Notes and Comments

Plant Defense and Density Dependence in the Population Growth of Herbivores

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ABSTRACT: Long-standing theory has predicted that plant defensive and nutritional traits contribute to the population dynamics of insect herbivores. To examine the role of plant variation in density dependence, I took a comparative approach by conducting density manipulation experiments with the specialist aphid, *Aphis nerii*, on 18 species of milkweed (*Asclepias* spp.). The strength of density dependence varied on the plant species. Variation in plant secondary compounds (cardenolides), trichomes, leaf carbon and nitrogen concentrations, and seed mass of the milkweed species predicted the R_{max} of aphid populations, while specific leaf weight, carbon concentration, latex, water content, and trichome density were significant predictors of the strength of density dependence. Thus, plant traits that probably evolved for primary and defensive functions contribute to the ecological dynamics of herbivore populations.

Keywords: Aphis nerii, *Asclepias* spp., cardenolides, aphid population dynamics, milkweed, plant-insect interactions.

About one-half of the world's macroscopic biodiversity is dominated by plants and their insect herbivores, and herbivory is perhaps the dominant species interaction on earth (Strong et al. 1984). Given that herbivory is the conduit of energy transfer between autotrophic plants and the rest of the food web, there long has been the view that plants and herbivores have an antagonistic coevolutionary relationship resulting in adaptations (e.g., defense and offense traits; Fraenkel 1959; Ehrlich and Raven 1964; Fox 1981; Rhoades 1985; Karban and Agrawal 2002). Despite this view and much accumulated data on coevolution, less progress has been made on understanding the ecological consequences of such adaptations (e.g., Yoshida et al. 2003).

There has been great speculation on the consequences of resource quality, as determined by plant nutritional and defensive traits, on population dynamics of insect herbivores (Haukioja and Hakala 1975; Rhoades 1983; White 1984; Karban 1992; Underwood and Rausher 2002). In particular, these ideas were generated from the observation that plant traits can have strong impacts on the preference and performance of individual herbivores. Although it is reasonable to expect that these effects scale up to herbivore population dynamics, definitive experimental tests of the effects of plant traits on insect populations and density dependence are largely lacking. Although some studies have linked plant defense traits to population growth of herbivores (e.g., Cole 1997; Ciepiela and Sempruch 1999), the link to variation in the strength of density dependence has been made much more rarely (Underwood and Rausher 2000, 2002).

The existence of density dependence has been debated historically (Andrewartha and Birch 1954; Strong 1984; Cappuccino and Price 1995), though current progress in understanding population dynamics is focused on understanding the conditions that drive strong versus weak density dependence (Krebs 2002). In this study, I examined effects of experimentally varying densities of an aphid, *Aphis nerii*, on individual milkweed plants from 18 species of *Asclepias* to assess density dependence in population growth of a herbivore. I then measured 10 defensive, nutritional, and life-history characteristics of the milkweed species to explore predictors of the intrinsic rate of increase (or maximum population growth rate, R_{max}) and the slope of density dependence. By taking a broad comparative approach to understanding the effect of plant quality on aphids, my goal was to take advantage of the major variation in defensive traits across the genus *Asclepias*. Also, by examining population dynamics of aphids across plant species, I link the (co)evolutionary history between the plants and aphids, which may have generated defensive traits, with contemporary consequences for populations.

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Material and Methods

Plant and Insect Biology

Aphis nerii is a cosmopolitan aphid species that typically reproduces by parthenogenesis; it has a limited host range and feeds only on *Nerium* spp. and plants in the Asclepiadaceae (oleander and milkweeds), from which it sequesters toxic cardiac glycosides (Rothschild et al. 1970). Individuals typically grow in clusters, and the progeny of a single adult are especially aggregated. Although individuals are not very mobile and do not usually walk off a suitable host plant, under dense conditions winged dispersal morphs are produced (Groeters 1989). I started a colony of *A. nerii* from a single individual on *Asclepias syriaca*, collected from the Koffler Scientific Reserve, at Jokers Hill in southern Ontario, 44°03′N, 79°29′W (http://www.zoo.utoronto.ca/jokershill). The colony was maintained by serial transfers on *A. syriaca* in a laboratory growth chamber.

The genus *Asclepias* contains over 130 species in North America (M. Fishbein, personal communication; Woodson 1954). This study was conducted with 18 species of *Asclepias* (table A1 in the online edition of the *American Naturalist*), all of which are hosts to *A. nerii*. Milkweeds possess several putative resistance traits including toxic cardenolides, sticky latex, and trichomes (Malcolm 1991; Agrawal and Malcolm 2002), and species of *Asclepias* vary qualitatively and quantitatively in the production of these traits (table A1). In addition to these three defensive traits, I measured seven other physiological and life-history parameters that may pertain to resistance: seed mass, aboveground biomass after 1 mo of growth, leaf carbon concentration, leaf nitrogen concentration, leaf water content, specific leaf mass, and leaf toughness. Plant traits were measured from undamaged (i.e., not infested) plants grown in a common garden (field) or together in a large growth chamber (lab). All plants were grown from seeds collected from southern Ontario or were purchased from seed suppliers. All plant traits were measured on at least five individuals of each species, and methodological details are outlined in the appendix in the online edition of the *American Naturalist*.

Experimental Procedures

For the density manipulation experiments, a separate group of plants from above was grown in 500-mL pots in high-light growth chambers with Pro-Mix BX soil (Red Hill, Pa.), supplemented with ~0.6 g of slow-release Nutricote fertilizer pellets (13N : 13P : 13K; Vicksburg Chemical, Vicksburg, Miss.). Plants were grown for approximately 1 mo (until they had 8–10 leaves) before use in experiments. Chambers were set to 24°C : 20°C on a 16L : 8D cycle. Plants were grown and maintained in a completely randomized design in a large growth chamber, and aphid density was experimentally manipulated on each plant species (range of 1–15 aphids per plant, $n \approx 15$ per species). Because only 150 plants could be comfortably grown in the chamber without leaves of the plants touching, the experiment was conducted in two blocks, each employing 10 milkweed species. The experiment was conducted with eight unique species in each block, while repeating *A. syriaca* and *Asclepias tuberosa* in each block.

I manipulated the density of aphids by placing two adult gravid aphids near the apex of each plant. After 2 d, the adults were removed and the offspring were thinned to the randomly assigned number between 1 and 15 aphids. Plants were checked the following day and any minor adjustments to meet the assigned initial aphid densities were made. After 11 d, representing two to three generations, the number of aphids was counted on each plant and the per capita growth rate was calculated. The per capita growth rate dN/Ndt was calculated as $(\ln N2 - \ln N1)/(t2 - t1)$, where $N1$ and $N2$ are the population densities at time $t1$ and $t2$, respectively. The density dependence of the per capita growth rate was described by the logistic model as $dN/Ndt = R_{max}(1 - N/K)$, where R_{max} is the maximum per capita growth rate, and K is the carrying capacity.

Analyses

I calculated the strength of density dependence as the slope of the regression of per capita population growth rate versus initial aphid density for each *Asclepias* spp. Although the strength of density dependence may be overestimated by the regression of rates of population growth on population density in time series analysis, the manipulative approach employed in this study is an uncompromised method for estimating effects of density (Prairie and Bird 1989). Growth rate R_{max} was estimated as the y-intercept of each regression.

Variation in the slope of density dependence was assessed by the interaction term between milkweed species and initial density on per capita growth rates of the aphids. Block was maintained in the analysis to factor out potential differences between the two trials. Type III sums of squares were employed because of the unbalanced design with only two plant species being represented in both blocks (Sokal and Rohlf 1995). To examine the potential causes of variation in R_{max} and the slopes of density dependence on the different species of milkweed, I employed multiple regression using the 10 defensive, nutritional, and life-history characteristics of the milkweeds (see table A1 for means and standard errors). A stepwise backwards elimination procedure was used in Sys-

tat (version 9) for the multiple regression with a P to remove value of 0.15 (Wilkinson 1997). Covariances between the predictor variables were also checked. In the multiple regressions, I employed data only from *A. syriaca* and *A. tuberosa* from the first block.

Results and Discussion

I detected negative density dependence of aphids on 17 of the 18 species of milkweed (fig. 1; table A2 in the online edition of the *American Naturalist*). Only on *Asclepias fruticosa* did initial aphid density not significantly influence per capita growth rate (fig. 1; table A2). The slopes of density dependence varied across the species (see interaction term in table 1), indicating that the magnitude of the decrease in aphid population growth per additional initial aphid depended on the plant species on which the aphids were growing. I have reported similar variation in density dependence when *Aphis nerii* is grown on *Asclepias syriaca* under varying environmental conditions (Agrawal et al. 2004).

Multiple regression revealed that the most important predictors of aphid population dynamics were cardenolide content and percent leaf carbon, together explaining 20% of the variation in R_{max} (table 2; fig. 2). The slope of density dependence was strongly influenced by specific leaf weight and leaf carbon, latex, and water content (table 2; fig. 3). In total, five of the 10 defensive, nutritional, and life-history traits of milkweed accounted for 88% of the variation in R_{max} (table 2; overall model $F = 13.182$, df $= 6, 11$, $P < .001$). Despite the specialized nature of *A. nerii* and its ability to sequester plant toxins (Rothschild et al. 1970), concentrations of cardenolides strongly negatively correlated with the R_{max} of aphid populations. Future research will examine the role of induced defense (cardenolides) in the density dependence demonstrated (e.g., Agrawal 1998; Martel and Malcolm 2004). The effect of putative defense chemicals on specialist herbivores has been debated, although the general belief has been that specialists are relatively unaffected by such compounds (Blau et al. 1978; Rhoades 1983; Van Der Meijden 1996). However, a recent accumulation of evidence demonstrates that chemical defenses do affect specialist herbivores (Berenbaum et al. 1989; Adler et al. 1995; Agrawal and Kurashige 2003), suggesting an ongoing evolutionary interaction between plants and herbivores.

Consistent with theory that herbivores, and phloem suckers in particular, are limited by nitrogen (White 1984), I found a positive association between nitrogen content and R_{max} of aphids. The negative association between percent carbon and R_{max} of aphids may represent a "dilution" effect. Because nitrogen is typically limiting for aphids and is extracted from carbon-rich phloem, plants having

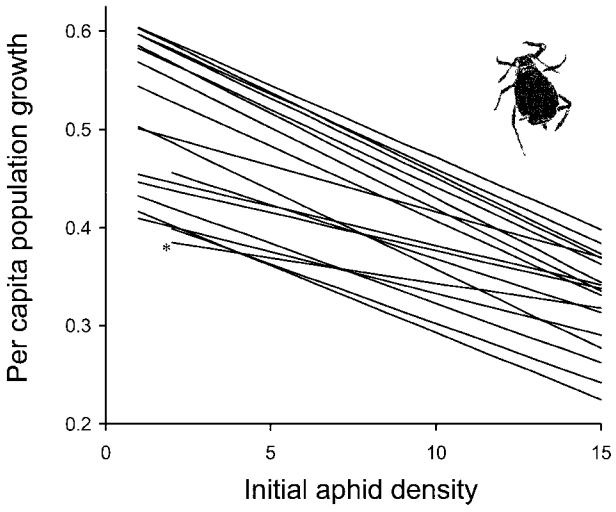

Figure 1: Density-dependent population growth of aphids on 18 species of milkweed. For clarity, raw data are not shown. All slopes are significantly different from 0, except the one marked by an asterisk.

greater carbon content may make it more difficult for aphids to extract nitrogen (see also Simpson and Raubenheimer 2001). As for most of the traits measured, nitrogen and carbon content were not correlated (table A3 in the online edition of the *American Naturalist*). There were few variables that were correlated and survived the stepwise removal procedure (tables 2, A3). The positive correlation between trichomes and R_{max} of aphid populations was unexpected and may have to do with trichomes providing a beneficial microclimate for aphid proliferation.

Factors that affect population growth may not necessarily predict dynamic parameters such as the strength of density dependence. In multiple regression analyses of the traits on the slope of density dependence, there was little overlap between the factors influencing R_{max} (only carbon and trichomes). Together with the other major factors, specific leaf weight, latex, and water content of leaves, the overall model explained 84% of the variation in the strength of density dependence (table 2; fig. 3; $F = 9.367$, df $= 6, 11$, $P < .001$). When a factor has a positive correlation with the slope of density dependence (such as carbon concentration), high values are associated with shallower slopes or a weaker strength of density dependence. Although cause and effect are difficult to distinguish in such analyses, it is clear that different factors are contributing to R_{max} and the strength of density dependence in these aphid populations.

The slope of density dependence was predicted by the R_{max} achieved by aphids on each host species ($R^2 = 0.635$, $F = 27.804$, df $= 1, 16$, $P < .001$). As intrinsic population growth rate increased, density dependence be-

Table 1: ANOVA for effects of the 18 species of milkweed, initial aphid density, and block on per capita growth rate of the aphid populations

	Type III sum of squares	df	MS	*F*	*P*
Species	.134	17	.008	4.278	<.001
Initial density	.776	1	.776	420.087	<.001
Species × initial density	.057	17	.003	1.804	.028
Block	.078	1	.078	42.505	<.001
Error	.467	253	.002		

comes proportionally more negative. Although the relationship between the slope of density dependence and R_{max} may be biased by an artifactual covariance between the two variables (because they are estimated from the same data), I have shown elsewhere that statistical correction of this bias for the data presented in this article still results in a strong negative relationship (Agrawal et al. 2004). Basic population models (Pearl and Reed 1920; MacArthur 1970; Abrams 1998) and recently derived data (Krüger et al. 2002; Agrawal et al. 2004) demonstrate that variation in the slope of density dependence may be predicted by growth rate of a population. This relationship depends on the scaling of the intrinsic rate of increase (R_{max}, *y*-intercept of regressions) and the carrying capacity (*K*, *x*-intercept of regressions); when the two scale proportionally, R_{max} will be uncorrelated with the strength of density dependence because the slopes will be equal (i.e., parallel regressions; Agrawal et al. 2004). Given the association between R_{max} and density dependence, it is interesting that two traits influential in predicting R_{max} (cardenolides and nitrogen content) were not associated with the slope of density dependence. High levels of carbon and trichomes appear to reduce intraspecific competition at higher population densities (influencing density dependence), while other traits that affect R_{max} apparently did not influence intraspecific competition.

Although a few studies have taken a comparative approach to understanding the role that plant traits play in determining insect herbivory (Coley 1983; Becerra 1997; Marquis et al. 2001), no previous study has applied this to understanding population dynamics. Recent work by Underwood and Rausher (2000, 2002) has contrasted the effects of different types of plant resistance traits (constitutive vs. induced resistance), employing varieties of soybean, on herbivore population dynamics. The benefit of the comparative approach I took is that large differences in (uncorrelated) plant traits between species could be quantitatively linked to herbivore population dynamic parameters. However, I have not yet addressed the role of natural enemies of aphids in generating variable population dynamics across host plants, and this will likely be a fruitful avenue of research. For example, Helms et al. (2004) have recently reported an effect of milkweed hostplant species on tritrophic interactions involving *A. nerii*. Ultimately, field studies will be needed to understand the dynamic ecological impacts of plant defense.

One limitation of my analysis is that I did not attempt to factor out a phylogenetic signal of plant traits on insect populations; although no modern phylogeny is available for *Asclepias*, a qualitative analysis with the phylogenetic relationships proposed by Woodson (1954) suggests that most defensive traits were labile even within closely related groups. Woodson's phylogenetic groupings were based on eight series in the subgenus *Asclepias*. For example, Woodson's Incarnate series contained *Asclepias curassavica, Asclepias fascicularis, Asclepias incarnata,* and *Asclepias verticillata*. These species show considerable variation in the traits measured in the current study (table A1). The same is true for the Purpurascentes series containing *Asclepias hallii, Asclepias sulvantii,* and *Asclepias variegata*.

Table 2: Stepwise multiple regressions for the effects of 10 defensive, nutritional, and life-history traits of milkweed on the R_{max} of aphid populations and slopes of density dependence

Effect	Coefficient	SE	R^2	*t*	*P*
R_{max}:					
Constant	1.760	.412		4.271	.001
Trial	−.090	.021	.212	−4.372	.001
Cardenolides	−.228	.075	.103	−3.038	.011
Carbon	−.031	.010	.099	−2.990	.012
Trichome density	.001	.001	.080	2.686	.021
Nitrogen	.069	.032	.052	2.172	.053
Seed mass	−.003	.001	.052	−2.153	.054
Slope:					
Constant	−.093	.034		−2.734	.019
Specific leaf weight	.001	.001	.342	4.799	.001
Carbon	.003	.001	.340	4.784	.001
Latex	−.002	.001	.179	−3.472	.005
Percent water	−.062	.023	.110	−2.728	.020
Trichome density	.001	.001	.075	−2.245	.046
Toughness	.001	.001	.048	1.800	.099

Note: Effects are ordered by the variation explained (R^2), calculated as the difference in R^2 with and without that factor in the complete model. The only factor excluded from both stepwise analyses was plant biomass.

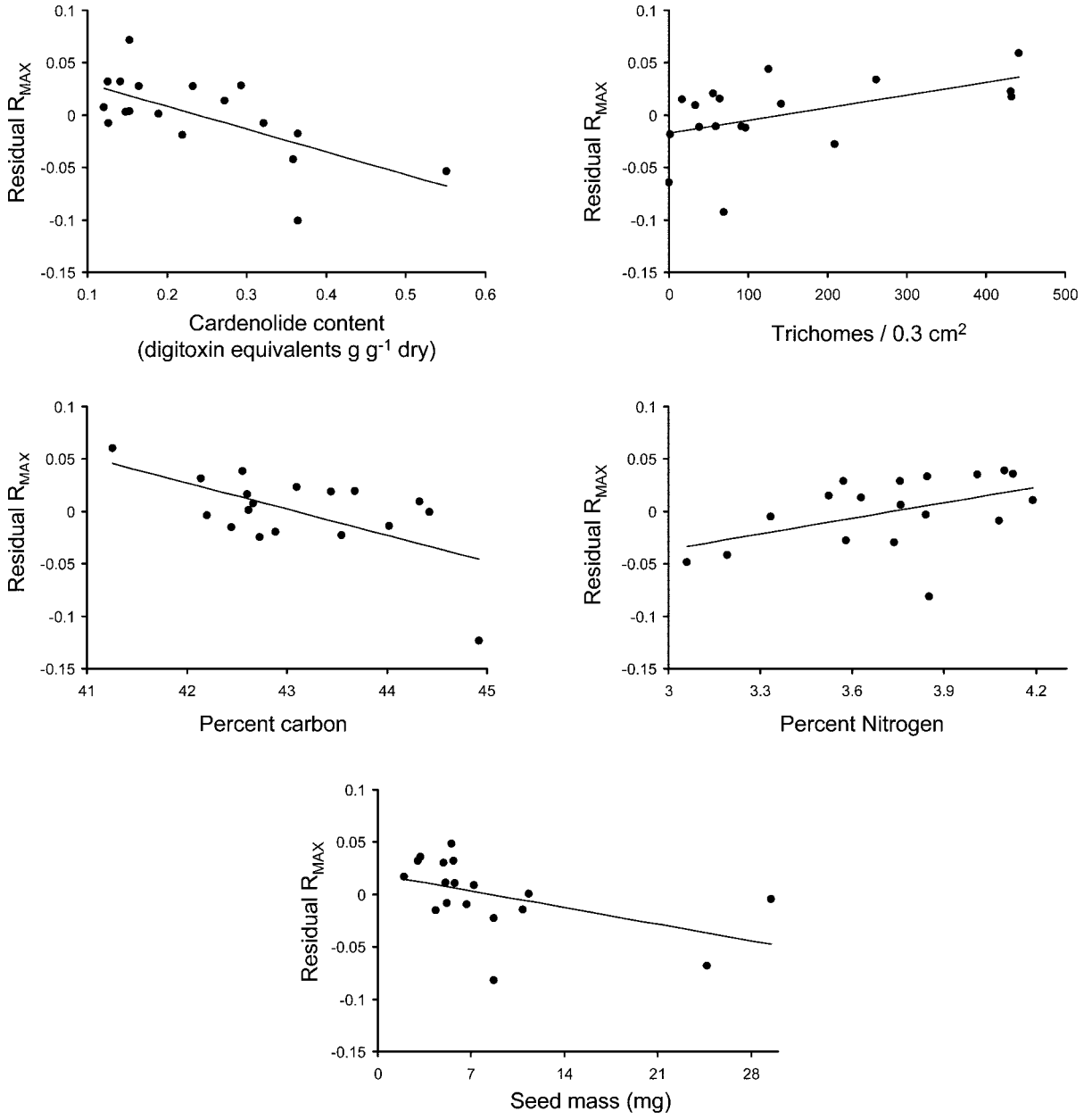

Figure 2: Predictors of aphid population growth. Bivariate relationships between the significant defensive and nutritional traits and residual population growth rate (R_{max} residuals with the other traits included in the model) of aphids on 18 species of milkweed. Each point represents the parameters estimated from aphid density manipulation on one species of *Asclepias* and the plant traits on at least five uninfested plants.

Determining the population consequences of plant defensive traits bears directly on the long-standing view that plant resources are fundamental to the process of insect population regulation (Haukioja and Hakala 1975; White 1984; Karban 1992). Although density dependence is being increasingly reported from manipulative experiments, the mechanisms that generate these effects are not well understood. Plant primary and secondary compounds were strongly implicated in determining the growth rate and slope of density dependence of aphid populations. The discovery of plant traits that affect the population dynamics of herbivores suggests a strong link between historical evolutionary dynamics and contemporary processes (Johnson and Agrawal 2003). Ultimately the feedback of

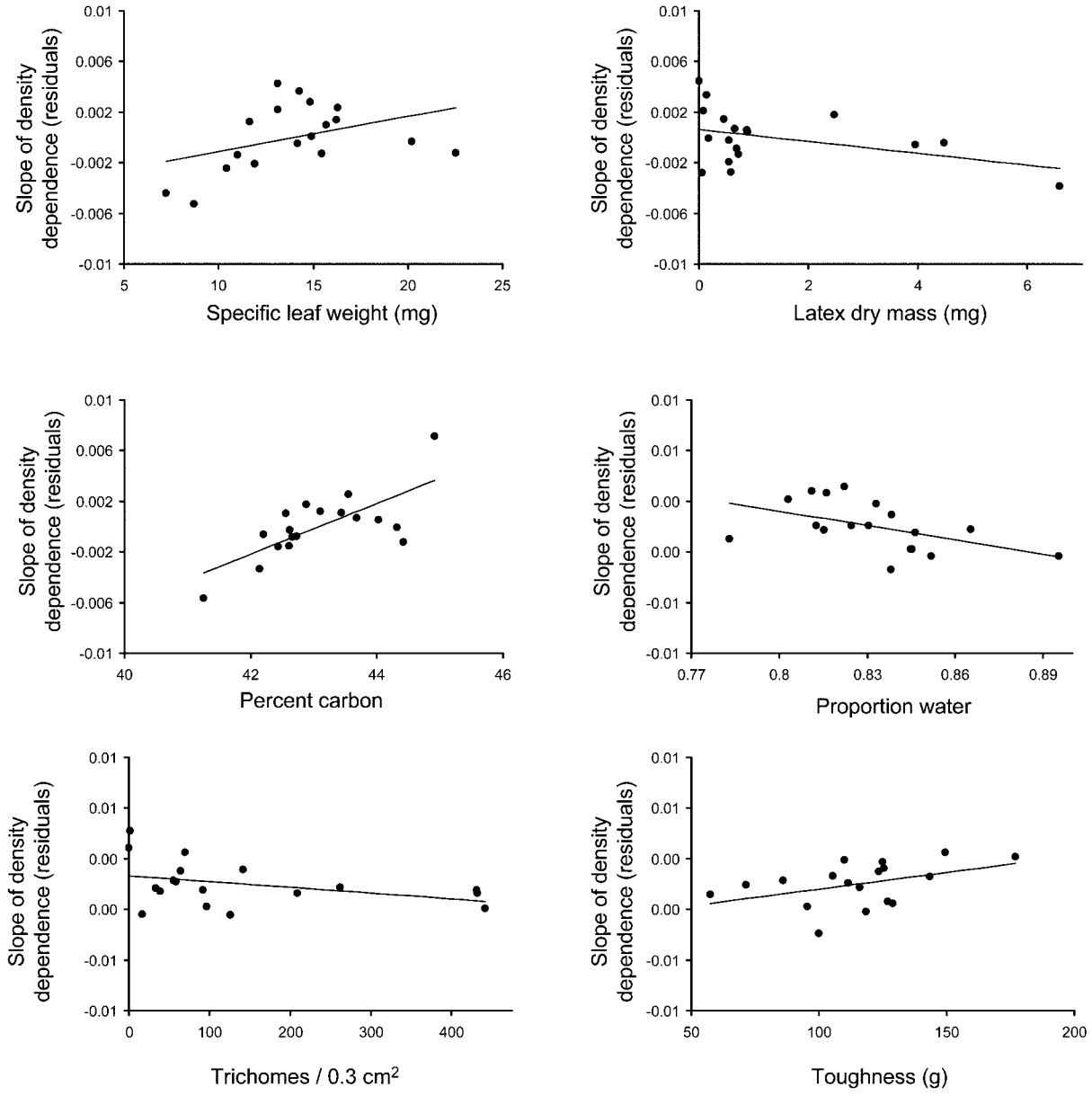

Figure 3: Predictors of the strength of density dependence in aphid populations. Bivariate relationships between the significant defensive and nutritional traits and residual slope of density dependence (residuals with the other traits included in the model) of aphids on 18 species of milkweed. Each point represents the parameters estimated from aphid density manipulation on one species of *Asclepias* and the plant traits on at least five uninfested plants.

the impacts of herbivore populations on plants will determine the coevolutionary trajectory of plant-insect interactions.

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Literature Cited

Abrams, P. A. 1998. High competition with low similarity and low competition with high similarity: exploitative and apparent competition in consumer-resource systems. American Naturalist 152:114–128.

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 (Berlin) 101: 75–85.

Agrawal, A. A. 1998. Induced responses to herbivory and increased plant performance. Science 279:1201–1202.

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 111:48–53.

Agrawal, A. A., N. Underwood, and J. R. Stinchcombe. 2004. Intraspecific variation in the strength of density dependence. Ecological Entomology (in press).

Andrewartha, H. G., and L. C. Birch. 1954. The distribution and abundance of animals. University of Chicago Press, Chicago.

Becerra, J. X. 1997. Insects on plants: macroevolutionary chemical trends in host use. Science 276:253–256.

Berenbaum, M. R., A. R. Zangerl, and K. Lee. 1989. Chemical barriers to adaptation by a specialist herbivore. Oecologia (Berlin) 80:501–506.

Blau, P. A., P. Feeny, L. Contardo, and D. S. Robson. 1978. Allylglucosinolate and herbivorous caterpillars: a contrast in toxicity and tolerance. Science 200:1296–1298.

Cappuccino, N., and P. W. Price. 1995. Population dynamics. Academic Press, San Diego, Calif.

Ciepiela, A. P., and C. Sempruch. 1999. Effect of L-3,4-dihydroxyphenylalanine, ornithine and gamma-aminobutyric acid on winter wheat resistance to grain aphid. Journal of Applied Entomology 123:285–288.

Cole, R. A. 1997. The relative importance of glucosinolates and amino acids to the development of two aphid pests *Brevicoryne brassicae* and *Myzus persicae* on wild and cultivated brassica species. Entomologia Experimentalis et Applicata 85:121–133.

Coley, P. D. 1983. Herbivory and defensive characteristics of tree species in a lowland tropical forest. Ecological Monographs 53:209–233.

Ehrlich, P. R., and P. H. Raven. 1964. Butterflies and plants: a study in coevolution. Evolution 18:586–608.

Fox, L. R. 1981. Defense and dynamics in plant-herbivore systems. American Zoologist 21:853–864.

Fraenkel, G. S. 1959. The raison d'être of secondary plant substances. Science 129:1466–1470.

Groeters, F. R. 1989. Geographic and clonal variation in the milkweed-oleander aphid, *Aphis nerii* (Homoptera, Aphididae), for winged morph production, life-history, and morphology in relation to host plant permanence. Evolutionary Ecology 3:327–341.

Haukioja, E., and T. Hakala. 1975. Herbivore cycles and periodic outbreaks: formation of a general hypothesis. Reports of the Kevo Subarctic Research Station 12:1–9.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between an herbivore and its parasitoid. Ecological Entomology 29:44–51.

Johnson, M. T. J., and A. A. Agrawal. 2003. The ecological play of predator-prey dynamics in an evolutionary theatre. Trends in Ecology & Evolution 18:549–551.

Karban, R. 1992. Plant variation: its effects on populations of herbivorous insects. Pages 195–215 *in* R. S. Fritz and E. L. Simms, eds. Plant resistance to herbivores and pathogens: ecology, evolution, and genetics. University of Chicago Press, Chicago.

Karban, R., and A. A. Agrawal. 2002. Herbivore offense. Annual Review of Ecology and Systematics 33:641–664.

Krebs, C. J. 2002. Two complementary paradigms for analysing population dynamics. Philosophical Transactions of the Royal Society of London B 357:1211–1219.

Krüger, O., R. Liversidge, and J. Lindström. 2002. Statistical modelling of the population dynamics of a raptor community in a semi-desert environment. Journal of Animal Ecology 71:603–613.

MacArthur, R. H. 1970. Species packing and competitive equilibria for many species. Theoretical Population Biology 1:1–11.

Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores. Pages 251–296 *in* G. A. Rosenthal and M. R. Berenbaum, eds. Herbivores: their interactions with secondary plant metabolites. 2d ed. Vol. 1. Academic Press, San Diego, Calif.

Marquis, R. J., I. R. Diniz, and H. C. Morais. 2001. Patterns and correlates of interspecific variation in foliar insect herbivory and pathogen attack in Brazilian cerrado. Journal of Tropical Ecology 17:127–148.

Martel, J. W., and S. B. Malcolm. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. Journal of Chemical Ecology 30:545-561.

Pearl, R., and L. J. Reed. 1920. On the rate of growth of the population of the United States since 1790 and its mathematical representation. Proceedings of the National Academy of Sciences of the USA 6:275–288.

Prairie, Y. T., and D. F. Bird. 1989. Some misconceptions about the spurious correlation problem in the ecological literature. Oecologia (Berlin) 81:285–288.

Rhoades, D. F. 1983. Herbivore population dynamics and

plant chemistry. Pages 155–220 *in* R. F. Denno and M. S. McClure, eds. Variable plants and herbivores in natural and managed systems. Academic Press, New York.

———. 1985. Offensive-defensive interactions between herbivores and plants: their relevance in herbivore population-dynamics and ecological theory. American Naturalist 125:205–238.

Rothschild, M., J. V. Euw, and T. Reichste. 1970. Cardiac glycosides in Oleander aphid, *Aphis nerii*. Journal of Insect Physiology 16:1141–1145.

Simpson, S. J., and D. Raubenheimer. 2001. The geometric analysis of nutrient-allelochemical interactions: a case study using locusts. Ecology 82:422–439.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. 3d ed. Freeman, New York.

Strong, D. R. 1984. Density-vague ecology and liberal population regulation in insects. Pages 313–327 *in* P. W. Price, C. N. Slobodchikoff, and W. S. Gaud, eds. A new ecology: novel approaches to interactive systems. Wiley, New York.

Strong, D. R., J. H. Lawton, and R. Southwood. 1984. Insects on plants. Harvard University Press, Cambridge, Mass.

Underwood, N., and M. Rausher. 2000. The effects of host-plant genotype on herbivore population dynamics. Ecology 81:1565–1576.

———. 2002. Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. American Naturalist 160:20–30.

Van Der Meijden, E. 1996. Plant defence, an evolutionary dilemma: contrasting effects of (specialist and generalist) herbivores and natural enemies. Entomologia Experimentalis et Applicata 80:307–310.

White, T. C. R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia (Berlin) 63:90–105.

Wilkinson, L. 1997. Systat 7.0: statistics. SPSS, Chicago.

Woodson, R. E. 1954. The North American species of *Asclepias* L. Annals of the Missouri Botanical Garden 41: 1–211.

Yoshida, T., L. E. Jones, S. P. Ellner, G. F. Fussmann, and N. G. Hairston, Jr. 2003. Rapid evolution drives ecological dynamics in a predator-prey system. Nature 424: 303–306.

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Appendix. Methods for measuring defensive, nutritional, and life-history traits of the 18 species of *Asclepias*.

Field measurements:

All measures were taken from newly expanded, undamaged leaves of plants in a common garden. Plants were grown from seed in growth chambers and out-planted to a plowed field at the Koffler Scientific Reserve at Jokers Hill. Cardenolide concentrations were measured as digitoxin equivalents (grams per gram dry tissue) extracted from 50 mg dry leaf tissue; I employed a spectrophotometric assay modified Nelson (1993). I adapted the assay for sampling using a microplate reader (PowerWave X, Bio-Tek Instruments, Winooski, Vermont) and sampled 5 randomly selected replicates from each species. 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 (MCT 200-C, AXYGEN Scientific, Union City, California). To each tube, 1.9 ml of 95% ethanol was added, tubes were then vortexed, and floated in a sonicating water bath (65 ^{o}C) for 10 minutes. I then centrifuged the tubes at 5000 rpm for five minutes at room temperature. Two 45 µl 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 (Sigma Chemical Co., 0.125 mg/ml to 3 mg/ml). I then added 90 µl of ethanol to the blanks and 90 µl of 0.15% 2,2'4,4'-tetranitrodiphenyl (TNDP) in ethanol to the active samples. Finally, 70 µl of 0.1 M 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.

I measured latex from 5 replicates from each species by cutting the tip off (0.5 cm) of an intact leaf in the field and collecting the exuding latex onto a 1cm disc of filter paper (#1 Whatman International, Maidstone, U.K.). Latex stopped flowing after ≈10 seconds, all latex was absorbed on the filter paper, and this disc was placed 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.

I assessed the trichome density of 5 replicate plants from each species by counting the tops and bottoms of leaf discs (28 mm^2) under a dissection microscope. Leaf discs were taken from the tips of leaves.

I measured leaf toughness on 5 replicates from each species with a force gauge penetrometer (Type 516, Chatillon Corp., NY) that measures the grams of force needed to penetrate a surface. I sandwiched the leaf between two pieces of plexiglass, 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, I 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 and nitrogen concentration was measured from 5 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).

Laboratory measurements:

Seed mass was measured by weighing at least 5 seeds of each species. Plant biomass was estimated by growing at least 5 replicates of each species, randomly mixed throughout large growth chambers, in 500 ml pots and potting mix soil. After one month of growth, plants were harvested by drying the aboveground plant parts in a drying oven and weighing the biomass. Specific leaf weight was estimated as the wet mass of leaf discs (28 mm^2, from a newly expanded leaf) from each of 5 chamber grown plants of each species. Proportion water was calculated as (wet mass – dry mass)/wet mass of the same leaf discs.

Literature Cited

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, eds. Biology and conservation of the monarch butterfly. Los Angeles, CA, Natural History Museum of Los Angeles County.

Electronic table 1. Species means and standard errors (in parentheses) for ten defensive, nutritional, and life-history characteristics of the milkweeds. Data on these traits represent the measurement of at least 5 undamaged individuals of each species grown in a common environment and differences between species are highly significant for all traits (AAA, unpublished).

Asclepias species	Seed mass (mg)	biomass (g)	Latex (mg)	Trichomes (per 0.3 cm^2)	Leaf toughness (g)	Cardenolides (g g-1 dry digitoxin equivalents)	Carbon (% dry mass)	Nitrogen (% dry mass)	Specific leaf weight (mg)	Proportion water
asperula	5.10	0.51	0.78	91.71	116.00	0.031	42.14	3.63	11.92	0.82
	(0.23)	(0.06)	(0.25)	(12.01)	(18.21)	(0.014)	(0.37)	(0.19)	(0.60)	(0.01)
cordifolia	24.69	0.13	0.98	0.17	57.50	0.007	42.62	4.08	13.14	0.83
	(0.70)	(0.02)	(0.16)	(0.17)	(4.90)	(0.007)	(0.69)	(0.20)	(0.88)	(0.01)
curassavica	3.00	5.91	0.74	141.96	86.00	0.012	42.56	3.76	108.53	0.90
	(0.09)	(0.54)	(0.27)	(10.75)	(4.88)	(0.012)	(0.72)	(0.11)	(15.35)	(0.01)
curassavica var. silky gold	3.19	2.18	0.17	55.42	143.50	0.019	43.10	3.85	14.84	0.83
	(0.09)	(0.17)	(0.11)	(10.24)	(19.54)	(0.014)	(0.55)	(0.21)	(1.40)	(0.03)
erosa	29.52	2.10	6.68	441.88	129.00	0.016	43.68	4.13	22.53	0.85
	(1.10)	(0.23)	(1.30)	(132.94)	(9.24)	(0.007)	(0.48)	(0.10)	(0.89)	(0.01)
fascicularis	5.71	0.96	0.66	16.50	100.00	0.007	44.43	4.10	11.02	0.78
	(0.28)	(0.11)	(0.22)	(4.43)	(13.27)	(0.005)	(0.55)	(0.29)	(0.71)	(0.02)
fruticosa	8.72	1.99	0.23	69.58	110.00	0.019	44.92	3.85	13.13	0.84
	(0.39)	(0.31)	(0.14)	(25.87)	(6.67)	(0.009)	(0.12)	(0.17)	(1.17)	(0.02)
hallii	6.69	1.69	0.97	208.75	111.50	0.009	42.67	4.19	14.17	0.85
	(0.45)	(0.13)	(0.21)	(37.46)	(11.21)	(0.007)	(0.88)	(0.23)	(0.66)	(0.01)
hirtella	11.32	0.32	0.27	33.54	177.00	0.075	42.61	3.33	10.43	0.81
	(0.55)	(0.03)	(0.08)	(8.45)	(29.88)	(0.014)	(0.37)	(0.10)	(0.89)	(0.01)
incarnata	4.97	1.87	0.54	64.11	125.50	0.031	43.44	4.01	11.63	0.80
	(0.40)	(0.12)	(0.16)	(7.43)	(7.73)	(0.007)	(0.64)	(0.26)	(0.41)	(0.01)
oenotheroides	5.57	2.37	1.05	38.83	129.50	0.035	42.24	3.42	13.84	0.87
	(0.32)	(0.27)	(0.42)	(12.33)	(16.42)	(0.007)	(0.41)	(0.16)	(1.73)	(0.01)

speciosa ssp. davis	10.87	1.89	4.56	431.71	123.50	0.009	42.20	3.58	20.22	0.87
	(0.66)	(0.22)	(1.28)	(98.33)	(9.63)	(0.005)	(0.52)	(0.20)	(1.50)	(0.01)
sullivantii	8.70	0.39	4.04	1.70	149.50	0.035	42.89	3.19	16.29	0.81
	(0.54)	(0.03)	(1.25)	(1.07)	(8.31)	(0.007)	(0.43)	(0.36)	(0.64)	(0.01)
syriaca	4.84	2.15	1.61	427.13	71.50	0.052	42.73	3.74	15.48	0.83
	(0.18)	(0.12)	(0.58)	(48.33)	(4.22)	(0.007)	(0.43)	(0.17)	(1.00)	(0.01)
tuberosa	5.54	1.46	0.15	139.00	95.50	0.035	44.32	3.57	8.54	0.84
	(0.32)	(0.13)	(0.14)	(28.97)	(14.61)	(0.007)	(0.65)	(0.23)	(0.11)	(0.01)
variegata	4.14	0.37	1.32	38.42	127.00	0.028	42.44	3.06	15.89	0.82
	(0.06)	(0.04)	(0.41)	(7.14)	(16.40)	(0.005)	(0.84)	(0.18)	(0.82)	(0.01)
verticillata	2.00	0.91	0.63	58.96	105.56	0.038	44.03	3.76	7.24	0.82
	(0.11)	(0.11)	(0.17)	(12.78)	(9.63)	(0.009)	(0.25)	(0.14)	(1.34)	(0.01)
viridis	5.80	1.31	0.81	96.46	118.50	0.064	41.26	3.52	15.46	0.84
	(0.34)	(0.14)	(0.23)	(17.35)	(6.41)	(0.009)	(0.60)	(0.19)	(1.07)	(0.01)

Electronic table 2. Individual regression analyses for *Aphis nerii* on 18 species of *Asclepias*. The first 10 species listed below were employed in the first block of the experiment.

Species	SS	Df	MS	F	P	R^2	Y-intercept	Slope
asperula	0.073	1	0.073	20.927	0.001	0.617	0.519	-0.016
	0.045	13	0.003					
speciosa ssp. davis	0.072	1	0.072	25.312	0.001	0.661	0.620	-0.016
	0.037	13	0.003					
erosa	0.061	1	0.061	13.746	0.003	0.514	0.592	-0.014
	0.057	13	0.004					
fruticosa	0.004	1	0.004	1.916	0.196	0.161	0.395	-0.005
	0.023	10	0.002					
verticillata	0.065	1	0.065	49.123	0.001	0.791	0.559	-0.015
	0.017	13	0.001					
viridis	0.090	1	0.090	28.470	0.001	0.670	0.602	-0.017
	0.044	14	0.003					
curassavica	0.073	1	0.073	38.244	0.001	0.746	0.613	-0.016
	0.025	13	0.002					
syriaca	0.065	1	0.065	155.181	0.001	0.922	0.612	-0.015
	0.005	13	0.001					
hallii	0.060	1	0.060	74.696	0.001	0.852	0.619	-0.015
	0.011	13	0.001					
tuberosa	0.077	1	0.077	61.906	0.001	0.826	0.585	-0.017
	0.016	13	0.001					
cordifolia	0.059	1	0.059	20.939	0.001	0.636	0.430	-0.014
	0.034	12	0.003					
curassavica	0.016	1	0.016	25.946	0.001	0.684	0.462	-0.008
(silky gold)	0.008	12	0.001					
fascicularis	0.027	1	0.027	14.185	0.002	0.522	0.478	-0.011
	0.025	13	0.002					
hirtella	0.033	1	0.033	24.872	0.001	0.675	0.423	-0.012
	0.016	12	0.001					

physocarpa	0.014	1	0.014	9.468	0.011	0.463	0.454	-0.008
	0.016	11	0.001					
sullvantii	0.020	1	0.020	17.039	0.001	0.549	0.418	-0.008
	0.017	14	0.001					
variegata	0.034	1	0.034	24.086	0.001	0.667	0.445	-0.12
	0.017	12	0.001					
incarnata	0.026	1	0.026	16.197	0.001	0.536	0.510	-0.009
	0.022	14	0.002					
syriaca	0.016	1	0.016	27.276	0.001	0.713	0.463	-0.008
(trial 2)	0.006	11	0.001					
tuberosa	0.067	1	0.067	45.824	0.001	0.806	0.512	-0.015
(trial 2)	0.016	11	0.001					

Electronic Table 3. Pearson product moment correlations among the 10 traits included in the multiple regression. N=18. *P<0.05, **P<0.01, ***P<0.001.

	Seed mass	Plant mass	Specific leaf weight	Percent water	Carbon	Nitrogen	Latex	Trichomes	Toughness	Cardenolides
Seed mass	1.00									
Plant mass	-0.19	1.00								
Specific leaf weight	*0.49	0.25	1.00							
Percent water	0.13	**0.62	*0.49	1.00						
Carbon	0.02	0.04	-0.36	-0.36	1.00					
Nitrogen	0.32	0.28	0.01	-0.07	0.37	1.00				
Latex	**0.61	0.02	***0.79	0.23	-0.12	0.02	1.00			
Trichomes	0.30	0.37	**0.66	0.38	-0.10	0.23	**0.64	1.00		
Toughness	-0.07	-0.24	0.11	-0.20	-0.10	-0.44	0.11	-0.14	1.00	
Cardenolides	-0.02	0.09	0.06	0.04	-0.10	-0.02	-0.10	0.09	0.29	1.00