statistical analyses of phylogenetic and nonphylogenetic trait associations*

Pairwise correlations among traits were calculated using raw trait values and phylogenetically independent

contrasts (PICs) (Felsenstein 1985). We calculated PICs using the maximum-likelihood (ML) estimate of the phylogeny of the 24 species of *Asclepias* (Fig. 2) for which defense traits were measured (Table 2), based on noncoding plastid DNA (cpDNA) sequences (*rp116* intron and *trnC-rpoB* intergenic spacer; M. Fishbein et al., *unpublished manuscript*). We obtained sequences directly, using standard protocols, and manually aligned them (M. Fishbein et al., *unpublished manuscript*). The best fitting nucleotide substitution model under the ML criterion (general time reversible plus invariant sites plus gamma [GTR+I+ $\Gamma$ ]) was selected using hierarchical likelihood ratio tests and ML trees were sought using a standard heuristic search strategy (using PAUP\*4.0b10; Swofford 2001) (see Fishbein et al. 2001). Clade support was assessed with Bayesian probabilities estimated under the GTR+I+ $\Gamma$  model, assuming uninformative priors for model parameters and tree topologies (using MrBayes 3.0b4, Ronquist and Huelsenbeck 2003) (see Fishbein and Soltis 2004). Markov chains were iterated for  $5 \times 10^5$  generations with trees sampled during the first  $10^5$  generations discarded as transitory samples. The ML tree for these 24 species did not differ significantly from relationships found in a comprehensive analysis of 105 species of *Asclepias* (M. Fishbein et al., *unpublished manuscript*; results not shown).

We calculated PICs using Felsenstein's (1985) method, as implemented in COMPARE (*available online*).<sup>6</sup> We have no a priori evidence concerning rates of evolution of defense traits. Thus, we conducted PIC analyses under the assumptions of (1) equal branch lengths, corresponding to a speciation model of evolution (Mooers et al. 1999), and (2) branch lengths estimated from cpDNA sequences. However, the branch lengths estimated from molecular data caused pathological behavior in the PIC analysis: several exceedingly short internodes (Fig. 2) resulted in widely inflated

<sup>6</sup> (<http://compare.bio.indiana.edu>)

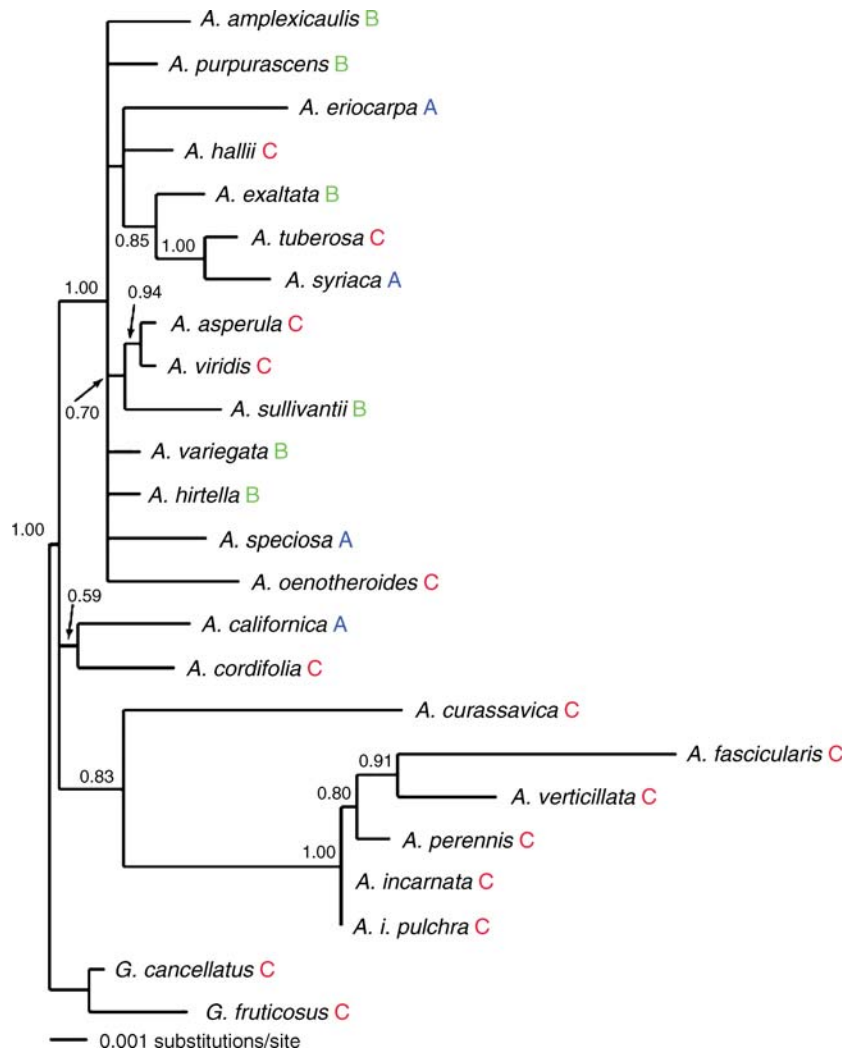


FIG. 2. Maximum-likelihood phylogram of the 24 species of *Asclepias* employed in this study. Numbers indicate Bayesian posterior probabilities. Letters to the right of taxon names represent defense syndrome clusters (see Fig. 4 and *Results: Assessing defense syndromes*).

estimates of contrasts involving these branches. Thus, the few data points involving these nodes drove the pattern of correlation among contrasts (results not shown), which often differed dramatically from those found in equal branch length analyses. Despite the drawbacks of assuming equal probabilities of change across all branches of the phylogeny, this seems preferable to alternative assumptions about unknown rates of evolution. Thus, we only present the results based on assuming equal branch lengths.

The nodes of the phylogeny subtended by extremely short branches correspond to areas of uncertainty in the phylogeny of *Asclepias* (see posterior clade probabilities in Fig. 2). A consequence of this uncertainty is possible error in the PIC analysis, due to using an incorrect phylogenetic estimate. To examine this source of error, we conducted PIC analyses on alternative topologies and assessed the robustness of PIC-based correlations

(cf. Housworth and Martins 2001). Starting with a tree in which all clades with posterior probabilities <0.70 in Fig. 2 were collapsed, we generated 100 trees that randomly resolved all polytomies using MacClade 4.06 (Maddison and Maddison 2003). We present 95% confidence limits for our correlation coefficients by excluding the lowest and highest 2.5% of the  $r$  values. We conducted PIC analyses, as described, with COMPARE on each of these 100 trees.

We used a hierarchical cluster analysis to produce a defense phenogram (cf. Becerra's "chemograms") (Becerra 1997, Legendre and Legendre 1998) to group species by expression of defense traits. We used the mean values for each of the seven plant traits measured for each species to generate a phenogram. Mean trait values were transformed to Z scores (mean = 0, SD = 1) so that they were measured on a comparable scale. Following Becerra's (1997) model, we employed Ward's method for

TABLE 3. Pairwise correlations of defense-related traits among *Asclepias* species.

	Latex (mg)	Trichomes (no. hairs/cm <sup>2</sup> )	Toughness (g)	C:N	Water content (%)	Cardenolides (% dry mass)	SLA (cm <sup>2</sup> /mg)
Latex (mg)		<b>0.60**</b>	0.10	0.04	-0.21	-0.13	<b>-0.59**</b>
Trichomes (no. hairs/cm <sup>2</sup> )	<b>0.62**</b> (0.47–0.70)		-0.10	-0.17	0.04	-0.05	<b>-0.43*</b>
Toughness (g)	-0.06 (-0.25–0.10)	0.05 (-0.27–0.18)		0.32	-0.19	0.06	-0.26
C:N	0.03 (-0.25–0.10)	-0.18 (-0.43 to -0.115)	<b>0.43*</b> (0.18–0.455)		-0.25	-0.25	-0.26
Water content (%)	-0.29 (-0.32 to -0.045)	-0.01 (-0.08–0.165)	-0.34† (-0.35 to -0.13)	-0.17 (-0.30 to -0.01)		0.27	0.39†
Cardenolides (% dry mass)	-0.08 (-0.16–0.02)	0.1 (0.015–0.195)	-0.01 (-0.095–0.105)	-0.24 (-0.32 to -0.14)	0.16 (0.065–0.265)		0.15
SLA (cm <sup>2</sup> /mg)	<b>-0.57**</b> (-0.58 to -0.42)	<b>-0.45*</b> (-0.495 to -0.295)	-0.15 (-0.24–0.055)	-0.07 (-0.215–0.01)	<b>0.57**</b> (0.46–0.61)	0.21 (0.12–0.27)	

Notes: Correlation coefficients ( $r$ ) above the diagonal are uncorrected for bias due to phylogenetic history; those below the diagonal are phylogenetically independent contrasts (Felsenstein 1985) assuming a speciation model of evolution (with the 95% confidence limits, based on random resolution of poorly supported nodes, shown in parentheses). SLA (last column) is specific leaf area. None of the significant correlations represents a trade-off in defenses (see *Results*).

\*  $P < 0.05$ , \*\* $P < 0.01$ ; these values are also highlighted in boldface type;  $df = 22$ .

†  $P \leq 0.1$ ;  $df = 22$ .

linkage and Euclidean distances, which combines subgroups (initially building from one species) at each iteration so as to minimize the within-cluster ANOVA sum of squares (Wilkinson 1997). Alternate joining algorithms provided qualitatively similar relationships. Clusters were designated by distances from nodes; nodes separated by a distance of  $<0.5$  were included in the same cluster, whereas nodes separated by  $>0.5$  were placed in separate clusters (Wilkinson 1997). We further examined the strength to which particular traits contributed to differences among clusters using discriminant function analysis (Wilkinson 1997).

To investigate whether factors related to geographical distribution were associated with the repeated emergence of trait combinations, we conducted a preliminary test for an association between geography and membership in the hierarchical clusters (Thaler and Karban 1997, Becerra and Venable 1999). Biogeographical associations of defense clusters were investigated by contingency table analysis. The bulk of species we studied have ranges in eastern North American (United States and Canada, east of the Tall Grass Prairie–Great Plains ecotone) or western North American (west of the ecotone) (Table 1).

Congruence between the ML phylogeny of *Asclepias* and hierarchical clustering of species based on defense traits was assessed using the test of Shimodaira and Hasegawa (SH test) (Shimodaira and Hasegawa 1999, Goldman et al. 2000), as implemented in PAUP\* 4.0b10 (Swofford 2001), using RELI resampling to estimate site likelihoods. Statistical incongruence between the phylogeny of *Asclepias* and clustering of species by defense traits would indicate that the evolution of defensive traits does not passively track phylogeny, but instead is evolutionarily labile, and suggests phylogenetically independent derivations of associations among traits. This approach evaluates whether associations among traits generate hierarchical similarity among taxa

that is statistically independent of phylogenetic relationships. Stochastically evolving traits will generate clusters of taxa statistically indistinguishable from the estimated phylogeny. Significant deviations in the phenotypic clustering could be caused by convergent adaptation of traits or other processes that generate trait correlations that are independent of association due to phylogenetic history. This test is similar in aim to tests of trait conservatism (e.g., Ackerly and Donoghue 1998, Cavender-Bares et al. 2004). An advantage to our approach is the ability to consider the associations among all traits simultaneously, with the concomitant disadvantage that the conservatism of a single trait cannot be assessed.

#### Effects of defense traits on caterpillars

To assess how individual plant traits and defense syndromes affect the performance of one natural feeder of milkweeds, we conducted a bioassay with monarch caterpillars (*Danaus plexippus*) (Fig. 1). We placed single eggs of freshly hatched caterpillars on all plants ( $n \approx 12$  for each of the 24 plant species) in the field. Caterpillars were from a laboratory colony established from local individuals collected the previous summer and maintained on frozen milkweed foliage. Each caterpillar was caged in a spun polyester bag (Rockingham Opportunities, Reidsville, North Carolina, USA) on the apical meristem with four fully expanded leaves. After five days, we recorded mortality and collected and weighed each living caterpillar.

To assess whether plant species that were in the three different defense clusters (see *Results*) showed differential resistance to monarch caterpillars, we conducted a one-way ANOVA. The species cluster was used as the factor (each species was assigned to one cluster) and mean monarch mass and percentage survival on each species were the response variables (total  $n = 24$ ). In addition, we employed multiple regression to assess the

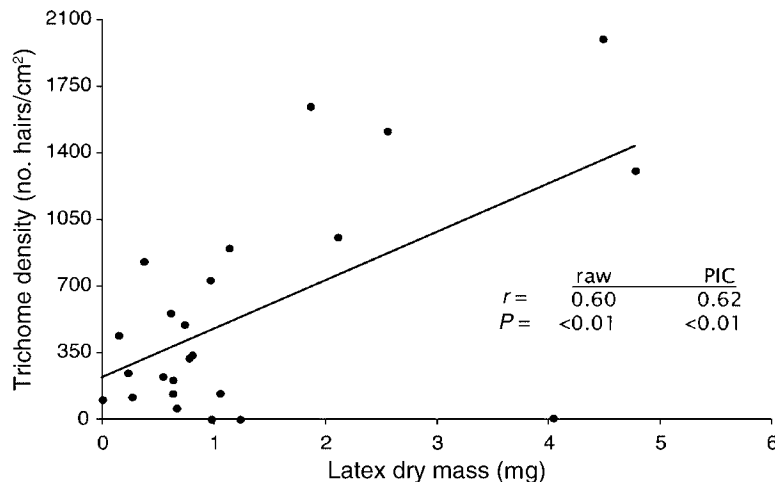


FIG. 3. Significant pairwise correlation between raw values of latex production and trichome density among 24 *Asclepias* species (see Table 2). Raw correlations and those of phylogenetically independent contrasts (PIC) (Felsenstein 1985) are significant and of similar magnitude.

effects of plant traits on monarch performance across the 24 species. For this analysis, we used species means for each of the seven traits (cardenolides, latex, trichomes, water content, specific leaf area, toughness, and C:N ratio) and mean caterpillar mass and percentage mortality as the response variables. We employed a stepwise multiple regression with backwards removal ( $P = 0.15$  to enter or remove) (Wilkinson 1997).

## RESULTS

### *Pairwise correlations between traits*

Three of the 21 raw pairwise correlations (and five based on phylogenetically independent contrasts, PICs) among seven defense-related traits were statistically significant (Table 3). This observed frequency of phylogenetically independent correlations is unlikely to have occurred by chance (binomial expansion test,  $P = 0.0028$ ) (Zar 1996). Each of the significant raw correlations involved just three traits showing complementary patterns of variation. Species with lower specific leaf areas (SLA) exhibited higher leaf trichome densities and latex production (Fig. 3). Species with lower SLA also exhibited lower water content ( $P = 0.06$ ), as expected (SLA is derived from leaf dry mass). Recall that SLA is a physiological plant trait, often indicative of rapid growth and high palatability to herbivores, and that low SLA might defend against herbivory. Leaves of species with high C:N ratios were also tougher, although this relationship was not significant ( $P = 0.13$ ). Thus, we found no indications of trade-offs among defense traits. Indeed, all correlations were “positive”; the significant negative correlations in Table 2 represent negative associations between nutritional quality and defense.

The phylogenetically independent correlations did not differ substantially from raw correlations (Table 3, Fig. 3). The phylogenetically independent correlation

between SLA and water content and between leaf C:N ratio and toughness were stronger and statistically significant compared to the raw correlations. In addition, a weak and nonsignificant negative correlation between leaf water content and toughness had a nearly significant phylogenetically independent correlation ( $P = 0.10$ ; Table 3).

Correlations based on PICs were generally robust to phylogenetic uncertainty (Table 3). For the latex–trichome, latex–SLA and SLA–percentage water correlations, every one of the randomly resolved topologies generated significant correlations. The trichome–SLA correlation was significant in just 41% of the random resolutions, although the upper bound of the 95% confidence limit ( $r = -0.295$ ) was still strongly negative. The C:N–toughness correlation was less robust, with only 12% of the random resolutions remaining significant. The only instance of a nonsignificant PIC correlation that was found to be significant in randomly resolved topologies was the C:N–trichome correlation, but this occurred in only 3% of the resolutions. Overall, little bias can be attributed to using the ML phylogeny in the face of weak support for a number of clades.

### *Assessing defense syndromes*

Our hierarchical cluster analysis of the seven defense-related traits revealed three distinct clusters (Fig. 4). For illustrative purposes only, in post hoc analyses, we determined that clusters differed strongly from one another (MANOVA results from discriminant function analysis: Wilks’ lambda = 0.033,  $F_{14,30} = 9.688$ ,  $P < 0.001$ ; see Appendix B). The analysis also indicates how each trait correlated with each discriminant function (Table 4); although most traits contributed substantially to at least one function, cardenolides and leaf toughness contributed the least to both. We also identified which traits were significantly heterogeneous among the three

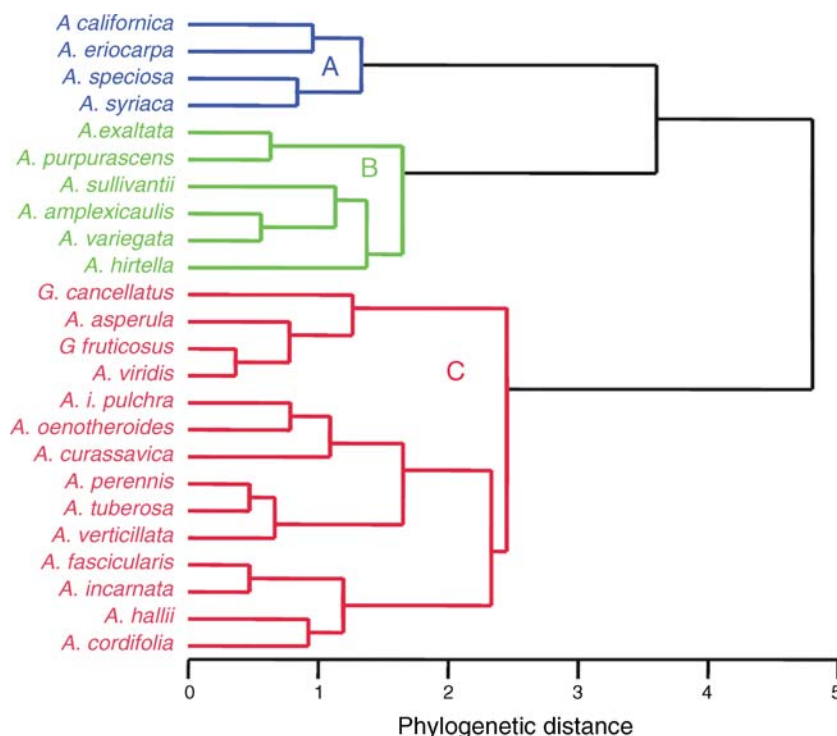


FIG. 4. A defense phenogram that depicts similarity among 24 species of *Asclepias* generated by hierarchical cluster analysis of seven defense-related traits. Tightly clustered species are defensively similar and can be considered to form defense syndromes, A–C (see *Results: Assessing defense syndromes*).

clusters (Table 4). These analyses revealed that most traits were expressed differently in the three defense clusters, and again cardenolides and leaf toughness were the least distinct.

The three clusters (Fig. 4, Table 4) represent species with combinations of: (A) high nitrogen (i.e., low C:N) coupled with high physical defense traits (trichomes, latex), (B) very high C:N ratio, coupled with tough leaves and low water content (hard to eat, little reward), and (C) low C:N and SLA coupled with high cardenolides. Although differences in cardenolides were not significantly different between clusters (Table 3), we found 17% and 50% higher levels in cluster C than in A

or B, respectively. Clusters A and C are strategies that both represent the coupling of a trait increasing the reward to herbivores (nitrogen or SLA) with high defense allocation. The three defense clusters were not significantly associated with distribution in eastern vs. western North America ( $\chi^2 = 8.77$ ,  $df = 5$ ,  $P = 0.12$ ).

#### Phylogenetic congruence test

The phylogenetic relationships suggested by the defense trait cluster analysis (Fig. 4) are significantly incongruent with relationships estimated from non-coding cpDNA sequences (Fig. 2). The difference in log likelihood [ $\ln(L)$ ] between the maximum likelihood

TABLE 4. The relationship between seven defensive traits and defensive clustering of milkweed species. Coefficients of standardized canonical discriminant functions (CDFs) indicate how each trait contributes to the two factors generated by discriminant analysis (Wilkinson 1997). Trait values (mean  $\pm$  SE) appear for the three defense syndromes (clusters) identified in this study. Significance of differences among traits tested by ANOVA.

	CDF1	CDF2	Cluster A (n = 4)	Cluster B (n = 6)	Cluster C (n = 14)	P
Latex (mg)	-0.495	0.404	3.13 $\pm$ 0.72	1.28 $\pm$ 0.56	0.31 $\pm$ 0.09	<0.001
Trichomes (no. hairs/cm <sup>2</sup> )	-0.907	0.316	1630.08 $\pm$ 146.57	355.40 $\pm$ 185.22	337.47 $\pm$ 67.11	<0.001
Toughness (g)	-0.089	0.285	111.08 $\pm$ 17.93	131.00 $\pm$ 12.01	104.86 $\pm$ 5.53	0.136
C:N	0.637	0.703	11.48 $\pm$ 0.08	14.09 $\pm$ 0.32	11.66 $\pm$ 0.21	<0.001
Water content (%)	0.102	-0.388	0.83 $\pm$ 0.01	0.81 $\pm$ 0.01	0.84 $\pm$ 0.01	0.088
Cardenolides (% dry mass)	-0.011	-0.008	4.97 $\pm$ 0.67	3.89 $\pm$ 0.76	5.85 $\pm$ 1.11	0.508
Specific leaf area (mm <sup>2</sup> /mg)	-0.456	-0.284	9.98 $\pm$ 0.69	10.41 $\pm$ 0.99	15.80 $\pm$ 1.02	0.002

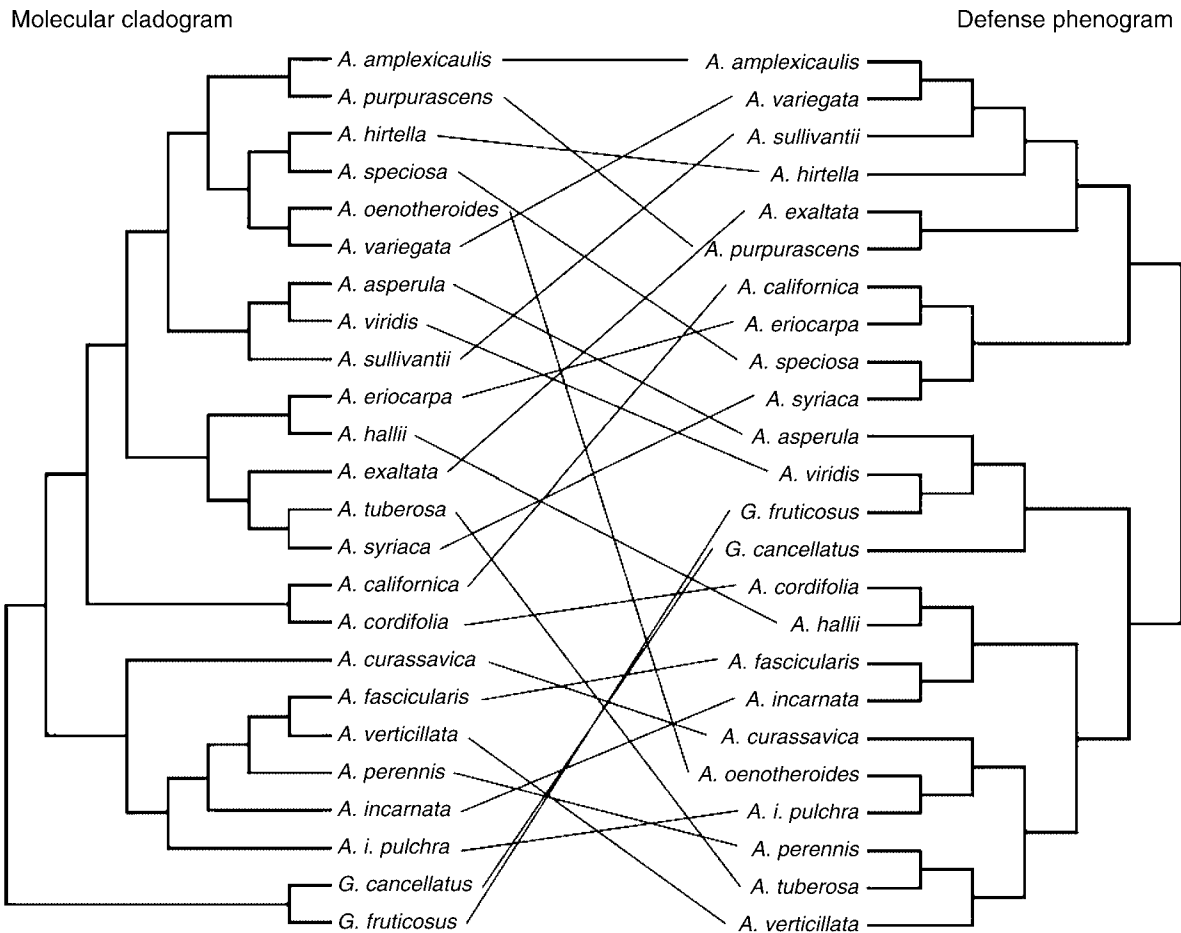


FIG. 5. Schematic depiction of the lack of congruence between the molecular phylogeny of *Asclepias* and the defense trait phenogram (see Fig. 4).

tree for the sequence data ( $-\ln(L) = 4225.61$ ) and the highest likelihood tree congruent with the phenogram ( $-\ln(L) = 4532.10$ ) was 306.49, which was highly significant (SH test:  $P < 0.05$ ). Thus, multivariate defense clusters did not come about only as a consequence of tracking the speciation history of *Asclepias* (Fig. 5).

#### *Effects of defense traits on caterpillars*

When plant species were classified by defense cluster (Fig. 4), we found no difference in the performance of monarch caterpillars among groups (mass,  $F_{2,20} = 2.460$ ,  $P = 0.111$ ; survival,  $F_{2,21} = 2.080$ ,  $P = 0.150$ ). Nonetheless, in multiple regression analyses, plant traits significantly explained monarch performance (Fig. 6) (overall model for mass,  $R^2 = 0.33$ ,  $F_{2,20} = 4.906$ ,  $P = 0.018$ , trichome coefficient =  $-0.130$ ,  $P = 0.022$ , toughness coefficient =  $-0.698$ ,  $P = 0.041$ ; overall model for survival,  $R^2 = 0.26$ ,  $F_{1,22} = 7.566$ ,  $P = 0.012$ , latex coefficient =  $0.060$ ,  $P = 0.012$ ). The latter positive correlation between latex and percentage of caterpillar

survival is difficult to explain and is discussed below (see the *Discussion*).

#### DISCUSSION

The concept of convergent expression of suites of traits acting as syndromes has been a useful framework for conceptualizing adaptations in ecological communities. For example, plant ecologists have proposed that specific suites of traits might be associated with particular types of environmental stress (Grime 1977, Chapin et al. 1993). In animal ecology, the guild concept is used to characterize species that exploit the same type of resources in a similar manner (Root 1967). Species forming a guild can share a common set of traits, but are explicitly not necessarily unified by phylogenetic relatedness. Nonetheless, in the past there was limited ability to disentangle the role of phylogenetic history and convergence in generating such species groups. For example, in the plant pollination literature, phylogenetic analyses of convergence on plant traits associated with bee and hummingbird pollination have only recently been employed (McDade 1992, Fenster et al. 2004,

Wilson et al. 2004). Other assessments of syndromes have also recently adopted a phylogenetically explicit approach (Cunningham et al. 1999, Fine et al. 2004). Thus, we see our concept of plant defense syndromes as an extension and phylogenetic modernization of classic plant defense theory (e.g., Feeny 1976).

#### Correlations between traits

In our study of *Asclepias*, none of the measured defense-related traits showed the bivariate trade-off predicted by traditional redundancy theory. Indeed, all of the pairwise correlations between defensive traits were positive; the significant correlations with a negative sign were between nutritional quality and defense, thus, not indicating a trade-off among traits providing resistance. Using phylogenetically independent contrasts among cotton (*Gossypium*) species, Rudgers et al. (2004) also found no trade-off between the production of extrafloral nectaries (EFNs) and trichomes, or between EFNs and chemical defense glands; however, they did find a negative association between trichomes and glands. This negative correlation could reflect allocation costs of the traits or redundancy in their ecological functions. Given that the costs and ecological functions of most defense-related traits are unknown, however, in this paper we have argued that any two traits should not be a priori predicted to negatively covary. This same conclusion has been reached in a recent meta-analysis of intraspecific genetic correlations of defense traits (Koricheva et al. 2004).

We found a positive (phylogenetically independent) correlation between plant production of latex and trichomes (Fig. 3). In an analysis of the correlation between the same two traits across 23 genetic families of *A. syriaca*, we found no correlation (Agrawal 2005). Although other intraspecific correlations are needed, Armbruster et al. (2004) and others have argued that such correlations across species, but not within species, are suggestive of adaptation over constraint. We speculate that this association could be due to the fact that latex is a water-intensive defense, and the protection from water loss by trichomes facilitates use of latex. Additionally, latex has widely been reported to function as a defense against monarch caterpillars and other milkweed herbivores (Dussourd and Eisner 1987, Zalucki et al. 2001, Agrawal and Van Zandt 2003, Agrawal 2004b, 2005), suggesting that the two strategies might be employed in concert to produce a synergistic defense. Although we did not detect a negative effect of latex on monarchs in the current study, it is perhaps in this situation where plants derive benefits from employing dual strategies. We speculate that the positive correlation we observed between latex production and percentage caterpillar survival is an artifact of a correlation between latex and some unmeasured trait. It is also possible that plant species with higher levels of latex prevented caterpillars from feeding, which protected them from death due to other plant defenses; in

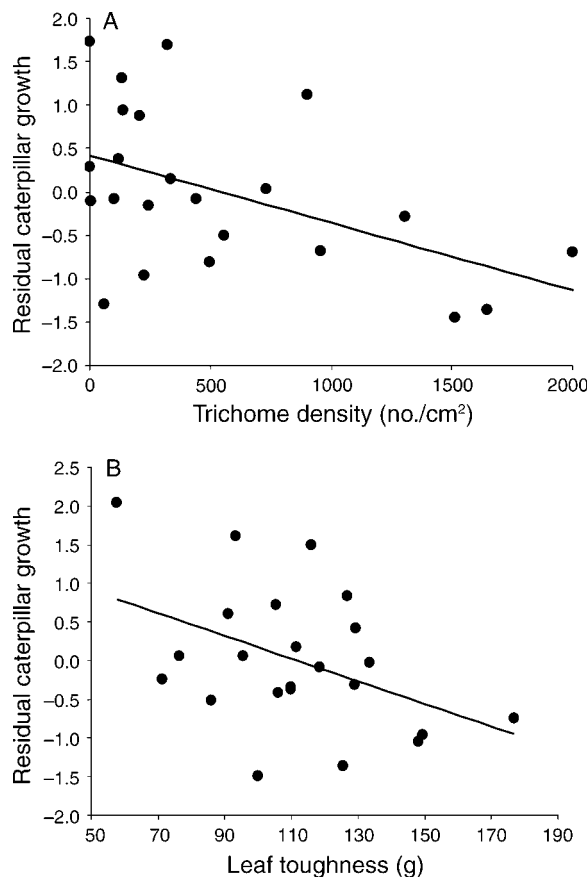


FIG. 6. Effects of milkweed defensive traits on monarch caterpillar growth. Seven plant traits were assayed, and only the significant predictors are shown: (A) trichome density and (B) leaf toughness. Residual growth refers to the residuals from the statistical model with the other factor in the analysis.

our short-term assays this protective effect is, thus, perhaps an artifact.

A notable outlier in the positive correlation between trichomes and latex (Fig. 3) was *A. sullivantii*, which has high latex production but no trichomes. Two other possible independent origins of species with high latex production coupled with the absence of trichomes are *A. humistrata* (southeastern USA) and a small clade of Mexican species (*A. glaucescens*, *A. elata*, *A. mirifica*, and *A. lynchiana*), neither of which were sampled in the current study. These species (including *A. sullivantii*) all have pronounced depositions of epicuticular wax (Fishbein 1996), which we speculate may be a substitute for trichomes in a physical defense syndrome.

Thus, in summary, the admittedly adaptationist hypothesis under the defense syndrome concept is not of specific a priori trade-offs, but a prediction that the multivariate trait complex is grouped such that costs are minimized and defense is maximized, given the biotic and abiotic environment of the species. Defense syndromes

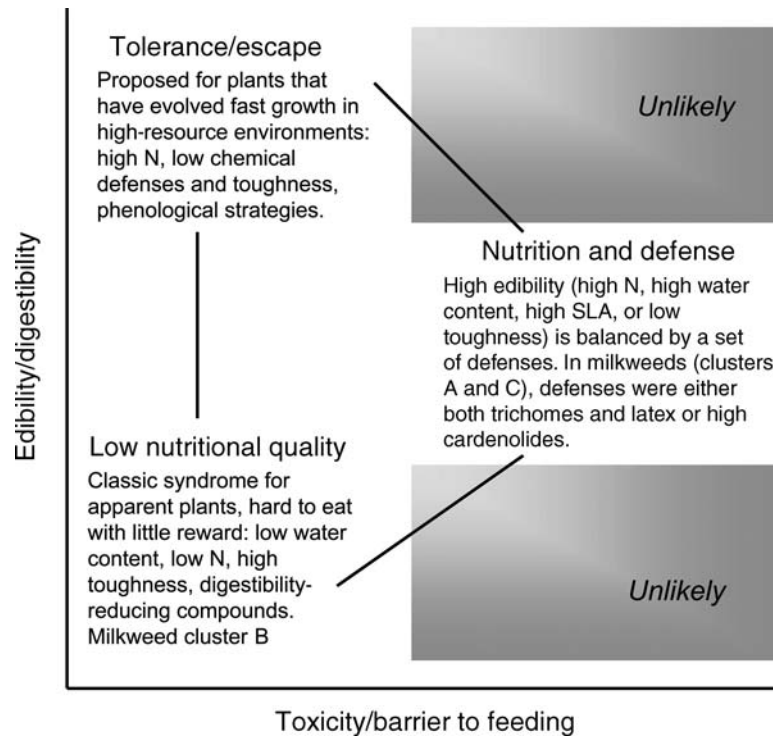


FIG. 7. The plant defense syndrome triangle. Low nutritional defense syndrome is consistent with that outlined for apparent plants by Feeny (1976); a similar group was found in this study. Tolerance follows the fast-growth and high-edibility pattern outlined by Coley (Coley et al. 1985, Kursar and Coley 2003). Nutrition and defense is a strategy that couples a toxic defense or barrier to feeding with relatively high edibility and digestibility. SLA denotes specific leaf area.

themselves might trade-off if they represent multivariate strategies targeted at alternative ecological interactions.

#### *Defense syndromes in milkweeds and beyond*

We distinguished three syndromes (clusters) in our multivariate analysis of seven defense-related traits, which grouped as plant species employing (A) high physical defenses, (B) low nutritional quality, and (C) putatively high chemical defense (Figs. 1 and 3). We found that, overall, our clustering of plant species by defense traits was not congruent with the molecular phylogeny of the group. At a minimum, this means that the defense characteristics of *Asclepias* are evolutionarily labile, and do not simply track phylogenetic history. For example, in the well-resolved group of three species that co-occur in eastern North America (*A. syriaca*, *A. tuberosa*, and *A. exaltata*) all three clusters are represented. In addition, it is apparent that some taxa, although distantly related, have converged on similar defense phenotypes.

Although we have not reconstructed the relative timing of changes in plant nutritional quality and expression of defense traits, we assume that when such traits are associated, changes in defense traits follow after divergences in nutrition traits rather than the reverse. For two of the three defense clusters (A and C in Fig. 4) there is an association between high resource

quality (from the herbivore's perspective) and high expression of defense traits. In cluster A, low C:N ratio (i.e., relatively high nitrogen levels) is coupled with high latex production and trichome density. In cluster C, both a low C:N ratio and high specific leaf area (SLA; i.e., relatively concentrated plant tissue) is coupled with higher levels of cardenolides than the other clusters. In the case of the cluster-A species, repeated evolution of a latex-trichome associated syndrome is statistically supported (Table 3, Fig. 3)

Although this analysis of *Asclepias* focused primarily on resistance, plant growth traits and tolerance are likely to be an important component of particular defense syndromes (van der Meijden et al. 1988, Fornoni et al. 2004). For example, Kursar and Coley (2003) made the argument that a trade-off between defense and growth promoted divergent strategies among tropical tree species. We offer the "defense syndrome triangle" hypothesis to include all defense categories (Fig. 7). Presumably other traits, such as plant traits that affect herbivore preference and attraction or facilitation of enemies of herbivores will also be important to integrate into the concept of plant defense syndromes.

Nearest to the origin of the two axes of edibility/digestibility and toxicity/barriers is the low nutritional quality syndrome (Fig. 7). This syndrome, empirically demonstrated in *Asclepias* (Syndrome B in Fig. 4, Table

4), very closely mirrors Feeny's (1976) grouping of defensive traits for apparent plants, and Coley et al.'s (1985, Kursar and Coley 2003) "defense syndrome" for plants that have evolved in low-resource environments. Note that the defense syndrome concept does not implicate a particular selective agent in generating the suite of adaptations. Feeny hypothesized that the selective agents were herbivores under the constraint of the probability of being found; Coley also hypothesized that herbivores were the selective agent; however, this was presumed to be constrained by the abiotic (resource) environment. The tolerance or escape syndrome (proposed by Kursar and Coley [2003], and not investigated in the current study) predicts a lack of resistance traits in very fast-growing and nutritive plants. Finally, our study revealed two syndromes with intermediate levels of edibility coupled with barriers to consumption (Syndromes A and C, Fig. 4, Table 4). In some ways, this mixed strategy is similar to Feeny's prediction for unapparent plants (i.e., qualitative defenses that cannot be overcome by generalist herbivores, but specialists feed on these plants with impunity). The important distinction is the implicit recognition that these toxins or barriers to consumption (latex, trichomes, cardenolides, etc), typically have a dose-dependent (or quantitative) effect on specialist herbivores (Berenbaum et al. 1989, Adler et al. 1995, Agrawal and Kurashige 2003, Agrawal and Van Zandt 2003, Agrawal 2004a, b, 2005). Plants with high levels of toxicity or barriers to feeding, however, are not predicted to have particularly high or low levels of nutritional quality (Fig. 7). We reach this conclusion based on the reasoning that plants with extreme investments in toxins should not have the ability to produce very nutritive tissues; likewise plants with very low nutritive quality should not need to invest in toxins.

#### Caveats

The performance of monarch butterfly caterpillars on the 24 milkweed species did not differ on plants from the three syndromes. This suggests that plant species might have alternate mechanisms to achieve similar levels of defense, and these might be driven by environmental differences in the habitats in which these species live naturally. Alternatively, our analysis should be viewed as preliminary and is limited by (1) our somewhat arbitrary distinction of clusters (see *Materials and methods: Effects of defense traits on caterpillars*), (2) unmeasured defense traits (epicuticular waxes, proteases, etc.), (3) a limited sample of species, and (4) limited information on the ecology of each species that would be informative for why particular defense syndromes were expressed across these species. Thus, further analyses are needed. In addition, our approach was to sample broadly across *Asclepias*, but more detailed analyses of well-resolved clades would also be valuable. Such fine-scale sampling would better control for the confounding effects of unmeasured traits by

limiting the time of divergence among species and permitting denser taxon sampling.

It is important to bear in mind that our working hypothesis for the phylogeny of *Asclepias* (M. Fishbein et al., *unpublished manuscript*) is not highly resolved with strong statistical support (see Fig. 2). The lack of resolution reflects the reality of the apparently rapid initial radiation of *Asclepias*. It is also important to consider the effect of our assumption of speciation evolution of morphology (i.e., equal morphological branch lengths across the tree) in calculating phylogenetically independent contrasts (PICs). Both of these factors could bias our estimates of evolutionary correlations among traits. We found that estimated correlations among PICs were very robust to uncertainty regarding the correct relationships in poorly supported regions of the tree. Conversely, the assumption concerning the mode of evolution of defense traits had a dramatic effect on the sign and magnitude of correlations. Ideally, an individual model of evolution should be developed for each trait in order to accurately estimate correlations (e.g., Pagel 1999, Lewis 2001). Because of the relatively small number of taxa (i.e., low sample size) and uncertainty regarding the correct phylogenetic relationships, we did not pursue individualized fitting of evolutionary models for the defense traits. We did attempt to analyze correlations assuming that rates of morphological evolution were accurately estimated by branch lengths inferred from the rates of nucleotide substitution in the cpDNA sequences used to estimate the tree. Correlations estimated under this assumption differed substantially from those reported here.

Although we are not comfortable assuming equal branch lengths across the tree (which is almost certainly incorrect), we prefer this assumption to the use of molecular branch lengths. Generally, there is little evidence for correspondence between rates of molecular and morphological evolution (Bromham et al. 2002). Specifically, the extreme branch length heterogeneity for the molecular data is likely unreasonable for the morphological traits under study here. Species of *Asclepias* that exhibit very low levels of divergence in cpDNA sequences can be dramatically different in morphology, whereas species that differ only subtly (*A. fascicularis*, *A. verticillata*, *A. perennis*, and *A. incarnata*) are separated by branches that are orders of magnitude longer than those separating morphologically diverse species (see Fig. 2). Given the apparently poor correspondence between rates of molecular and morphological evolution, we prefer the assumption of speciation changes in morphology over any assumption about specific rates across lineages.

#### Directions

We see the identification of syndromes as a starting point to test alternative hypotheses for why plant defenses have converged. In this regard, the biotic and

abiotic environment could conspire to drive particular syndromes. For example, plant trichomes are known to have many functions, including defense against herbivory as well as a barrier against evapotranspirative water loss (e.g., Woodman and Fernandes 1991, Agrawal and Spiller 2004). Thus, trichomes might be particular to plant species in environments with low water availability (i.e., deserts) and subject to particular types of herbivores. We have shown here that trichomes are a significant barrier to growth of monarch caterpillars, but benefit some aphids (Agrawal 2004a). Thus, trichomes might only be favored in particular combinations of biotic and abiotic conditions.

We suggest working toward formalizing hypotheses about the particular types of herbivores that likely drive the evolution of particular syndromes. It might be that plants that share guilds of herbivores (i.e., those that attack the same plants in a similar way) defend in a similar manner. The example described initially (see *Introduction*), that of plants defending against vertebrate megafaunal grazers vs. insect herbivores, serves as a starting point. For North American *Asclepias*, the herbivore fauna of some species is >12 insect herbivore species that eat every plant part, from the flowers and seed pods, to the stems and leaves, to the phloem and roots.

But all milkweeds are not subject to herbivory by all insect species, and levels of attack presumably vary considerably. For example, the very damaging stem weevil (*Rhysomatus lineaticollis*) is primarily known from *A. syriaca*, and there apparently are not analogous herbivores on other milkweeds (Agrawal and Van Zandt 2003). Twenty-four species of root-boring *Tetraopes* beetles attack a single species of milkweed each; apparently the ~100 other North American milkweeds are not attacked in this way. Monarch butterflies only attack a subset of the milkweed flora, probably because their flight paths do not overlap with all species, and some species inhabit forest understories or occur as widely scattered individuals. Given that herbivore guilds vary on plant species and there is a high level of specificity in the effects of particular plant defenses on particular herbivores (e.g., Da Costa and Jones 1971, Giamoustaris and Mithen 1995, Van Dam and Hare 1998, Agrawal and Karban 2000, Van Zandt and Agrawal 2004a, b), we predict that future studies will find that certain herbivore species or guilds have been critical in generating patterns of defense. In identifying such convergent plant defense syndromes, we may finally understand the evolutionary association between herbivore communities and adaptive variation in plant species.

#### ACKNOWLEDGMENTS

We thank Rowan Barrett, Erin Douthit, Dana Farmer, Karen Hooper, Deborah Hopp, Shelley McMahon, Michael Moody, Lisa Plane, Ana Lilia Reina G., Karin Rotem, Victor Steinmann, Deborah Tam, Jennifer Thaler, Tom Van Dender, Pete Van Zandt, and Sergio Zamudio for help in the lab

and field; Robie Mason-Gamer and Steve Lynch for contributions to the phylogenetic study of *Asclepias*; David Goyder, Mark Chase, and Marlin Bowles for providing DNA samples; Bobby Gendron for seeds; Steve Malcolm for discussions on cardenolide analysis; and Paul Fine, Marc Johnson, Rick Karban, Marc Lajeunesse, Peter Price, Jennifer and Jon Thaler, and Cam Webb for comments and discussion. This research and our laboratories are supported by Natural Science and Engineering Research Council of Canada, the U.S. National Science Foundation, and the U.S. Department of Agriculture.

#### LITERATURE CITED

- Ackerly, D. D., and M. J. Donoghue. 1998. Leaf size, sapling allometry, and Corner's rules: phylogeny and correlated evolution in maples (*Acer*). *American Naturalist* **152**:767–791.
- Adler, L. S., J. Schmitt, and M. D. Bowers. 1995. Genetic variation in defensive chemistry in *Plantago lanceolata* (Plantaginaceae) and its effect on the specialist herbivore *Junonia coenia* (Nymphalidae). *Oecologia* **101**:75–85.
- Agrawal, A. A. 2004a. Plant defense and density dependence in the population growth of herbivores. *American Naturalist* **164**:113–120.
- Agrawal, A. A. 2004b. Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. *Ecology* **85**:2118–2133.
- Agrawal, A. A. 2005. Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. *Evolutionary Ecology Research* **7**:651–667.
- Agrawal, A., and R. Karban. 2000. Specificity of constitutive and induced resistance: pigment glands influence mites and caterpillars on cotton plants. *Entomologia Experimentalis et Applicata* **96**:39–49.
- Agrawal, A. A., and N. S. Kurashige. 2003. A role for isothiocyanates in plant resistance against the specialist herbivore *Pieris rapae*. *Journal of Chemical Ecology* **29**:1403–1415.
- Agrawal, A. A., and S. B. Malcolm. 2002. Once upon a milkweed. *Natural History* **111**(7):48–53.
- Agrawal, A. A., and D. A. Spiller. 2004. Polymorphic buttonwood: effects of disturbance on resistance to herbivores in green and silver morphs of a Bahamian shrub. *American Journal of Botany* **91**:1990–1997.
- Agrawal, A. A., and P. A. Van Zandt. 2003. Ecological play in the coevolutionary theatre: genetic and environmental determinants of attack by a specialist weevil on milkweed. *Journal of Ecology* **91**:1049–1059.
- Armbruster, W. S., C. Pélabon, T. F. Hansen, and C. P. H. Mulder. 2004. Floral integration, modularity, and accuracy. Pages 23–49 in M. Pigliucci and K. Preston, editors. *Phenotypic integration*. Oxford University Press, Oxford, UK.
- Beardsley, P. M., A. Yen, and R. G. Olmstead. 2003. AFLP phylogeny of *Mimulus* section *Erythranthe* and the evolution of hummingbird pollination. *Evolution* **57**:1397–1410.
- Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. *Science* **276**:253–256.
- Becerra, J. X., and D. L. Venable. 1999. Macroevolution of insect–plant associations: the relevance of host biogeography to host affiliation. *Proceedings of the National Academy of Sciences (USA)* **96**:12626–12631.
- Becerra, J. X., D. L. Venable, P. H. Evans, and W. S. Bowers. 2001. Interactions between chemical and mechanical defenses in the plant genus *Bursera* and their implications for herbivores. *American Zoologist* **41**:865–876.
- Bennett, R. N., and R. M. Wallsgrove. 1994. Tansley review no. 72. Secondary metabolites in plant defence mechanisms. *New Phytologist* **127**:617–633.
- Berenbaum, M. R. 2001. Chemical mediation of coevolution: phylogenetic evidence for Apiaceae and associates. *Annals of the Missouri Botanical Garden* **88**:45–59.

- Berenbaum, M. R., J. K. Nitao, and A. R. Zangerl. 1991. Adaptive significance of furanocoumarin diversity in *Pastinaca sativa* (Apiaceae). *Journal of Chemical Ecology* **17**:207–215.
- Berenbaum, M. R., A. R. Zangerl, and K. Lee. 1989. Chemical barriers to adaptation by a specialist herbivore. *Oecologia* **80**:501–506.
- Berenbaum, M. R., A. R. Zangerl, and J. K. Nitao. 1986. Constraints on chemical coevolution: wild parsnips and the parsnip webworm. *Evolution* **40**:1215–1228.
- Blakley, N. R., and H. Dingle. 1978. Competition: butterflies eliminate milkweed bugs from a Caribbean island. *Oecologia* **37**:133–136.
- Bollwinkel, C. W. 1969. A revision of the South American species of *Asclepias* L. Dissertation. Southern Illinois University, Carbondale, Illinois, USA.
- Broadway, R. M., and S. S. Duffey. 1988. The effects of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors. *Journal of Insect Physiology* **34**:1111–1118.
- Bromham, L., M. Woolfit, M. S. Y. Lee, and A. Rambaut. 2002. Testing the relationships between morphological and molecular rates of change along phylogenies. *Evolution* **56**:1921–1930.
- Brower, L. P., P. B. McEvoy, K. L. Williamson, and M. A. Flannery. 1972. Variation in cardiac glycoside content of monarch butterflies from natural populations in eastern North America. *Science* **177**:426–429.
- Brower, L. P., J. Van Zandt, and J. M. Corvino. 1967. Plant poisons in a terrestrial food chain. *Proceedings of the National Academy of Science (USA)* **57**:893–898.
- Bryant, J. P., F. S. Chapin, and D. R. Klein. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* **40**:357–368.
- Castellanos, M. C., P. Wilson, and J. D. Thomson. 2003. Pollen transfer by hummingbirds and bumblebees, and the divergence of pollination modes in *Penstemon*. *Evolution* **57**:2742–2752.
- Castellanos, M. C., P. Wilson, and J. D. Thomson. 2004. “Anti-bee” and “pro-bird” changes during the evolution of hummingbird pollination in *Penstemon* flowers. *Journal of Evolutionary Biology* **17**:876–885.
- Cavender-Bares, J., D. D. Ackerly, D. A. Baum, and F. A. Bazzaz. 2004. Phylogenetic overdispersion in Floridian oak communities. *American Naturalist* **163**:823–843.
- Chapin, F. S., K. Autumn, and F. Pugnaire. 1993. Evolution of suites of traits in response to environmental stress. *American Naturalist* **142**:S78–S92.
- Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* **53**:209–233.
- Coley, P. D. 1987. Interspecific variation in plant anti-herbivore properties: the role of habitat quality and rate of disturbance. *New Phytologist* **106**:251–264.
- Coley, P. D., J. P. Bryant, and F. S. Chapin. 1985. Resource availability and plant antiherbivore defense. *Science* **230**:895–899.
- Conway-Morris, S. 2003. *Life's solution: inevitable humans in a lonely universe*. Cambridge University Press, Cambridge, UK.
- Cunningham, S. A., B. Summerhayes, and M. Westoby. 1999. Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecological Monographs* **69**:569–588.
- Da Costa, C. P., and C. M. Jones. 1971. Cucumber beetle resistance and mite susceptibility controlled by the bitter gene in *Cucumis sativus* L. *Science* **172**:1145–1146.
- Dobzhansky, T. G. 1970. *Genetics of the evolutionary process*. Columbia University Press, New York, New York, USA.
- Duffey, S. S., and M. J. Stout. 1996. Antinutritive and toxic components of plant defense against insects. *Archives of Insect Biochemistry and Physiology* **32**:3–37.
- Dussourd, D. E., and T. Eisner. 1987. Vein-cutting behavior: insect counterploy to the latex defense of plants. *Science* **237**:898–900.
- Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. *Evolution* **18**:586–608.
- Fægri, K., and L. van der Pijl. 1979. *The principles of pollination ecology*. Third edition. Pergamon Press, New York, New York, USA.
- Farrell, B. D., D. E. Dussourd, and C. Mitter. 1991. Escalation of plant defense: Do latex and resin canals spur plant diversification? *American Naturalist* **138**:881–900.
- Farrell, B. D., and C. Mitter. 1993. Phylogenetic determinants of insect/plant community diversity. Pages 253–266 in R. Ricklefs and D. Schluter, editors. *Historical and geographical determinants of community diversity*. University of Chicago Press, Chicago, Illinois, USA.
- Farrell, B. D., and C. Mitter. 1998. The timing of insect–plant diversification: Might *Tetraopes* (Coleoptera: Cerambycidae) and *Asclepias* (Asclepiadaceae) have co-evolved? *Biological Journal of the Linnean Society* **63**:553–577.
- Feeny, P. P. 1976. Plant apparency and chemical defense. Pages 1–40 in J. W. Wallace and R. L. Mansell, editors. *Biochemical interaction between plants and insects*. Plenum, New York, New York, USA.
- Felsenstein, J. 1985. Phylogenies and the comparative method. *American Naturalist* **125**:1–15.
- Fenster, C. B., W. S. Armbruster, M. R. Dudash, P. Wilson, and J. D. Thomson. 2004. Pollination syndromes and floral specialization. *Annual Review of Ecology and Systematics* **35**:375–403.
- Fine, P. V. A., I. Mesones, and P. D. Coley. 2004. Herbivores promote habitat specialization by trees in Amazonian forests. *Science* **305**:663–665.
- Fine, P. V. A., Z. J. Miller, I. Mesones, S. Irazuzta, H. M. Appel, M. H. H. Stevens, I. Sääksjärvi, J. C. Schultz, and P. D. Coley. 2006. The growth–defense trade-off and habitat specialization by plants in Amazonian forests. *Ecology* **87**:S150–S162.
- Fishbein, M. 1996. *Phylogenetic relationships of North American Asclepias L. and the role of pollinators in the evolution of the milkweed inflorescence*. Dissertation, University of Arizona, Tucson, Arizona, USA.
- Fishbein, M., C. Hirsch-Jetter, D. E. Soltis, and L. Hufford. 2001. Phylogeny of Saxifragales (angiosperms, eudicots): analysis of a rapid, ancient radiation. *Systematic Biology* **50**:817–847.
- Fishbein, M., and D. E. Soltis. 2004. Further resolution of the rapid radiation of Saxifragales (angiosperms, eudicots) supported by mixed-model Bayesian analysis. *Systematic Botany* **29**:883–891.
- Fordyce, J. A., and S. B. Malcolm. 2000. Specialist weevil, *Rhyssomatus lineaticollis*, does not spatially avoid cardenolide defenses of common milkweed by ovipositing into pith tissue. *Journal of Chemical Ecology* **26**:2857–2874.
- Fornoni, J., P. L. Valverde, and J. Núñez-Farfán. 2004. Population variation in the cost and benefit of tolerance and resistance against herbivory in *Datura stramonium*. *Evolution* **58**:1696–1704.
- Giamoustaris, A., and R. Mithen. 1995. The effect of modifying the glucosinolate content of leaves of oilseed rape (*Brassica napus* ssp. *oleifera*) on its interaction with specialist and generalist pests. *Annals of Applied Biology* **126**:347–363.
- Goldman, N., J. P. Anderson, and A. G. Rodrigo. 2000. Likelihood-based tests of topologies in phylogenetics. *Systematic Biology* **49**:652–670.
- Grime, J. P. 1977. Evidence for existence of three primary strategies in plants and its relevance to ecological and evolutionary theory. *American Naturalist* **111**:1169–1194.
- Gunasena, G. H., S. B. Vinson, H. J. Williams, and R. D. Stipanovic. 1988. Effects of caryophyllene, caryophyllene oxide, and their interaction with gossypol on the growth and

- development of *Heliothis virescens* (F) (Lepidoptera, Noctuidae). *Journal of Economic Entomology* **81**:93–97.
- Haddad, N. M., and W. M. Hicks. 2000. Host pubescence and the behavior and performance of the butterfly *Papilio troilus* (Lepidoptera: Papilionidae). *Environmental Entomology* **29**: 299–303.
- Heil, M., T. Delsinne, A. Hilpert, S. Schurkens, C. Andary, K. E. Linsenmair, M. S. Sousa, and D. McKey. 2002. Reduced chemical defence in ant-plants? A critical re-evaluation of a widely accepted hypothesis. *Oikos* **99**:457–468.
- Heil, M., S. Greiner, H. Meimberg, R. Kruger, J.-L. Noyer, G. Heubl, K. E. Linsenmair, and W. Boland. 2004. Evolutionary change from induced to constitutive expression of an indirect plant resistance. *Nature* **430**:205–208.
- Heil, M., C. Staehelin, and D. McKey. 2000. Low chitinase activity in *Acacia* myrmecophytes: A potential trade-off between biotic and chemical defences? *Naturwissenschaften* **87**:555–558.
- Hermis, D. A., and W. J. Mattson. 1992. The dilemma of plants: to grow or defend. *Quarterly Review of Biology* **67**:283–335.
- Housworth, E. A., and E. P. Martins. 2001. Random sampling of constrained phylogenies: conducting phylogenetic analyses when the phylogeny is partially known. *Systematic Biology* **50**:628–639.
- Janzen, D. H. 1974. Tropical blackwater rivers, animals, and mast fruiting by the Dipterocarpaceae. *Biotropica* **6**:69–103.
- Koricheva, J., H. Nykänen, and E. Gianoli. 2004. Meta-analysis of trade-offs among plant antiherbivore defenses: Are plants jacks-of-all-trades, masters of all? *American Naturalist* **163**:E64–E75.
- Kursar, T. A., and P. D. Coley. 2003. Convergence in defense syndromes of young leaves in tropical rainforests. *Biochemical Systematics and Ecology* **31**:929–949.
- Lavoie, B., and K. S. Oberhauser. 2004. Compensatory feeding in *Danaus plexippus* (Lepidoptera: Nymphalidae) in response to variation in host plant quality. *Environmental Entomology* **33**:1062–1069.
- Legendre, P., and L. Legendre. 1998. Numerical ecology. Second English edition. Elsevier Science BV, Amsterdam, The Netherlands.
- Lewis, P. L. 2001. Maximum likelihood phylogenetic inference: modeling discrete morphological characters. *Systematic Biology* **50**:913–925.
- Maddison, D. R., and W. P. Maddison. 2003. MacClade. Version 4.06. Sinauer Associates, Sunderland, Massachusetts, USA.
- Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores. Pages 251–296 in G. A. Rosenthal and M. R. Berenbaum, editors. *Herbivores: their interactions with secondary plant metabolites*. Volume 1. Academic Press, San Diego, California, USA.
- Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca* L. *Journal of Chemical Ecology* **15**:819–853.
- Mattson, W. J., Jr. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology and Systematics* **11**:119–161.
- McDade, L. A. 1992. Pollinator relationships, biogeography, and phylogenetics. *BioScience* **42**:21–26.
- Mooers, A. Ø., S. M. Vamosi, and D. Schluter. 1999. Using phylogenies to test macroevolutionary hypotheses of trait evolution in cranes (Gruinae). *American Naturalist* **154**:249–259.
- Morse, D. H. 1985. Milkweeds and their visitors. *Scientific American* **253**:112–119.
- Nelson, C. J. 1993. Sequestration and storage of cardenolides and cardenolide glycosides by *Danaus plexippus* and *D. chrysippus petila* when reared on *Asclepias fruticosa*: with a review of some factors that influence sequestration. Pages 91–105 in S. B. Malcolm and M. P. Zalucki, editors. *Biology and conservation of the monarch butterfly*. Natural History Museum of Los Angeles County, Los Angeles, California, USA.
- Page, M. 1999. The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Systematic Biology* **48**:612–622.
- Price, P., and M. Willson. 1979. Abundance of herbivores on six milkweed species in Illinois. *American Midland Naturalist* **101**:76–86.
- Rapini, A., M. W. Chase, D. J. Goyder, and J. Griffiths. 2003. Asclepiadeae classification: evaluating the phylogenetic relationships of New World Asclepiadoideae (Apocynaceae). *Taxon* **52**:33–50.
- Rehr, S. S., P. P. Feeny, and D. H. Janzen. 1973. Chemical defence in Central American non-ant-Acacias. *Journal of Animal Ecology* **42**:405–416.
- Romeo, J. T., J. A. Saunders, and P. Barbosa, editors. 1996. *Phytochemical diversity and redundancy in ecological interactions*. Plenum Press, New York, New York, USA.
- Ronquist, F., and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**:1572–1574.
- Root, R. B. 1967. The niche exploitation pattern of the Blue-gray Gnatcatcher. *Ecological Monographs* **37**:317–350.
- Rothschild, M., J. V. Ew, and T. Reichste. 1970. Cardiac glycosides in Oleander aphid, *Aphis nerii*. *Journal of Insect Physiology* **16**:1141–1145.
- Rudgers, J. A., S. Y. Strauss, and J. F. Wendel. 2004. Trade-offs among anti-herbivore resistance traits: insights from Gossypieae (Malvaceae). *American Journal of Botany* **91**: 871–880.
- Schädler, M., G. Jung, H. Auge, and R. Brandl. 2003. Palatability, decomposition and insect herbivory: patterns in a successional old-field plant community. *Oikos* **103**:121–132.
- Schemske, D. W., and H. D. Bradshaw. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (*Mimulus*). *Proceedings of the National Academy of Sciences (USA)* **96**:11910–11915.
- Schluter, D. 2000. *The ecology of adaptive radiation*. Oxford University Press, Oxford, UK.
- Seigler, D. S., and J. E. Ebinger. 1987. Cyanogenic glycosides in ant-Acacias of Mexico and Central America. *Southwestern Naturalist* **32**:499–503.
- Shimodaira, H., and M. Hasegawa. 1999. Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Molecular Biology and Evolution* **16**:1114–1116.
- Silvertown, J., and M. Dodd. 1996. Comparing plants and connecting traits. *Philosophical Transactions of the Royal Society of London B* **351**:1233–1239.
- Simberloff, D., and T. Dayan. 1991. The guild concept and the structure of ecological communities. *Annual Review of Ecology and Systematics* **22**:115–143.
- Stapley, L. 1998. The interaction of thorns and symbiotic ants as an effective defence mechanism of swollen-thorn acacias. *Oecologia* **115**:401–405.
- Steward, J. L., and K. H. Keeler. 1988. Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae)? *Oikos* **53**:79–86.
- Swofford, D. L. 2001. PAUP\*. Phylogenetic analysis using parsimony (\*and other methods). Version 4. Sinauer Associates, Sunderland, Massachusetts, USA.
- Thaler, J. S., and R. Karban. 1997. A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium*. *American Naturalist* **149**:1139–1146.
- Thomson, J. D., P. Wilson, M. Valenzuela, and M. Malzone. 2000. Pollen presentation and pollination syndromes, with special reference to *Penstemon*. *Plant Species Biology* **15**:11–29.
- Tilman, D., and S. Pacala. 1993. The maintenance of species richness in plant communities. Pages 13–25 in R. Ricklefs and

- D. Schuster, editors. Species diversity in ecological communities. University of Chicago Press, Chicago, Illinois, USA.
- Twigg, L. E., and L. V. Socha. 1996. Physical versus chemical defence mechanisms in toxic *Gastrolobium*. *Oecologia* **108**: 21–28.
- Van Dam, N. M., and J. D. Hare. 1998. Differences in distribution and performance of two sap-sucking herbivores on glandular and non-glandular *Datura wrightii*. *Ecological Entomology* **23**:22–32.
- van der Meijden, E., M. Wijn, and H. J. Verkaar. 1988. Defense and regrowth, alternative plant strategies in the struggle against herbivores. *Oikos* **51**:355–363.
- Van Zandt, P. A., and A. A. Agrawal. 2004a. Specificity of induced plant responses to specialist herbivores of the common milkweed, *Asclepias syriaca*. *Oikos* **104**:401–409.
- Van Zandt, P. A., and A. A. Agrawal. 2004b. Community-wide impacts of herbivore-induced plant responses in milkweed (*Asclepias syriaca*). *Ecology* **85**:2616–2629.
- Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. *Ecology* **77**:1043–1060.
- Weiss, H. B., and E. L. Dickerson. 1921. Notes on milkweed insects in New Jersey. *Journal of the New York Entomological Society* **20**:123–145.
- Wilbur, H. 1976. Life history evolution in seven milkweeds of the genus *Asclepias*. *Journal of Ecology* **64**:223–240.
- Wilkinson, L. 1997. SYSTAT 7.0: the system for statistics. SPSS, Chicago, Illinois, USA.
- Wilson, P., M. C. Castellanos, J. N. Hogue, J. D. Thomson, and W. S. Armbruster. 2004. A multivariate search for pollination syndromes among penstemons. *Oikos* **104**:345–361.
- Woodman, R. L., and G. W. Fernandes. 1991. Differential mechanical defense: herbivory, evapotranspiration, and leaf hairs. *Oikos* **60**:11–19.
- Woodson, R. E., Jr. 1954. The North American species of *Asclepias* L. *Annals of the Missouri Botanical Garden* **41**:1–211.
- Zalucki, M. P., L. P. Brower, and A. Alonso. 2001. Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology* **26**:212–224.
- Zar, J. H. 1996. Biostatistical analysis. Prentice Hall, Englewood Cliffs, New Jersey, USA.

#### APPENDIX A

Means and standard errors for seven defense-related traits of the 24 species of milkweed employed in this study (*Ecological Archives* E087-116-A1).

#### APPENDIX B

The three defense clusters separated in multivariate space (*Ecological Archives* E087-116-A2).

Ecological Archives Appendix A. Means and standard errors (in parentheses) for seven defense related traits of the 24 species of milkweed employed in this study.

	Latex (mgs)	Trichomes (hairs/cm <sup>2</sup> )	Toughness (g force)	C-N Ratio	% Water	Cardenolides (% dry mass)	Specific leaf area (mm <sup>2</sup> /mg)
<i>A. amplexicaulis</i>	1.23 (0.15)	0.00 (0.00)	129.00 (9.54)	14.42 (0.91)	80.92 (1.27)	0.15 (0.10)	11.05 (0.61)
<i>A. asperula</i>	0.78 (0.25)	324.17 (42.44)	116.00 (18.21)	11.72 (0.52)	82.47 (1.10)	0.72 (0.09)	13.96 (1.03)
<i>A. californica</i>	1.86 (0.58)	1660.30 (217.88)	139.00 (10.54)	11.65 (0.26)	82.39 (0.97)	0.24 (0.03)	10.93 (1.53)
<i>A. cordifolia</i>	0.98 (0.16)	0.59 (0.59)	57.50 (4.90)	10.52 (0.37)	83.31 (1.04)	0.16 (0.04)	13.73 (1.92)
<i>A. curassavica</i>	0.74 (0.27)	501.80 (38.00)	86.00 (4.88)	11.36 (0.31)	89.53 (0.39)	0.29 (0.07)	19.14 (2.26)
<i>A. eriocarpa</i>	4.78 (0.96)	1320.40 (307.53)	133.50 (6.24)	11.47 (0.50)	80.99 (1.53)	0.36 (0.07)	8.38 (0.47)
<i>A. exaltata</i>	1.04 (0.26)	994.76 (243.32)	93.50 (9.31)	15.19 (0.89)	78.84 (0.46)	0.13 (0.03)	9.57 (0.32)
<i>A. fascicularis</i>	0.66 (0.22)	58.32 (15.65)	100.00 (13.27)	11.08 (0.85)	78.30 (1.68)	0.18 (0.03)	12.40 (1.22)
<i>A. hallii</i>	0.97 (0.21)	737.89 (132.43)	111.50 (11.21)	10.30 (0.56)	84.64 (0.34)	0.18 (0.04)	13.26 (0.60)
<i>A. hirtella</i>	0.27 (0.08)	118.56 (29.86)	177.00 (29.88)	12.82 (0.36)	81.27 (0.87)	0.41 (0.08)	14.90 (0.66)
<i>A. incarnata incarnata</i>	0.54 (0.16)	226.61 (26.28)	125.50 (7.73)	10.97 (0.53)	80.32 (0.61)	0.16 (0.04)	12.49 (0.49)
<i>A. incarnata pulchra</i>	0.37 (0.10)	836.57 (161.63)	130.00 (14.24)	12.93 (1.48)	86.21 (0.44)	0.12 (0.02)	11.88 (1.82)
<i>A. oenotheroides</i>	1.05 (0.42)	137.27 (43.59)	129.50 (16.42)	12.46 (0.61)	87.05 (1.26)	0.19 (0.04)	18.12 (2.57)
<i>A. perennis</i>	0.00 (0.17)	104.42 (10.29)	76.50 (7.82)	12.47 (0.45)	84.15 (0.78)	0.31 (0.10)	21.72 (2.06)
<i>A. purpurascens</i>	2.11 (0.27)	966.04 (175.57)	110.00 (11.43)	13.72 (1.17)	78.81 (2.98)	0.19 (0.03)	7.91 (0.14)
<i>A. speciosa</i>	4.49 (0.30)	2019.26 (355.36)	91.00 (8.19)	11.29 (0.29)	85.17 (0.47)	0.19 (0.04)	9.30 (0.45)
<i>A. sullivantii</i>	4.04 (1.25)	6.01 (3.77)	149.50 (8.31)	14.35 (2.10)	81.11 (0.33)	0.19 (0.04)	9.26 (0.40)
<i>A. syriaca</i>	1.61 (0.58)	1509.81 (170.85)	71.50 (4.22)	11.52 (0.48)	83.15 (0.55)	0.27 (0.03)	11.30 (0.68)
<i>A. tuberosa</i>	0.15 (0.14)	491.34 (102.40)	95.50 (14.61)	12.59 (0.71)	83.80 (0.98)	0.19 (0.04)	19.76 (2.86)
<i>A. variegata</i>	1.32 (0.41)	135.80 (25.22)	127.00 (16.40)	14.01 (0.60)	81.68 (0.83)	0.15 (0.03)	9.74 (0.06)
<i>A. verticillata</i>	0.63 (0.17)	208.41 (45.18)	105.56 (9.63)	11.79 (0.50)	81.52 (1.07)	0.21 (0.05)	23.35 (2.22)
<i>A. viridis</i>	0.81 (0.23)	340.96 (61.33)	118.50 (6.41)	11.82 (0.54)	84.50 (1.45)	0.34 (0.06)	12.54 (1.25)
<i>G. cancellatus</i>	0.61 (0.08)	562.77 (120.72)	106.00 (11.54)	11.48 (0.48)	87.49 (0.95)	0.93 (0.06)	14.71 (1.65)
<i>G. fruticosus</i>	0.23 (0.14)	245.96 (91.46)	110.00 (6.67)	11.76 (0.54)	83.85 (1.61)	0.47 (0.06)	14.15 (1.17)

Anurag A. Agrawal and Mark Fishbein. 2006. Plant defense syndromes. Ecology

Ecological Archives Appendix B. The three defense clusters separated in multivariate space. A discriminant function analysis explained among-cluster variation in the seven defensive traits with two factors (shown on the X and Y axis). Letters inside the clouds correspond to the syndromes identified in the text.

