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Competition among herbivores of *Solanum carolinense* as a constraint on the evolution of host-plant resistance

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Abstract Interspecific competition (or facilitation) between herbivores sharing a host plant species can result in negative (or positive) correlations in damage levels, independent of a plant's genetic covariance in resistance to the herbivores. Just like genetic correlations in resistance to herbivory, these "environmental correlations" in damage may affect the evolution of resistance in the host plant. In a field study of 960 ramets and 40 genets of the herbaceous plant *Solanum carolinense*, I looked for evidence of such environmental correlations in damage caused by 11 species of herbivores, including 10 insects and 1 mammal. There were 28 significant correlations in damage levels between species (21 negative and 7 positive) after plant genetic influences on resistance were statistically removed. Negative environmental correlations were more likely between species that fed upon the same type of plant organs than between those that fed on different types of organs, and the magnitudes of the correlations were inversely proportional to the abundance of the organ types. Taken together, these results offer strong evidence that competition is largely responsible for the pattern of environmental correlations in damage. Environmental correlations were just as common as genetic correlations in resistance, but the environmental correlations tended to be lower in magnitude, were more likely to be negative, and were more evenly spread out among the herbivore community than the genetic correlations. Damage levels by all 11 species were negatively correlated with damage by at least one other species. Thus the selective advantage a plant would receive from increased resistance any of these herbivores would be partially negated by increased damage by competing herbivores. As a result, competition has the potential to be an important constraint on the evolution of resistance in *S. carolinense*.

Keywords Environmental correlations · Genetic correlations · Horsenettle · Multiple-herbivore community · Plant–insect interactions · Resistance to herbivory

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Introduction

Investigators of the evolutionary ecology of plant resistance to herbivory have increasingly stressed the importance of a community-level approach; that is, simultaneously studying the influence of multiple herbivores on a shared host species (Anderson and Paige 2003; Poitrineau et al. 2003; Strauss and Irwin 2004; Johnson and Agrawal 2005; Strauss et al. 2005). When more than one herbivore is considered, factors that either facilitate or constrain the evolution of resistance may emerge that would not be evident in pairwise plant-herbivore studies. These emergent factors include genetic covariances in resistance to different herbivores, nonadditive impacts of multiple herbivores on host-plant fitness, and ecological interactions among herbivores (e.g., competition) that change the relative likelihood of damage among plants, and thus result in correlations in damage among herbivore species (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997; Strauss et al. 2005).

Progress in illuminating the evolutionary dynamics of resistance to multiple herbivores has been accomplished in several plant species using the phenotypic or genotypic selection analyses described by Lande and Arnold (1983) and Rausher (1992): (e.g., Rausher and Simms 1989; Núñez-Farfán and Dirzo 1994; Mauricio and Rausher 1997; Juenger and Bergelson 1998; Stinchcombe and Rausher 2001; Wise 2003). In such analyses, multiple regressions generate selection gradients, which quantify the direction and strength of natural selection acting directly on resistance to each herbivore, independent of damage by other herbivores. To estimate the evolutionary response of resistance to each herbivore, a vector of selection gradients can be multiplied by a matrix containing the genetic variances for resistance to each herbivore and the genetic covariances for resistance between herbivores (i.e., the ***G***-matrix). This selection-response approach treats correlations in damage that are not the direct result of genetically controlled resistance traits as environmental noise. If these non-genetic correlations in damage (hereafter referred to as "environmental correlations") are random or transient, then they are not expected to affect evolutionary trajectories of resistance. However, if they are consistent and predictable, then they may play an important role in the dynamics of the evolution of resistance.

A primary potential cause for consistent, predictable environmental correlations in damage levels is interspecific interactions between herbivores. For instance, studies have documented that some herbivores prefer plants that have previously been damaged by other species of herbivores (Damman 1989; Tscharntke 1989; Pilson 1992; Cappuccino and Martin 1994; Agrawal and Sherriffs 2001). Such facilitative interactions between herbivore species can magnify the fitness differences among resistant and susceptible plants and thus strengthen natural selection for resistance, at least against the species causing the initial damage. Even more studies have shown that herbivores can prefer plants that have received less prior damage by other species (Damman 1993; Denno et al. 1995; Agrawal 1999; González-Megías and Gómez 2003; Van Zandt and Agrawal 2004). In these cases, the selective benefit a plant might achieve from decreased damage by an herbivore could be offset by the loss of fitness due to increased damage by succeeding herbivores (Gould 1988). To the extent that such facilitation and competition commonly occur between herbivore species, environmental correlations in damage levels may promote or constrain the evolution of resistance in multiple-herbivore communities.

Although documentation of interactions between focal pairs of insects reveals that environmental correlations in damage sometimes have the potential to affect the evolution of host-plant resistance, isolated cases do not give a good sense of how prevalent such interactions are. There is a good chance that researchers choose species that are likely to interact, and studies in which no interactions are found are probably less likely to be published (Schoener 1983; Denno et al. 1995). To gain more insight into the role of environmental correlations in a host plant's evolution, it is important to look simultaneously at as many of the plant's herbivores as possible.

To address this need, I measured damage levels of 11 herbivores of the perennial herb *Solanum carolinense* L. (Solanaceae) in an experimental field population composed of 24 ramets (stems) of 40 genets (genetic individuals). These herbivores included 10 insects and 1 mammal, and each herbivore left diagnostic damage to leaves, stems, flowers, and/or fruits. After statistically partitioning the damage into plant genetic and non-genetic influences, I calculated environmental correlations in damage between each pair of herbivores. I addressed four main questions with these data: (1) What are the frequencies of significant negative and positive correlations in damage between species? (2) How do correlations for herbivores that feed on the same type of plant organ compare with correlations for herbivores of different plant organs? (3) Are negative correlations stronger for scarcer plant organs (e.g., fruits > flowers > leaves), a result that would suggest competition? and (4) How do the frequency, signs, and magnitudes of environmental correlations in damage compare with genetic correlations in resistance?

Materials and methods

Study organisms

Solanum carolinense, or "horsenettle," is a perennial herbaceous weed native to the southeastern United States, and it has become a crop and pasture pest throughout the US and in areas of Europe and Asia (Bassett and Munro 1986; Imura 2003; NAPPO 2003). Horsenettle spreads vegetatively through a rapidly expanding lateral root system, and it reproduces sexually with showy white and purple flowers that form yellow berries averaging ~1.5 cm in diameter (Ilnicki et al. 1962; Nichols et al. 1991; Wise and Sacchi 1996).

Although seemingly well armed with spines, trichomes, and toxic alkaloids, horenettle is regularly attacked by a diverse community of herbivores (Wise 2007b). This study focused on 11 of the most damaging species, which included one mammal (the meadow vole) and 10 insects from four taxonomic orders (Table 1). Details on the natural history of the herbivores and methods for damage assessment can be found in Wise (2003, 2007b). Importantly, each species produces a unique damage pattern that can be used to identify its feeding well after the herbivores are gone.

Experimental design

The data analyzed in this article came from the same experiment described in Wise (2003, 2007a), and readers may consult those papers for further experimental detail. The study involved measuring herbivore damage on 960 transplanted horsenettle ramets (stems) in an experimental population in 2001 at Blandy Experimental Farm in Clarke County, Virginia, USA (39° N 78° W). The transplanted ramets comprised 24 clonal replicates of each of 40 genetic individuals (genets), collected from four populations in and around Blandy Farm in the spring of 1997 and propagated in pots in commercial growing medium (Wesco growing media III, Wetsel Seed Company, Harrisburg, Virginia) each year through 2001. These

Table 1 Taxonomy and damage measurement units for horsenettle herbivores

Order species (family)	Common name	Feeding mode and site	Damage measurement units
Hemiptera			
Gargaphia solani Heidemann (Tingidae)	Eggplant lace bug	Sucks leaf mesophyll cells	Presence of eggs
Coleoptera			
Epitrix fuscula Crotch (Chrysomelidae)	Eggplant flea beetle	Adults chew leaves	Relative leaf area eaten (grid)
Leptinotarsa juncta (Say) (Chrysomelidae)	False potato beetle	Chews leaves, flowers, and fruits	(1) % leaf area eaten (2) No. of flowers destroyed (3) No. of fruits destroyed
Gratiana pallidula (Boheman) (Chrysomelidae)	Eggplant tortoise beetle	Chews leaves	No. of leaves with damage
Trichobaris trinotata (Say) (Curculionidae)	Potato stalk borer	Larvae bore stems	Presence of borer or damage
Anthonomus nigrinus Boheman (Curculionidae)	Potato bud weevil	Larvae feed inside flower buds	No. of flower buds destroyed
Diptera			
Prodiplosis longifila Gagné (Cecidomyiidae)	Citrus gall midge	Larvae feed in rolled leaves	No. of leaves with damage
Zonosemata electa (Say) (Tephritidae)	Pepper maggot	Larvae feed on fruit pulp	No. of fruits infested
Lepidoptera			
Frumenta nundinella (Zeller) (Gelechiidae)	N/A	Larvae eat ovules inside fruits	No. of flowers destroyed
Tildenia inconspicuella (Murtfeldt) (Gelechiidae)	Eggplant leafminer	Larvae mine leaves	No. of leaves with damage
Rodentia			
Microtus pennsylvanicus (Cricetidae)	Meadow vole	Chews flowers and fruits	(1) No. of flowers destroyed (2) No. of fruits destroyed

propagation procedures served to create large quantities of root material for clonal replicates and to purge plants of potential carryover effects from their source environments (Roach and Wulff 1987).

In spring of 2001, at least 38 root segments (each 2 cm^3) from each of the 40 genets were planted individually in 3.8-l plastic pots. Twenty-four pots for each genet were placed outdoors on wooden pallets in a randomized-block design with three blocks. Unhealthy or damaged ramets, and ramets that had begun to flower before potential exposure to floral herbivores, were replaced with alternates of the appropriate genet. Between 28 June and 2 July, the ramets were transplanted into three blocks of 10 rows each within an established horsenettle population in an old field at Blandy Farm. Ramets were placed 1.5 m apart within a row and 2 m apart between rows. The transplanted ramets blended very well with the native horsenettle, and the density of transplanted to native horsenettle ramets in the field was roughly 1:30 (Wise 2007a).

Damage measurements

Data on floral herbivory (florivory) were recorded for each ramet every 3–5 days, beginning just after transplantation and ending in late September when flowering was complete. I checked for fruit damage (frugivory) at the same time, and I collected fruits as they ripened to prevent dispersal. The fruits were kept in a growth chamber in plastic cups to allow insects to emerge. Once emergence had ceased, I dissected each fruit to look for other evidence of infestation. Data on the six leaf feeders (folivores) were taken in two rounds: damage by eggplant flea beetles, false potato beetles, and eggplant leaf miners (*Epitrix*, *Leptinotarsa*, and *Tildenia*) was measured in August, while damage by eggplant tortoise beetles, citrus gall midges, and eggplant lace bugs (*Gratiana*, *Prodiplosis*, and *Gargaphia*) was measured in early September. This timing ensured that the measurements occurred after most of the damage accrued but before leaves had senesced. At the end of the season, I dissected all stems to look for evidence of stem-borer damage. The damage metric used for each herbivore is shown in Table 1, and more details on these metrics are reported in Wise (2003, 2007a).

Data analysis

Before the analyses, I converted raw damage measurements into "operational resistance" values that could more readily be compared among ramets and among herbivore species. The size of a plant (e.g., the number of leaves or flowers it produces) will obviously affect the absolute amount of damage it can receive. Typically, this fact has been taken into account by estimating resistance using the proportion of the available tissues eaten. For example, to calculate resistance of a plant to a florivore, one might use the equation *resistance* = 1 − *flowers eaten/flowers available*. This estimate is reasonable as long as the number of flowers available is relatively large. If it is not always large, then the plants with the fewest flowers are bound to have the most extreme resistance estimates, because they are more likely to have all or none of their flowers eaten. These extreme estimates may drive the patterns found in analyses of resistance, even though it is for these plants that the resistance estimate is actually least reliable.

Instead of using the proportion of a ramet's flowers eaten by a species of florivore to calculate resistance, I accounted for the total number of flowers the ramet produced by running a linear regression of the number of flowers eaten on the total number of flowers and saving the residuals for each ramet. The more resistant a ramet is to the florivore, the more negative the residual will be. These residuals were then multiplied by -1 so that the higher numbers corresponded to the plants with the greater resistance. I ran similar regressions for the frugivores and for those folivores for which the raw damage measures were numbers of leaves damaged. Finally, I applied power transformations (such as the log-transformation) to the resistance values if they improved the normality of their distributions and reduced outliers prior to subjecting them to further analysis.

Phenotypic correlations in damage levels between different herbivores are due to both genetically controlled plant differences in resistance and environmental causes, such as interactions between herbivores. The use of clonal replicates of many different genets in this experiment allowed partitioning of genetic and environmental covariances among the operational-resistance values. To accomplish this partitioning, I used a mixed-effects procedure in SAS version 9.1 for Windows (SAS Institute, Cary, NC, USA), adapting code presented by Fry (2004) to an experiment with clonal replicates (see also Messina and Fry

2003). A separate model was run for each pair of herbivores. For simplicity, all 40 genets were considered simultaneously (ignoring source population), and block differences in herbivore damage were removed from the operational-resistance measurements (using ANOVA) prior to running the mixed-effects models.

I calculated genetic and environmental correlations from the covariance estimates for each species pair, and the pairwise significance of the correlations were obtained from Z-values calculated by restricted-maximum-likelihood tests on the respective genetic and environmental covariances. For herbivores that fed on more than one type of plant organ (viz., *Leptinotarsa* and *Microtus*), correlations were calculated separately for each organ type. The analysis yielded 91 pairwise environmental correlations (87 between species and 4 within species but between organ types) and 91 pairwise genetic correlations.

To determine whether damage levels by herbivores that fed on the same organ types (either leaves, stems, flowers, or fruits) were more likely to be environmentally correlated, I performed a Mantel test (Sokal and Rohlf 1995). This test involved creating a design matrix analogous to the species-pair matrix (Table 2), except with 1's in the cells for species that fed on the same organ types and 0's in the cells for different organ types. From these two matrices, I calculated the Mantel Z, also known as the Hadamard product of two matrices, which is the sum of the products of corresponding off-diagonal elements. I then repeatedly randomized the rows and columns of the design matrix and calculated Mantel values for 2,000 random permutations. The position of the observed Mantel Z in the distribution of random Mantel values determined its P-value.

The genetic and environmental correlations were compared in several ways. I compared the relative frequencies of positive and negative correlations for genetic vs. environmental correlations using χ^2 tests, first for all of the 87 interspecific comparisons, then only for those correlations significant at a pairwise alpha of 0.05. I tested for differences in means of genetic and environmental correlations using t-tests assuming unequal variances. To further assess the relationships among the types of correlations, I calculated product-moment correlations between the sets of genetic and phenotypic correlations, between the sets of environmental and phenotypic correlations, and between the sets of genetic and environmental correlations. All of these analyses were performed with JMP-IN 4.0.4 (SAS Institute, Cary, NC, USA). The significance of these correlations was determined by Mantel tests (Sokal and Rohlf 1995). I calculated the Mantel Z values for the three pairs of matrices (G × P, E × P, and G × E), then compared these observed values with distributions of Mantel Z's created from 1,000 random permutations of the matrices.

Results

Environmental correlations in resistance were widespread among the herbivores of horsenettle: 28 of the 87 interspecific correlations were significantly different from zero, with 21 negative and 7 positive correlations (Table 2). If the damage levels were completely independent, then with 87 correlations calculated, only two positive and two negative correlations would be expected by chance at a significance level of 0.05 (i.e., due to type 1 error). The negative correlations in damage levels were rather widespread, with the damage level by every species being negatively environmentally correlated with damage by at least one other species.

Damage levels by herbivores that fed on the same plant-organ type were significantly more negative than would be expected based on chance (Mantel $Z = -1.85$, $P = 0.003$). While 42% of the within-organ correlations were significantly negative, only 17% of the

Table 2 Environmental correlations[a] in damage levels between herbivore species

Herbivore (organ fed on)	*Gratiana* (leaves)	*Prodiplosis* (leaves)	*Leptinotarsa* (leaves)	*Epitrix* (leaves)	*Gargaphia* (leaves)	*Trichobaris* (stem)	*Anthonomus* (flowers)	*Leptinotarsa* (flowers)	*Frumenta* (flowers)	*Microtus* (flowers)	*Leptinotarsa* (fruits)	*Zonosemata* (fruits)	*Microtus* (fruits)
Tildenia (leaves)	0.05	**0.08**	0.02	−0.04	−**0.11**	0.04	−0.02	0.06	0.00	0.03	−0.05	−0.03	0.06
Gratiana (leaves)	–	**0.12**	−0.01	−0.01	0.06	−0.07	0.04	−**0.08**	0.00	**0.14**	−0.06	−0.06	**0.14**
Prodiplosis (leaves)	–	–	0.01	−0.02	0.02	0.03	−**0.07**	0.03	**0.07**	**0.11**	−0.01	−0.04	**0.10**
Leptinotarsa (leaves)	–	–	–	−**0.09**	−0.05	0.05	−0.06	(**0.31**)	−**0.14**	−0.01	(**0.34**)	−**0.14**	0.02
Epitrix (leaves)	–	–	–	–	−**0.13**	0.04	−0.01	−**0.12**	0.02	0.04	−**0.12**	−0.02	0.06
Gargaphia (leaves)	–	–	–	–	–	−0.02	0.02	0.01	0.02	−0.01	−0.02	−0.01	0.03
Trichobaris (stem)	–	–	–	–	–	–	0.01	0.02	0.01	−**0.07**	0.02	−0.01	−0.03
Anthonomus (flowers)	–	–	–	–	–	–	–	−**0.28**	0.06	−**0.24**	−0.02	0.04	−0.02
Leptinotarsa (flowers)	–	–	–	–	–	–	–	–	−0.06	−**0.19**	(**0.32**)	−**0.11**	−0.04
Frumenta (flowers)	–	–	–	–	–	–	–	–	–	−**0.08**	−0.02	0.01	−**0.10**
Microtus (flowers)	–	–	–	–	–	–	–	–	–	–	−**0.10**	−**0.09**	(**0.32**)
Leptinotarsa (fruits)	–	–	–	–	–	–	–	–	–	–	–	−**0.27**	−**0.25**
Zonosemata (fruits)	–	–	–	–	–	–	–	–	–	–	–	–	−**0.47**

[a] Boldface values are significant at a pairwise $P < 0.05$. Underlined values are significant at $P < 0.05$ with a Dunn-Šidák correction for multiple comparisons, which corresponds to a pairwise significance of $P < 0.00056$ (Sokal and Rohlf 1995). Values in parentheses are correlations within a species feeding on different plant organs

between-organ correlations were. Overall, the means of the within-organ-type correlations were: −0.006 for folivores, −0.13 for florivores, and −0.33 for frugivores. The mean of the between-organ correlations was −0.008. If florivores and frugivores are grouped together as a single reproductive-organ-feeding category, then the mean of the between-organ correlations would be −0.001.

The genetic correlations among herbivore species tended to be more positive than the environmental correlations (Table 3). Overall, the ratio of positive-to-negative genetic correlations between species was 49:38, while the ratio for environmental correlations was 38:49. This difference in ratios was not statistically significant ($X^2 = 2.304$, $P = 0.13$). However, when only considering the significant correlations (at pairwise $P < 0.05$), the genetic correlations were significantly more likely to be positive (11:7) than the environmental correlations (7:21) ($X^2 = 6.030$, $P = 0.014$.). The mean (±1 SE) of all the genetic correlations was 0.06 ± 0.05, while the mean of all the environmental correlations was −0.03 ± 0.01 ($t = 1.79$, $P = 0.08$). Considering only the significant correlations, the mean environmental correlation was −0.09 ± 0.03, and the mean genetic correlation was 0.16 ± 0.17 ($t = 1.49$, $P = 0.15$).

The mean phenotypic correlation in damage levels between species was −0.03 ± 0.01, and there were roughly the same number of positive as negative values (42 vs. 45, respectively). The phenotypic correlations were strongly correlated with both the genetic correlations ($r = 0.45$, Mantel $Z = 1.62$, $P < 0.001$) and the environmental correlations ($r = 0.94$, Mantel $Z = 1.62$, $P < 0.001$). The genetic and environmental correlations were also positively correlated with each other ($r = 0.25$, Mantel $Z = 0.75$, $P = 0.017$).

Discussion

Partitioning of covariances

In this study, phenotypic covariances in damage levels among horsenettle herbivores were partitioned into genetic covariances and environmental covariances. The phenotypic covariances in damage among species were just as likely to be positive as negative, while the genetic covariances were slightly more likely to be positive, and the environmental covariances were slightly more likely to be negative. The phenotypic covariances appeared to be controlled more strongly by the environmental than the genetic covariances, as the correlation between the phenotypic and environmental correlations was greater ($r = 0.94$) than the correlation between the phenotypic and genetic correlations ($r = 0.45$). Both were highly significant, however. It is also important to note that the genetic and environmental correlations were themselves positively correlated ($r = 0.25$). Such a correlation could imply that the genetic correlations still contained some environmental influence, and that the partitioning of the phenotypic covariance was not complete.

Although I am aware of no other attempts to isolate genetic and environmental correlations in damage in multiple-herbivore communities, two studies on multiple-herbivore communities have examined both phenotypic and genetic correlations in damage. Maddox and Root (1990) studied abundances of 17 insect herbivores of tall goldenrod, *Solidago altissima* L. (Asteraceae), and Roche and Fritz (1997) studied abundances of 12 species of leaf- and stem-feeding insects on silky willow, *Salix sericea* Marshall (Salicaceae). Whereas the significant environmental correlations among horsenettle herbivores in the current study were predominantly negative (75%), the significant phenotypic correlations in these other studies were predominantly positive (26:6 positive-to-negative for goldenrod

Table 3 Genetic correlations[a] in horsenettle's resistance to herbivores

Herbivore (organ fed on)	*Gratiana* (leaves)	*Prodiplosis* (leaves)	*Leptinotarsa* (leaves)	*Epitrix* (leaves)	*Gargaphia* (leaves)	*Trichobaris* (stem)	*Anthonomus* (flowers)	*Leptinotarsa* (flowers)	*Frumenta* (flowers)	*Microtus* (flowers)	*Leptinotarsa* (fruits)	*Zonosemata* (fruits)	*Microtus* (fruits)
Tildenia (leaves)	0.33	0.26	−0.90	−0.50	0.18	0.15	0.43	0.70	0.43	0.41	0.41	0.45	−0.33
Gratiana (leaves)	–	0.48	0.29	−0.35	**0.50**	0.03	0.08	0.31	0.12	0.33	0.47	0.00	0.28
Prodiplosis (leaves)	–	–	0.27	−0.02	−0.40	−0.15	−0.27	−0.09	−0.20	0.48	0.14	−0.09	0.31
Leptinotarsa (leaves)	–	–	–	**0.82**	−0.69	−0.22	−0.20	(0.12)	−0.20	−0.63	(0.60)	0.16	0.61
Epitrix (leaves)	–	–	–	–	**−0.72**	**−0.16**	−0.36	−0.44	**−0.60**	−0.10	**−0.82**	−0.29	0.45
Gargaphia (leaves)	–	–	–	–	–	0.07	0.30	0.35	**0.52**	0.06	**0.65**	0.23	−0.30
Trichobaris (stem)	–	–	–	–	–	–	**0.23**	0.07	0.07	−0.07	0.04	0.15	−0.05
Anthonomus (flowers)	–	–	–	–	–	–	–	0.48	**0.58**	−0.31	0.31	0.36	−0.35
Leptinotarsa (flowers)	–	–	–	–	–	–	–	–	**0.82**	−0.29	(**0.86**)	**0.83**	**−0.78**
Frumenta (flowers)	–	–	–	–	–	–	–	–	–	−0.27	**0.92**	**0.63**	−0.51
Microtus (flowers)	–	–	–	–	–	–	–	–	–	–	0.01	−0.28	(0.40)
Leptinotarsa (fruits)	–	–	–	–	–	–	–	–	–	–	–	**1.24**	**−0.77**
Zonosemata (fruits)	–	–	–	–	–	–	–	–	–	–	–	–	**−0.73**

[a] Boldface values are significant at a pairwise $P < 0.05$. Values in parentheses are correlations within a species feeding on different plant organ

herbivores, and 22:0 and 31:0 positive-to-negative for silky willow herbivores in 2 years of study).

In Maddox and Root's (1990) goldenrod study, the phenotypic correlations were strongly correlated with the genetic correlations ($r = 0.75$), while Roche and Fritz (1997) did not find the phenotypic correlations to be correlated with genetic correlations in silky willow. Thus, the preponderance of positive phenotypic correlations in the latter study may be a better reflection of environmental correlations in damage. As such, it appears that the herbivores of silky willow did not compete, and it is possible that the herbivores facilitated each other's damage. However, it is also likely that the silky willow herbivores responded similarly to microhabitat differences or variation in host-plant vigor (cf., Fritz et al. 2000, 2003). It is also important to note that Roche and Fritz (1997) found silky willow's heritability for resistance to herbivory to be very low, thus making a high correlation between phenotype and genotype statistically unlikely.

Evidence for interspecific competition

The importance of interspecific competition on the ecology and evolution of herbivorous insects has long been an issue of debate (Hairston et al. 1960; Schoener 1982; Strong et al. 1984; Denno et al. 1995; González-Megías and Gómez 2003). Even if direct competition is not commonly strong enough to affect herbivore fitness, subtle interactions between herbivores have the potential to affect their host plant's evolution of resistance (Gould 1988; Hougen-Eitzman and Rausher 1994; Agrawal 2005). For example if an herbivore prefers plants with less prior damage by another species, then the gain in fitness that a plant might achieve from increased resistance to the one herbivore could be negated by the loss of fitness caused by the second herbivore (Gould 1988). Because competition among herbivores has the potential to be an important constraint on the evolution of host-plant resistance, it is worth asking whether the patterns of environmental correlations observed in the horsenettle-herbivore community are likely a result of interspecific competition.

Because this study did not involve herbivore manipulations, the environmental correlations cannot unambiguously be attributed to interactions between herbivores (Hastings 1987; Damman 1993). However, because the experimental design minimized microhabitat differences among plants, the correlations are less likely to be an artifact of herbivores responding similarly to an unmeasured environmental factor. In addition, there are some data from manipulative studies on this system that show competition between herbivores. Specifically, false potato beetles avoid oviposition on plants with greater eggplant flea beetle damage (Wise and Weinberg 2002), and eggplant flea beetles prefer plants with less damage by eggplant lace bugs, and vice versa (Wise, unpublished data).

The caveats of correlational studies notwithstanding, there are three main lines of evidence in this study that strengthen the conclusion that interspecific competition among herbivores is the main cause of the negative environmental correlations in damage. First, if the environmental correlations were due to another factor, such as variation in nutrient levels or plant vigor, it is likely that there would have been at least as many positive correlations as negative. However, the majority (75%) of the significant environmental correlations in damage between horsenettle herbivores were negative, as would be expected if they were caused by competition.

A second expectation if herbivores are competing is that the correlations in damage would be more strongly negative for pairs of herbivores that feed on the same type of plant organ than for herbivores that feed on different organ types. (For simplicity, "guild" will be used to refer to herbivores that feed on the same type of organ.) This is exactly the pattern seen among the horsenettle herbivores. While 42% of the within-guild environmental correlations in damage were significantly negative, only 17% of the inter-guild correlations were significantly negative. Closer inspection suggests that all but two of these significant inter-guild correlations may have occurred because the diet breadth of the species actually overlapped. For example, damage by adult flea beetles, which feed almost exclusively on leaves, was negatively correlated with flower and fruit damage by potato beetles. However, these between-guild correlations were likely a result of the fact that potato beetles also feed on leaves, and that their damage to leaves was strongly positively correlated with their damage to flowers and fruits. Thus, the only unambiguously significant inter-guild correlation was between the leaf-feeding citrus gall midges and the flower-feeding potato bud weevils, and between flower-feeding voles and the potato stalk borer. However, with 63 such inter-guild correlations, at least two would be expected to be found significantly negative at an alpha of 0.05 due to chance alone (i.e., due to Type I error). Finally, the herbivore whose damage was environmentally correlated with the fewest other herbivores was the potato stalk borer, which is the only member of the stem-feeding guild and is thus the least likely to compete with other herbivores.

The third line of evidence relates to the expectation that if competition was the cause of the environmental correlations, the correlations would be more strongly negative for food types that are less abundant. In this study, most of the leaves received at least a small amount of damage, but leaves were plentiful, and the total proportion of leaf tissue removed was rather low (Wise 2007b). Damage to flowers was more intense, with half of the flowers destroyed by herbivores. Fruits were much less numerous than flowers, and roughly three-quarters were destroyed by herbivores.

The pattern of correlations in this study was completely consistent with this third competition expectation. The negative correlations between folivorous species were the smallest of these three guilds, with a mean correlation of −0.006. The fact that one-third of the correlations between folivores were significant, however, suggests that leaf feeding by an herbivore was often influenced by the amount of damage by other folivores. Of course, even if leaf tissue appears abundant, leaves vary in age and quality and they are not all going to be suitable for the specific needs of each folivore. The magnitude of the mean correlation between flower feeders (−0.13) was greater than for folivores, and the mean correlation between frugivores (−0.33) was more than twice as strong as for flower feeders. In fact, all of the environmental correlations among the three species of frugivores were highly significantly negative.

Taken together, the evidence argues that interspecific competition among herbivores played a large role in the negative environmental correlations found in this study. Correlations in damage resulting from competition are likely to be consistent and predictable any time competing species are present in the same host-plant population. Consequently, competition may play a significant role in the evolutionary dynamics of horsenettle resistance, as well as in any other plant species that host multiple herbivores.

Evolutionary effects of correlations in damage

The genetic correlations in resistance reached larger magnitudes than the environmental correlations in damage between species. While only one of the environmental correlations had an absolute value greater than 0.3, 16 of the significant genetic correlations had an absolute value of at least 0.5. In addition, 31 other genetic correlations had magnitudes greater than 0.3 but were not statistically significant. This finding is likely indicative of the greater uncertainty in estimating the genetic correlations than the environmental correlations, as there were only 40 different plant genets and 960 ramets. If the difference in magnitudes of the environmental and genetic correlations is reliable, then genetic correlations might be expected to have a stronger influence on the evolutionary dynamics of horsenettle resistance than ecological interactions among herbivores.

Significant positive genetic correlations in resistance to different herbivores were slightly more common than negative genetic correlations (11 positive vs. 7 negative). Thus, genetic correlations are likely to facilitate the evolution of resistance to some of the herbivores and slow the evolution of resistance to other herbivores. In contrast, the environmental correlations were much more likely to be negative than positive, suggesting that competition between herbivores was more influential than facilitation. Therefore, the environmental correlations would tend to constrain the evolution of resistance more often than genetic correlations.

Negative environmental correlations tended to be more widespread across the herbivore community than the negative genetic correlations in resistance. Although resistances to 7 of the 11 species were negatively genetically correlated with resistance to at least one other species, all of the significant negative genetic correlations involved either eggplant flea beetles or meadow voles. In contrast, damage levels by all 11 species were negatively environmentally correlated with damage by at least one other species, and damage levels by the majority of the species were correlated with multiple species. Therefore, even though the magnitude of the environmental correlations tended not to be as great as the genetic correlations, the evolution of resistance to a greater proportion of the herbivore community is likely to be constrained by competition among herbivores than by genetic correlations in resistance.

With the potential established for ecological interactions to affect the evolution of resistance, the next step is to quantify more rigorously the magnitude of this effect on the evolutionary response of plants. A typical selection analysis applied to plant's resistance to multiple herbivores would quantify the impact of each herbivore's damage on plant fitness. The result of the analysis would be a vector of linear selection gradients ($\boldsymbol{\beta}$), which estimates the direction and strength of selection acting directly on resistance to each herbivore, independent of the effects of other herbivores. Through quadratic selection analysis, one can calculate correlational selection gradients, which take into account whether the *fitness impact* of damage by each herbivore depends on the amount of damage by the other herbivores. However, neither the linear nor the correlational gradients take into account whether the *amount* of damage caused by an herbivore will depend on the amount of damage by other herbivores. I have previously outlined a procedure for adjusting the linear selection gradients for environmental correlations in damage (Wise 2003). However, it may be more straightforward to adjust the predicted response to selection instead of adjusting the selection gradients themselves.

The effect of genetic correlations in resistance on the evolutionary response to selection is taken into account by the off-diagonal elements of the $\boldsymbol{G}$-matrix (the genetic variance-covariance matrix), which can be multiplied by the vector of selection gradients, $\boldsymbol{\beta}$, to predict the evolutionary change in mean resistance levels after one generation. Correlations in damage that are not caused by genetic covariance in resistance are treated as transient, environmental noise that cannot affect evolution. To incorporate the influence of herbivore–herbivore facilitation and competition, which would not be transient noise if the herbivores persist on the plants for multiple plant generations, one might consider multiplying the vector of selection gradients first by the $\boldsymbol{G}$-matrix, then by an analogous square matrix (***E***) with 1's on the diagonals and the environmental covariances on the off-diagonal elements. A comparison of the evolutionary change predictions from this product, $\mathbf{E}(\mathbf{G}\boldsymbol{\beta})$, with the traditional product, $\boldsymbol{G\beta}$, should yield a simple indication of the potential evolutionary significance of herbivore interactions in the evolution of plant resistance.

Regardless of how it is quantified, the effect of environmental correlations on the evolutionary response will also depend on the strength of natural selection for the interacting herbivores. In the horsenettle-herbivore system, the species most likely to compete are also the ones for which direct selection to increase resistance is the greatest. In a separate selection analysis (Wise 2003), selection to increase resistance was significant for all seven frugivores and florivores and for two of the folivores (flea beetles and leafminers). The standardized phenotypic selection gradients to increase resistance against the frugivores were on average almost five times greater than for the florivores, which were themselves nearly three times greater than for the folivores. The only species for which selection acted to decrease resistance was the potato stalk borer, which was the species least affected by interactions among the other herbivores. This pattern of stronger natural selection for resistance against species for which competition is the strongest makes the constraining potential for interactions among herbivores all the more significant.

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References

Agrawal AA (1999) Induced responses to herbivory in wild radish: effects on several herbivores and plant fitness. Ecology 80(5):1713–1723

Agrawal AA (2005) Natural selection on common milkweed (*Asclepias syriaca*) by a community of specialized insect herbivores. Evol Ecol Res 7:651–667

Agrawal AA, Sherriffs MF (2001) Induced plant resistance and susceptibility to late-season herbivores of wild radish. Ann Entomol Soc Am 94(1):71–75

Anderson LL, Paige KN (2003) Multiple herbivores and coevolutionary interactions in an *Ipomopsis* hybrid swarm. Evol Ecol 17:139–156

Bassett IJ, Munro DB (1986) The biology of Canadian weeds. 78. *Solanum carolinense* L. and *Solanum rostratum* Dunal. Can J Plant Sci 66:977–991

Cappuccino N, Martin M-A (1994) Eliminating early-season leaf-tiers of paper birch reduces abundance of mid-summer species. Ecol Entomol 19:399–401

Damman H (1989) Facilitative interactions between two lepidopteran herbivores of *Asimina*. Oecologia 78:214–219

Damman H (1993) Patterns of interaction among herbivore species. In: Stamp NE, Casey TM (eds) Caterpillars. Ecological and evolutionary constraints on foraging vol. Chapman & Hall, New York, pp 132–169

Denno RF, McClure MS, Ott JR (1995) Interspecific interactions in phytophagous insects: competition reexamined and resurrected. Annu Rev Entomol 40:297–331

Fritz RS, Crabb BA, Hochwender CG (2000) Preference and performance of a gall-inducing sawfly: a test of the plant vigor hypothesis. Oikos 89:555–563

Fritz RS, Crabb BA, Hochwender CG (2003) Preference and performance of a gall-inducing sawfly: plant vigor, sex, gall traits and phenology. Oikos 102:601–613

Fry JD (2004) Estimation of genetic variances and covariances by restricted maximum likelihood using proc mixed. In: Saxton AM (ed) Genetic analysis of complex traits using SAS vol. SAS Institute Inc., Cary, pp 11–34

González-Megías A, Gómez JM (2003) Consequences of removing a keystone herbivore for the abundance and diversity of arthropods associated with a cruciferous shrub. Ecol Entomol 28:299–308

Gould F (1988) Genetics of pairwise and multispecies plant-herbivore coevolution. In: Spencer KC (ed) Chemical mediation of coevolution vol. Academic Press, Inc., San Diego, pp 13–55

Hairston NG, Smith FE, Slobodkin LB (1960) Community structure, population control, and competition. Am Nat 94(879):421–425

Hastings A (1987) Can competition be detected using species co-occurrence data? Ecology 68(1):117–123

Hougen-Eitzman D, Rausher MD (1994) Interactions between herbivorous insects and plant-insect coevolution. Am Nat 143(4):677–697

Ilnicki RD, Tisdell TF, Fertig SN, Furrer AH Jr (1962) Life history studies as related to weed control in the Northeast – horse nettle, Rep. no. 368. Agricultural Experimental Station, University of Rhode Island, Kingston, Rhode Island

Imura O (2003) Herbivorous arthropod community of an alien weed *Solanum carolinense* L. Appl Entomol Zool 38(3):293–300

Iwao K, Rausher MD (1997) Evolution of plant resistance to multiple herbivores: quantifying diffuse coevolution. Am Nat 149(2):316–335

Johnson MTJ, Agrawal AA (2005) Plant genotype and environment interact to shape a diverse arthropod community on evening primrose (*Oenothera biennis*). Ecology 86(4):874–885

Juenger T, Bergelson J (1998) Pairwise versus diffuse natural selection and the multiple herbivores of scarlet gilia, *Ipomopsis aggregata*. Evolution 52(6):1583–1592

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Maddox GD, Root RB (1990) Structure of the encounter between goldenrod (*Solidago altissima*) and its diverse insect fauna. Ecology 71(6):2115–2124

Mauricio R, Rausher MD (1997) Experimental manipulation of putative selection agents provides evidence for the role of natural enemies in the evolution of plant defense. Evolution 51(5):1435–1444

Messina FJ, Fry JD (2003) Environment-dependent reversal of a life history trade-off in the seed beetle *Callosobruchus maculatus*. J Evol Biol 16:501–509

NAPPO (2003) PRA/Grains panel facts sheet – *Solanum carolinense* L. North American Plant Protection Organization, Ottawa, ON

Nichols RL, Cardina J, Gaines TP (1991) Growth, reproduction and chemical composition of horsenettle (*Solanum carolinense*). Weed Technol 5(3):513–520

Núñez-Farfán J, Dirzo R (1994) Evolutionary ecology of *Datura stramonium* L. in central Mexico: natural selection for resistance to herbivorous insects. Evolution 48:423–436

Pilson D (1992) Aphid distribution and the evolution of goldenrod resistance. Evolution 46(5):1358–1372

Poitrineau K, Brown SP, Hochberg ME (2003) Defence against multiple enemies. J Evol Biol 16:1319–1327

Rausher MD (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. Evolution 46:616–626

Rausher MD, Simms EL (1989) The evolution of resistance to herbivory in *Ipomoea purpurea*. I. Attempts to detect selection. Evolution 43(3):563–572

Roach DA, Wulff RD (1987) Maternal effects in plants. Annu Rev Ecol Syst 18:209–235

Roche BM, Fritz RS (1997) Genetics of resistance of *Salix sericea* to a diverse community of herbivores. Evolution 51(5):1490–1498

Schoener TW (1982) The controversy over interspecific competition. Am Sci 70:586–895

Schoener TW (1983) Field experiments on interspecific competition. Am Nat 122(2):240–285

Sokal RR, Rohlf FJ (1995) Biometry, 3rd edn. W.H. Freeman and Company, New York

Stinchcombe JR, Rausher MD (2001) Diffuse selection on resistance to deer herbivory in the ivyleaf morning glory, *Ipomoea hederacea*. Am Nat 158(4):376–388

Strauss SY, Irwin RE (2004) Ecological and evolutionary consequences of multispecies plant-animal interactions. Annu Rev Ecol Evol Syst 35:435–466

Strauss SY, Sahli HF, Conner JK (2005) Toward a more trait-centered approach to diffuse (co)evolution. New Phytol 165:81–90

Strong DR Jr, Lawton JH, Southwood R (1984) Insects on plants: community patterns and mechanisms. Harvard University Press, Cambridge

Tscharntke T (1989) Attack by a stem-boring moth increases susceptibility of *Phragmites australis* to gall-making by a midge: mechanisms and effects on midge population dynamics. Oikos 55(1):93–100

Van Zandt PA, Agrawal AA (2004) Community-wide impacts of herbivore-induced plant responses in milkweed *Asclepias syriaca*. Ecology 85(9):2616–2629

Wise MJ (2003) The ecological genetics of plant resistance to herbivory: evolutionary constraints imposed by a multiple-herbivore community. Ph.D. dissertation, Duke University, Durham, North Carolina

Wise MJ (2007a) Evolutionary ecology of resistance to herbivory: an investigation of potential genetic constraints in the multiple-herbivore community of *Solanum carolinense*. New Phytol 175:773–784

Wise MJ (2007b) The herbivores of horsenettle, *Solanum carolinense*, in northern Virginia: natural history and damage assessment. Southeast Nat 6:505–522

Wise MJ, Sacchi CF (1996) Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*. Oecologia 108:328–337

Wise MJ, Weinberg AM (2002) Prior flea beetle herbivory affects oviposition preference and larval performance of a potato beetle on their shared host plant. Ecol Entomol 27:115–122