Evolutionary Ecology (2005) 19: 79–101
DOI: 10.1007/s10682-004-8090-4

Research article

Scarlet gilia resistance to insect herbivory: the effects of early season browsing, plant apparency, and phytochemistry on patterns of seed fly attack

THOMAS JUENGER[1,*], TIMOTHY C. MORTON[2], RICK E. MILLER[3] and JOY BERGELSON[2]

[1]*Section of Integrative Biology, The University of Texas at Austin,1 University Station C0930, Austin, TX 78712, USA;* [2]*Department of Ecology and Evolutionary Biology, University of Chicago, 1101 E. 57th Street, Chicago, IL 60637, USA;* [3]*Department of Biological Sciences, Southeasten Louisiana University, Hammond, LA 70402, USA (*author for correspondence, e-mail: tjuenger@mail.utexas.edu)*

Received 27 August 2004; Accepted 17 December 2004

Co-ordinating editor: J. Tuomi

Abstract. Pollen-limited plants are confronted with a difficult tradeoff because they must present showy floral displays to attract pollinators and yet must also minimize their apparency to herbivores. In these systems, traits that increase pollinator visitation may also increase herbivore oviposition and overall plant resistance may therefore be constrained to evolve largely as a correlated response to selection on plant apparency or vigor. We used a family-structured quantitative genetic experiment to evaluate the importance of ungulate browsing, flowering date and plant height (traits that are related to overall vigor), and variation in a putative phytochemical defense (cucurbitacin production) on patterns of seed fly attack in a scarlet gilia population. We found significant genetic variation in the amount of insect damage plants experience in the field, providing evidence that resistance may evolve. In addition, we found that browsing reduced seed fly attack and that oviposition is strongly related to plant size and flowering date; large, early flowering plants experience high attack. In addition, we found that high cucurbitacin production was correlated with low seed fly damage, although this effect was relatively weak.

We found directional selection on final plant height and flowering date; tall, early flowering plants had the highest reproductive success. In addition, we found negative directional selection on cucurbitacin production, which may indicate a high cost of cucurbitacin or other functions of this phytochemical. Although seed fly herbivory arguably decreases plant fitness, we found an unexpected positive relationship between damage and fitness. A negative relationship between fitness and damage may be masked in this system through strong positive indirect correlations between patterns of damage and levels of pollinator visitation. Finally, we found significant genetic variation in flowering date, plant height, and cucurbitacin production. Resistance to seed flies may evolve in this population, but largely as a non-adaptive correlated response to selection on overall plant vigor. Phytochemicals may play a more important role in defense in years with high seed fly attack, or when pollen-limitation is less severe.

Key words: apparency, bio-model, cucurbitacin, *Delia* sp., *Ipomopsis*, plant-vigor, quantitative genetics, scarlet gilia

Introduction

Plants are generally attacked by herbivores and this damage usually causes a decrease in plant fitness (Crawley, 1983; Marquis, 1992). Since plants cannot flee attack, they are expected to evolve resistance traits that reduce herbivory. Numerous plant traits are hypothesized to have evolved as defenses against herbivory. For example, sharp spines, thorns, and plants hairs are common and often mechanically deter attack (Young, 1987; Marquis, 1992; Mauricio and Rausher, 1997; Elle *et al.*, 1999). Similarly, metabolically produced phytochemicals often deter damage by making plant tissue unpalatable, costly to metabolize, or toxic to herbivores. Numerous optimality models have been developed which attempt to predict the equilibrium level of resistance that should evolve in natural populations (reviewed in Simms, 1992). Generally, these models predict that resistance should evolve to balance the benefits and allocation costs of defensive traits, and suggest the maintenance of intermediate levels of resistance if defense is costly. These ideas have had a persuasive influence on evolutionary studies of plant–herbivore interaction.

A perspective based only on relative costs and benefits of defense to plants is phytocentric in that it considers herbivores passive actors in the interaction. Insects are expected to evolve oviposition preferences that increase their relative fitness. This argument is similar to the plant vigor hypothesis (Price, 1991), which predicts that insect herbivores with a tight link between adult oviposition and larval feeding sites will preferentially feed on robust plants. A consequence of this pattern of insect attack is that overall levels of plant resistance or susceptibility may be positively correlated with plant traits directly related to functions other than defense (e.g., floral display, plant size, or reproductive phenology) (Augspurger, 1981; De Steven, 1983; Hainsworth *et al.*, 1984; Abrahmson and Weiss, 1997). In other words, overall patterns of insect attack may be largely driven by plant characters unrelated to defense.

Pollen-limited plants are confronted with a particularly difficult situation because they must present showy floral displays to attract limiting pollinators, while minimizing herbivory of reproductive structures. When host plants are pollen-limited, herbivores may ensure food resources for their seed-eating larvae by evolving oviposition cues based on the same cues that attract pollinators; traits that increase pollinator visitation (e.g., large floral display, patterns of flowering phenology) may thus also attract herbivore oviposition. In these systems, the evolution of plant resistance to herbivory will depend on the relative strength of selection imposed by pollen-limitation and loss to seed predators.

In addition to plant defense and insect preference, numerous ecological factors can influence patterns of insect oviposition. For example, plants are generally attached by several herbivores simultaneously. Herbivores may compete directly on a shared host plant (Lawton and Strong, 1981; Lawton and Hassel, 1984; Karban, 1989; Fritz, 1992), and these interactions may reduce or facilitate patterns of insect oviposition or feeding. Similarly, early season herbivores may influence late-season herbivores by altering phenology, tissue quality, or architecture of host plant (Schultz and Baldwin, 1982; Williams and Myers, 1984; Harrison and Karban, 1986; Pilson, 1992; Alonso and Herrera, 1996), or by altering susceptibility to predators (Thaler, 1999). Our work on interactions between ungulate browsing and subsequent insect attack in scarlet gilia, *Ipomopsis aggregata,* has documented striking effects of early season vertebrate browsing (Juenger and Bergelson, 1998). In this system, browsed plants receive significantly less seed fly and caterpillar attack than plants that escaped early season browsing. Despite the generality of vertebrate browsing, the importance of browsing on the evolution of plant–insect interactions has been largely unexplored. This is unfortunate because the relative importance of extrinsic (e.g., spatial microhabitat, recent herbivore history, interaction with other natural enemies) vs. intrinsic factors (e.g., phytochemical defense) on the distribution of herbivore attack may potentially constrain or facilitate the evolution of plant defense.

We utilized a family-structured quantitative genetic experiment along with surveys of natural insect occurrences to explore patterns of herbivory in a scarlet gilia population at both the phenotypic and genetic level. The primary goals of this study were to determine (1) is there genetic variation in resistance to seed fly attack in a natural scarlet gilia population, (2) are there interactions between simulated early season browsing and subsequent seed fly attack, (3) the relative role of putative defense traits (phytochemicals) and plant vigor or apparency (flowering phenology, and plant height) on patterns of insect herbivory, and (4) whether there is significant genetic variation in specific plant traits that influence patterns of herbivory.

Materials and methods

Experimental organisms and study site

We studied a large population of scarlet gilia, *Ipomopsis aggregata* ssp. *candida* (Polemoniaceae), located near the Colorado Mountain Research Station from 1995 to 1998. After germination, scarlet gilia grows as a hardy rosette for 2–5 years until it bolts an indeterminate flowering stalk, reproduces, and dies. Front Range scarlet gilia flowers are hermaphroditic, protandrous, and self-sterile. Subspecies *candida* is primarily pollinated by the white-lined hawkmoth (Sphingidae: *Hyles lineata*), and to a lesser extent by resident and migratory hummingbirds (Elam and Linhart, 1988; Juenger and Bergelson, 1997). Several field experiments have demonstrated experimentally that scarlet gilia fruit set and seed production can be pollen limited under natural conditions (Hainsworth *et al.*, 1985; Paige and Whitham, 1987; Campbell, 1991, Campbell and Halama, 1993; Juenger and Bergelson, 1997). In this population, fruit set is often low due to limited pollinator visitation (Juenger and Bergelson, 1997).

At this site, up to 44% of bolting scarlet gilia shoots experience early season grazing (May–early June) by mule deer (*Odocoileus hemionus*) and occasionally elk (*Cervus elaphus*) (Bergelson *et al.,* 1996). Grazing occurs in the early developmental stage of the growing inflorescence shoot, and subsequent late season grazing is rare. Later in the reproductive season, plants are attacked by several insect herbivores (Juenger and Bergelson, 1998), including a specialist seed fly (Anthomyiidae: *Delia* sp.), a specialist lepidopteran fruit predator (Tortricidae: *Olethreutes* sp.), a generalist lepidopteran fruit predator (Noctuidae: *Heliothis phloxiphaga*), unidentified green aphids, and an unidentified dipteran root borer.

The present study focuses on factors influencing patterns of seed fly attack. Seed flies oviposit under the calyx of developing scarlet gilia floral buds and flowers, and the newly emerged larvae burrow into the developing ovary of pollinated flowers. Oviposition begins with the first developing flowers and continues throughout the season. Normally a single egg is laid on each flower, and the resulting larvae consume all of the developing ovules and seeds in a single fruit (Hainsworth *et al.*, 1984; Campbell, 1991; Brody, 1992a, b, 1997; Brody and Mitchell, 1997). After completing development, the larva exits through the ovary wall, drops to the ground, and pupates (usually in late July–August) (Zimmerman, 1980a, b). Larvae are sessile, thus the plant tissue they can consume is limited to the fruit on which they hatch. It is relatively easy to count the number of successful seed fly hatches from a given plant by inspecting fruits for characteristic damage, frass within the spent fruit, and the characteristic larval exit hole. The hatching success of seed fly eggs is contingent on the pollination of the flower on which it was laid, in addition to any effect due to the quality of fruit tissue. Previous studies with *Ipomopsis aggregata* ssp. *aggregata* at the Rocky Mountain Biological Research Station have demonstrated that seed predation may be influenced by final plant height (Hainsworth *et al.*, 1984), the spatial pattern of flowering (Brody, 1992a), floral morphology, and inflorescence size (Brody and Mitchell, 1997; Brody and Morita, 2000). It has also been suggested that seed fly attack may influence the evolution of the flowering phenologies of both *Ipomopsis* and *Polemonium* (Zimmerman and Gross, 1984; Brody and Waser, 1985, Brody, 1997). There is no evidence that seed flies pollinate scarlet gilia flowers.

For additional information on the natural history of Front Range scarlet gilia see Wilken and Allard (1986), Elam and Linhart (1988) and Juenger and Bergelson (1997).

Experimental design

In the spring of 1995, 30 bolting scarlet gilia rosettes were randomly chosen from our 40-acre field site to act as parents in a partial diallel cross. Twenty individuals were used as both pollen and seed parents, and the remaining 10 individuals were used exclusively as seed parents. Two exclusively maternal plants lodged, reducing the number of dams in the design to 28. We attempted all possible crosses between the 20 sires and 28 dams (including reciprocal crosses but excluding selfs). This asymmetrical crossing design was necessary as the availability of flowers on plants limited the number of crosses that could be performed. Numerous cells in the design (specific Sire × Dam crosses) are missing from the experiment due to the crossing scheme or cross failures. Overall, the final design is composed of 253 full sib families comprising 20 sire and 28 dam half sib families. An important benefit of this breeding design is that it produces six kin classes (full sibs, maternal and paternal half sibs, reciprocal full sibs, reciprocal half sibs), allowing complete partitioning of phenotypic variance under the full 'bio-model' of Cockerham and Weir (1977).

After 6 weeks of cold–wet stratification, seeds were germinated and 10–15 seeds from each full-sib family were planted in 12.7 × 12.7 cm plastic pots at the University of Chicago greenhouse. These seedlings were grown for 4 months under standard greenhouse conditions. In the late spring of 1996, these seedlings were transported to Colorado and planted into a randomized block design composed of 28 (10 m × 12 m) rectangles, each containing 120 rosettes separated by 1 m^2. The entire set of spatial blocks was surrounded by a solar powered electric fence to exclude browsing by elk and deer. By late November 1996, the surviving transplants had substantial new root and leaf growth and had entered winter dormancy.

In April 1997, over 2200 plants had survived the transplant, overwintered, and bolted. Approximately one third of all transplants had been damaged by small mammal (probably *Thomomys* sp.) herbivory to the apical bud of rosettes. This damage occurred very early in the season (before April 1, 1997) and occurred irrespective of parents (Fisher's Exact Test; Sire, $p > 0.15$; Dam, $p > 0.30$). In May 1997, we evenly and randomly divided the remaining undamaged bolting plants into a control group and an artificial grazing treatment. Plants that were allocated to the clipping treatment were clipped with scissors on May 28–29 so that 1 cm of the growing inflorescence remained. The timing of this treatment coincided with natural mule deer browsing in the surrounding natural population. Hereafter, we refer to treatment plants that experienced either natural damage or experimental clipping as plants from the browsing treatments. All plants in the experiment experienced natural levels of insect attack. We scored plants for absolute levels of seed fly damage (the number of successfully hatched seed flies) at the end of the season.

We recorded data on the date of first flowering, final plant height, and total fruit production for each plant in the experiment. We consider date of first flowering and final plant height as general indicators of plant 'vigor' as previous studies have documented strong relationships between these characters and pollinator visitation (Campbell, 1991; Mitchell, 1994, Brody and Mitchell, 1997), seed fly damage (Hainsworth *et al.*, 1984; Brody, 1992a; Brody and Mitchell, 1997), and fitness (Campbell, 1992; Mitchell, 1994; Juenger and Bergelson, 1998, 2000) in scarlet gilia. Flowering date was measured on a scale that set a value of one to represent the earliest flowering individuals in the population and spanned a 44 day period (July 3–August 15). Total fruit production is a reasonable estimate of lifetime maternal fitness in this system because fruit set and seed production are tightly associated at our field site (1994: $R^2 = 0.826, p < 0.0001$; 1995: $R^2 = 0.917$, $p < 0.000l$; 1996: $R^2 = 0.943$, $p < 0.001$). Relative fruit production was calculated as the fruit production of each individual plant divided by the mean fruit production of all experimental plants from a given treatment. In addition, we harvested several mature fruits from each experimental plant to estimate the production of putative defensive compounds. The details of fruit collection and phytochemical methods are described below. This experiment has previously been analyzed to evaluate genetic variation in tolerance to simulated browsing (Juenger and Bergelson, 2000).

To further investigate the function of plant traits on herbivory, we randomly selected 100 naturally occurring plants for a survey of seed fly attack rates in 1998. On May 29, we randomly allocated half of these plants to a clipping treatment (to mimic natural early season browsing) and left the remaining plants as undamaged controls. We surveyed all of the buds and flowers of these experimental plants for seed fly eggs every three days throughout the reproductive season. We recorded the date of first flower and final plant height for all experimental plants.

Fruit collection

In 1997, we collected ≈ 3–4 fruits (≈ 0.5 g tissue) from each plant in the quantitative genetics experiment directly into pre-weighed 8 ml amber glass vials. Fruits were randomly collected across the entire inflorescence stalk of each experimental plant over a 3-day period (August 15–18). After collection, vials were transported to the University of Colorado Mountain Research Station, re-weighed, and filled with 80% methanol.

Chemical analysis of fruit samples

Separation and quantification of fruit chemistry was performed at the University of Chicago. An aliquot from each sample vial was transferred to a

1.8 ml amber glass auto-sampler vial for HPLC injection. Separation was performed on an Hewlett Packard 1100 series HPLC using photodiode array detection and a 15 cm Zorbax SB-C8 rapid resolution column (Zorbax C8 pre-column, flow rate 1.0 ml/min, 10 μl injection, monitoring 230 nm with ref 360 nm). The column was loaded with H_2O, 4 min hold, and peaks were resolved using an acetonitrile gradient (65% ACN for 24 min, 80% ACN 21 min, 3 min final hold) at column temperature 40 °C. Quantification was conducted by peak area integration. Data used in subsequent statistical analyses were standardized to mAU (absorbance units) per gram of fresh fruit tissue. This specific HPLC protocol was developed to provide reliable, repeatable, and efficient separation and quantification of two oxygenated tetracyclic triterpenoids (cucurbitacin B and D). Cucurbitacins are the most bitter-tasting compounds known and have demonstrated oviposition and feeding deterrence against a variety of insect herbivores (Miro, 1995; Tallamy *et al.*, 1997). Cucurbitacin B and D were previously reported from gross extractions of scarlet gilia tissue (Arisawa *et al.*, 1984). Cucurbitacin B and D standards were provided by Dr D. Tallamy and Dr P. Gorski (U. of Delaware) from tissue of *Cucubita andreana*.

Statistical analysis

Quantitative genetic analysis

We partitioned phenotypic variation in seed fly damage, relative fruit production, flowering date, plant height, and phytochemical production into genetic and environmental components of variation using a mixed model approach under a full biological model (Cockerham and Weir, 1977; Shaw, 1987; McLean *et al.*, 1991; Searle *et al.*, 1992; Lynch and Walsh, 1998). This model partitions the total phenotypic variance into the following six casual components:

$$V_{\mathrm{P}} = V_{\mathrm{A}} + V_{\mathrm{D}} + V_{\mathrm{mat}} + V_{\mathrm{pat}} + V_{\mathrm{K}} + V_{\mathrm{E}};$$

where V_{p} represents the total phenotypic variance. Under standard interpretation of the bio-model, V_{A} is additive genetic variance (variance caused by the simple additive action of nuclear alleles), V_{D} is dominance variance (variance caused by the interaction of alleles at a nuclear locus), V_{mat} is maternal variance caused by contributions other than those from nuclear genes (maternal environmental or cytoplasmic effects), V_{pat} is paternal variance caused by contributions other than those from nuclear genes (paternal environmental effects), V_{K} is variance caused by parental interactions other than among nuclear genes (nuclear–extranuclear and extranuclear–extranuclear interactions), and V_{E} is the environmental variance. In addition, we included spatial blocking and the grazing treatment of the design as fixed factors in the model. Computing limitations restricted evaluation of interactions between spatial blocking and the grazing treatment on other factors in the model. The total phenotypic variance (V_P) for each trait was obtained by summing all of the variance components for the trait.

Because our design was unbalanced, we utilized an iterative restricted maximum likelihood method (REML) for significance testing and the estimation of variance components using PROC MIXED in SAS (Shaw, 1987; Searle *et al.*, 1992; Littell *et al.*, 1996). Likelihood tests were used to evaluate the statistical significance of individual components of variance. We evaluated models for control, clipped, and naturally damaged plants in three independent models as well as models where clipped and naturally damaged plants were pooled into one browsing treatment category. We found very similar results between these approaches, and so present the latter (pooled) models. Example programming code for the REML partitioning of a partial diallele using PROC MIXED is available from the authors on request.

Count data on seed fly damage ranged from 0 to 21 successful seed fly hatches, had an excess of zeros (no damage), and was very right skewed. Similarly, count data on fruit production was bimodal including an excess of zeros. The seed fly damage data was square-root ($1 + y$) transformed and the relative fruit production data was log($1 + y$) transformed. The residuals from full bio-model analyses far these traits following transformation were normally distributed and exhibited no systematic pattern and therefore should have little impact on our REML estimation and significance tests. Furthermore, we found similar results for analyses under generalized mixed linear models (GLIM) that modeled count data as poisson distributed (analyses not presented). Nonetheless, given these departures the variance component estimates and tests of significance for these traits should be viewed as approximations. The remaining traits were normally distributed and analyses were performed on raw data sources.

We investigated phenotypic correlations among this set of plant traits using a standard Pearson product–moment correlation between the paired raw data from the large quantitative genetics experiment. We evaluated genetic correlations as the Pearson product–moment correlation between additive genetic breeding values as estimated by best linear unbiased prediction (BLUPs) under the full bio-model analysis. The significance of phenotypic and genetic correlations was determined by t-tests after z-transformation of the correlation coefficient. Levels of significance for phenotypic and genetic correlations were not adjusted for multiple tests.

The role of plant apparency traits and phytochemical production on seed fly oviposition and damage

We used GLIM models to explore the role of plant apparency traits (final plant height and flowering date) and early season browsing on patterns of seed fly oviposition from the survey experiment (1998 field season). GLIM is an extension of the traditional general linear model (GLM) that is applicable to a wider range of data analysis problems and error structures (Crawley, 1993). A generalized linear model consists of a linear predictor, a link function and response variables that have a defined probability distribution (e.g., poisson, binomial, normal). This analysis used a poisson error distribution and a log link function to model variation in eggs counts with PROC GENMOD in SAS. Our initial model tested the main and interactive effect of plant height, flowering date, squared terms, and the clipping treatment on egg occurrences using a Type 3 analysis. The inclusion of squared terms for the independent variables in these models provides a means for assessing non-linear relationships between the plant traits and patterns of seed fly attack. Significance levels from Type 3 tests are based on the change in deviance upon deletion from the maximal model (SAS Institute, 1997). Because we detected significant interactions between the browsing treatment and the effect of plant traits, we evaluated models for control and clipped plants in independent analyses. Non-significant higher-order interactions were removed from the models in a hierarchical manner.

We also used GLIM models to explore the role of spatial block, plant apparency traits, and putative phytochemical defenses on seed fly damage from the quantitative genetics experiment (1997 field season). This analysis used a poisson error distribution and a log link function. Total cucurbitacin (cucurbitacin B + cucurbitacin D) was used as an independent variable instead of cucurbitacin B or D singly, as these phytochemicals were highly phenotypically and genetically correlated (see Tables 2 and 3). First, we investigated the effects of spatial location and plant apparency on damage by fitting models including spatial block, plant height, flowering date, interactions between traits, and squared terms for plant traits using Type 3 tests. Because of the strong effect of early season browsing on patterns of attack, we performed separate analyses for damaged and control plants. Second, we tested whether data on total cucurbitacin production significantly improved the fit of the models predicting damage. We fit full models including main effects and interactions between plant height, flowering date, total cucurbitacin, and squared terms using Type 3 tests. Non-significant higher-order terms were removed from the model in a hierarchical manner. We calculated the generalized coefficient of determination (R^2) from our final GLIM models as the ratio of the change in deviance due to the regression model (SSR) divided by the deviance of a model fitting only an intercept (SST). Variance inflation factors were low in all of our multiple regression models, suggesting little collinearity among independent variables.

Because our analysis detected complicated higher-order interactions between continuous predictors, we utilized a non-parametric cubic spline to fit bivariate surfaces describing the relationship between independent variables and seed fly oviposition and seed fly emergence (damage imposed by seed flies) using STATISTICA (Schluter and Nychka, 1994; StatSoft, 1995). These surfaces

Table 1. Estimates of variance components for phenotypic traits from REML analysis of a full bio-model

Source	Seed fly damage	Relative fruit production	Cucurbitacin B	Curcurbitacin D	Flowering date	Height
V_A	2.0* ± 0.9	0	0.47** ± 0.16	12.88*** ± 4.55	1.25***3 ± 0.43	5.24*** ± 1.95
V_D	0	0.73 ± 0.76	0	0	0	0
V_{mat}	0	1.04 ± 1.73	0.15 ± 0.16	1.51 ± 2.4	0	0
V_{pat}	0	0	0.06 ± 0.16	0	0.05 ± 0.17	0
V_K	0.061 ± 2.0	0.16 ± 0.20	0	0	0	0
V_E	179.5*** ± 9	19.6*** ± 6	6.66*** ± 0.30	143.89*** ± 6.42	20.10*** ± 0.71	187.6*** ± 6.41
−2 Res LL	2050.8	2598.1	5017.5	8188.6	9749.7	14,136.5

Each variance component is followed by 1 SE as estimated by asymptotic theory. Variance components that were initially estimated as zero were removed from the final model and final variance component estimation. Significance of the individual variance components was obtained by comparing the log-likelihood value for the full-model versus models constraining the variance component in question to zero: the value of −2 times the log-likelihood of the full model is presented for each trait. Asterisks represent significance levels – $*p < 0.01$; $**p < 0.001$; $***p < 0.0001$.

V_A, additive genetic variance; V_D, dominance genetic variance; V_{mat}, maternal variance; V_{pat}, paternal variance; V_K, parental interaction variance; V_E, environmental variance.

Table 2. Phenotypic correlations between traits in both the control and damage treatments

	Fly damage	Fruit #	CucB	CucD	Flowering date	Height
Control plants						
Fly damage	1.00					
Fruit #	**0.57**	1.00				
CucB	**0.39**	0.69	1.00			
CucD	**0.47**	0.73	**0.94**	1.00		
Flowering date	**−0.53**	**−0.53**	**0.19**	0.12	1.00	
Height	**0.83**	0.83	**0.50**	**0.51**	**−0.34**	1.00
Damaged plants						
Fly damage	1.00					
Fruit #	**0.37**	1.00				
CucB	−0.08	**−0.23**	1.00			
CucD	−0.10	**−0.33**	**0.83**	1.00		
Flowering date	**−0.16**	**−0.43**	**0.23**	**0.30**	1.00	
Height	**0.25**	0.54	−0.10	−0.10	**−0.36**	1.00

Phenotypic correlations were estimated as the standard Pearson product-moment correlation between the paired raw data from the large quantitative genetics experiment. The significance of each phenotypic correlation was evaluated using a t-test after z-transformation of the correlation coefficient. Boldface type indicates significant correlations at $p < 0.05$.

represent only the relationship between the two factors in question and damage, and therefore do not completely represent the relationships evaluated in the overall poisson regression model. Moreover, we were unable to fit confidence limits on the features of these surfaces, and therefore they should be viewed as an exploratory approach.

Natural selection on plant apparency and phytochemical production

We performed a phenotypic selection analysis to estimate the shape and form of natural selection on plant apparency (final plant height and flowering date) and total cucurbitacin production. Standardized linear and nonlinear selection gradients were estimated as the partial linear and quadratic regression coefficients of relative fitness on each plant trait in each treatment category. We standardized each independent variable in this analysis to have a mean of zero and a variance of one to facilitate comparisons and interpretation of the coefficients across the damage treatment. Following Lande and Arnold (1983) and Mitchell-Olds and Shaw (1987), selection gradients were estimated using untransformed relative fitness, and significance was tested using log transformed relative fitness. In addition, we evaluated the relationship between seed fly damage and fitness (while controlling for overall plant apparency) using a multiple regression approach.

An important caveat to this study is that our exploration of trait function is based solely on a phenotypic correlational approach using multiple regression modeling. We acknowledge the limitations of this approach (Mitchell-Olds and

Shaw, 1987). In particular, a regression analysis that does not include all of the important correlated traits may give a misleading representation of the underlying function or importance of the particular traits included in the analysis. This may be a serious problem in terms of our use of plant height and flowering date as an indicator of plant 'apparency', as these measures of overall growth and reproduction may be significantly correlated to numerous other plant traits. In addition, our functional analyses necessarily excluded plants that set no fruit, as these individuals could not express the phytochemical traits (cucurbitacin production/gram fresh fruit) under study. Episodes of selection removing individuals from an experimental population prior to either quantitative genetic or phenotypic selection analyses may bias results with respect to the actual base population of study (Falconer and Mackay, 1996; Lynch and Walsh, 1998). Finally, any investigation based solely on multiple regression (and considerable model building) should be viewed as an exploratory approach that must be followed by more direct tests using manipulative experiments.

Results

Genetic variation in insect attack rates and plant traits

The results of the quantitative genetic analysis of seed fly damage, relative fruit production, phytochemical production, flowering date, and plant height are presented in Table 1. We detected significant additive genetic variation for seed fly damage, flowering date, final plant height, and the production of cucurbitacin B and D. In contrast, we found no additive genetic variation in relative fruit production under the bio-model analysis. Similarly, we found no evidence of dominance variance, maternal or paternal environmental effects, or non-additive interaction variation for any of the measured traits. We estimated the overall heritability of these traits as $4V_A/V_P$; seed fly damage $= 0.05$, cucurbitacin B $= 0.26$; cucurbitacin D $= 0.33$; flowering date $= 0.23$; plant height $= 0.11$, respectively. We found relatively large spatial block effects for all of the measured response variables.

Consistent with previous studies (Juenger and Bergelson, 1998), we detected a significant effect of early season browsing on seed fly damage. Browsed (clipped or naturally damaged) plants experienced lower levels of seed fly damage when compared to controls. In addition, we found a significant effect of the browsing treatment on fruit production, flowering date, final plant height, and cucurbitacin B production. Clipped plants produced $\approx 40\%$ fewer fruits, flowered later ($\approx$ 5 day delay), were $\approx 33\%$ shorter, and produced slightly more cucurbitacin B than undamaged controls. Although we detected significant browsing induction of cucurbitacin B, the effect was small (less than a 10% increase in the average cucurbitacin B content in damaged plants). There was no effect of the browsing treatment on cucurbitacin D production.

We detected significant phenotypic correlations between all of the measured traits in both the control and damaged environment (Table 2), with only a few exceptions. We found no phenotypic correlation between curcurbitacin D production and flowering date in the control environment and between cucurbitacin B and D production and plant height in the damaged environment. Likewise, we found no phenotypic correlation between cucurbitacin production and seed fly damage in the browsed environment. In general, phenotypic correlations were potitive and of moderate strength. We detected four significant genetic correlations (Table 3). We detected a large positive genetic correlation between cucurbitacin B and D production ($r_G = 0.87$). Moreover, we found a significant positive genetic correlation between seed fly damage and plant fruit produttion ($r_G = 0.39$). Here, genotypes with lower resistance to seed fly attack had higher fruit production when compared to genotypes with higher resistance. We also found a significant negative genetic correlation between seed fly damage and flowering date ($r_G = -0.33$) and between fruit production and flowering date ($r_G = -0.45$). In this case, earlier flowering genotypes had a higher incidence of seed fly damage as well as higher fitness when compared to later flowering genotypes.

Patterns of oviposition in naturally occurring plants

We detected a significant interaction between final plant height and flowering date on seed fly oviposition for control plants (Table 4, Fig. 1). Inspection of the spline damage surface reveals that the majority of insect attack occurs on tall, early flowering plants. These two predictors, and their interaction, explain ≈ 47% of the variation in seed fly oviposition. For browsed plants, we found a significant relationship between final plant height and seed fly oviposition.

Table 3. Genetic correlations between traits in the average environment

	Fly damage	Fruit #	CucB	CucD	Flowering date	Height
Fly damage	1.00					
Fruit #	**0.39**	1.00				
CucB	0.31	−0.04	1.00			
CucD	0.15	−0.11	**0.87**	1.00		
Flowering date	**−0.33**	**−0.45**	0.09	0.20	1.00	
Height	0.25	0.23	0.18	0.26	0.23	1.00

Genetic correlations were estimated as the standard Pearson product–moment correlation between the additive BLUPs estimated from the full bio-model analyses. The significance of each genetic correlation was evaluated using a *t*-test after *z*-transformation of the correlation coefficient. Boldface type indicates significant correlations at $p < 0.05$.

Table 4. GLIM model of the relationship between putative resistance traits and seed fly oviposition

Source	df	Estimate	χ^2	*p*-value
Control plants				
Height	1	0.761	0.7614	0.3829
Flowering date	1	−0.119	8.7520	0.0031
Height × flowering date	1	−0.008	4.9382	0.0263
Clipped plants				
Height	1	0.060	10.9206	0.0010

The full model was reduced using a hierarchical, backward elimination approach. The scale parameter was estimated as the square root of the deviance/df.

Again, inspection of the spline surface suggests that the majority of insect attack occurs on tall, early flowering plants (see Fig. 1). Final plant height explained ≈ 19% of the variation in seed fly oviposition in browsed plants.

Patterns of insect damage

We found similar effects of plant size and flowering date on patterns of damage for both control and browsed plants; tall, early flowering plants experienced the most seed fly damage (Table 5, Fig. 2). There was also significant effect of spatial block on seed fly damage in both treatment categories. These models explained ≈ 40% and 22% of the variation in damage for control and browsed plants, respectively.

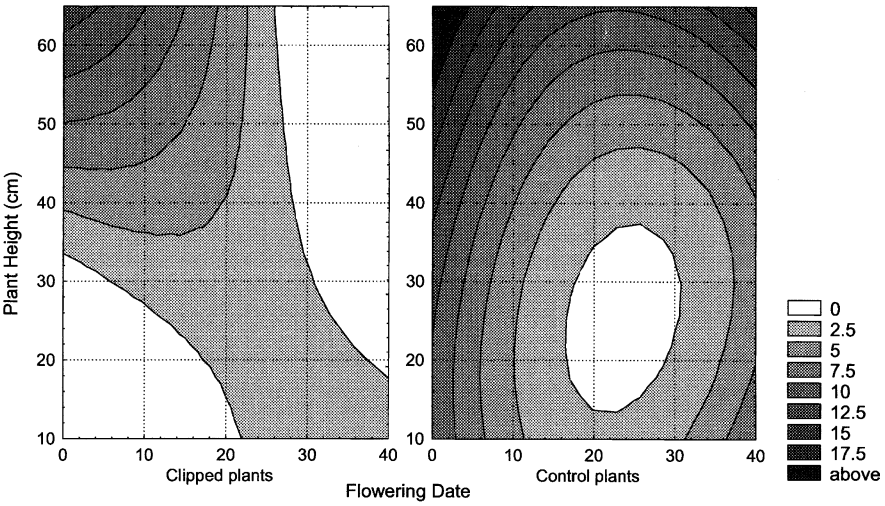

Figure 1. The spline surface depicting the relationship between final plant height, flowering date (days since flowering began), and seed fly oviposition for control and experimentally clipped plants. Dark shading represents areas of the surface with high seed fly attack.

Table 5. GLIM of putative resistance traits on seed fly damage scored at the end of the reproductive season

Source	df	χ^2	p-value
Control plants			
Block	28	134.14	0.0001
Height	1	63.80	0.0001
Flowering date	1	23.77	0.0001
Cucurbitacin	1	15.16	0.0001
Height * flowering date	1	41.65	0.0001
Height * cucurbitacin	1	10.12	0.0015
Height * height	1	39.48	0.0001
Damaged plants			
Block	28	47.01	0.0099
Height	1	6.81	0.0091
Flowering date	1	3.58	0.0583
Cucurbitacin	1	9.55	0.0022
Height * flowering date	1	6.07	0.0137
Height * cucurbitacin	1	7.59	0.0059

The full model was reduced using a hierarchical, backward elimination approach. The scale parameter was estimated as the square root of the deviance/df.

We significantly improved the fit of models predicting seed fly damage by including total cucurbitacin production and higher-order interactions with plant apparency (see Table 5). For example, we detected a significant interaction between plant height and total cucurbitacin production on damage for

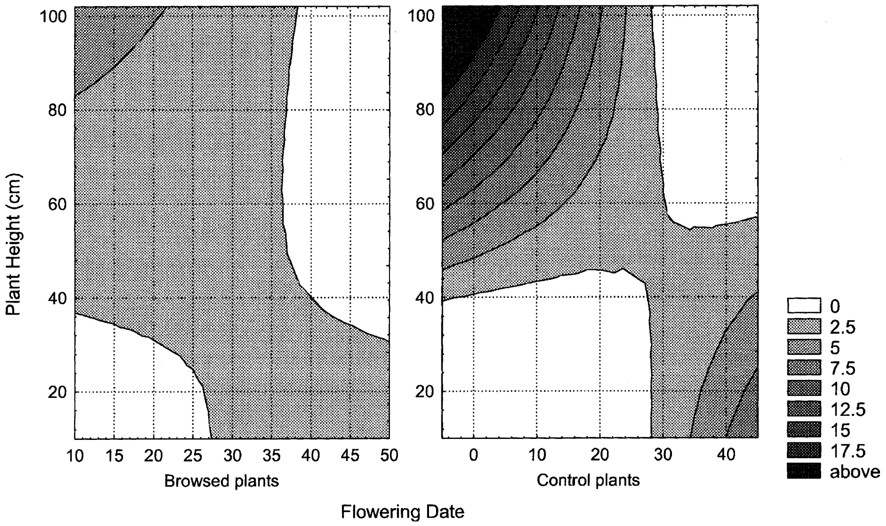

Figure 2. The spline surface depicting the relationship between final plant height, flowering date (days since flowering began), and seed fly damage for controij and browsed plants (both clipped and naturally damaged). Dark shading represents areas of the surface with high seed fly damage.

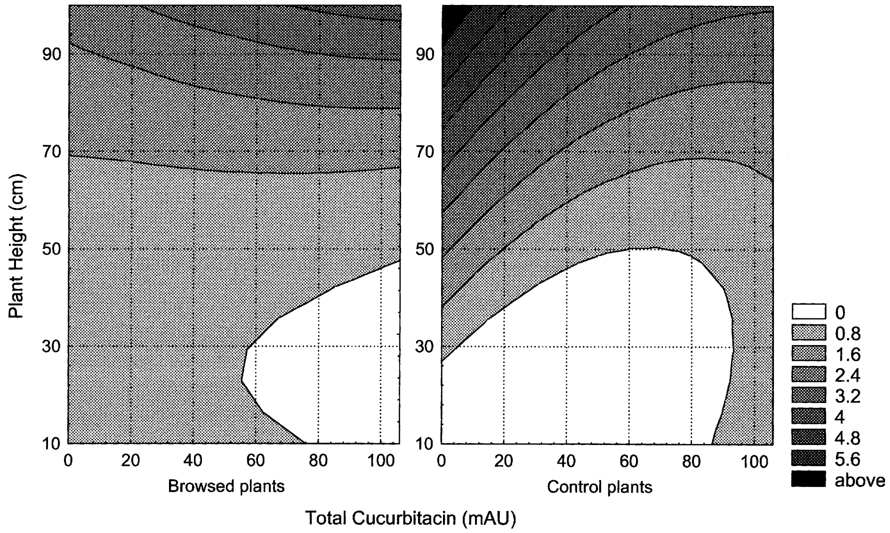

Figure 3. The spline surface depicting the relationship between final plant height, total cucurbitacin production, and seed fly damage for control and browsed plants (both clipped and naturally damaged). Dark shading represents areas of the surface with high seed fly damage.

control plants; tall plants with the low cucurbitacin production experienced the greatest seed fly damage (see Fig. 3). Furthermore, we detected significant curvature to the relationship between plant height and damage. The final model including block, plant apparency, cucurbitacin production, and significant interactions explained ≈ 46% of the variation in seed fly damage for control plants. For browsed plants, we found a significant two-way interaction between final plant height and total cucurbitacin production on seed fly damage (see Table 5, Fig. 3). As before, tall plants with low cucurbitacin production experienced the greatest damage. The final model including plant apparency, total cucurbitacin, and interactions explains ≈ 24% of the variation in seed fly damage for plants that experienced early season damage.

Natural selection on plant apparency and total cucurbitacin production

We detected significant linear selection for increased plant height, earlier flowering, and decreased cucurbitacin production in both the control and damaged treatments (see Table 6). Selection on plant height and flowering date was stronger for browsed plants than for control plants (significantly different β based on non-overlapping 95% confidence intervals) (Sokal and Rohlf, 1981), suggesting that browsing imposes selection on these traits. The strength of selection on total cucurbitacin production was similar across the browsing treatment.

Table 6. Phenotypic selection analysis on final plant height, flowering date, in and total cucurbitacin for control (Panel A) and damaged (Panel B) plants

Source	Linear		Nonlinear
Panel A: Control selection gradients	(β_0)	(β)	(γ_{ii})
HT	**0.27** ± 0.02	**0.27** ± 0.02	**0.03** ± 0.01
FD	−0.12 ± 0.01	**−0.14** ± 0.03	0.00 ± 0.02
C	**−0.17** ± 0.02	**−0.22** ± 0.03	0.01 ± 0.01
	Correlational (γ_{ij})		
HT × FD	−0.03 ± 0.02		
HT × C	−0.07 ± 0.02		
FD × C	−0.02 ± 0.03		
Panel B: Browsed selection gradients			
HT	**0.35** ± 0.03	**0.34** ± 0.02	−0.02 ± 0.02
FD	−0.19 ± 0.03	−0.24 ± 0.03	**0.04** ± 0.02
C	−0.14 ± 0.03	−0.23 ± 0.04	0.03 ± 0.02
	Correlational (γ_{ij})		
HT × FD	−0.04 ± 0.03		
HT × C	−0.08 ± 0.03		
FD × C	0.02 ± 0.03		

β_0 is the standardized linear selection gradient estimated from a multiple regression model including only linear terms. β and γ are the standardized linear and nonlinear selection gradients estimated from a regression model including all terms in the model. Significant coefficients (from models predicting log transformed relative fitness) are indicated in bold type.

We detected a significant positive (concave upward/disruptive-like) component of non-linear selection on final plant height in control plants, and a significant negative (concave downward/stabilizing-like) component of non-linear selection on final plant height in the browsed plants. In addition, we detected a significant positive (concave upward) component of non-linear selection on flowering date in the browsed plants. Inspection of plots of fitness against second order polynomials for final height and flowering date suggest there was no intermediate fitness minimum or maximum in any case of non-linear selection. We detected significant non-linear correlational selection on plant height and cucurbitacin production in the browsed plants. In this instance, the correlational selection gradients indicate how the slope of the relationship between height and cucurbitacin production and fitness changes across the range of variation in the other characters. Together, the linear and correlational selection gradients (and inspection of the spline surfaces) on plant height and cucurbitacin production indicate that tall plants with low cucurbitacin production had the highest fitness.

Finally, we detected a significant positive relationship between seed fly damage and fitness in both the control and browsed treatments, while simultaneously controlling for final plant height and flowering date (see Table 7).

Table 7. The results of a regression analysis of plant heigt, flowering date, and seed fly damage on plant fitness

Source	df	MS	*F*-value	*p*-value
Control plants				
Height	1	46.51	134.69	0.0001
Flowering date	1	11.96	34.62	0.0001
Damage	1	6.50	18.81	0.0001
Error	512	0.34		
Damaged plants				
Height	1	8.53	131.02	0.0001
Flowering date	1	0.67	75.41	0.0001
Damage	1	4.57	32.30	0.0001
Flowering date × flowering date	1	1.04	7.33	0.0070
Error	549	0.14		

Relative fitness was $\log(y + 1)$ transformed to improve normality and to homogenize variances.

Discussion

Resistance or susceptibility to herbivores may change in plant populations by the evolution of defensive traits, by the evolution of characters that attract herbivores, or by herbivore evolution itself. Generally, herbivore damage will be detrimental and therefore the evolution of defense is expected to dominate plant responses. However, the evolution of defense may be more complicated in situations where herbivore preferences have evolved to favor vigorous (high fecundity) plants (Price, 1991; Abrahamson and Weiss, 1997). In this case, the mean fitness of a population may increase under strong selection for general vigor at the same time as the mean herbivore damage level increases. This situation may be common in systems with pollen-limited host plants, as plants must provide extensive floral display to attract limiting pollinators, and these displays may subsequently be used as cues by herbivores. We evaluated the relative importance of two apparency traits and phytochemical production on damage and plant fitness in a natural population of the pollen-limited wildflower, scarlet gilia.

We used multiple regression to investigate the extent to which data on plant size and flowering date predict patterns of seed fly oviposition. In general, we found a very strong association between overall plant apparency and vigor on herbivore attack. A simple model including plant height, flowering date, and their interaction explained ≈ 47% of the variation in seed fly oviposition for control plants. Likewise, plant size and flowering date explained ≈ 19% of the variation in seed fly oviposition for browsed plants. We believe seed flies preferentially oviposition tall, early flowering plants because these plants generally receive increased pollinator visitation (Hainsworth *et al.*, 1984;

Mitchell, 1994; Juenger and Bergelson, 1997; Brody and Morita, 2000), and therefore provide a more reliable food resource for developing seed fly larvae. These results support the plant vigor hypothesis (Price, 1991) by demonstrating the potential role of plant size and flowering date as oviposition cues for seed flies. As suggested by Brody (1992a), variation in floral morphology and the density of neighboring plants may further explain patterns of seed fly oviposition in scarlet gilia.

We found that the strong effects of plant apparency on oviposition translated into the patterns of damage observed at the end of the reproductive season. In particular, tall, early flowering plants experience the majority of seed fly damage. Spatial block, plant height, flowering date, the interaction between height and flowering date, and height squared explained 40% and 22% of the observed variation in seed fly damage for control and browsed plants, respectively. After controlling for the effect of apparency, we found that plants with high cucurbitacin production in fruit tissue experienced reduced levels of seed fly damage ($\approx$ 6% and 2% increase in R^2 for control and browsed plants, respectively). These results provide preliminary evidence for the defensive function of cucurbitacin in scarlet gilia. We speculate that high cucurbitacin production in fruit tissue potentially reduces seed fly larval survivorship, possibly as an antagonist of insect developmental hormone receptors (Blackford and Dinan, 1997; Dinan *et al*., 1997, 1999). Several recent studies have documented that cucurbitacins compete with ecdysteroids for the ligand binding sites of the insect steroid hormone receptor (reviewed in Dinan *et al*., 1999), with subsequent effects on larval development. This hypothesis needs conformation by experimentally manipulating cucurbitacin levels and investigating subsequent effects on developing seed fly larvae. Variation in pollinator visitation, stress from environmental variation (e.g., desiccation), differential predation (Brody and Waser, 1995), selective fruit abortion (Hainsworth *et al*., 1984), or other unmeasured defensive plant traits may further explain variation in seed fly damage.

It seems reasonable that shifts in flowering date or reductions in plant height would increase resistance to seed fly attack in this scarlet gilia population. However, we detected strong selection on final plant height and flowering date in the opposite direction. Tall, early flowering plants had the highest reproductive success, probably due to variation in pollinator visitation (Campbell, 1992; Mitchell, 1994; Juenger and Bergelson, 1997) despite apparently experiencing higher insect loads. Resistance via escape in either space or time seems relatively constrained in this population because of the overwhelming necessity of maintaining pollination. Likewise, increases in cucurbitacin production may be expected to lead to increased resistance. However, we detected strong negative phenotypic selection on cucurbitacin production in both treatments. This result is somewhat surprising in that cucurbitacin production is negatively correlated with seed fly damage (when controlling for plant height and flowering date) and seed fly damage can arguably decrease plant fitness (Juenger and Bergelson, 1998). It is possible that a high cost of cucurbitacin production outweighed any apparent benefit to cucurbitacin production in this year, that cucurbitacins are under strong direct negative selection by some other agent of selection (e.g., perhaps acting as an attractant for some other herbivore), or experience strong indirect selection through some other genetically correlated characters. Alternatively, the true pattern of selection on curcurbitacins may be different than that detected through a phenotypic analysis, due to unobserved environmental correlations. Additional experimental manipulations of seed fly attack will provide a more rigorous partitioning of the selection imposed by seed fly damage on phytochemistry (Mauricio and Rausher, 1997) and a more direct test of costs of cucurbitacin production. Finally, we found an unexpected positive relationship between seed fly damage and fitness at both the phenotypic and genetic level. It is possible that the expected negative relationship between damage and fitness was obscured in this system by an indirect positive correlation between damage and levels of pollination in this pollen-limited year (T.E. Juenger, personal observation). This result stresses that seed flies may impose relatively weak selection in contrast to pollinators, particularly in pollen-limited years.

In conclusion, we have found significant genetic variation in resistance to anthomyiid seed fly attack in a natural plant population. Herbivory is highly correlated with two indicators of plant apparency, possibly due to strong selection on herbivores to oviposit on plants with a high probability of receiving pollination. Despite the overall importance of apparency, we also detected subtle effects of plant chemistry on damage. We found additive genetic variation for all of the traits measured in this study, with the exception of relative fruit production, suggesting that they may evolve in response to selection imposed by either herbivores or pollinators. The overwhelming importance of apparency on plant fitness and levels of damage suggests that overall resistance or susceptibility to seed fly herbivory may evolve primarily as an indirect correlated response to strong selection on plant apparency.

Acknowledgements

We thank S. Machula, L. Berk, N. Cochran, and J. Juenger for help with field work. The US Forest Service and D. and M. Morroni kindly provided access to our field populations. We thank D. Tallamy and P. Gorski for their advice on quantifying cucurbitacins and for generously providing cucurbitacin standards. This work was supported by a Hinds Fund grant, a Shortgrass Steppe

Long Term Ecological Research Station Graduate Student Fellowship, an Environmental Protection Agency STAR Fellowship, and a Miller Institute Postdoctoral Fellowship to TJ, a Dropkin Foundation Postdoctoral Fellowship to TM, and a Packard Fellowship JB.

References

Abrahamson, W.G. and Weiss, A.E. (1997) *Evolutionary Ecology Across Three Trophic Levels: Goldenrods, Gallmakers, and Natural Enemies*. Monographs in Population Biology 29. Princeton University Press, Princeton, NJ.

Alonso, C. and Herrera, C.M. (1996) Variation in herbivory within and among plants of *Daphne laureola* (Thymelaeaceae): correlation with plant size and architecture. *J. Ecol.* **84**, 495–502.

Arisawa, M., Pezzuto, J.M., Kinghorn, A.D., Cordell, G.A. and Farnsworth, N.R. (1984) *Plant Pharma. Sci.* **73**, 411–413.

Augspurger, C.K. (1981) Reproductive synchrony of a tropical shrub: experimental studies on effects of pollinators and seed predators on *Hybanthus prunifolius* (Violaceae). *Ecology* **62**, 775–788.

Bergelson, J., Juenger, T. and Crawley, M.J. (1996) Regrowth following herbivory in *Ipomopsis aggregata:* compensation but not overcompensation. *Am. Nat.* **148**, 744–755.

Blackford, M. and Dinan, L. (1997) The effect of ingested ecdysteroid agonists (20-hydroxyecdysone, RH5849, RH5992) and an ecdysteroid antagonist (cucurbitacin B) on larval development of two polyphagus lepidopterans *(Acherontia atropos* and *Laconobia oleraceae)*. *Entomol. Experimentalis et Applica* **83**, 263–276.

Brody, A.K. (1992a) Oviposition choices by a pre-dispersal seed predator *(Hylemya* sp.) I. correspondance with hummingbird pollinators, and the role of plant size, density and floral morphology. *Oecologia* **91**, 56–62.

Brody, A. K. (1992b) Oviposition choices by a pre-dispersal seed predator *(Hylemya* sp.) II. a positive association between female choice and fruit set. *Oecologia* **91**, 63–67.

Brody, A.K. (1997) Effects of pollinators, herbivores, and seed predation on flowering phenology. *Ecology* **78**, 1624–1631.

Brody, A. K. and Mitchell, R.J. (1997) Effects of experimental manipulation of inflorescence size on pollination and pre-dispersal seed predation in the hummingbird-pollinated plant *Ipomopsis aggregata*. *Oecologia* **110**, 86–93.

Brody, A.K. and Morita, S.I. (2000) A positive association between oviposition and fruit set: female choice or manipulation. *Oecologia* **124**, 418–425.

Brody, A.K. and Waser, N.M. (1995) Oviposition pattern and larval success of a pre-dispersal seed predator attacking two confamilial host plants. *Oikos* **74**, 477–452.

Campbell, D.R. (1991). Effects of floral traits on sequential components of fitness in *Ipomopsis aggregata*. *Am. Nat.* **137**, 713–737.

Campbell, D.R. and Halama, K.J. (1993) Resource and pollen limitations to lifetime seed production in a natural plant population. *Ecology* **74**, 1043–1051.

Cockerham, C.C. and Weir, B.S. (1977) Quadratic analysis of reciprocal crosses. *Biometrics* **33**, 187–203.

Crawley, M.J. (1983) *Herbivory: The Dynamics of Animal-Plant Interactions*. Blackwell Scientific, Oxford, UK.

Crawley, M.J. (1993) *GLIM for Ecologists. Methods in Ecology*. Blackwell Scientific Publications, Oxford, UK.

De Steven, D. (1983) Reproductive consequences of insect seed predation in *Hamamelis virginiana*. *Ecology* **64**, 89–98.

Dinan, L., Whiting, P., Girault, J.P., Lafont, R., Dhadialla, T.S., Gess, D.E., Mugat, B., Antoniewski, C. and Lepesant, J.A. (1997) Cucurbitacins are insect steroid hormone antagonists acting at the ecdysteroid receptor. *Biochem. J.* **327**, 643–650.

Dinan, L., Savchenko, T., Whiting, P. and Sarker, S.D. (1999) Plant natural products as insect steroid receptor agonists and antagonists. *Pest. Sci.* **55**, 331–335.

Elam, D.R. and Linhart, Y.B. (1988) Pollination and seed production in *Ipomopsis aggregata:* differences among and within flower color morph. *Am. J. Botany* **75**, 1262–1274.

Elle, E., van Dam, N. and Daniel Hare, J. (1999) Cost of glandular trichomes, a "resistance" character in *Datura wrightii* Regel (Solanaceae). *Evolution* **53**(1), 22–35.

Falconer, D.S. and Mackay, T.F.C. (1996) *Introduction to Quantitative Genetics,* 4th edn., Longman Group, Essex, England.

Fritz, R.S. (1992) Community structure and species interactions of phytophagous insects on resistant and susceptible host plants. In R.S. Fritz and E.L Simms (eds) *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*. University of Chicago Press, Chicago, pp. 240–277.

Hainsworth, F.R., Wolf, L.L., and Mercier, T. (1984) Polination and pre-dispersal seed predation: net effects on reproduction and inflorescence characteristics in *Ipomopsis aggregata*. *Oecologia* **63**, 405–409.

Hainsworth, F.R., Wolf, L.L. and Mercier, T. (1985) Pollen limitation in a monocarpic species, *Ipomopsis aggregata*. *J. Ecol.* **73**, 263–270.

Harrison, S. and Karban, R. (1986) Effects of an early-season folivorous moth on the success of a late-season species, mediated by a change in the quality of a shared host, *Lupinus arboreus* Sims. Oecologia **69**, 352–359.

Juenger, T. and Bergelson, J. (1997) Pollen and resource limitation of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*. *Ecology* **78**, 1684–1695.

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

Juenger, T. and Bergelson, J. (2000) The evolution of compensation to browsing in scarlet gilia, *Ipomopsis aggregata:* herbivore-imposed natural selection and quantitative genetics of tolerance characters. *Evolution* **54**, 764–777.

Karban, R. 1989. Community organization of *Erigeron glaucus* folivores: effects of predation, competition, and host plant. *Ecology* **70**, 1028–1039.

Lande, R. and Arnold, S.J. (1983) The measurement of selection on correlated characters. *Evolution* **37**, 1210–1226.

Lawton, J.H. and Hassell, M.P. (1984) Interspecific competition in insects. In C.B. Huffaker and R.C. Rabbs (eds) *Ecological Entomology*. Wiley, New York.

Lawton, J.H. and Strong, D.R. (1981) Community patterns and competition in folivorous insects. *Am. Nat.* **118**, 317–338.

Littell, R.C., Milliken, G.A., Stroup, W.W. and Wolfinger, R.D. (1996) *SAS System for Mixed Models*. SAS Institute Inc., Cary, NC, 633 pp.

Lynch, M. and Walsh, B. (1998) *Genetics and the Analysis of Quantitative Traits*, Sinauer Associates, Inc., Sunderland, MA.

Marquis, R.J. (1992) The selective impact of herbivores. In Fritz and Simms (eds) *Plant Resistance to Herbivores and Pathogens; Ecology, Evolution, and Genetics*. The University of Chicago Press, Chicago, pp. 301–325.

Mauricio, R. and Rausher, M.D. (1997) Experimental manipulation of putative selective agents provides evidence for the role of natural enemies in the evolution of plant defense. *Evolution* **51**, 1435–1444.

McLean, R.A., William, S. and Stroup, W.W. (1991) A unified approach to mixed linear models. *Am. Stat.* **45**, 54–63.

Miro, M. (1995) Cucurbitacins and their pharmological effects. *Phytotherapy Research* **9**, 159–168.

Mitchell, R. (1994) Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. *Am. Nat.* **143**, 871–889.

Mitchell-Olds, T. and Shaw, R.G. (1987) Regression analysis of natural selection: statistical inference and biological interpretation. *Evolution* **41**, 1149–1161.

Paige, K.N. and Whitham, T.G. (1987) Flexible life-history traits: shifts by scarlet gilia in response to pollinator abundance. *Ecology* **68**, 1691–1695.

Pilson, D. (1992) Aphid distribution and the evolution of goldenrod resistance. *Evolution* **46**, 1358–1371.

Price, P.W. (1991) The plant vigor hypothesis and herbivore attack. *Oikos* **62**, 244–251.

SAS Institute. (1997) *SAS/STAT Software: Changes and Enhancements Through Release 6.12*. SAS Institute, Inc. Cary, NC.

Schluter, D. and Nychka, M.L. (1994) Exploring fitness surfaces. *Am. Nat.* **143**, 597–616.

Schultz, J.C. and Baldwin, I.T. (1982) Oak leaf quality declines in response to defoliation by gypsy moth larvae. *Science* **217**, 149–151.

Searle, S.R., Casella, G. and McCulloch, C.E. (1992) *Variance Components*. Wiley Series in Probability and Mathematical Statistics. John Wiley and Sons, New York.

Shaw, R. (1987) Maximum-likelihood approaches applied to quantitative genetics of natural populations. *Evolution* **41**, 812–826.

Simms, E. (1992) Costs of plant resistance to herbivory. In R.S. Fritz and E.L. Simms (eds) *Plant Resistance to Herbivores and Pathogens: Ecology, Evolution, and Genetics*. The University of Chicago Press, Chicago, IL.

Sokal, R.R. and Rohlf, F.J. (1981) *Biometry: The Principles and Practice of Statistics in Biological Research*, 2nd edn. W.H. Freemon and Company, New York.

StatSoft (1995) *Statistica. Volume II: Graphics*. StatSoft, Inc., Tulsa, OK.

Tallamy, D., Stull, J., Ehresman, N.P., Gorski, P.M. and Manson, C.E. (1997) Cucurbitacins as feeding and oviposition deterrents to insects. *Environ. Entomol.* **26**, 678–683.

Thaler, J.S. (1999) Jasmonate-inducible plants defences cause increased parasitism of herbivores. *Nature* **399**, 686–687.

Wilken, D.H. and Allard, S.T. (1986) Intergradation among populations of the *Ipomopsis aggregata* complex in the Colorado Front Range. *Syst. Botany* **11**, 1–13.

Williams, K.S. and Meyers, J.H. (1984) Previous herbivore attack of red alder may improve food quality for fall webworm larvae. *Oecologia* **63**, 166–170.

Young, T.P. (1987) Increased thorn length in *Acacia drepanolobium* – an induced response to browsing. *Oecologia* **71**, 436–438.

Zimmerman, M. (1980a) Reproduction in *Polemonium:* pre-dispersal seed predation. *Ecology* **61**(3), 502–506.

Zimmerman, M. (1980b) Selective deposition of an oviposition-deterring pheromone by *Hylemya*. *Environ. Entomol.* **9**, 321–324.

Zimmerman, M. and Gross, R.S. (1984) The relationship between flowering phenology and seed set in an herbaceous perennial plant, *Polemonium foliosissimum* Gray. *Am. Midl. Nat.* **111**, 185–191.