

Diane R. Campbell · Matthew Crawford
Alison K. Brody · Tara A. Forbis

Resistance to pre-dispersal seed predators in a natural hybrid zone

Received: 5 June 2001 / Accepted: 31 January 2002 / Published online: 5 April 2002
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Abstract Plant hybrids can be more, less, or equally resistant to herbivores compared to their parental species. These patterns in resistance can be critical determinants of the fitness of plant hybrids and may also influence distribution of the herbivore. We examined resistance to a pre-dispersal seed predator by natural and experimental hybrids between *Ipomopsis aggregata* and *I. tenuituba*. These species and their hybrid offspring differed primarily in ability to avoid oviposition by *Hylemya* sp. (Diptera: Anthomyiidae) rather than in reducing damage to seeds by a developing larva. Plants of *I. tenuituba* had the lowest frequency of fly eggs and were thus the most successful at avoiding damage. Hybrids were either intermediate to or less resistant than both parental species. Because these patterns persisted in experimental arrays of interspersed potted plants, they cannot be attributed to ongoing differences in the environment between hybrid and parental sites. In experimental arrays, the frequency of fly eggs correlated positively with corolla width, a dimension of flower size that also influences the rate of pollination, suggesting seed predators can generate selection on reproductive traits of hybrids. Furthermore, in one of the arrays, oviposition on F2 hybrids exceeded the average for the F1 and the midparent. Our results underscore the need to consider genetic background of hybrids in assessing plant responses to herbivores.

Keywords Herbivory · Hybrids · *Hylemya* · *Ipomopsis* · Seed predation

Introduction

The fate of natural hybrids can depend on interactions with other organisms, including natural enemies. Whitham (1989) suggested that plant hybrids might be particularly susceptible to herbivory, so that hybrid zones serve as sinks for pests. Since then a large number of investigators have examined patterns of herbivory and parasitism in natural hybrid zones (reviewed by Strauss 1994; Whitham et al. 1999). Although hybrids were more susceptible than parents in many cases, intermediate abundances of herbivores on hybrids were also common. In yet other cases, hybrids received the same or fewer herbivores as one or both parental species. As resistance to herbivores can contribute to plant fitness (Rausher and Feeny 1980; Louda and Potvin 1995), these patterns in herbivore susceptibility have the potential to determine the fitness of hybrids relative to parental species (Arnold and Hodges 1995). Hybrid fitness, in turn, is a critical factor distinguishing among the major theories for stability of hybrid zones (Barton and Hewitt 1989). One prominent theory suggests hybrids are intrinsically unfit but that selection against hybrids is balanced by gene flow (Key 1968; Barton and Hewitt 1989). An alternative is that selection is environmentally dependent (Endler 1977), with the possibility that hybrids are most fit in certain environments (Anderson 1948; Moore 1977; Arnold 1997). In addition to influencing plant fitness, the susceptibility of hybrids to herbivores can influence whether hybrids facilitate expansion of herbivore host range (Floate and Whitham 1993; Pilson 1999).

Interpreting patterns in herbivory between parental species and hybrids is complicated by two factors. First, the genetic background of natural hybrids is often unknown, and yet genetic identity is likely to influence herbivory. For example, if susceptibility or resistance to herbivores depends on epistatic effects of nuclear genes,

D.R. Campbell (✉)
Department of Ecology and Evolutionary Biology,
University of California, Irvine, CA 92697, USA
e-mail: drcampbe@uci.edu
Fax: +1-949-8242181

A.K. Brody
Department of Biology, University of Vermont, Burlington,
VT 05405, USA

D.R. Campbell · M. Crawford · A.K. Brody · T.A. Forbis
Rocky Mountain Biological Laboratory, Crested Butte,
CO 81224, USA

Present address:

T.A. Forbis, Department of EPO Biology, University of Colorado,
Boulder, CO 80309, USA

such effects will not appear until the F2 generation when recombinant genotypes are produced (Fritz 1999). Densities of herbivores on F1 and F2 or backcross hybrids may therefore differ (Dungey et al. 2000). By conducting crosses to produce hybrids of known genotype, and exposing them to herbivores, the impact of genetic differences can be revealed. The second complication is that the distribution of natural hybrids usually differs from that of the parental species. Therefore, in the absence of controlled experiments, it is difficult to determine whether herbivores choose hybrid genotypes or simply differ in abundance across environments. This complication can be overcome by the use of common gardens and reciprocal transplants. By employing experimental crosses to produce hybrids of different genetic background and planting them into common gardens, one can separate genetic from environmental effects and compare different genetic classes of hybrids. Some recent studies have employed common gardens (Messina et al. 1996; Fritz et al. 1998; Graham et al. 2001), but to our knowledge only one compared herbivores on F1 and F2 hybrids (Dungey et al. 2000).

Seed predators are a special type of herbivore that consume developing plant offspring. Despite their high potential to influence plant fitness (Louda and Potvin 1995), they have received remarkably little attention in natural hybrid zones. Most information concerning herbivores on hybrids comes from galling insects or those that damage leaves (Strauss 1994). However, in one observational study, hybrid cattails had densities of seed-eating lepidopterans intermediate to or lower than densities on the two parental plant species (Eisenbach 1996). In one unusual common garden experiment, Cummings et al. (1999) found that hybrids received higher levels of pre-dispersal seed predation than the parental species, but in this case the hybrids were between a crop and related wild sunflowers, not naturally hybridizing taxa.

Here we examine pre-dispersal seed predation on natural and synthetic hybrids between *Ipomopsis aggregata* (scarlet gilia) and *I. tenuituba*. These two herbaceous plant species hybridize naturally in many areas of the western United States (Grant and Wilken 1986). At our study area, average survival to flowering is as high for F1 hybrids as it is for the mean of the two parental species (Campbell and Waser 2001). Hybrids also receive more pollen than *I. tenuituba* (Campbell et al. 2002). However, we do not yet know how hybrids compare in resistance to seed predators. Both species are subject to pre-dispersal seed predation by the fly *Hylemya* sp. (Anthomyiidae). *Hylemya* destroys up to 80% of fruits on individual *I. aggregata* plants (Brody 1991). Thus, the seed predator can greatly impact total seed production by a plant of this species (Juenger and Bergelson 1998) and presumably has the potential to influence hybrid fitness as well.

Resistance to seed predators includes two components. Plants may be less attractive to ovipositing females and thus avoid predation, or they can reduce herbivore performance and therefore damage to seeds by

the developing larva (antibiosis; Futuyma 1983; Tiffin 2000). We ask the following two questions. First, how do avoidance and antibiosis vary among *I. aggregata*, hybridizing, and *I. tenuituba* populations in a natural hybrid zone? Second, if flies are given a simultaneous choice in a common environment, how do natural hybrids and known F1 and F2 hybrids compare with plants of the parental species in avoidance of oviposition? To answer this question, we constructed experimental arrays of potted plants. Although our experiments did not allow complete isolation of genetic effects from all environmental effects, they did allow us to eliminate effects of abundance of the seed predator or other aspects of the immediate environment in our comparisons of resistance.

Materials and methods

Study system

Our study system comprised plants across an elevational transect at Poverty Gulch in Gunnison County, Colorado. *Ipomopsis tenuituba* grows at elevations above 3,100 m and *I. aggregata* below 2,900 m. Between these parental sites are a series of hybridizing populations that show clinal variation in floral traits (Campbell et al. 1997). For further description of populations in this study see Campbell et al. (1997) and Campbell and Waser (2001).

In this area, individuals of both plant species are monocarpic with rare exception, spending on average 5 years as a vegetative rosette before blooming during a single season, setting seed, and dying (Campbell 1997; Campbell and Waser 2001). In comparison to *I. tenuituba* flowers, *I. aggregata* flowers have relatively broad and short floral tubes that are intensely red-colored, rather than white or pale pink, and relatively wide sepals. In most years both species are visited primarily by hummingbirds, although hummingbirds prefer *I. aggregata* and hybrids over *I. tenuituba* (Campbell et al. 1997). In this region, the only known pre-dispersal seed predator of *Ipomopsis* is the fly *Hylemya* (Brody 1992). Female *Hylemya* lay eggs individually on the inside of the sepals of an elongated flower bud or flower, and the developing larva usually consumes all seeds in the fruit before emerging as a pupa (Campbell 1991). *Hylemya* does not pollinate the flowers on which it lays eggs.

Patterns of resistance in natural populations

To determine the pattern in avoidance of seed predators, we estimated the frequency of flowers with fly eggs in sites across the hybrid zone. We also recorded the total percentage of fruits eaten by flies as an indicator of potential impact on plant fitness. These studies were conducted in 1992 and 1999. In 1992, we sampled plants in each of 12 populations that span the hybrid zone (populations A-L in Campbell et al. 1997). In 1999, we compared three populations, one *I. aggregata* (population L), one population in the center of the hybrid zone (population I), and one *I. tenuituba* population (DP in Campbell and Waser 2001).

In 1992, we sampled plants by establishing a transect through the population and then choosing the nearest plant to each 3 m mark. We sampled 9–13 plants in each of the 12 populations. At two times during the season, 14 July and 29–30 July, we counted open flowers and recorded how many had an *Hylemya* egg. We also noted any evidence of other herbivory, including stem browsing by ungulates and the presence of stem-boring microlepidopteran caterpillars (either eggs or larvae). At the end of the season we collected the fruiting stalks. From these we determined the number of fruits with mature seeds, the number of fruits damaged by *Hylemya*, and the number of flowers that failed to set fruit. Fruits

damaged by *Hylemya* are easily identified even if the fly pupa has already exited, as they contain frass and remnant pieces of seeds along with a characteristic exit hole.

For each plant we calculated the proportion of flowers with an egg on each census date and the proportion of fruits that were eaten. We compared eggs per flower across populations using a repeated measures ANOVA, with the two time intervals providing the repeated measures (Proc GLM in SAS version 6.12; SAS Institute, Cary, N.C.), and proportion of fruits eaten using one-way ANOVA. Values were arcsin transformed prior to analysis. Although the exact genetic identity of plants in introgressed and hybrid populations was unknown, we constructed a morphological hybrid index using canonical discriminant analysis to examine possible correlations with proportion of fruits eaten. For ten plants per population, we measured three floral traits: corolla length, corolla width, and length of the longest stamen (Campbell 1989, 1997). We sampled three flowers on each plant and used the mean values for plants from populations A and L, the highest *I. tenuituba* population and lowest *I. aggregata* population, in a canonical discriminant analysis to find the linear combination of traits that maximized variance between the species. For each of the other populations, we used its class mean on the first canonical variable as a morphological index of similarity to *I. aggregata*.

In 1999, we sampled 15 plants in each of the three populations studied that year by choosing the nearest plant to each 1 m interval along a transect. At three times during the season (12 July, 21–23 July, and 26–28 July), we recorded the number of open flowers and number with a *Hylemya* egg and any loss of stem to browsing. Beginning in August we collected fruits every 2–3 days just prior to dehiscence so that we could determine fruit set and the proportion eaten by *Hylemya*. Eggs per flower (arcsin transformed) was analyzed with a repeated measures ANOVA comparing the three populations. We also used ANCOVA to examine proportion of fruits eaten as a function of the class variable population and the continuous variable eggs per flower.

To determine patterns in reduction of herbivore performance (antibiosis) across the hybrid zone, in the summer of 2000 we examined the fate of flowers with a fly egg on 15 plants at each of *I. aggregata* site L, hybrid site I, and *I. tenuituba* site DP. Because we anticipated that performance of the seed predator might depend on pollination success (Brody 1992), and pollinators visit more frequently in *I. aggregata* sites (Campbell et al. 1997), we performed an experiment that crossed presence versus absence of a fly egg with supplemental hand-pollination versus natural pollination. On 11 dates between 10 July and 4 August 2000, we chose up to four flowers on each plant that had a fly egg (if available) and four flowers without a fly egg. We hand-pollinated half of the flowers with and without eggs using pollen from 2–3 *Ipomopsis* plants located within 1–10 m, following Campbell and Halama (1993). Treated flowers were marked with one of four colors of ink on the sepals. At the end of the flowering season we collected the fruits or aborted fruits from these flowers every 2–3 days. For each plant and treatment combination (45 plants \times 4 treatments) we determined the mean undamaged seeds per flower as an index of plant fitness. Some undamaged fruits had dehisced and released seeds prior to collection; we estimated their seed number as the average number of seeds for other uneaten fruits in that plant-treatment combination. For treatments with a fly egg, we also determined the proportion of expanded fruits whose seeds were eaten and the proportion of all flowers, whether they produced an expanded fruit or not, that resulted in successful pupation of the fly. Proportion of fruits eaten and frequency of successful pupation provided measures of herbivore performance, with the latter variable including any variation due to level of pollination. Some plants produced few flowers or died, but in all we collected 656 fruits or abortions from our marked plants. We included in our analyses only plant-treatment combinations with information from at least two flowers.

We analyzed undamaged seeds per flower using a three-way ANOVA with the factors: fly egg (present vs absent), pollination (supplemental vs natural), and population (*I. aggregata* vs hybrid vs *I. tenuituba*). We also performed separate two-way ANOVAs

for the subset of plants that received natural levels of pollination and the subset receiving supplemental pollen. Given natural levels of pollination, a fly egg \times population interaction would indicate that populations of the three types of plants differ in antibiosis. To further evaluate herbivore performance, we used two-way ANOVAs to compare the three populations and two levels of pollination with respect to the proportion of fruits with seeds eaten and the frequency of successful pupation.

Avoidance of seed predators in experimental arrays

Differences in the rate of oviposition among populations in the hybrid zone could result from differences in abundance of the fly or differences attributable to the plant phenotype. To separate these causes we assembled two experimental arrays of potted plants at a site 1 km south of the Rocky Mountain Biological Laboratory (RMBL) and approximately 10 km from the natural hybrid zone. The 1999 experiment used natural hybrids collected from the center of the hybrid zone; the 2000 experiment used F1 and F2 hybrids.

The 1999 array consisted of 12 *I. aggregata* plants from population L, 12 *I. tenuituba* plants from populations A-C and 12 plants from hybrid population I. Plants were potted into 15-cm-diameter flower pots after the flowering stem had begun to elongate, and were given N:P:K fertilizer before they were placed in the field. The 2000 array was identical except that we used known F1 and F2 plants (6 of each) instead of natural hybrids. These hybrid plants were produced by crosses between parents potted from populations C and L. The 6 F1 plants included 4 unrelated full sib families. The F2 were a subset of plants from a diallel breeding design; none were more closely related than half-sibs. The F1 and F2 were raised in pots inside a Weatherport used as a greenhouse during the summers and recessed into the ground from September to June at RMBL. All plants in each array received regular water. The 36 plants in each array were arranged in a square 6 \times 6 grid with a spacing of 0.5 m, with individuals assigned at random to the 36 grid locations for the duration of the experiment.

These same arrays were used in an experiment on pollen dispersal (Campbell et al. 2002). Due to constraints of that study, plants were set out in the array during three time periods over the summer and returned to the greenhouse in between. In 1999, plants were exposed in the field during 19–21 July, 24–26 July, and 29–31 July. At the end of each cycle, we inspected all open flowers for *Hylemya* eggs the following morning. In 2000, plants were exposed in the field during 5–7 July, 10–12 July, and 18–20 July, and we counted *Hylemya* eggs on the day of return to the greenhouse or the following day. To explore the relationship between frequency of fly eggs and specific phenotypic traits, we also measured corolla length, corolla width, sepal length, and sepal width on 3–5 flowers on each plant and calculated the plant mean for each trait. We chose these traits because previous studies of *Hylemya* had uncovered associations of corolla and sepal dimensions with either *Hylemya* oviposition or pupation success (Brody 1992; Zimmerman and Brody 1998).

For each year, we analyzed the proportion of flowers with eggs (arcsin transformed) using a repeated measures ANOVA with type of plant as the categorical factor and the time intervals providing the repeated measures. We used multiple regression to examine the relationship of egg frequency to the four floral traits. For the 2000 array, we also tested three a priori contrasts about the effects of hybridization on avoidance of seed predators. Provided that environmental effects are absent, each of these contrasts would coincide with a particular genetic hypothesis. Our first contrast (“heterosis”) compared the F1 mean to the average of the two parental species. The null hypothesis corresponds to the additive hypothesis for herbivore response to hybrids described by Fritz et al. (1994). A departure from the null expectation can indicate dominance or epistasis (Lynch and Walsh 1998). A second contrast (“hybrid breakdown”) compared the F2 with the mean of the F1 and average of the two parental species, its expectation in the absence of epistasis. Because the hybrids and parental species

were raised under different conditions, we cannot distinguish whether such differences in avoidance truly result from genetic effects rather than environmental effects that act during early growth. However, we can eliminate the possibility that they are due to differences in the current environment including abundance of *Hylemya*. Our final contrast compared the F1 and F2 hybrids. Since they were raised under the same conditions, this contrast tested for genetic differences between the two hybrid generations.

Results

Patterns of resistance in natural populations

In natural populations across the hybrid zone, 90–95% of plants (in 1992 and 1999 respectively) had *Hylemya* eggs during our observation windows. For comparison, only 9% (1999) to 22% (1992) of plants were browsed at some point during the entire season and 6% (1992) had a stem-boring caterpillar. These low rates of browsing and caterpillar damage were uncorrelated with plant morphology as indexed by the canonical variable in 1992 ($P > 0.05$), and presence of the caterpillar is unrelated to oviposition rate by *Hylemya* (Brody, personal observation).

During 1992, we detected a strong interaction between population and time of census in the proportion of flowers with a fly egg ($P = 0.0075$), but no average variation among populations ($P = 0.1554$, Table 1). In the earlier part of the season, populations differed significantly in proportion of flowers with an egg (univariate ANOVA, $F_{11, 81} = 2.12$, $P = 0.0278$). To assess whether damage in a population related to plant morphology, we examined correlations with the mean canonical variable. Our canonical discriminant analysis easily separated *I. tenuituba* population A from *I. aggregata* population L (Wilks Lambda, $P < 0.0001$) and also distinguished hybrid population I from parental populations (Fig. 1). The mean canonical variable representing similarity to *I. aggregata* correlated highly with the average proportion of flowers with eggs during the first census ($r = 0.75$, $n = 12$, $P = 0.0054$). During the second census, the overall proportion of fly eggs dropped, from 0.27 to 0.19 on average ($P < 0.0001$), and populations no longer differed detectably in proportion of flowers with eggs ($P = 0.1278$). Although populations varied in the proportion of fruits that were eaten ($F_{11, 81} = 1.92$, $P = 0.0483$), this measure of predation was unrelated to similarity to *I. aggregata* as indexed by the canonical variable (Fig. 1).

In 1999, the *I. aggregata* population consistently received the most fly eggs (Fig. 2, population effect in Table 1, $P = 0.0023$). Just as in 1992, we observed strong effects of phenology, as indicated by a time \times population interaction (Table 1) in which the attack rate on *I. aggregata* relative to that on hybrids fell as the season progressed (Fig. 2). The hybrid population was always intermediate to those of the two parental species in avoidance of seed predators (Fig. 2).

Plants with a higher proportion of flowers with eggs also had a higher proportion of fruits eaten (Fig. 3).

Table 1 Repeated measures ANOVAs comparing the proportion of flowers with a fly egg across 12 natural populations in 1992 and 3 populations (*Ipomopsis aggregata* population L, hybrid population I, and *I. tenuituba* population DP) in 1999. Analyses employed type III SS. Because the 1999 data set departed from sphericity, P values were corrected using the Greenhouse-Geisser method (SAS Ver 6.12). MANOVA test criteria gave similar results (Wilks Lambda, population $P = 0.0003$, time $P = 0.0828$, time \times population $P = 0.0479$)

Year of study	Source of variation	df	MS	F	P
1992	Population	11	0.198	1.48	0.1554
	Plant (population)	81	0.134		
	Time	1	1.522	20.68	0.0001
	Time \times population	11	0.189	2.57	0.0075
	Error (time)	81	0.074		
1999	Population	2	1.612	10.28	0.0003
	Plant (population)	35	0.157		
	Time	2	0.232	3.87	0.0345
	Time \times population	4	0.171	2.84	0.0471
	Error (time)	70	0.060		

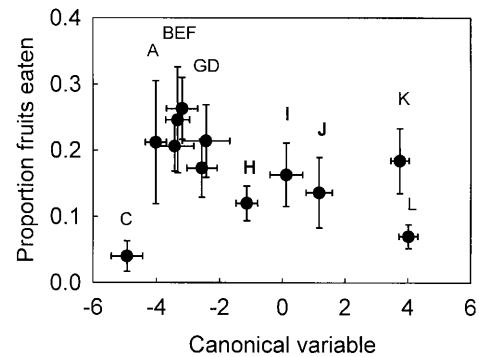


Fig. 1 Proportion of fruits eaten by *Hylemya* larvae in 1992 in 12 natural populations spanning the natural hybrid zone. Symbols indicate means and error bars indicate 1 SE. The canonical variable was obtained from a canonical discriminant analysis on floral traits separating *Ipomopsis tenuituba* population A from *I. aggregata* population L. It had a negative coefficient for corolla length and positive coefficients for corolla width and stamen length. Letters indicate the populations in order from highest elevation (A) to lowest elevation (L)

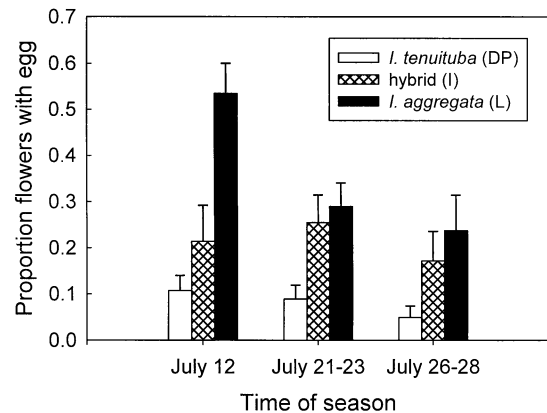


Fig. 2 Proportion of flowers with an *Hylemya* egg (mean \pm SE) in three natural populations in 1999

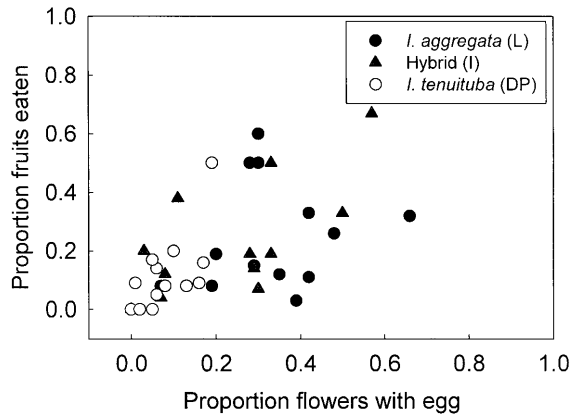


Fig. 3 Proportion of fruits eaten as a function of the proportion of flowers with an egg in three natural populations in 1999

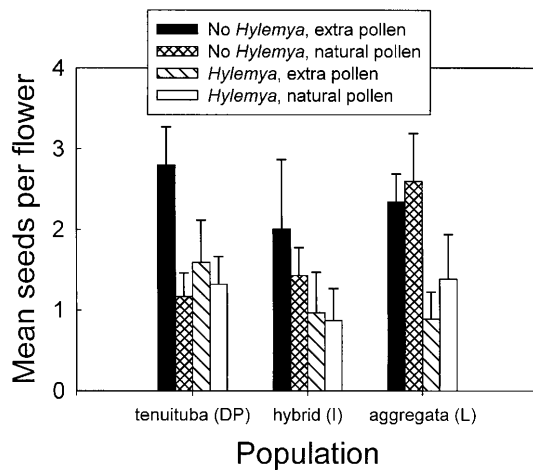


Fig. 4 Effects of an *Hylemya* egg and of addition of extra pollen on mean undamaged seeds produced per flower in three natural populations in 2000. Histograms indicate means across plants, and error bars indicate 1 SE

ANCOVA revealed a strong effect of fly eggs in predicting seed predation within a population ($F_{1, 34}=8.78$, $P=0.0055$) with no additional variation among the three natural populations ($F_{2, 34}=0.18$, $P=0.8339$). The populations of *I. aggregata* and *I. tenuituba* were strikingly different in the proportion of flowers with an egg, while plants in the hybrid population spanned most of the entire range (Fig. 3).

In our 2000 field study, presence of a fly egg strongly reduced the number of undamaged seeds per flower (Fig. 4, Table 2, $P=0.0034$ for main effect in three-way ANOVA). Dividing the data by level of pollination, the *Hylemya* effect was easily detected in hand-pollinated flowers ($F_{1, 48}=7.12$, $P=0.0104$) but not in flowers with natural levels of pollen ($F_{1, 54}=2.05$, $P=0.1577$). For natural levels of pollination, we saw no interaction between presence of a fly egg and population ($F_{2, 54}=1.31$, $P=0.2789$). A significant interaction would have indicated that antibiosis differed among the populations of two species and hybrids. Nor did populations differ

Table 2 Three-way ANOVA on mean seeds per flower. The design crossed population with addition of pollen and presence/absence of an *Hylemya* egg on the flower

Source of variation	df	Type III MS	F	P
Population	2	3.951	0.87	0.4202
Pollen	1	2.409	1.07	0.3042
Hylemya	1	20.318	9.00	0.0034
Population×pollen	2	4.531	2.01	0.1398
Population×Hylemya	2	1.663	0.74	0.4813
Pollen×Hylemya	1	3.110	1.38	0.2434
Population×pollen×Hylemya	2	0.899	0.40	0.6728
Error	102	2.259		

significantly in the proportion of fruits with seeds eaten by *Hylemya* ($F_{2, 22}=0.26$, $P=0.7712$) or frequency of successful fly pupation ($F_{2, 42}=0.20$, $P=0.8171$). Mean values for pupation success were 0.24, 0.28, and 0.27 in the *I. aggregata*, hybrid, and *I. tenuituba* populations, respectively, given control levels of pollen. For flowers without a fly egg, hand-pollination significantly increased seeds per flower in the *I. tenuituba* population ($P<0.05$) but not in the others (Fig. 4, sequential Bonferroni test controlling for multiple comparisons in three populations).

Avoidance of damage in experimental arrays

In the common array of 1999, *I. aggregata* plants received the highest proportion of fly eggs and *I. tenuituba* the lowest, with natural hybrids intermediate in avoidance of damage (Fig. 5A, population effect in Table 3, $P=0.0023$). Frequency of fly eggs was positively related to corolla width in a multiple regression ($F_{1, 19}=6.81$, $P=0.0172$) but not to other aspects of floral morphology ($P>0.05$). Phenological effects were in the same direction as observed in natural populations, with oviposition on *I. aggregata* dropping slightly below that on hybrids during the third census, producing a time×population interaction (Table 3). However, this change in fly oviposition was not associated with a change in the relative sizes of floral displays produced by the three types of plants. We detected no time×population interaction in number of open flowers ($P=0.3184$). *I. tenuituba* had the highest number of flowers and *I. aggregata* the least at every census.

In the 2000 array, *I. tenuituba* once again had the lowest proportion of flowers with a fly egg (Fig. 5B). However, in this year F2 hybrids were oviposited on more often than *I. aggregata*. While the F1 hybrids did not differ significantly from the average of the two parental species, F2 hybrids received more fly eggs than the average of the F1 and the mid-parent, the expectation in the absence of epistasis (a priori contrast $P=0.0076$, Table 3). As in the 1999 array, frequency of fly eggs increased with corolla width in a multiple regression ($P=0.0361$). In this case, a positive slope on corolla width was evident even within a particular type of plant (ANCOVA

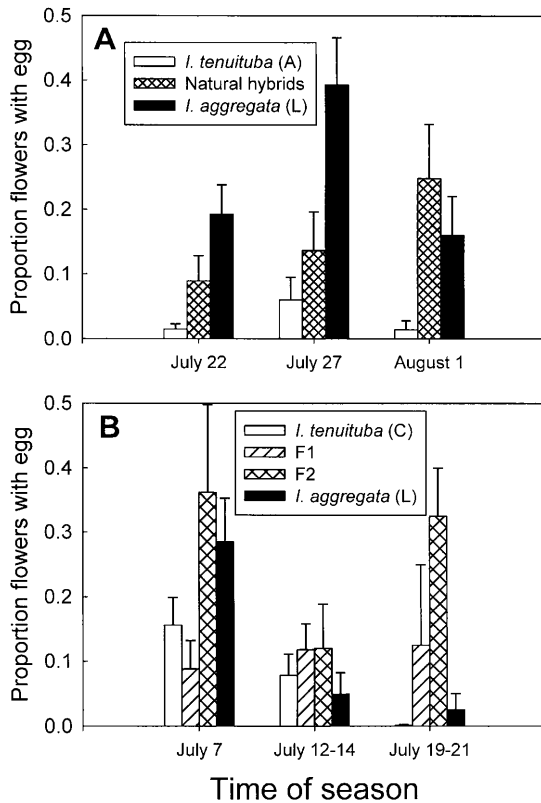


Fig. 5A, B Proportion of flowers with an *Hylemya* egg (mean+SE) for potted plants in the experimental arrays. **A** 1999 comparison of natural hybrids with the two species of *Ipomopsis*. **B** 2000 comparison of the two species of *Ipomopsis* and known F1 and F2 hybrids

Table 3 Repeated measures ANOVA of the array experiments. The assumption of sphericity was met for both sets of data ($P>0.25$). The 1999 array included plants from an *I. aggregata*, a hybrid, and an *I. tenuituba* population. The 2000 array included *I. aggregata*, *I. tenuituba*, F1, and F2 hybrids. Three a priori hypotheses were tested: F1 versus average of the parental species (heterosis), F2 versus average of F1 and the mid parent (hybrid breakdown), and F1 versus F2

Year of experiment	Source of variation	df	MS	F	P
1999	Population	2	0.734	7.728	0.0023
	Plant (population)	26	0.095		
	Time	2	0.133	1.655	0.2011
	Time×population	4	0.214	2.659	0.0429
	Error (time)	52	0.081		
2000	Type	3	0.382	5.77	0.0045
	Plant (type)	22	0.066		
	Time	2	0.526	8.90	0.0006
	Time×type	6	0.195	3.30	0.0090
	Error (time)	44	0.059		
A priori contrasts	Heterosis	1	0.064	0.97	0.3360
	Hybrid breakdown	1	0.572	8.64	0.0076
	F1 vs F2	1	0.239	3.61	0.0706

$P=0.0026$). Although sepal width also correlated with the frequency of fly eggs ($P<0.05$), it had no detectable effect once its correlation with corolla width was accounted for in the multiple regression. As in 1999, we observed a strong phenological effect, as indicated by a

time × type interaction (Table 3). The relative susceptibility of the two parental species to fly eggs decreased at the end of the season, while that of F1 and F2 hybrids remained high (Fig. 5B). During the last census, the F2 were significantly more susceptible than the F1 (contrast $P=0.0046$) although they were not detectably so overall ($P=0.0706$, Table 3). The relative number of open flowers on plants of the four types did not change over the experiment (time×type interaction, $P=0.9409$), indicating similar flowering phenologies.

Discussion

Our studies were designed to compare the relative resistance of plant hybrids to pre-dispersal seed predators in natural populations, and in experimental arrays that eliminated any ongoing environmental differences. We examined attack rates by the fly *Hylemya* on flowers of *Ipomopsis aggregata*, *I. tenuituba*, and their hybrids. Plants of *I. tenuituba* usually had the lowest frequency of fly eggs and thus avoided damage most successfully. This pattern was seen in populations across a natural hybrid zone in Gunnison County, Colorado in 1999 and in experimental arrays of potted plants. In 1992, we observed this pattern early in the season, although oviposition rates did not differ among populations later in the season. The ability of hybrids to avoid attack, and thus damage, ranged from intermediate between the species (Figs. 2, 5A) to lower than both parental species (Fig. 5B). We can thus reject the hypothesis that hybrids are the best at avoiding damage. Instead our results are consistent with the additive hypothesis as posed by Fritz et al. (1994) since F1 hybrids had egg frequencies statistically equal to the average for the parents. As these patterns of low attack on *I. tenuituba* and intermediate or sometimes high attack on hybrids were observed not only in the field but also in experimental arrays of potted plants, they cannot be attributed to changes in population size of the flies across the hybrid zone. Instead they must arise either from genetic differences between the two species and hybrids, or from differences in the early environment experienced prior to bolting when plants were potted for use in the array.

The proportion of flowers with fly eggs showed strong phenological effects in all of our studies. Oviposition dropped off late in the season on flowers of *I. aggregata* while remaining high on flowers of hybrids. This pattern cannot be explained by phenology of the fly since the pattern also occurred in our experimental arrays where all plants were presented in flower simultaneously. In principle, a difference in flowering phenology could produce such a shift (Floate et al. 1993). Since the percentage of fruits eaten is higher on *I. aggregata* plants with a larger number of flowers open (Brody and Mitchell 1997), if *I. aggregata* bloomed earlier *Hylemya* might preferentially oviposit on it early in the season and then shift to hybrids as they came into full bloom. However, flowering phenologies of all plant types were indistinguishable in our experimental arrays, ruling out this

hypothesis. Alternative possibilities include different phenologies for production of defensive chemicals and/or plant stress levels (Louda and Collinge 1992) in hybrids compared to the parental species.

Some of the variability in egg laying on hybrids likely results from variation in genetic background. For example, whereas oviposition on natural hybrids was intermediate to that on the parental species, oviposition on F2 hybrids exceeded the average for the parental species in the 2000 array. Those F2 were also more susceptible than the average level of the F1 and the midparent. This pattern is consistent with the hypothesis that recombination disrupts gene combinations that interact favorably to resist herbivores (Whitham 1989). However, these results must be interpreted with caution since the F1 and F2 plants were raised in a different environment for the early part of their lifecycle from the environment experienced by parental plants and the natural hybrids used in 1999. The only comparison that eliminates effects of being raised in the greenhouse versus the field as an alternative explanation is the contrast between the F2 and F1 plants in the 2000 array. These F2 were more susceptible than the F1 during the last census, suggesting genetic differences are involved. However, only a small number of families were tested, and an expanded study would be necessary to decide whether the result is general or whether certain F2 recombinant genotypes have high resistance even while others show low resistance. We are now directly testing the hypothesis of epistatic effects on resistance to seed predators with individuals planted as seeds into multiple common gardens in a reciprocal transplant design across the natural hybrid zone. Even our current results, however, underscore the need to consider genetic background of hybrids in assessing herbivore response. We note that few studies have compared hybrids of different genetic background with one notable exception. In a study of eucalypts (Dungey et al. 2000), insect species richness was higher on F1 than F2 hybrids suggesting, in that system, no F2 hybrid breakdown in resistance.

Rate of oviposition correlated strongly with corolla width, both within plant-type and between the narrow-tubed *I. tenuituba* and the wider-tubed hybrids and *I. aggregata*. In contrast, other aspects of floral morphology including sepal width had no independent effect on oviposition. Using flowers of *I. aggregata*, Zimmerman and Brody (1998) also found a correlation of *Hylemya* eggs with corolla width at one of two sites tested. Apparently, the female flies use either corolla width or some unmeasured correlated trait in choosing where to oviposit. Hummingbird pollinators also preferentially visit plants with wider corollas (Campbell et al. 1997). In our 2000 experiment, the proportion of flowers with eggs correlated positively ($r=0.47$, $P=0.0046$) with hummingbird visits per flower per hour measured on the same plants by Campbell et al. (2002). Moreover, both fly eggs and pollen receipt were highest on *I. aggregata* plants in our 1999 array and on F2 hybrids in 2000 (here, and Campbell et al. 2002). Thus there was close correspondence between damage by seed predators and pollination, as

has been suggested within-species (Brody and Waser 1995). As a result, some floral traits in this hybrid zone may experience opposing selective pressures through pollination and seed predation. Given spatio-temporal variation in these two sources of selection (Campbell et al. 1997; Zimmerman and Brody 1998), the balance between them may differ between years, conceivably contributing to variation in corolla width.

The effect of an herbivore on hybrid fitness can depend not only on ability to avoid the herbivore but also on the ability of plants to reduce damage (antibiosis) and to tolerate damage. Most studies of herbivores in hybrid zones have focused on abundances of herbivores on individual plants and thus provide information on avoidance. Although we did not explicitly study tolerance (Juenger and Bergelson 2000), we did examine ability to reduce damage by developing flies along with avoidance of oviposition. Antibiosis appears to vary little between natural populations of the two species and their hybrids. In 2000 when we traced the fate of flowers with and without *Hylemya* eggs, we found no evidence for differences between populations of the two species and hybrids in pupation success of the flies or in the effect of an egg on seed production. Although low sample sizes could have reduced the statistical power, our observational study in 1999 also supports the hypothesis of similar antibiosis in all the natural populations. In 1999 we detected no additional variation among populations in proportion of fruits eaten once frequency of fly eggs was taken into account. We do not yet know how the two species and hybrids compare in antibiosis in a common garden, but the main difference in resistance between natural populations of these taxa seems to lie in avoiding oviposition rather than reducing damage.

Resistance to seed predators is only one component of plant fitness. In this hybrid zone other types of herbivory are relatively uncommon compared to levels in many other *Ipomopsis* populations (Bergelson et al. 1996; Juenger and Bergelson 1998). However, fitness depends on more than herbivory, and understanding the role of resistance in the dynamics of hybrid zones will require investigators to study other fitness components. Rates of seed predation are generally higher on *I. aggregata* and hybrids, but evaluating the impact of the flies on fitness is complicated by interactions with level of pollination (this study) and other herbivores (Juenger and Bergelson 1998). Despite susceptibility to *Hylemya*, plants of *I. aggregata* and hybrids appear to have the highest net reproductive success, as measured by the number of undamaged seeds produced. *I. aggregata* had the highest seed set per flower in an experimental array study (Meléndez-Ackerman and Campbell 1998). Similarly, in a reciprocal transplant, total production of undamaged seeds by plants that bloomed during their first 5 years was higher for *I. aggregata* and F1 hybrids than for *I. tenuituba* (Campbell and Waser 2001). The high seed production of *I. aggregata* probably results from its superior ability to attract hummingbird pollinators (Campbell et al. 1997). Our current finding that hand-pollinated flowers of *I. tenuituba* produced more seeds is

consistent with the hypothesis that those high elevation populations are pollen-limited except in unusual years when hawkmoth pollinators appear in addition to hummingbirds (Campbell et al. 1997). At the same time, *I. tenuituba* has higher survival than *I. aggregata* in the high elevation sites (Campbell and Waser 2001). Thus, while *Hylemya* reduces the number of viable seeds produced by a flower, seed predation is just one of many sources of selection in this hybrid zone. A definitive test of its role in influencing hybrid fitness relative to other factors awaits further study.

Acknowledgements We thank G. Pederson and N. Waser for assistance with the array experiments. Financial assistance was provided by NSF grants DEB-9806547 to D.C. and DBI-9200040 and DBI-9987953 to R.M.B.L.

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