Research article

Multiple herbivores and coevolutionary interactions in an *Ipomopsis* hybrid swarm

LYNN L. ANDERSON[1] and KEN N. PAIGE[2,*]
[1]*Department of Natural Resources and Environmental Sciences, University of Illinois, W-503 Turner, 1102 S. Goodwin, Urbana, IL 61801, USA;* [2]*Department of Ecology, Ethology and Evolution, University of Illinois, 515 Morrill Hall, 505 S. Goodwin, Urbana, IL 61801, USA*
(*author for correspondence, tel.: + 1-217-244-6606; fax: + 1-217-244-4565;
e-mail: k-paige@uiuc.edu)*

Abstract. Studies focusing on pairwise interactions between plants and herbivores may not give an accurate picture of the overall selective effect of herbivory, given that plants are often eaten by a diverse array of herbivore species. The outcome of such interactions may be further complicated by the effects of plant hybridization. Hybridization can lead to changes in morphological, phenological and chemical traits that could in turn alter plant–herbivore interactions. Here we present results from manipulative field experiments investigating the interactive effects of multiple herbivores and plant hybridization on the reproductive success of *Ipomopsis aggregata formosissima* X *I. tenuituba*. Results showed that ungulate herbivores alone had a net positive effect on plant relative fitness, increasing seed production approximately 2-fold. Caterpillars had no effect on plant relative fitness when acting alone, with caterpillar-attacked plants producing the same number of flowers, fruits and seeds as the uneaten controls. Caterpillars, however, significantly reduced flower production of ungulate browsed plants. Flower production in these plants, however, was still significantly greater (approximately 1.7-fold greater) than uneaten controls, likely leading to an increase in reproductive success through the paternal component of fitness given that fruit and seed production was not significantly different from that of herbivore-free controls. Although results suggest that herbivore imposed selection is pairwise, ungulates likely have a large influence on the abundance of, and hence the amount of damage caused by, caterpillar herbivores. Thus, because of the ecological interactions between ungulates and caterpillars, selection on *Ipomopsis* may be diffuse rather than pairwise, assuming such interactions translate into differential effects on plant fitness as herbivore densities vary. Plant hybridization had no significant effect on patterns of ungulate or caterpillar herbivory; i.e., no significant interactions were detected between herbivory and plant hybridization for any of the fitness traits measured in this study nor did plant hybridization have any significant effect on host preference. These results may be due to patterns of introgression or the lack of species-specific differences between *I. aggregata formosissima* and *I. tenuituba*. Plant hybridization per se resulted in lowered reproductive success of white colored morphs due in part to the effects of pollination. Although it appears that there would be strong directional selection favoring darker flower colors due to the lower reproductive success of the white colored morphs in the short run, the natural distribution of hybrids suggest that over the long run selection either tends to average out or there are no fitness differences among morphs in most years due to the additive fitness effects of hawkmoth and hummingbird pollinators.

Key words: *Cervus elaphus, Heliothis phloxiphaga,* herbivory, hybridization, *Ipomopsis aggregata formossissma, Ipomopsis tenuituba, Odocoileus hemionus,* pairwise vs. diffuse coevolutionary interactions

Introduction

Studies focusing on pairwise interactions between plants and herbivores may give an inaccurate picture of the overall selective effect of herbivory, given that plants are almost always eaten by a diverse array of herbivore species (Strauss, 1991; Pilson, 1996; Juenger and Bergelson, 1998). Therefore, many investigators have argued that plants evolve diffuse (Janzen, 1980; Fox, 1981) or generalized responses to diverse suites of herbivores, rather than tightly adapted specific responses to single herbivores (Juenger and Bergelson, 1998).

Hougen-Eitzman and Rausher (1994) argued that in multispecies systems plant–herbivore coevolution proceeds in a diffuse rather than a pairwise manner when there are non-additive effects of herbivory on plant fitness or when resistances to different herbivores are genetically correlated. Ecological mechanisms that determine whether the fitness effects of multiple herbivores are non-additive, might include direct interactions among herbivores that could enhance or reduce the effects of each herbivore on plant fitness (Karban, 1989; Fritz, 1992; Karban and Strauss, 1993; Juenger and Bergelson, 1998), or herbivore induced changes in plant phenology, architecture or quality which could indirectly alter plant interactions with other herbivores (Schultz and Baldwin, 1982; Brown and Weiss, 1995; Pilson, 1996; Juenger and Bergelson, 1998).

The outcome of such interactions may be further complicated by the effects of plant hybridization. Hybridization can lead to a number of changes in plant life history, morphology, phenology, and chemistry compared to parental species, and may result in the production of new, novel traits (Rieseberg and Ellstrand, 1993; Fritz, 1999). In particular, hybridization can result in the alteration of plant resistance (Fritz, 1999). Recent studies have shown that different herbivore species often respond differently to hybrid plants within a population (Boecklen and Larson, 1994; Morrow et al., 1994; Strauss, 1994; Fritz et al., 1994, 1996, 1998). Studies addressing the interactive effects of hybridization and multiple herbivore species on host–plant fitness, however, are few (e.g., there is no mention of the interactive effects of hybridization and herbivory on host–plant fitness in Rieseberg and Carney's (1998) review of hybridization). Such studies would contribute greatly to our understanding of the distribution, abundance, and dynamics of plant hybrids observed in nature and the coevolutionary interactions of plants and herbivores in general. This is particularly important given that plant hybridization is quite common in nature (Whitham et al., 1991; Ellstrand et al., 1996).

For more than a decade we have been conducting studies on the ecological and evolutionary consequences of ungulate herbivory on the growth and reproductive success of scarlet gilia (Paige and Whitham, 1987; Paige, 1992, 1994, 1999; Gronemeyer et al., 1997) in pure and hybrid populations of

Ipomopsis aggregata formosissima and *I. aggregata formosissima* X *I. tenuituba*. Although *Ipomopsis* is known to interact with a diverse suite of insect herbivores, including lepidopteran fruit predators (*Heliothis phloxiphaga*: Noctuidae and *Olethreutes* sp.: Tortricidae), a specialist seed fly (*Hylemya* sp.: Anthomyiidae), a stem boring moth (*Sparganothis belfrageana*: Noctuidae), an unidentified green aphid, and an unidentified dipteran root borer (Paige, 1992; Juenger and Bergelson, 1998), the high elevation hybrid populations that we have been studying in Arizona have been relatively depauperate of insect herbivores. Only green aphids and a lepidopteran fruit predator, *Heliothis phloxiphaga*, have been observed, and at exceptionally low levels ($\ll$1% of plants). Thus, the primary focus of our studies have centered predominantly on ungulate herbivory.

Since 1997, however, Heliothid moths have substantially increased in numbers. Because the effects of any one herbivore may not give an accurate picture of the overall selective effect of herbivory (Strauss, 1991; Pilson, 1996; Wise and Sacchi, 1996), it would be misleading to consider the effects of ungulate herbivores in the absence of *Heliothis*. Thus, it becomes important to assess the interactive fitness effects of moth and ungulate herbivores on *Ipomopsis*. Furthermore, it is important to assess the interactive effects of plant hybridization and herbivory, given that hybridization may alter the selective effect of these diverse herbivores. Studies to date have been conducted independent of the potential effects of hybridization per se on *Ipomopsis*.

Here, we assess the interactive effects of herbivores and hybridization on the reproductive success of *Ipomopsis aggregata formosissima* X *I. tenuituba*. Specifically we address the following five questions. (1) What are the independent and interactive effects of Heliothid moths and ungulate herbivores on the reproductive success of *Ipomopsis*? (2) Are interactions pairwise or diffuse? (3) How do the interactive effects of hybridization and herbivory influence host–plant fitness in this hybrid swarm? (4) How does hybridization per se affect plant fitness? (5) What can be inferred about the dynamics of this hybrid swarm?

Methods and materials

Study sites and organisms

Field studies were conducted during the summer of 1999 in a large population (>150,000 individuals) of *Ipomopsis* in the San Francisco Peaks region of north central Arizona, near Flagstaff. This population is composed of a hybrid swarm of two species of *Ipomopsis*, *Ipomopsis aggregata formosissima* and *Ipomopsis tenuituba*.

Ipomopsis is a monocarpic, hermaphroditic, biennial/perennial herb that flowers from early to mid July through late September. Following germination in the spring, *Ipomopsis* develops into a leafy rosette. After 1–8 years, the rosette sends up a single inflorescence, flowers, reproduces once and then dies. Given that it reproduces only once, it makes an ideal plant for acquiring measures of lifetime reproduction (Gronemeyer *et al.*, 1997). *Ipomopsis* is self-incompatible and pollinated by two hummingbird species, the rufous hummingbird, *Selasphorus rufus*, and the broad-tailed hummingbird, *S. platycercus* – and a single species of hawkmoth, the white-lined sphinx, *Hyles lineata* (Paige and Whitham, 1985).

Previous studies (Paige and Whitham, 1987; Paige, 1992, 1994, 1999; Gronemeyer *et al.*, 1997) on *Ipomopsis* in Arizona have shown that during the period of stem elongation 77% (range: 64–91%) of all plants are browsed by mule deer, *Odocoileus hemionus*, and elk, *Cervus elaphus*. No ungulate herbivory occurs prior to inflorescence elongation when scarlet gilia is a leafy rosette (Paige and Whitham, 1987). In addition, previous experimental studies have shown no significant differences in the survival of rosettes generated from browsed vs. unbrowsed parents (19.0 ± 1.5% vs. 15.8 ± 2.1% for rosettes of browsed vs. unbrowsed parents, respectively, $t = 0.378$, d.f. $= 8$, $p = 0.715$) (Paige, 1994). On the average, 80% of all browsed plants have approximately 95% of their aboveground biomass consumed, being browsed down to a height of 1–2 cm. Removal of gilias' single inflorescence results in the production of, on the average, five new inflorescences from dormant lateral buds at the base of the remaining portion of the plant's stem. This change in plant architecture results in an increase in flower and fruit production. Because there are no significant differences between browsed and unbrowsed individuals in the number of seeds produced per fruit, seed mass, germination success, or subsequent seedling survival, an increase in total fruits produced by browsed plants results in an increase in maternal fitness. In addition, we have recently completed studies demonstrating that ungulate herbivory also results in an increase in paternal as well as maternal fitness through an increase in flower, and hence, pollen production (Gronemeyer *et al.*, 1997).

Lepidopteran fruit-predators, *Heliothis phloxiphaga*, are common at lower elevation sites but have been exceedingly rare at higher elevation sites over 14 of 17 years of study (Paige, 1992; Paige, personal observation) with fewer than 150 estimated caterpillars observed at this site each year. Over the past 3 years, however, populations have become substantially larger at this high elevation site with approximately 6% of all plants containing these lepidopteran herbivores (an estimated 9000 caterpillars). The increase in the number of *Heliothis* caterpillars may be a result of milder, warmer, drier winters, which have enhanced the survival of overwintering pupae located in the soil.

In terms of its life-cycle, adult *Heliothis* lay eggs singly on rosettes that are starting to bolt in early spring (mid-late May). Following egg hatch, first instar larvae (<1 mm in length) begin to feed on rosette tissues. As the plants bolt and mature, and the larvae develop into larger instars, caterpillars begin to feed on flowers and developing fruits (by mid-late July). By early to mid August, the larvae begin to drop to the ground and burrow into the soil where they pupate and overwinter, emerging as adults the following spring.

Relationship between flower color, morphology and hybridization

Hybridization in *Ipomopsis* is manifested primarily by differences in flower color and shape. Flowers of pure *I. aggregata formosissima* are predominantly red in color with relatively short, wide corollas (15–23 mm long), while flowers of *I. tenuituba* are predominantly white with relatively long, narrow corollas (19–45 mm long) and more strongly included anthers and stigma (Grant and Wilken, 1986). Hybridization between these two species produces an array of intermediate pinks with intermediate morphologies (Grant and Wilken, 1988; Melendez-Ackerman, 1997; Melendez-Ackerman *et al.*, 1997). In this study, plants were assigned to one of five color categories using a standarized corolla color scale (see Paige and Whitham, 1985); color categories included plants with red, dark pink, pink, light pink or white flowers, covering the range of variation observed. To assess the relationship between flower color, morphology and the relative degree of hybridization, we measured four floral traits on each of 100 randomly chosen plants from within the hybrid swarm, taken from two flowers per plant on each of 20 plants per color category. Traits included the degree of anther exsertion, stigma length, corolla length and corolla width. Measures were averaged over the two flowers and compared across color categories using a discriminant function analysis. Results of the discriminant function analysis showed that 65% of reds and 75% of whites were correctly classified as 'parentals' based on morphology. Thus, red and white morphs within the hybrid swarm under study here, appear to maintain some semblance of their parental traits. Dark pinks, pinks and light pinks were correctly classified back to their respective categories in 60, 30 and 35% of cases and as intermediate hybrids in 85, 75 and 70% of cases based on morphology. The close association between flower color and morphology may be due to linkage disequilibrium among these traits.

Interactive effects of herbivores, herbivory and hybridization

To assess how herbivores, herbivory and hybridization interact in affecting plant growth and reproduction, in mid-July 300 plants were selected within a 125×60 m grid covering approximately 75% of the study population. The grid

was divided into 25 columns spaced 10 m apart. Within each column, 12 individuals were selected approximately 5 m apart and placed into one of four treatment categories. To accomplish this, treatments and color categories were randomly drawn and the individual plant nearest the 5 m mark fitting this category was incorporated into the study. Each column contained three replicates of all four treatments. Each treatment also contained 15 plants from each of five color categories (i.e., 15 reds, 15 dark pinks, 15 pinks, 15 light pinks and 15 whites) for a total of 75 plants per treatment or a total of 300 plants across the entire experiment. Treatments included: (1) uneaten controls, (2) ungulate browsed, (3) caterpillar herbivory, and (4) ungulate browsed and caterpillar herbivory. Due to comparatively lower numbers of red and white individuals, some of these had to be chosen off transect or off grid. Uneaten control plants are easily identified by the fact that they are all single stemmed with no flower or fruit damage. Ungulate browsed plants are always multiply stemmed with a clear stem scar, indicative of ungulate browsing. No other herbivores release apical dominance at this site. Caterpillar treatments are described below.

In this study, almost all browsed individuals (plants that had been naturally eaten by mule deer and elk) were cropped down to a height of ≤1 cm. Previous studies (Paige and Whitham, 1987; Paige, 1994, 1999), including those within hybrid swarms (Paige, 1992), have failed to show significant differences in relative fitness between naturally browsed and experimentally clipped plants (which were chosen from the pool of uneaten individuals following natural herbivory). These results argue that plant selectivity by herbivores had no effect on gilias' ability to compensate or, alternatively, that herbivores were not selective. Thus, the naturally browsed plants used in this study represent unbiased samples of all plants. No plants were protected from subsequent herbivory by mule deer and elk given that the majority of ungulate feeding occurs early in the season. However, in the few cases where ungulate herbivory occurred on a non-ungulate browsed treatment, plants were removed from the study.

Caterpillar treatments included those that already had an individual present (approximately 20%) or one that was added to the designated treatment from a haphazardly chosen plant in the population. Plants rarely (<1%) harbored more than a single individual. We checked plants one day following the transplant to ensure that the caterpillar remained on the plant. If a caterpillar moved off of the treatment plant we would replace the caterpillar and again check the following day until a caterpillar remained for at least a day. After remaining a day we assumed movements were natural and not due to an effect of transplant, given that caterpillars will move plant to plant over a period of time as they feed. Caterpillar transplant experiments were initiated during the third week of July when plants were flowering and beginning to set some fruit. Caterpillars averaged approximately 30 mm in length (3rd–4th instar larvae) at

the time of transplant and were left undisturbed until they either pupated or moved off of the target plant. Impacts of caterpillars in the transplant experiment likely reflect overall natural levels given that the majority of damage occurs when caterpillars reach sizes large enough to substantially impact a plant (3rd–4th instar stage). In addition, plants were checked on a daily basis to ensure that caterpillars had not moved onto non-caterpillar treated plants. In the few cases in which this occurred (approximately 2% of cases), caterpillars were removed before any feeding occurred.

At the end of the flowering season, in late September, all plants were harvested and returned to the University of Illinois. For each of the experimental plants we recorded the number of flowers and fruits produced, number of seeds produced per fruit (from a sub-sample of bagged fruits), seed mass, and stem diameter just above the root crown at the base of the plant. In addition, 1000 seeds, 50 from each of the five color categories for each of the four treatments, were germinated on #3 Whatman filter paper in moist petri plates to assess germination success. Pollen counts were conducted on a single flower from each of 25 haphazardly selected plants per treatment (five per treatment per color combination). Flowers were collected at the initiation of anther dehiscence and placed in vials for pollen counts. Pollen counts were conducted using a Beckman Z1 Coulter Counter (Harder et al., 1985; Gronemeyer et al., 1997).

Censuses were also conducted in early August to determine natural patterns of ungulate and lepidopteran herbivory, and the interactive effects of hybridization and herbivory. Censuses of 375 plants within the study site were conducted by extending a string down each of the 25 designated transects and noting the status (color and type of herbivory) of the plant nearest the string at 5 m intervals.

Statistical analysis

Because intermediate hybrids (in particular pinks and light pinks) did not class back to their respective flower color categories in the majority of cases (30 and 35% for pinks and light pinks, respectively) but did class as intermediate hybrids in the majority of cases (85, 75 and 70% for dark pinks, pinks and light pinks, respectively) we collapsed the three hybrid color categories into one category for purposes of analysis. However, the fact that hybrid categories were collapsed into a single category or analyzed as separate categories did not have any significant effect on the outcome or interpretation of our results.

Potential differences in relative fitness were assessed using an analysis of covariance and Type III sums of squares with three treatment factors (ungulate herbivory, caterpillar herbivory and hybridization/color). Although there were no significant differences in stem diameter among the four treatment groups

($p = 0.88$), basal stem diameter was used as a covariate to further adjust for potential plant size differences. The initial ANCOVA model contained all possible interactions between stem diameter and all treatment factors. Non-significant interactions involving stem diameter were deleted. Non-significant interactions between the treatments were not removed given that they were of particular interest. Tests were run on log-transformed or $\log(Y + 1)$-transformed data to equalize variances. Percentages were arcsin transformed. Data were analyzed using Systat 5.0.5.

Non-additivity, or the potential for diffuse coevolutionary interactions, is indicated by significant statistical interactions between herbivores in an analysis-of-variance model in which each herbivore species is considered a treatment factor (Hougen-Eitzman and Rausher, 1994; Wise and Sacchi, 1996). Additive effects or conditions promoting pairwise coevolution between a plant and its herbivores would be indicated in the absence of statistical interactions between herbivores on plant relative fitness (Hougen-Eitzman and Rausher, 1994; Wise and Sacchi, 1996).

Results

Effects of herbivores on plant relative fitness

Ungulate herbivores had an overall net positive effect on plant relative fitness, significantly increasing flower and fruit production (Tables 1 and 2, Fig. 1A and B). In contrast, the combined effects of ungulate and caterpillar herbivores resulted in a significant decrease in flower production in relation to the effects of ungulates alone (Fig. 1A). Nonetheless, flower production was still significantly greater in plants eaten by both ungulates and caterpillars than in plants that were caterpillar-damaged or herbivore-free controls, potentially leading to enhanced fitness through the male component (see Gronemeyer *et al.*, 1997). The decrease in flower production due to the combined effects of ungulate and caterpillar herbivores did not translate into a significant difference in fruit set when compared to ungulate browsed or uneaten control plants. However, fruit set of plants eaten by both ungulates and caterpillars was significantly greater than for plants eaten by caterpillars alone (Fig. 1B). No significant differences in flower or fruit production were detected when comparing caterpillar-damaged plants to herbivore-free controls (Fig. 1A and B). Furthermore, no significant differences or significant interactions in the number of seeds produced per fruit ($p \geq 0.11$), seed mass ($p \geq 0.28$), or germination success ($p \geq 0.06$) among the treatments were found.

There was also a significant effect of stem diameter on flower and fruit production (Tables 1 and 2) and a significant interaction between stem dia-

Table 1. Analysis of covariance for number of flowers produced per plant

Source of variation	Sum of squares	d.f.	F-ratio	Significance level
Ungulate	1.563	1	32.448	0.0001
Caterpillar	0.238	1	4.950	0.027
Color	0.729	2	7.564	0.0006
Ungulate × caterpillar	0.061	1	1.256	0.263
Ungulate × color	0.118	2	1.230	0.294
Caterpillar × color	0.157	2	1.625	0.199
Ungulate × caterpillar × color	0.110	2	1.137	0.322
Covariate-stem diameter	0.815	1	16.915	0.0001
Stem diameter × ungulate	0.253	1	5.253	0.023
Residual	13.196	274		

Data were log transformed to equalize variances.

Table 2. Analysis of covariance for numbers of fruit produced per plant

Source of variation	Sum of squares	d.f.	F-ratio	Significance level
Ungulate	2.692	1	14.584	0.0002
Caterpillar	0.482	1	2.609	0.107
Color	6.279	2	17.009	0.0001
Ungulate × caterpillar	0.002	1	0.009	0.928
Ungulate × color	0.881	2	2.388	0.094
Caterpillar × color	0.062	2	0.169	0.845
Ungulate × caterpillar × color	0.817	2	2.214	0.111
Covariate-stem diameter	3.919	1	21.231	0.0001
Residual	50.756	275		

Data were log transformed to equalize variances.

meter and ungulate herbivory on flower production (Table 1). In this case, there was a steeper relationship between size and flower production for uneaten controls in comparison to ungulate browsed plants.

Pairwise vs. diffuse coevolutionary interactions

No significant ungulate × caterpillar interactions were detected in this study (Tables 1 and 2, Fig. 1A and B), suggesting that coevolutionary interactions are pairwise in this population. Thus, the effect of ungulate and caterpillar herbivory on plant reproduction is 'additive'. Of course this assumes that resistance traits for different herbivores are genetically uncorrelated; a condition that is often met for plant–herbivore systems (see Hougen-Eitzman and Rausher, 1994) and, although yet unknown, likely for the *Ipomopsis* system as well, given the taxonomically disparate nature of these herbivores.
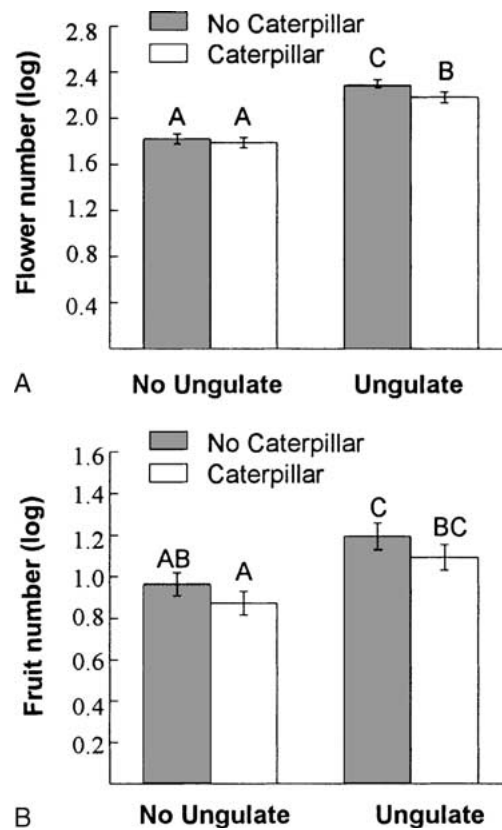
Figure 1. The interactive effects of caterpillars and ungulates on (A) flower and (B) fruit production in *Ipomopsis.* Letters indicate significant differences among treatments. Each bar represents the mean ± 1 SE. Data shown are log transformed.

Interactive effects of herbivory and plant hybridization

No significant interactions were detected between herbivory and plant hybridization for any fitness trait measured in this study (Tables 1–3). Plant hybridization (color) per se, however, had a significant effect on flower and fruit production and the percentage of fruits set (Tables 1–3). Intermediate pinks produced significantly more flowers than either 'parental' reds or whites (Fig. 2A). However, 'parental' whites set significantly fewer fruits than any of the other color morphs (Fig. 2B). In terms of the percentage of fruits set, white morphs also set significantly fewer fruits than intermediate pinks or reds (Fig. 2C).

Natural patterns of herbivory and plant hybridization

Census results showed that 37.6% of all plants were ungulate browsed, 5.6% of all plants were caterpillar damaged and 3.5% of these were eaten by both.

Table 3. Analysis of covariance for percent fruit set

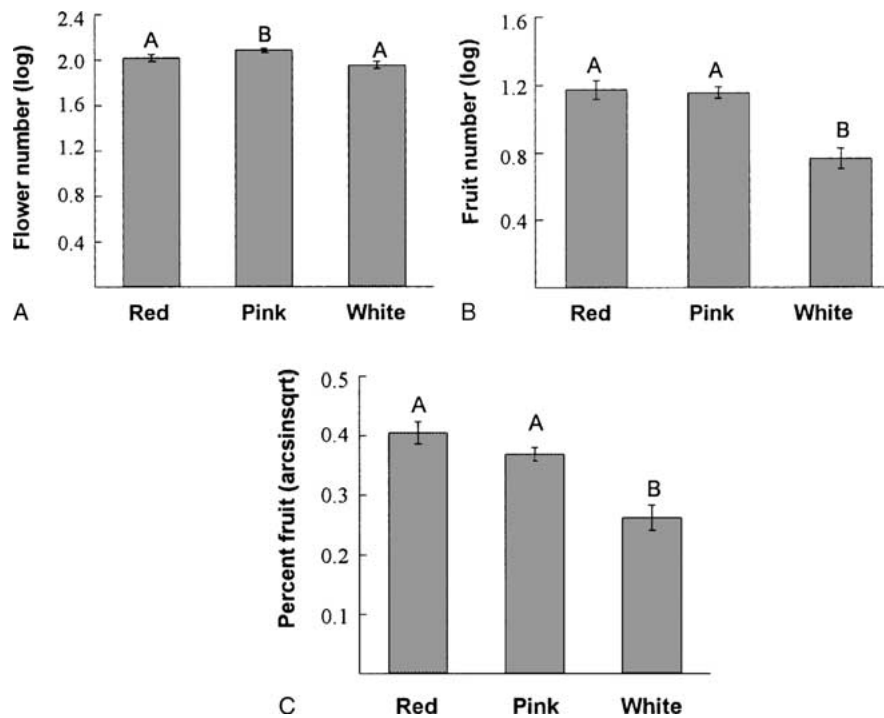| Source of variation	Sum of squares	d.f.	*F*-ratio	Significance level
Ungulate	0.0264	1	2.321	0.129
Caterpillar	0.0143	1	1.263	0.262
Color	0.2235	2	9.836	0.0001
Ungulate × caterpillar	0.0004	1	0.036	0.851
Ungulate × color	0.0248	2	1.091	0.337
Caterpillar × color	0.0168	2	0.741	0.478
Ungulate × catepillar × color	0.0247	2	1.088	0.338
Residual	3.238	285		

Percent data were arcsin sort transformed.

Figure 2. The effects of plant hybridization on (A) flower number, (B) fruit number and (C) the percentage of fruits set in *Ipomopsis*. Letters indicate significant differences among color categories. Each bar represents the mean ± 1 SE. Data shown are log or arcsinsqrt transformed.

Ungulate and caterpillar herbivores showed no preference for any of the hybrid or parental color morphs, taking plants in equal proportions relative to their natural patterns of occurrence in the field ($X^2 = 0.13$, d.f. = 2, $p > 0.90$ and

$X^2 = 0.26$, d.f. $= 2$, $p > 0.80$ for ungulate browsed and caterpillar grazed plants, respectively).

Discussion

Fitness effects of herbivory

As in past studies (Paige and Whitham, 1987; Paige, 1992, 1994, 1999; Gronemeyer et al., 1997), ungulate herbivores had a positive effect on plant relative fitness, increasing flower and fruit production by approximately 2.0-fold over that of the uneaten controls. Because there were no significant differences or significant interactions in the number of seeds produced per fruit, seed mass, or germination success among the treatment groups, total fruit production represents an accurate measure of plant maternal reproduction.

Heliothid moths alone had no significant effect on plant reproductive success, producing the same number of flowers, fruits and seeds as the uneaten controls. These results are likely due to the fact that caterpillars tend to move from plant to plant, moving on average every 2 days. Given the compensatory capabilities of scarlet gilia and the fact that few flowers and fruits are eaten in a 2 day period impacts are negligible. These results are consistent with the findings of Juenger and Bergelson (1998), who allowed caterpillars to naturally choose plants. Because their results were virtually identical to those found here, we conclude that our results were not due to treatment effects caused by placement of caterpillars on plants.

Heliothid moths, however, significantly reduced flower production of ungulate browsed plants. There are at least two possible reasons for the differences in impact on plants when caterpillars occur alone vs. when they occur in combination with ungulate browsers. First, because caterpillars move from stem to stem and plant to plant while feeding, there is a higher probability that caterpillars will move off the single-stemmed unbrowsed plants than the multiply stemmed ungulate browsed plants, increasing their impacts on multiply stemmed plants due to intraplant movements (recall that ungulate browsed plants produce an average of five new inflorescences following herbivory while unbrowsed plants produce only a single inflorescence). Second, there may be differences in plant chemistry between browsed and unbrowsed plants that could alter caterpillar preference; for example, ungulate herbivory may induce chemicals that attract caterpillars or stimulate feeding. In fact, the proportion of caterpillars found on browsed individuals was greater than expected given the natural distribution of unbrowsed vs. browsed plants (3.4 vs. 9.2% caterpillars on unbrowsed vs. browsed individuals; $X^2 = 5.18$, d.f. $= 1, p = 0.013$). Although the phytochemistry of Ipomopsis has not been extensively investi-

gated, it is known to contain a number of phenolic compounds including scopoletin, hydroquinone, related coumarin derivatives, and three forms of cucurbitacin B (Munehisa *et al.*, 1984a, b; Paige, 1992; Juenger and Bergelson, 1998). Nonetheless, to our knowledge nothing is known about the responses of *Heliothis phloxiphaga* to host plant chemistry in *Ipomopsis*. Experimental manipulations and insect observations will be necessary to determine the precise mechanism(s) underlying this interaction.

Although flower production was significantly reduced by Heliothid caterpillars in plants that were ungulate browsed, flower production was significantly greater (approximately 1.7-fold greater) than uneaten controls or plants damaged solely by caterpillars. Because there were no significant differences in the amount of pollen produced per flower among treatments ($p = 0.29$), the potential for an increase in fitness through the paternal component exists for plants eaten by both ungulate and caterpillar herbivores. Previous studies (Gronemeyer *et al.*, 1997) within this population have shown that an increase in flower, and hence pollen, production directly translated into an increase in male reproductive success. Thus, it is likely that the combined effect of ungulate and caterpillar herbivory will lead to an approximate 1.7-fold increase in reproductive success through the paternal component of fitness given that fruit, although seed production was not significantly different from that of the uneaten controls.

Pairwise vs. diffuse coevolution

Although our results suggest that herbivore imposed selection is pairwise, given that no significant ungulate × caterpillar interactions were found in this study, ungulates likely have a large influence on the abundance of, and hence the amount of damage caused by, caterpillar herbivores. Hougen-Eitzman and Rausher (1994) argued that, 'if interactions among herbivores influence the densities of, and hence the damage caused by, those herbivores', then selection would be diffuse rather than pairwise. It is likely that early instar caterpillar larvae (approximately 1 mm in length) and eggs are ingested when plants are browsed by ungulate herbivores (recall that >90% of ungulate herbivory on scarlet gilia occurs early in the season during the period of stem elongation prior to flowering; Paige, personal observations; Paige, 1992, also see Juenger and Bergelson, 1998, their Fig. 1). Thus, the selective impact of caterpillar herbivory is likely contingent upon the level of ungulate herbivory. Because of the inadvertent predation of these insects by ungulates, selection on scarlet gilia may be diffuse rather than pairwise. Of course manipulative experiments will be necessary to assess whether these interactions truly translate into differential effects on plant fitness.

We might also entertain the notion that there is no genetic variation in attractiveness or tolerance to herbivores upon which natural selection can act. This is unlikely, given that there is variation in the degree of compensation (flower, fruit and seed set) following ungulate herbivory directly related to traits such as stem number and phenology known to have heritable genetic bases (Juenger and Bergelson, 2000). Thus, ungulates likely impose directional selection for increased stem number and earlier flowering. Earlier flowering in particular could potentially decrease the impacts caterpillars have on flower number in ungulate browsed plants as well, since that caterpillars do not impact plants until later in the flowering season.

Interactive effects of herbivory and plant hybridization

Plant hybridization had no significant effect on patterns of ungulate or caterpillar herbivory; i.e., no significant interactions were detected between herbivory and plant hybridization for any of the fitness traits measured in this study, nor did plant hybridization have any significant effect on host preference. At first glance these results seem surprising given that the majority of studies have found that herbivore species respond differently to hybrid plants within a population (Boecklen and Larson, 1994; Morrow *et al.*, 1994; Strauss, 1994; Fritz *et al.*, 1994, 1996) which could ultimately lead to differences in host plant fitness. One possible reason for the lack of any relative fitness differences among color morphs is that *I. aggregata* and *I. tenuituba* may not be good species and are thus perceived and treated similarly by herbivores. Genetic data from 23 allozyme loci have shown that populations of *I. tenuituba* cluster within *I. aggregata*. Furthermore, the genetic identity for the two subspecies studied here, *I. aggregata formosissima* and *I. tenuituba latiloba*, was 0.975 (Wolf *et al.*, 1991). This value is much higher than the mean value of 0.670 reported for other congeneric species (Gottlieb, 1981; Wolf *et al.*, 1991). In addition, no species-specific differences were found for restriction site data gathered from nuclear, chloroplast and mitochondrial DNA when comparing populations of *I. aggregata* and *I. tenuituba*, including Arizona populations of *I. aggregata formosissima* and *I. tenuituba latiloba* (Wolf *et al.*, 1997).

A second possible reason why herbivores perceive the color categories in a similar fashion deals with the level of introgression. In spite of maintaining morphological traits associated with the parental types, red and white morphs, along with all of the intermediate pink morphs, may be products of considerable introgression, wherein biochemical traits associated with herbivore resistance or attraction have been genetically homogenized. This may be why ungulates show no preference for any particular hybrid category.

Effects of plant hybridization

Although no significant interactions between herbivory and plant hybridization were found, there were differences in reproductive success among the hybrid color categories. First, some evidence for hybrid vigor was found, with dark pinks, pinks and light pinks producing significantly, or on the average, more flowers than 'parental' reds and whites (Fig. 2A). Second, the lower number and percentage of fruits set among white colored morphs (Fig. 2B and C) may be due, at least in part, to the effects of pollination. In the year of this study, low numbers of hawkmoths, which selectively forage on white morphs of *Ipomopsis* (Paige and Whitham, 1985), may have limited the number of fruits, and hence seeds, produced.

Concluding remarks

Overall, we can conclude that ungulate herbivores alone lead to an approximate 2-fold increase in plant reproductive success, caterpillars alone had no significant effect on reproductive success and the combined effects of ungulate and caterpillar herbivory resulted in a significant decrease in flower production in relation to the effects of ungulates alone. In spite of a reduction, flower production was still significantly greater in plants eaten by both ungulates and caterpillars than in plants that were strictly caterpillar damaged or those that were not eaten, potentially leading to enhanced fitness through the male component. Given that 37.6% of plants were ungulate browsed, a conservative 5.6% were grazed by caterpillars and 3.5% were eaten by both, we estimate that herbivory would lead to an approximate 22% increase in seed production at the population level, due primarily to the effects of ungulate herbivory.

Coevolutionary interactions appear to be pairwise given that there were no significant statistical interactions between caterpillar and ungulate herbivores on plant reproduction in this study. However, because of the ecological interactions between ungulates and caterpillars selection on scarlet gilia may be diffuse rather than pairwise; whether such interactions translate into differential effects on plant fitness in different years as densities and herbivory vary will require additional manipulative experiments.

In terms of the dynamics of the hybrid swarm, it appears that there would be strong directional selection favoring darker flower colors due to the lower reproductive success of the lighter colored morphs. Although, there were no significant interactions between plant hybridization and herbivory, there was a marginally significant interaction between ungulate herbivory and plant hybridization in fruit production wherein dark pink and pink morphs tended to produce higher numbers of fruits than any of the other color morphs. Such

interactions would further contribute to selection for darker flower colors. However, the natural distribution of hybrids approximates a normal distribution; 2.4% of plants are red, 25.9% are dark pink, 52.0% are pink, 19.2% are light pink and 0.5% are white within this population. Selection may fluctuate from year to year favoring darker flower colors in some years and lighter flower colors in other years, given the distribution of color morphs. Thus, over the long run selection either tends to average out or, in most years, there are no fitness differences among morphs due to the additive fitness effects of hawkmoth and hummingbird pollinators (see Paige and Whitham, 1985).

Overall, these results add to the limited number of studies that have assessed the effects of multispecies interactions on host plant fitness, the complex nature of selection imposed (Strauss, 1991; Hougen-Eitzman and Rausher, 1994; Pilson, 1996; Wise and Sacchi, 1996; Juenger and Bergelson, 1998) and the effects of plant hybridization. Although no significant interactions between plant hybridization and herbivory were found in this study, the nature of the effects of hybridization on herbivory may be contingent upon the degree of introgression, which could, in turn, create a geographic mosaic pattern of coevolutionary interactions (Thompson, 1994).

Acknowledgements

References

Boecklen, W.J. and Larson, K.C. (1994) Gall-forming wasps (Hymenoptera: Cynipidae) in an oak hybrid zone: testing hypotheses about hybrid susceptibility to herbivores. In P.W. Price, W.J. Mattson and Y.N. Baranchikov (eds) *The Ecology and Evolution of Gall-forming Insects*. North Central Forest Experiment Station, Forest Service, US Department of Agriculture, Saint Paul, Minneasota, USA, pp. 110–120.

Brown, D.G. and Weiss, A.E. (1995) Direct and indirect effects of prior grazing of goldenrod upon the performance of a leaf beetle. *Ecology* **76**, 426–436.

Campbell, D.R., Waser, N.M. and Wolf, P.G. (1998) Pollen transfer by natural hybirds and parental species in an *Ipomopsis* hybrid zone. *Evolution* **52**, 1602–1611.

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

Ellstrand, N.C., Whitkus, R. and Rieseberg, L.H. (1996) Distribution of spontaneous plant hybrids. *Proc. Nation. Acad. Sci.* **93**, 5090–5093.

Fox, L.R. (1981) Defense and dynamics in plant–herbivore systems. *Am. Zool.* **21**, 853–864.

Fritz, R.S. (1992) Community structure and species interactions of phytophagus 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, pp. 240–277.

Fritz, R.S. (1999) Resistance of hybrid plants to herbivores: genes, environment or both? *Ecology* **80**, 382–391.

Fritz, R.S., Nichols-Orians, C.M. and Brunsfeld, S.J. (1994) Interspecific hybridization of plants and resistance to herbivores: hypotheses, genetics, and variable responses in a diverse community. *Oecologia* **97**, 106–117.

Fritz, R.S., Roche, B.M. and Brunsfeld, S.J. (1998) Genetic variation in resistance of hybrid willows to herbivores. *Oikos* **83**, 117–128.

Fritz, R.S., Roche, B.M., Brunsfeld, S.J. and Orians, C.M. (1996) Interspecific and temporal variation in herbivore responses to hybrid williows. *Oecologia* **108**, 121–129.

Gottlieb, L.D. (1981) Electrophoretic evidence and plant populations. *Progress in Phytochemistry* **7**, 1–46.

Grant, V. and Wilken, D.H. (1986) Taxonomy of the *Ipomopsis aggregata* group (Polemoniaceae). *Bot. Gaz.* **147**, 359–371.

Grant, V. and Wilken, D.H. (1988) Natural hybridization between *Ipomopsis aggregata* and *I. tenuituba* (Polemoniaceae). *Bot. Gaz.* **149**, 213–221.

Gronemeyer, P.A., Dilger, B.J., Bouzat, J.L. and Paige, K.N. (1997) The effects of herbivory on paternal fitness in scarlet gilia: better moms also make better pops. *Am. Nat.* **150**, 592–602.

Harder, L.D., Thomson, J.D., Cruzan, M.B. and Unnasch, R.S. (1985) Sexual reproduction and variation in floral morphology in an ephemeral vernallily, *Erythonium americanum*. *Oecologia* **67**, 286–291.

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

Janzen, D.H. (1980) When is it coevolution? *Evolution* **34**, 611–612.

Juenger, T. and Bergelson, J. (1998) Pairwise vs. 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 herbivory in scarlet gilia, *Ipomopsis aggregata*; herbivore-imposed natural selection and the quantitative genetics of tolerance. *Evolution* **54**, 764–777.

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

Karban, R. and Strauss, S.Y. (1993) Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* **74**, 39–46.

Melendez-Ackerman, E.J. (1997) Patterns of color and nectar variation across an *Ipomopsis* (Polemoniaceae) hybrid zone. *Am. J. Bot.* **84**, 41–47.

Melendez-Ackerman, E.J., Campbell, D.R. and Waser, N.M. (1997) Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis*. *Ecology* **78**, 2532–2541.

Morrow, P.A., Whitham, T.G., Potts, B.M., Ladiges, P., Ashton, D.H. and Williams, J.B. (1994) Gall-forming insects concentrate on hybrid phenotypes of *Eucalyptus*. In P.W. Price, W.J. Mattson and Y.N. Baranchikov (eds) *The Ecology and Evolution of Gall-Forming Insects*. North Central Forest Experiment Station, Forest Service, US Department of Agriculture, Saint Paul, Minneasota, USA, pp. 121–134.

Munehisa, A., Kinghorn, A.D., Cordell, G.A. and Farnsworth, N.R. (1984a) Ipomopsin, a new biscoumarin from *Ipomopsis aggregata*. *J. Nat. Prod. Chem.* **47**, 106–112.

Munehisa, A., Pezzuto, J.M., Kinghorn, A.D., Cordell, G.A. and Farnsworth, N.R. (1984b) Plant cancer agents. XXX. Cucurbitacins from *Ipomopsis aggregata* (Polemoniaceae). *J. Pharmaceut. Sci.* **73**, 411–413.

Paige, K.N. (1992) Overcompensation in response to mammalian herbivory: from mutualistic to antagonistic interactions. *Ecology* **73**, 2076–2085.

Paige, K.N. (1994) Herbivory and *Ipomopsis aggregata*: differences in response, differences in experimental protocol: a reply to Bergelson and Crawley. *Am. Nat.* **143**, 739–749.

Paige, K.N. (1999) Regrowth following ungulate herbivory in *Ipomopsis aggregata*: geographic evidence for overcompensation. *Oecologia* **118**, 316–323.

Paige, K.N. and Whitham, T.G. (1985) Individual and population shifts in flower color by scarlet gilia: a mechanism for pollinator tracking. *Science* **227**, 315–317.

Paige, K.N. and Whitham, T.G. (1987) Overcompensation in response to mammalian herbivory: the advantage of being eaten. *Am. Nat.* **143**, 739–749.

Pilson, D. (1996) Two herbivores and constraints on selection for resistance in *Brassica rapa*. *Evolution* **50**, 1492–1500.

Rieseberg, L.H. and Carney, S.E. (1998) Plant hybridization. *New Phytol.* **140**, 599–624.

Rieseberg, L.H. and Ellstrand, N.C. (1993) What can molecular and morphological markers tell us about plant hybridization? *Crit. Rev. Plant Sci.* **12**, 213–241.

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

Strauss, S.Y. (1991) Direct, indirect, and cumulative effects of three native herbivores on a shared host plant. *Ecology* **72**, 543–558.

Strauss, S.Y. (1994) Levels of herbivory and parasitism in host hybrid zones. *Trend. Ecol. Evol.* **9**, 209–214.

Thompson, J.N. (1994) *The Coevolutionary Process*. The University of Chicago Press, Chicago and London.

Whitham, T.G., Morrow, P.A. and Potts, B.M. (1991) Conservation of hybrid plants. *Science* **254**, 779–780.

Wise, M.J. and Sacchi, C.F. (1996) Impact of two specialist insect herbivores on reproduction of horse nettle, *Solanum carolinense*. *Oecologia* **108**, 328–337.

Wolf, P.G., Soltis, P.S. and Soltis, D.E. (1991) Genetic relationships and patterns of allozymic divergence in the *Ipomopsis aggregata* complex and related species (Polemoniaceae). *Am. J. Bot.* **78**, 515–526.

Wolf, P.G., Murray, R.A. and Sipes, S.D. (1997) Species-independent, geographical structuring of chloroplast DNA haplotypes in a montane herb *Ipomopsis* (Polemoniaceae). *Mol. Ecol.* **6**, 283–291.