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Evolution of Specialization: A Phylogenetic Study of Host Range in the Red Milkweed Beetle (*Tetraopes tetraophthalmus*)

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ABSTRACT: Specialization is common in most lineages of insect herbivores, one of the most diverse groups of organisms on earth. To address how and why specialization is maintained over evolutionary time, we hypothesized that plant defense and other ecological attributes of potential host plants would predict the performance of a specialist root-feeding herbivore (the red milkweed beetle, *Tetraopes tetraophthalmus*). Using a comparative phylogenetic and functional trait approach, we assessed the determinants of insect host range across 18 species of *Asclepias*. Larval survivorship decreased with increasing phylogenetic distance from the true host, *Asclepias syriaca*, suggesting that adaptation to plant traits drives specialization. Among several root traits measured, only cardenolides (toxic defense chemicals) correlated with larval survival, and cardenolides also explained the phylogenetic distance effect in phylogenetically controlled multiple regression analyses. Additionally, milkweed species having a known association with other *Tetraopes* beetles were better hosts than species lacking *Tetraopes* herbivores, and milkweeds with specific leaf area values (a trait related to leaf function and habitat affiliation) similar to those of *A. syriaca* were better hosts than species having divergent values. We thus conclude that phylogenetic distance is an integrated measure of phenotypic and ecological attributes of *Asclepias* species, especially defensive cardenolides, which can be used to explain specialization and constraints on host shifts over evolutionary time.

Keywords: chemical ecology, host range evolution, phylogenetic GLS, insect herbivore, plant defense, root herbivory.

Introduction

Most of the estimated 1–5 million phytophagous insect species are highly host specific, usually feeding on a small fraction of plant species they encounter over their lifetime (Strong et al. 1984; Mitter et al. 1988; Jaenike 1990). Why host plant specialization has evolved and been maintained

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as a very successful strategy has been extensively debated over the past half century (Dethier 1954; Ehrlich and Raven 1964; Krieger et al. 1971; Bernays and Graham 1988; Futuyma and Moreno 1988; Farrell 1998), and the existence of trade-offs in insect fitness on alternative hosts is the most widely held notion of why insects specialize (Gould 1979; Futuyma and Moreno 1988; Fry 2003; Agrawal et al. 2010; Kuwajima et al. 2010). Trade-offs are classically defined as the advantage of a trait, genotype, or species in one context being accompanied by a disadvantage in another context (Gould 1979; Jaenike 1985; Futuyma and Moreno 1988). Such trade-offs may be driven by many factors, including adaptation to plant defense (Krieger et al. 1971; Wheat et al. 2007), enemy-free space (e.g., Murphy 2004), or other factors such as habitat, mating site, or predation (Bernays and Graham 1988; Courtney 1988; Fry 1996).

Somewhat counterintuitively, generalist herbivores have often been employed to test for insect performance on different hosts. This may be due in part to the difficulty of forcing specialists to feed on nonhosts. For example, Mackenzie (1996) reported that among 77 clones of the broad generalist black bean aphid, *Aphis fabae*, a trade-off in fecundity was found on two out of three plant pairs. Three separate selection experiments with two-spot spider mites (*Tetranychus urticae*) found that selection lines adapted to novel and somewhat toxic hosts showed reduced fitness on the original host (Gould 1979; Fry 1996; Agrawal 2000). Studies of specialization and trade-offs with oligophages (e.g., herbivores restricted to one plant family) and specialists have also been conducted. Perhaps the best evidence comes from the oligophagous pea aphid (*Acyrthosiphon pisum*), where Via and Hawthorne (2002) identified the genetic basis of a trade-off in performance on two legume hosts. Nonetheless, other systems have pro-

vided limited evidence for trade-offs (e.g., James et al. 1988; Forister et al. 2007).

A few studies have also attempted to study the evolution of host plant associations by tying together aspects of micro- and macroevolution. For example, Futuyma et al. (1995) used specialist *Ophraella* beetles to test hypotheses about the evolution of host shifts and specialization. They employed the novel approach of assessing genetic variation for acceptance, feeding, and development on the typical host plant and the host plants of close beetle relatives. Where heritable variation was found, there was essentially no evidence for trade-offs in the use of alternative hosts. In another study, Ueno et al. (2003) used specialist *Epilachna* beetles to test hypotheses about the evolution of host shifts and specialization. Again, where heritable variation was found for using alternative hosts, there was no evidence for trade-offs in performance.

Here we take a phylogenetic and functional trait approach to assess the determinants of host range in the cerambycid beetle *Tetraopes tetraophthalmus* (Forster), a typically monophagous herbivore of common milkweed *Asclepias syriaca* L. (Apocynaceae). For 18 *Asclepias* species, we quantitatively assess the relationship between plant phylogenetic distance from the true host plant species and performance of root-feeding larvae. Our study stems from the prediction that the more closely related a plant species is to *A. syriaca*, the more phenotypically and ecologically similar it should be, and therefore the more suitable it would be as a host. Thus, although we do not directly address trade-offs in host use, we address whether specialization to one host plant species decreases the ability to use other species. The ability to use particular alternative hosts should decrease as their phylogenetic distance from the true host increases.

We measured plant traits relating to defense, life history, and habitat, assessed phylogenetic signal for each plant trait, and used phylogenetically controlled analyses to investigate the relative importance of the traits for host plant specialization. In particular, if we were to find a relationship between plant phylogenetic distance and larval performance, and yet none of the measured traits show phylogenetic signal (i.e., trait values vary independently from phylogeny), then we would conclude that the measured traits do not contribute to the association of phylogenetic distance with larval performance. However, if a trait showed at least some phylogenetic signal, this would be consistent with a potential contribution of the trait to the pattern of larval performance observed. Finally, phylogenetically controlled correlations between plant traits and larval performance were used to clarify the relationship between traits and larval performance independent of phylogenetic distance.

Material and Methods

Insect and Plants

In the field, *Tetraopes tetraophthalmus* typically feeds exclusively on *Asclepias syriaca*, a clonal, perennial plant commonly found in pastures and old fields in North America east of the Rocky Mountains (Wyatt 1996). During the summer and fall, larvae of *T. tetraophthalmus* feed underground on *A. syriaca* roots and rhizomes, and they overwinter as prepupae. Adults emerge in early summer synchronously with milkweed flowering (Matter et al. 1999) and begin to feed on milkweed buds, flowers, and foliage (Matter 2001). Males and females mate multiple times over the season, with females ovipositing 10–15 eggs per clutch into hollow stems of grasses (McCauley 1983). *Tetraopes tetraophthalmus* is nested within the New World clade of *Tetraopes*, with 24 species in North and Central America (Chemsak 1963; Farrell 2001). *Asclepias* includes about 130 species in North and Central America and the Caribbean and some 10 species in South America (Woodson 1954; Fishbein et al., forthcoming). Records of natural history suggest strong species-specific associations between many *Tetraopes* and *Asclepias* species, and Farrell and Mitter (1998) proposed that the plant and insect lineages cospeciated.

For all of our experiments, *T. tetraophthalmus* adults were collected on naturally occurring milkweed patches around Ithaca, New York, and kept in large ventilated containers (30 cm × 20 cm × 15 cm) in the laboratory. Males and females were provided with fresh milkweed leaves and oviposition sites (dried grass stems). The oviposition substrate was removed from the rearing boxes every third day and incubated in the dark at 27°C for 7–10 days. Newly hatched larvae were kept in large petri dishes (10 cm in diameter) on moist filter paper for a maximum of 24 h before the start of the experiments.

Common-Garden Experiment

We performed an experiment to test the predictive ability of (1) host plant phylogenetic distance, (2) four functional root traits (cardenolides, phenolics, carbon to nitrogen ratio [C/N ratio], clonality [number of reproductive root buds]), (3) specific leaf area (a measure of leaf function and/or habitat affiliation; see below), and (4) known associations with other *Tetraopes* species on the survival and performance of the specialist *T. tetraophthalmus* larvae on 18 species of *Asclepias* (table A1, available in the online edition of the *American Naturalist*). Plants were chosen out of a known comprehensive phylogeny of *Asclepias* (Fishbein et al., forthcoming). Out of the chosen 18 *Asclepias* species, nine regularly experience *Tetraopes* adult

feeding, and the other nine have no known *Tetraopes* association (Farrell 2001; Farrell and Mitter 1998; fig. 1).

The 18 milkweed species ($n = 6$ replicates per plant species) were germinated at room temperature after stratifying the seeds at 4°C on moist filter paper for 2 weeks. Individual seedlings were transplanted in potting soil (Metro-Mix, Sun Gro Horticulture Canada, Vancouver, British Columbia) in 10-cm-diameter pots and grown in a single growth chamber (10 h daylight, 26°C during the day, 17°C at night) for 3 weeks before being placed outside on a rooftop patio. Replicates were completely randomized. Plants were watered ad lib. and fertilized (N : P : K 21 : 5 : 20, 150 ppm N [mg/L]) once every 2 weeks. After a month of growth, plants were exposed to five first-instar *T. tetraophthalmus* larvae obtained as described above, by placing the larvae about 1 cm deep around the rhizosphere of the plant. Plants and herbivores were then left to grow for an additional month.

Subsequently, plants were removed from the pots, and by gently removing soil around the roots, we collected and counted the number of surviving larvae per plant. Larvae were weighed, and roots were washed with water. Average insect damage on roots was visually scored on a scale from 0 to 5 before roots were dried for chemical analysis (see below). *Asclepias* spp. span the range from being single stemmed to aggressively reproducing clonally via underground rhizomes. Because underground growth is the major resource for *Tetraopes* and it has been suggested that mode of plant reproduction can affect plant defense evolution (Johnson et al. 2009), we sought a measure of clonality for each of the 18 milkweed species. We thus sowed and grew an additional five plants per species as described above. After 2 months of growth, roots were washed and the number of dormant stem-producing buds on the roots was counted, as a measure of the clonal ability (A. A. Agrawal, personal observation).

We estimated specific leaf area (SLA) for each *Asclepias* species because it has been shown to be a reasonable predictor of leaf function and, to a lesser extent, habitat affiliation (Givnish 1987; Reich et al. 1999; Ackerly et al. 2002; Ackerly 2004; Cornwell and Ackerly 2009). For 13 *Asclepias* species that typically inhabit extreme habitats, we previously found that species living in the wettest environments had 70% higher SLA than species living in the driest environments ($r^2 = 0.439$; only one of the "dry" species had an SLA value overlapping with the "wet" species, summarized from Agrawal et al. 2009). Thus, in this study, we punched a 1-cm-diameter leaf disc from each replicate and report species means for SLA as area/dry mass.

Chemical Analysis of Roots

Roots of the 18 species of *Asclepias* from the common-garden experiment were washed, frozen, dried at 45°C for 3 days, and ground using a MM300 grinder (Retsch, Haan, Germany) in 10-mL steel grinding vessels at 27 Hz for 2 min. Between 50 and 100 mg of tissue was spiked with 20 μg of digitoxin (Sigma, 168 CAS:71-63-6) as the internal standard and extracted with 1 mL of pure methanol in a sonicating water bath at 55°C for 20 min. After centrifugation and filtration with a 45-μm pore size Millex filter (Millipore, Billerica, MA), high-performance liquid chromatography (HPLC) analysis was conducted by injecting 15 μL of the supernatant into an Agilent 1100 series HPLC, and compounds were separated on a Gemini C18 reversed-phase column (3 μm, 150 mm × 4.6 mm, Phenomenex, Torrance, CA).

Cardenolides were eluted on a constant flow of 0.7 mL/min with an acetonitrile −0.25% phosphoric acid in water gradient as follows: 0–5 min, 20% acetonitrile; 20 min, 70% acetonitrile; 20–25 min, 70% acetonitrile; 30 min, 95% acetonitrile; 30–35 min, 95% acetonitrile. Peaks were detected by a diode array detector at 218, 320, and 360 nm, and absorbance spectra were recorded from 200 to 400 nm. Peaks showing a symmetrical absorption band with a maximum between 217 and 222 nm were recorded
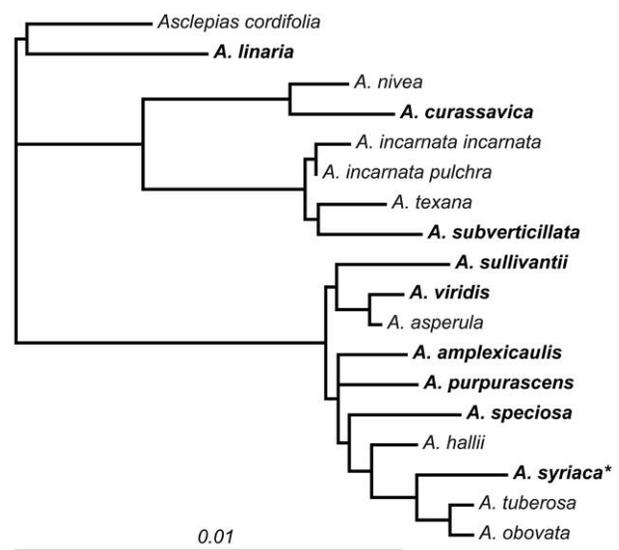

Figure 1: Phylogram of 18 species of *Asclepias* pruned from a comprehensive phylogeny of the genus (Fishbein et al., forthcoming). The complete phylogeny was the 50% majority-rule consensus of trees sampled from the stationary distribution of a Bayesian analysis of three noncoding cpDNA regions sampled from 145 taxa. Boldface indicates plant species having a known association with at least one *Tetraopes* species. The true host of *Tetraopes tetraophthalmus* is *Asclepias syriaca* (asterisk).

as cardenolides. Concentrations were calculated and standardized by peak areas of the known digitoxin concentration.

Additionally, phenolics (here summarized as the total sum of ferrulic acid–, p-coumaric acid–, caffeic acid–, and flavonoid-based compounds) were simultaneously extracted and analyzed with cardenolides and were recorded based on characteristic absorption spectra (Mabry et al. 1970; Bengoechea et al. 1995). Concentrations were calculated and standardized by peak areas of the known digitoxin concentration; total cardenolide and phenolic concentration were calculated as the sum of all individual peaks and reported as micrograms of digitoxin equivalents per milligram of dry mass tissue. Belowground tissue C/N ratio was assessed with ~3 mg of dried and powdered material by combustion at the Cornell University Stable Isotope Laboratory. All raw data for species trait means are provided in table A1.

Statistical Analysis

Branches from a comprehensive phylogeny were pruned to create a phylogram for the 18 taxa sampled for the common-garden experiment (fig. 1). A simple correlation was analyzed between larval survival rates (as the average number of larvae surviving per plant, out of five, and per species) and phylogenetic distance (i.e., total molecular branch length) from *A. syriaca*.

We tested the effect of phylogenetic history on trait covariation with larval survival in a maximum likelihood generalized least squares (GLS) framework using Pagel's Continuous implemented in BayesTraits (Pagel 1999; Freckleton et al. 2002). Using GLS, models of trait evolution differing in complexity (i.e., in the number of parameters) can be compared using a likelihood-ratio test (LR), in which LR $= -2$(log likelihood of the better-fitting model $-$ log likelihood of the worse-fitting model). Under the assumption of model equivalence, the LR statistic should be χ^2 distributed, with 1 df (because only a single parameter is altered between the models compared).

We first estimated phylogenetic signal of all continuous traits (larval survival, total cardenolides and phenolics, C/N ratio, SLA, and number of root buds) using Pagel's lambda (λ) in Continuous (Pagel 1999, 2007; Freckleton et al. 2002; Pagel and Meade 2007). The estimated λ is compared statistically to models where λ is forced to be either 0 or 1. A λ value of 1 indicates phylogenetic signal consistent with a random-walk model (i.e., trait similarity is directly proportional to the extent of shared evolutionary history). A λ value approaching 0 indicates no influence of shared ancestry on trait values (i.e., phylogenetic independence).

For phylogenetically independent correlation analyses

in Continuous (PGLS), the LR parameter was estimated from a random-walk model ($\lambda = 1$) as the difference between a model with and without an estimated covariance between traits. Finally, we used a phylogenetically corrected multiple regression to account for correlations between plant traits to predict larval performance. Specifically, using the regression module of Continuous, we compared the likelihood values of a full model (with all traits) with likelihood values of models lacking each trait separately.

Results

The amount of root damage and larval mass after a month of feeding both correlated with larval survival (root damage: Pearson correlation, $n = 18$, $r = 0.82$, $P < .0001$; larval mass: $n = 18$, $r = 0.66$, $P = .003$), and thus we focus on survival because it is a strong indicator of host range. Survival was highly dependent on plant species (one-way ANOVA, $F_{17,62} = 3.72$, $P < .0001$), ranging from zero survival to a maximum of 1.2 out of 5 (24%) larvae per plant on *A. syriaca*, the true host.

Results of larval survival support the "phylogenetic distance hypothesis," with decreasing survival as a function of increased total branch length (i.e., phylogenetic distance) from *Asclepias syriaca* (fig. 2; Pearson correlation, $n = 18$, $r = 0.758$, $P = .0003$; however, this relationship is lost when accounting for phylogenetic nonindependence: phylogenetic generalized least squares analysis [PGLS], LR $= 2.638$, $P = .104$). In our analyses, phylogenetic distance itself shows a phylogenetic signal ($\lambda = 1$), and thus the significance test of the phylogenetic dis-
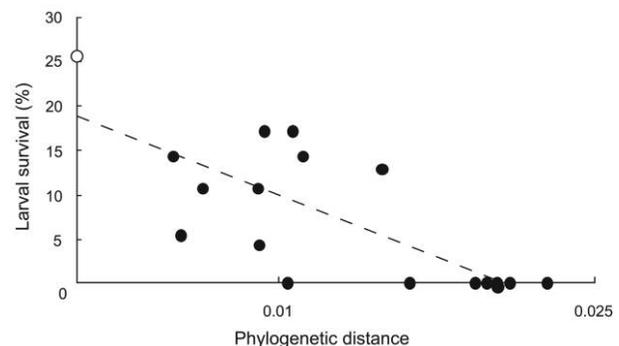

Figure 2: Mean *Tetraopes tetraophthalmus* larval survival is predicted by phylogenetic distance of 17 *Asclepias* plants species (*filled dots*) from the true host, *Asclepias syriaca*. Zero phylogenetic distance represents *A. syriaca* (*open dot*). This relationship (*dotted line*; $n = 18$, $r = 0.758$, $P = .0003$) holds significant also when removing the zero point ($n = 17$, $r = 0.676$, $P = .003$).

tance effect appears to be impacted by closely related species having values of distance similar to those of *A. syriaca*.

We next took a targeted functional trait approach to assess whether certain traits were important in determining *Tetraopes tetraophthalmus* survival (table A1). We first estimated the phylogenetic signal for each trait (table 1). Other than phylogenetic distance itself, the only parameter with phylogenetic signal consistent with Brownian motion evolution (i.e., $\lambda = 1$) was specific leaf area (table 1); phylogenetic signal for cardenolides was estimated at slightly less than 1 ($\lambda = 0.769$), but its interpretation is uncertain because it was not statistically distinguishable from 0 or 1. *Tetraopes* larval survival also carried some signal ($\lambda = 0.406$), significantly different from 0 and 1. All other traits measured showed little to no phylogenetic signal (table 1).

Subsequently, our phylogenetically controlled analysis of traits (cardenolides, phenolics, C/N ratio, and number of root buds) revealed the importance of toxic cardenolides as a predictor of larval survival (PGLS, LR = 4.808, $P = .028$ when assessed alone, $P = .019$ in phylogenetic multiple regression; table 1). Phenolics, number of buds, and C/N ratio of roots were not significant predictors of larval survival alone or in multiple regression (table 2).

To directly address whether the phylogenetic distance effect was driven by cardenolides, we conducted a PGLS multiple regression analysis with phylogenetic distance and cardenolides as predictors of larval survival. In this case, the effect of phylogenetic signal was minimal (LR = 3.370, $P = .066$), while the effect of cardenolides persisted

(LR = 4.575, $P = .032$; fig. 3; fig. A1 in the online edition of the *American Naturalist*).

Two additional ecological factors, SLA and association with other *Tetraopes* beetles, were also predictors of insect performance. Examination of larval survival against SLA values strongly suggested a quadratic function (fig. 4). Such a fit was significantly better than a linear model (comparison of linear vs. linear plus squared SLA, PGLS, $n = 18$, LR = 8.22, $P = .005$). This relationship was confirmed with an analysis of the pairwise differences (from *A. syriaca*) of larval survival against SLA (fig. A2 in the online edition of the *American Naturalist*; $n = 17$, $r = 0.646$, $P = .004$). In particular, *A. syriaca*, commonly found in fields and meadows, has an intermediate SLA value and maximum larval survivorship. Species with extreme SLAs and likely from extreme habitats (e.g., *Asclepias incarnata* from wetlands and *Asclepias linaria* from the desert) were poor hosts. Additionally, *Asclepias* species known to be associated with congeners of *T. tetraophthalmus* were better hosts for *T. tetraophthalmus* than *Asclepias* species without *Tetraopes* herbivores (fig. 5; PGLS, $n = 18$, LR = 4.66, $P = .031$).

Pairwise phylogenetically independent correlations among the three significant predictors of larval survival (cardenolides, SLA, and association with *Tetraopes*) show that species having higher SLA values have proportionally lower levels of total cardenolides (PGLS, $n = 18$, LR = 5.79, $P = .016$). Moreover, species having an association with *Tetraopes* have 23% lower levels of cardenolides than species without *Tetraopes* herbivores (PGLS, $n = 18$,

Table 1: Phylogenetic signal (Pagel's λ) for plant traits and insect survival (Pagel 1999)

Trait	$\lambda_{estimated}$	LR for $\lambda = 0$	LR for $\lambda = 1$	Interpretation
Cardenolides	.769	.820	.498	Ambiguous, but estimated λ indicates lower signal than Brownian motion
C/N ratio	.203	.212	5.256[a]	No phylogenetic signal
Number of buds	.150	.178	5.186[a]	No phylogenetic signal
Phenolics	.352	.408	9.724[a]	No phylogenetic signal
SLA	.902	5.134[a]	.56	Phylogenetic signal consistent with Brownian motion evolution
Larval survival	.406	3.382[a]	5.6[a]	Lower phylogenetic signal than Brownian motion
Phylogenetic distance from *Asclepias syriaca*	1	32.714[a]	<.0001	Phylogenetic signal consistent with Brownian motion evolution

Note: Shown are two times the differences in likelihood values (LR) between the estimated model and a model in which λ is fixed at either 1 or 0. Here we include phylogenetic distance from *A. syriaca* as a "trait" because we use a phylogram in our analysis (where branch lengths are proportional to the number of molecular substitutions) and closely related species do not necessarily show proportional distances to *A. syriaca* (as they would in a chronogram).

[a] Significant differences between the estimated model and the constrained model using a likelihood ratio test ($P < .05$).

Table 2: Phylogenetically independent multiple regression analysis of root buds, total root cardenolides, root C/N ratio, and total root phenolics as predictors of larval *Tetraopes tetraophthalmus* survival across 18 species of *Asclepias*

	Full model	−Root buds	−Cardenolides	−C/N ratio	−Phenolics
LH	−3.793	−4.15	−6.51	−3.842	−4.927
LR		.714	**5.434**	.098	2.268
P		.398	**.019**	.754	.132

Note: Probability values are derived from likelihood ratio (LR; two times the likelihood [LH] difference between the full model and model lacking each of the plant traits measured). A *P* value <.05 (in bold) indicates a significant effect of the trait on larval survival when all other traits and phylogenetic nonindependence are taken in account.

LR = 5.214, *P* = .022). Association with *Tetraopes* was not, however, correlated with SLA (PGLS, *n* = 18, LR = 0.072, *P* = .788).

Discussion

We have shown that adaptation and specialization of *Tetraopes tetraophthalmus* to *Asclepias syriaca* is associated with reduced performance on ever-increasing phylogenetically distant *Asclepias* species. As is universally assumed, phylogenetic distance is likely a reasonable estimate of phenotypic similarity and may frequently predict host associations (Perlman and Jaenike 2003; Krasnov et al. 2004; Poulin 2005). However, among the four plant traits we measured, only concentrations of toxic cardenolides predicted larval survival, and this effect of cardenolides was stronger than the phylogenetic distance effect. Additionally, our measures of milkweed species habitat affiliation and associations with other *Tetraopes* herbivores were also predictive of larval survival, but these measures were not independent of root cardenolides. Nonetheless, cardenolide concentration was the only root trait directly relevant to larval performance, and thus it appears that habitat affiliation, herbivore association, and root defense have repeatedly evolved in concert.

Estimating phylogenetic signal of the traits themselves allowed us to assess which of the traits we measured likely contributed to the mechanism underlying the phylogenetic distance effect. The two continuous traits predictive of larval survival (cardenolides and SLA) showed evidence of phylogenetic signal, confirming that both traits could contribute to the phylogenetic distance effect. Additionally, both traits appear to contribute to insect performance independent of phylogenetic signal, as suggested by their significant phylogenetically independent correlations with larval survival. The importance of SLA was confirmed by plotting pairwise differences (from *A. syriaca*) in larval survival against pairwise differences in SLA (fig. A2); however, the same analysis for total cardenolides was not significant, suggesting that detecting the impact of cardenolides is facilitated by accounting for phylogenetic

nonindependence. Indeed, in a PGLS multiple regression with phylogenetic distance and cardenolides as predictors of larval survival, cardenolide concentration was the best predictor of larval survival.

In our analyses, survival on *A. syriaca* and its cardenolide values are at an extreme end of the range (low cardenolides and highest survival). This likely contributes to the effect of both phylogenetic distance and cardenolides being predictive of larval survival. In other words, such a phylogenetic distance effect may not be expected for herbivores on plants with a less extreme defense phenotype. Nonetheless, four *Asclepias* species (*A. incarnata* ssp. *incarnata*, *A. incarnata* ssp. *pulchra*, *A. texana*, and *A. subverticillata*) showed a high phylogenetic distance but all share similar low cardenolide levels. Interestingly, these species traits (both phylogenetic distance and cardenolide levels) are not independent, as the four species all belong to a single clade (figs. 3, A1). In addition, the fact that no larvae survived on these four species suggests that these distant species have some trait other than cardenolides responsible for resistance. For instance, *Asclepias* species with intermediate SLA values (close to *A. syriaca*) showed the highest larval survival scores, and species' deviation from this intermediate value was associated with reduced performance, irrespective of the direction of this deviation (fig. A2). Thus, phylogenetic distance integrates multiple trait differences relevant to larval survival.

Macroevolution of Specialization

Many reasons have been hypothesized for why most species of insect herbivores are restricted to one or a few host plants. We advocate using specialists to test hypotheses about host range evolution, an approach that may be particularly powerful when studying closely related host plant species (James et al. 1988; Futuyma et al. 1993; Ueno et al. 2003; Forister et al. 2007). Additionally, we presented here an approach to test whether plant secondary metabolites, which function primarily as toxins against herbivores, are important traits maintaining host range. The cardenolide-containing *Asclepias* species are so well de-
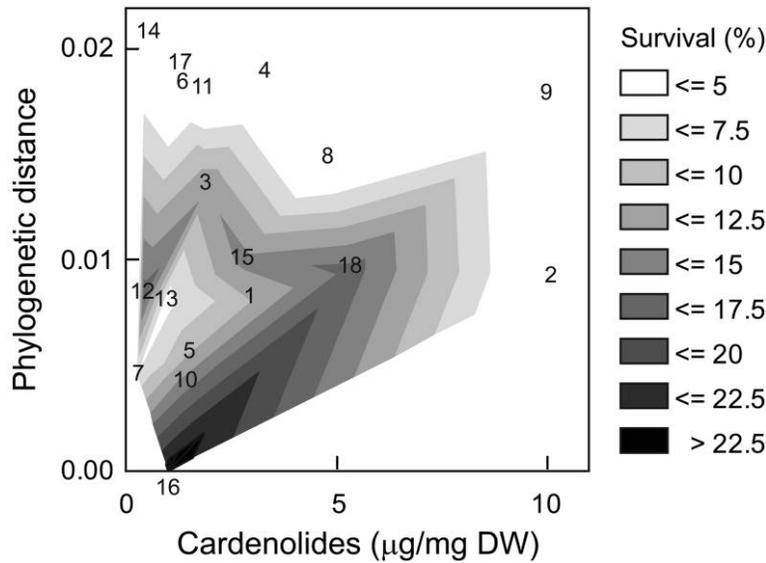
Figure 3: Mean *Tetraopes tetraophthalmus* larval survival (represented by different shades of gray) is predicted by levels of cardenolides of 18 species of *Asclepias* (*X*-axis), as well as by phylogenetic distance from *A. syriaca* (*Y*-axis). Each species is labeled by a number: *1, A. amplexicaulis; 2, A. asperula; 3, A. cordifolia; 4, A. curassavica; 5, A. hallii; 6, A. incarnata* ssp. *incarnata; 7, A. tuberosa; 8, A. linaria; 9, A. nivea; 10, A. obovata; 11, A. incarnata* ssp. *pulchra; 12, A. purpurascens; 13, A. speciosa; 14, A. subverticillata; 15, A. sullivantii; 16, A. syriaca; 17, A. texana;* and *18, A. viridis.*

fended that apparently all of their major herbivores are specialized, at least to the Apocynaceae (Agrawal 2005). It has long been hypothesized that physiological mechanisms to cope with potent chemical defenses such as the cardenolides come at a cost (Holzinger and Wink 1996) and that even highly adapted herbivores are somewhat sensitive to at least some cardenolides (Zalucki et al. 2001; Agrawal 2005). Several lines of evidence suggest that *T. tetraophthalmus* both sequesters cardenolides (Isman et al. 1977) and suffers from them (this study; Rasmann et al. 2011). In light of potential coevolution, it is no surprise that specialists are still affected by defensive plant traits (Agrawal and Kurashige 2003).

The integration of phylogenetic techniques at the community level has become a powerful tool in investigating insect community composition (and potentially specialization) on host plants. For example, Weiblen et al. (2006) demonstrated that a large proportion of herbivores found in tropical rain forest plant communities of New Guinea are clade specialists. This pattern was also found for beetles in the tropical forests of Panama (Ødegaard et al. 2005). Where herbivores specialize and utilize plant secondary metabolites as host-finding cues or where they are simply restricted to feeding on phylogenetically related clades, this pattern is expected (Jaenike 1990; Futuyma et al. 1993; Kursar et al. 2009). However, if the reverse was observed, that is, if herbivores preferred distantly related plant spe-

cies in the community, this pattern would suggest that herbivores are tracking convergent chemical, morphological, or ecological host traits (Becerra 1997). After dissecting phylogenetic distance into putative traits that might influence host use, we speculate that host range in *Tetraopes* may be determined both by trait conservatism in the
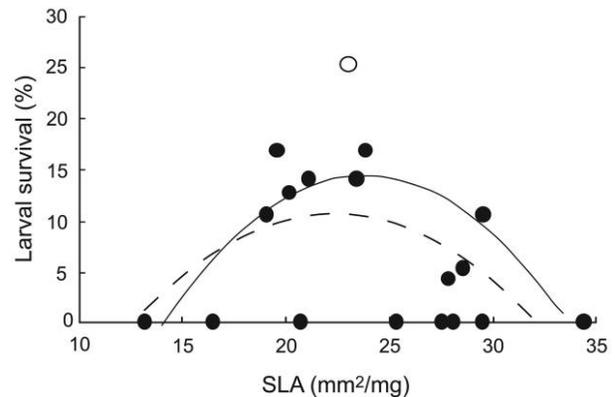

Figure 4: Mean *Tetraopes tetraophthalmus* larval survival across 18 species of *Asclepias* is predicted by specific leaf area (SLA) values for the species. Dotted line represents raw quadratic ($r^2 = 0.271$), whereas the solid line is the phylogenetically corrected relationship. Open dot represents *Asclepias syriaca*.
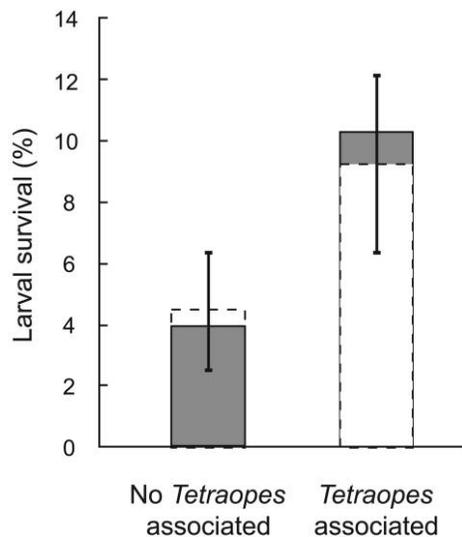
Figure 5: Mean *Tetraopes tetraophthalmus* larval survival averaged across nine *Asclepias* species with at least one recorded association with a *Tetraopes* species and nine *Asclepias* species with no recorded association with *Tetraopes* beetles. Dashed open bars are raw mean values ($\pm$ SE), whereas filled gray bars represent phylogenetically corrected mean values.

plants and by a pattern of species tracking convergent chemical or ecological traits. This conclusion, also reached by Becerra (1997), likely reflects the dual pattern of some phylogenetic signal and convergence seen for most traits.

Conclusion and Speculation

We have found that toxic secondary metabolites (i.e., low levels of cardenolides) predict host use in *T. tetraophthalmus*. A complete test of trade-offs would require similar experiments measuring a range of *T. tetraophthalmus* genotypes that have differential host use of *Asclepias* species. Alternatively, at a more macroevolutionary scale, perhaps reciprocal measures of the performance of other *Tetraopes* species on several *Asclepias* species could be used to test for trade-offs in this group, which may have cospeciated (Farrell and Mitter 1998). Nonetheless, we speculate that trade-offs arising from specialization should increase with phylogenetic distance from the true host.

From the plant's perspective, both functional traits and ecological conditions may dictate patterns of attack. Indeed, cardenolides in milkweed may be driven by plant habitat affiliations or associations with closely related herbivores. Not surprisingly, these characteristics of the plants are not independent, and others have convincingly shown that herbivory and habitat shape plant defenses in pairs of closely related species (Fine et al. 2004). We thus spec-

ulate that habitat or abiotic conditions may set the template for offensive-defensive coevolutionary interactions. For example, herbivores may prefer not to colonize particular habitats (e.g., root herbivores in wetlands, shady habitats, etc.), or particular habitats may favor some defensive strategies (Kursar and Coley 2003). Nonetheless, based on our common-garden experiment and phylogenetic analysis, investment in plant defense does appear to directly restrict the host range of a specialist herbivore.

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The red milkweed beetle (*Tetraopes tetraophthalmus*) spends its entire life on the common milkweed (*Asclepias syriaca*). *Left*, male and female adults feed on the leaves and flowers of the plant. Milkweed can reduce the feeding by secreting white sticky latex in the leaves (e.g., the droplet forming after a first bite in the middle vein of the leaf). *Right*, the larvae feed on the roots and rhizomes of the plant. Photographs: left by Anurag Agrawal and right by Sergio Rasmann.