

# Experimental Evidence That Selection Favors Character Displacement in the Ivyleaf Morning Glory

Robin Ann Smith<sup>1,\*</sup> and Mark D. Rausher<sup>2,†</sup>

1. Department of Biology and University Writing Program, Box 90025, Duke University, Durham, North Carolina 27708;

2. Department of Biology, Box 90338, Duke University, Durham, North Carolina 27708

Submitted May 21, 2007; Accepted August 10, 2007;  
Electronically published November 16, 2007

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**ABSTRACT:** While there is abundant evidence to suggest that pollinators influence the evolution of plant floral traits, there is little direct evidence that interactions between plant species shape the evolution of such characteristics. The purpose of this study was to determine whether the presence of the morning glory *Ipomoea purpurea* alters patterns of selection on floral traits of its congener, *Ipomoea hederacea*. We show that while selection on *I. hederacea* floral traits is effectively neutral when *I. purpurea* flowers are absent, selection acts to increase clustering of anthers about the stigma when *I. purpurea* flowers are present. Our results provide direct experimental evidence that the presence of flowers of a co-occurring congener can influence patterns of natural selection on floral traits that influence the mating system and contribute to prezygotic isolation. To the extent that this result is general, it also lends support to the claim that distributional patterns interpreted as ecological and reproductive character displacement in other plant species have been caused by natural selection generated by interactions among plant species.

**Keywords:** character displacement, natural selection, *Ipomoea hederacea*, *Ipomoea purpurea*.

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Although recent evidence suggests that plant-pollinator coevolution may not be as tight as once believed (Waser et al. 1996; Fenster et al. 2004), there is still abundant evidence that pollinators influence the evolution of plant floral and reproductive characteristics (Wilson and Thomson 1996). Less well understood is how, and to what extent,

interactions between plant species shape the evolution of such characteristics. While it has been frequently suggested that interactions between plant species, often mediated by common pollinators, generate selection that alters plant reproductive traits (Waser 1978, 1983; Rathcke 1983), current evidence for this type of effect is less compelling than that for the direct influence of pollinators.

Much of this evidence is in the form of differences in reproductive traits in areas where similar or closely related plant species co-occur. For example, temporal divergence of flowering time in areas of sympatry (Waser 1978, 1983; Rathcke 1983), divergence of flower color in areas of sympatry (Levin 1985), and increased selfing rates in areas of sympatry (Fishman and Wyatt 1999) have all been taken to indicate that interactions between plant species have generated selection to either alter mating-system characters or increase prezygotic reproductive isolation.

There are compelling reasons for expecting these types of pattern. Competition for pollination between co-occurring plant species is often intense (Rathcke 1983). One plant species may be able to attract pollinators away from another species, resulting in decreased pollinator visitation and reduced reproductive success. Even when pollinators visit coexisting species without discriminating between them, interspecific pollen transfer can result in loss of pollen by the donor to inappropriate mates (Campbell and Motten 1985; Caruso 1999), interference from heterospecific pollen on the stigmatic surface (Stucky 1985; Randall and Hilu 1990), or production of inviable or infertile hybrids (Rathcke 1983). To mitigate the costs of competition, selection is expected to favor the divergence of characters such as flowering phenology, attractiveness to specific pollinators, or selfing rates where competing plant species co-occur (Waser 1983).

Despite these expectations, other explanations for these patterns are possible. In particular, the presence in the range of a plant species of both areas where it co-occurs with a second plant species (sympatric areas) and areas where it does not (allopatric areas) suggests that some environmental conditions differ in areas of sympatry and allopatry. These are presumably the conditions that limit

\* E-mail: ras10@duke.edu.

† E-mail: mrausher@duke.edu.

the range of the second species. To the extent that these environmental factors also influence pollinator abundance or identity, any difference in floral traits between areas of sympatry and allopatry may reflect selection generated by these environmental differences rather than by any interaction between the two plant species.

The only way to differentiate conclusively between these explanations is to ascertain whether, under identical environmental conditions, the presence/absence of a second plant species alters the pattern of selection exerted on floral characteristics of the focal species. We know of only two studies in which this type of experiment has been conducted. Fishman and Wyatt (1999) found that outcrossing morphs of *Arenaria uniflora* suffered significantly greater reductions in fruit and seed set than did selfing morphs when in the presence of congener *Arenaria glabra*. They concluded that pollinator-mediated interspecific competition is likely to have contributed to the evolution of the highly selfing, cleistogamous populations of *A. uniflora* found in areas of overlap between the two species. Caruso (2000) examined the effect of a coflowering species, *Castilleja linariaefolia*, on selection on floral traits in hummingbird-pollinated *Ipomopsis aggregata*. She found that selection on corolla length was significantly stronger in populations containing both *I. aggregata* and *C. linariaefolia* than in populations containing *I. aggregata* alone. However, these experiments did not compare patterns of selection in the same environment with and without the competitor. Apart from these studies, there is very little direct experimental evidence indicating that interactions among co-occurring plant species can alter the pattern of selection on floral characters and drive their divergence.

The purpose of the investigation reported here was to determine whether the presence of the morning glory *Ipomoea purpurea* alters patterns of selection on floral traits of its congener *Ipomoea hederacea*. Results from previous investigations of the interactions between these two species provide reason for suspecting such an effect. The two species commonly co-occur throughout much of the southeastern United States. They share the same bumblebee pollinators, which account for more than 75%–95% of visits to both species (Stucky 1984; Wolfe and Sowell 2006), suggesting that they may compete for pollinator service. In addition, pollen flow from *I. purpurea* to *I. hederacea* has potentially detrimental effects on *I. hederacea* seed production because it germinates on *I. hederacea* stigmas and grows through the stylar tissue but results in hollow, inviable seeds (Guries 1978). By contrast, *I. hederacea* pollen does not fertilize *I. purpurea* ovules and has not been found to reduce *I. purpurea* seed set (Guries 1978; Stucky 1985).

*Ipomoea hederacea* belongs to a clade that includes *I. purpurea*, *Ipomoea nil*, *Ipomoea pubescens*, *Ipomoea lindheimeri*, and sister species *Ipomoea indica* (Miller et al.

2004). *Ipomoea hederacea* is unusual among species in this clade in that a flower's five anthers are tightly clustered around the stigma (Smith and Rausher 2007). Both Ennos (1981) and Stucky (1985) hypothesized that this arrangement constitutes an adaptation for minimizing the amount of detrimental heterospecific pollen flow by maximizing selfing rate and/or by acting as a barrier to incoming pollen. Smith and Rausher (2007) provided support for this hypothesis, using experimental arrays in which *I. hederacea* had anthers removed or intact. They found that the presence of *I. purpurea* reduced *I. hederacea* seed set if anthers were not present but did not reduce seed set if anthers were intact. However, this experiment is not definitive because it assumed that removal of anthers mimics a situation in which anthers are present but not clustered tightly about the stigma.

In this study, we first demonstrate that floral characteristics such as anther and stigma heights are genetically variable in an experimental field population of *I. hederacea*. We then manipulate experimentally whether flowers on co-occurring *I. purpurea* plants are available to pollinators and ask how this manipulation affects patterns of selection on *I. hederacea* floral traits. We show that while selection on *I. hederacea* floral traits is effectively neutral when *I. purpurea* flowers are unavailable, selection acts to increase clustering of anthers about the stigma when *I. purpurea* flowers are available. Our results provide direct experimental evidence that the presence of flowers of a co-occurring congener can influence patterns of natural selection on floral traits that influence the mating system and contribute to prezygotic isolation.

## Methods

### *Study System*

*Ipomoea hederacea* (L.) Jacquin and *Ipomoea purpurea* (L.) Roth (Convolvulaceae) are self-compatible annual vines that commonly co-occur as weeds of agricultural fields and along roadsides throughout the southeastern United States. The history of coexistence of these two species in North America is only partially known. Some authors believe that *I. purpurea* is native to Central America (Gray 1886; Barkley 1986; Hickman 1993), although it has been documented in the eastern United States since at least the early 1700s (Pursh 1814). There is some disagreement over whether *I. hederacea* is native to the United States (Mohr 1901; Stevens 1948) or was introduced from tropical America (Shreve et al. 1910; Strausbaugh and Core 1964; Long and Lakela 1971; Wunderlin 1982; Mahler 1984). Herbarium specimens show that *I. hederacea* has been in the United States for at least 150 years (Bright 1998).

Both species have trumpet-shaped flowers that are

showy but short-lived, typically opening before dawn and wilting by the afternoon of the same day. *Ipomoea hederacea* flowers are light to dark blue in color, with a white throat. *Ipomoea purpurea* flowers are slightly larger in size and range in color from blue to purple to bright pink to white. Flowers of both species have five stamens of varying heights and one style, but whereas in *I. purpurea* the stigma is generally exerted above the anthers, in *I. hederacea* the stigma is commonly found at the same level as, and tightly surrounded by, the anthers (Ennos 1981). These differences in proximity of anthers and stigma are thought to explain the differences in self-fertilization rate between these two species. While *I. purpurea* has a mixed mating system, with a selfing rate typically ranging between 65% and 70% (Ennos 1981; Brown and Clegg 1984), *I. hederacea* is highly selfing (selfing rate = 93%; Ennos 1981) and is capable of high levels of autonomous seed set in the absence of pollinators.

In North Carolina, seeds of both species germinate between May and August. Plants begin flowering about 6 weeks after germination and continue to produce flowers until they are killed by the first hard frost in the fall. Once fertilized, seeds take approximately 4 weeks to mature. Individual fruits typically contain one to six seeds, although fruits containing as many as eight seeds have been observed (R. A. Smith, personal observation).

Premating barriers between these two species are extremely weak. Although *I. hederacea* typically initiates blooming 1–2 weeks earlier than *I. purpurea* (R. A. Smith, personal observation), their flowering phenologies are largely overlapping, and both species are highly attractive to the bumblebees *Bombus pennsylvanicus* and *Bombus impatiens*, their primary pollinators. Results from pollinator preference studies are mixed and show no consistent tendency for pollinators to prefer either species (Ennos 1981; Stucky 1984; Iwao 1995), suggesting that interspecific pollen flow is common in natural populations. Results from hand pollination studies indicate that interspecific pollen flow is also costly, in particular for *I. hederacea*. Although viable hybrids have not been reported in nature, pollen from *I. purpurea* is able to germinate on and grow through the styles of *I. hederacea* but gives rise to inviable hybrid seeds (Guries 1978). Competition between conspecific and heterospecific pollen for fertilization in *I. hederacea* is also strong. When equal amounts of pollen from both species are placed simultaneously on the stigmas of emasculated *I. hederacea* plants, seed set is reduced by more than 50% relative to applications containing *I. hederacea* pollen alone (Stucky 1985). The consequences of heterospecific pollen transfer are asymmetrical, however, as *I. hederacea* pollen is ineffective at fertilization in *I. purpurea*, and mixed hand pollinations have no effect on *I. purpurea* seed set (Guries 1978; Stucky 1985). Asymmetrical crossing barriers are not

uncommon in angiosperms (Levin 1971; Tiffin et al. 2001). The asymmetry between *I. hederacea* and *I. purpurea* is not entirely surprising, as *I. purpurea* styles are generally longer than those of *I. hederacea*, and in hybrid crosses in plants, pollen from the longer-styled taxon frequently out-competes pollen from the shorter-styled taxon (Levin 1971; Kiang and Hamrick 1978; Gore et al. 1990; Williams and Rouse 1990).

#### Experimental Design

In the summer of 2004, we conducted a quantitative-genetics field experiment to compare patterns of selection on a suite of *I. hederacea* floral traits in the presence and absence of flowers of *I. purpurea*, using an approach similar to that originally described by Lande and Arnold (1983) for the measurement of selection on continuous traits. To avoid biases due to environmentally induced covariances between fitness and the floral traits measured, regression analysis involving genotypic values was used, as described by Rausher (1992).

*Ipomoea hederacea* and *I. purpurea* seeds for our experiment were drawn from a collection of ninth-generation single-seed-descent inbred lines, each originally collected as seed from a different individual in natural populations in central North Carolina. To generate our experimental seeds, 50 seeds from each of 14 different *I. purpurea* lines and one to three seeds from each of 40 *I. hederacea* lines were planted and grown under identical conditions in a climate-controlled greenhouse to minimize maternal effects. Each parental plant was allowed to self-fertilize to produce a self-sib family, which constituted the genetic units of our experiment. A total of 40 *I. hederacea* families and 14 *I. purpurea* families were used in this experiment, although because *I. hederacea* was the focal species, all analyses were performed on this species only. The family mean for a particular *I. hederacea* floral trait provided an estimate of the genotypic value of that trait for that family. We chose this design rather than a more conventional quantitative-genetics design (e.g., half-sib design) for several reasons. First, because *I. hederacea* is highly selfing, natural selection is expected to act on the total genetic variation for a trait rather than the additive variation (Roughgarden 1979), and our design measures selection on total genetic variation. In addition, the crosses necessary for measuring the additive component of variation would have created an experimental population of seeds with artificially high levels of heterozygosity that would be of questionable relevance to natural populations of this species (Mauricio 1998; Stinchcombe and Rausher 2002). Finally, our breeding design maximized our potential to detect variation for the measured traits among in-

bred lines by minimizing the amount of variation within an experimental family.

In June of 2004, a total of 2,000 seeds (1,000 *I. purpurea* seeds + 1,000 *I. hederacea* seeds, 25 seeds per *I. hederacea* family) were planted in a randomized block design consisting of 25 spatial blocks in a freshly plowed agricultural field in Orange County, North Carolina. Seeds were planted such that the two species were interspersed checkerboard fashion in a grid with 75 cm separating the rows and 100 cm separating the columns. To maintain a uniform interplant distance and to prevent plants from getting tangled with each other, each plant that germinated was allowed to twine up a 1.5-m-tall wooden stake placed 5–7 cm from the base of the plant. All nonexperimental *Ipomoea* plants that germinated in our site during the course of the experiment were removed, but other vegetation was left undisturbed.

Our experimental design included two treatments: (1) reproductive interference absent (RI–) and (2) reproductive interference present (RI+). The RI– treatment consisted of covering all *I. purpurea* plants with bags made of translucent bridal-veil mesh. This treatment had the effect of preventing pollinators from visiting *I. purpurea* flowers, and thus pollen flow from *I. purpurea* to *I. hederacea* was avoided. The RI+ treatment was identical to the RI– treatment except for the presence of the mesh bags, which were removed to allow for pollinator movement between the two species. In the RI+ treatment, pollinators could visit *I. purpurea* and transfer pollen to *I. hederacea*.

Over a 30-day period, we conducted three temporal replicates. A temporal replicate consisted of imposing one treatment for 2 days and then the other treatment for 2 days (fig. 1). *Ipomoea hederacea* and *I. purpurea* flowers last only one day, so individual flowers were exposed to only one treatment (RI+ or RI–) before wilting. Because the same site, experimental population, and plants were used for both treatments, there were no differences between treatments in the composition of the surrounding vegetation or in levels of soil and nutrient resources. The advantage of this experimental design is that it allowed us to alter the presence/absence of *I. purpurea* flowers in a way that controlled for differences in other environmental conditions, factors often confounded in studies of character displacement.

#### Data Collection

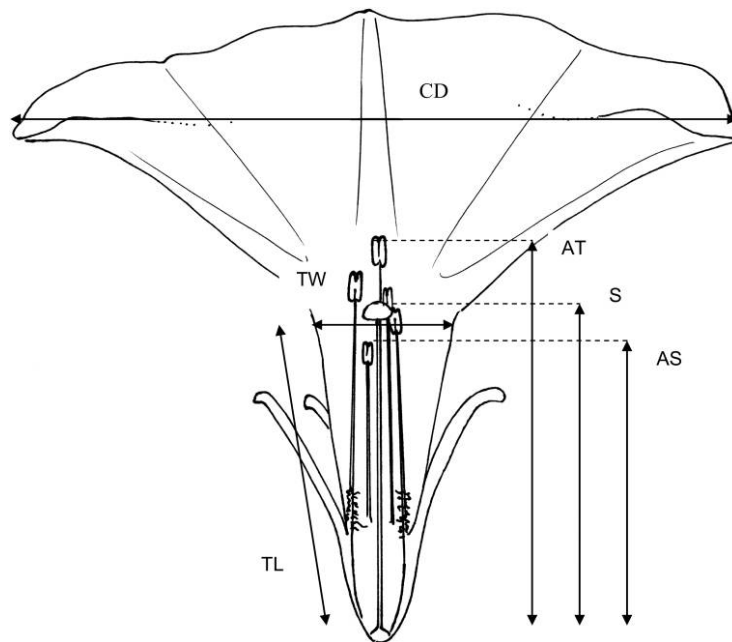
*Floral measurements.* On each *I. hederacea* plant in our experiment, we measured seven floral traits (fig. 2): (1) corolla diameter, defined as the diameter of one of the tubular flowers at the opening; (2) corolla tube length, measured from the base of the sepals to the top of the white corolla throat; (3) corolla tube width, measured as the outside diameter of the corolla tube at its distal end; (4) stigma position, measured from the base of the sepals to the tip of the stigma; (5, 6) two measures of anther position, based on the distance between the base of the sepals and the distal ends of both the shortest and tallest anthers; and (7) flower number. These traits were chosen because of their quantitative nature and because of their possible role in reproductive isolation.

Over the course of several weeks in August 2004, we measured one open flower per *I. hederacea* plant, passing down each row in our experimental population in turn until all *I. hederacea* plants had been measured. We swept through the population in this manner twice over the course of the flowering season, such that one or two flowers per plant were sampled. A pair of handheld digital calipers was used to measure all traits except flower number, which was estimated by counting the number of open flowers on a plant on the day of observation. The mean trait values for each plant in a family were then averaged to calculate a mean for that family, which was then used to approximate its genotypic value.

*Fitness data.* Female fitness was estimated for each plant and treatment as the average number of seeds per fruit. Each day of a given treatment, we examined each *I. hederacea* plant in the experiment, located a randomly positioned open flower, and loosely encircled the pedicel with a color-coded wire to indicate date and treatment. Once the fruits were mature, we collected the resulting seed capsules and counted the number of seeds per fruit. Obviously inviable seeds (seeds that were hollow or unusually small) and tagged flowers that ultimately did not produce any fruits at all were included in our estimates by assigning them a fitness value of 0. For each treatment, the means for each plant in a family were averaged to calculate a mean for that family, which was then used to approximate its genotypic value.

August 2004																						
Date	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Treatment			RI–			RI+				RI–			RI+					RI–			RI+	

**Figure 1:** August 2004 calendar. Dates when the RI– treatment was imposed are shown in light gray, and dates when the RI+ treatment was imposed are shown in dark gray. Other dates shown were spent installing or removing mesh bags or were avoided because of inclement weather.



**Figure 2:** Diagram of an *Ipomoea hederacea* flower, illustrating the morphological traits we measured. Flowers of *I. hederacea* have five stamens of variable length. *AS* = anther position<sub>short</sub>; *AT* = anther position<sub>tall</sub>; *CD* = corolla diameter; *S* = stigma position; *TL* = corolla tube length; *TW* = corolla tube width.

### Data Analysis

*Genetic variation for floral traits.* To determine whether the floral traits we measured were genetically variable, we performed a nested ANOVA. The assumptions of normality for all ANOVAs and ANCOVAs discussed in this article were tested using the Shapiro-Wilk statistic.

*Treatment effects on fitness.* To determine whether our two treatments had significantly different effects on fitness, we used ANOVA with Type III sums of squares. We report results of analyses using the appropriate means squares as error terms (Sokal and Rohlf 1995). The full model included treatment as a fixed effect; family, block, and replicate as random effects; and all two-way interactions. After the full model was run, nonsignificant interaction terms were eliminated. Results reported here are from a reduced model containing only main effects and significant interaction terms.

*Selection analysis.* Selection analyses were performed in much the same way as by Stinchcombe and Rausher (2001). We used Rausher's (1992) genetic-selection analysis, which is similar to that of Lande and Arnold (1983) but uses breeding or genotypic values for a trait rather than phenotypic values. This approach was used because it avoids biases due to environmentally induced covariances between fitness and the floral traits measured (Mitchell-Olds and Shaw 1987; Mauricio and Mojonier

1997). For our selection analyses, we used least squares family means of our trait data, after the effects of block were removed, and standardized these values to a mean of 0 and a standard deviation of 1 (Sokal and Rohlf 1995). We calculated relative fitness for each plant by dividing its mean viable seeds per fruit by the average viable seeds per fruit of all plants in that treatment (Lande and Arnold 1983). Directional selection gradients were estimated for each floral trait in each treatment by incorporating all fitness and floral-trait data into a single multiple-regression model and for each treatment by performing a multiple linear regression of the family mean of relative fitness on the family means of the traits. Each resulting partial-regression coefficient is an estimate of the magnitude of selection acting directly on a given trait, with the effects of selection on all other measured traits removed. We used ANCOVA to determine whether the patterns of selection on floral traits differed between temporal replicates and between the two treatments (Iwao and Rausher 1997; Juenger and Bergelson 1998; Stinchcombe and Rausher 2001).

### Results

*Descriptive statistics.* Of the 2,000 seeds that were planted, a total of 1,339 (~67%) survived to flowering. Of these, 683 were *Ipomoea hederacea* plants and 656 were *Ipomoea*

*purpurea* plants, such that the two species were in roughly equal frequencies at the time of data collection. A  $\chi^2$  test for equal proportions indicated that families did not differ in survivorship to flowering and thus were not differentially represented at the time of data collection ( $P = .99$ ).

*Treatment effects on fitness.* Mean *I. hederacea* seed set (seeds per fruit) was approximately 13% less in the RI+ treatment than in the RI- treatment (3.81 vs. 4.39 seeds per fruit, respectively). Although this difference was not significant ( $F = 1.03$ ,  $df = 1, 4$ ,  $P = .367$ ), probably because of substantial variation among replicates ( $F = 47.3$ ,  $df = 4, 2,242$ ,  $P < .0001$ ), the trend is consistent with results of previous hand pollination experiments, which have shown that pollen from *I. purpurea* reduces seed set in *I. hederacea* (Guries 1978; Stucky 1985).

*Genetic variation for floral traits.* Each of the floral traits we measured proved to be genetically variable, as evidenced by a significant family effect on each of the traits in our ANOVAs (table 1). Significant block effects showed that the floral traits we measured were also affected by spatial variation. We account for this in subsequent analyses by using only least squares means of each of the traits after the effects of block had been removed. Genetic variances and covariances among these traits are presented elsewhere (R. A. Smith and M. D. Rausher, unpublished manuscript).

*Selection analysis.* To estimate the magnitude of selection on the measured floral traits, we performed a family-mean multiple regression of fitness on the traits. Because preliminary tests for heterogeneity of slopes between replicates within treatments were negative, we pooled across replicates to compare regression coefficients between treatments. In the absence of reproductive interference (RI-

treatment), selection was not detectable on any of the *I. hederacea* floral traits measured ( $\beta = -0.038$  to  $0.028$ , nonsignificant for every trait). By contrast, in the presence of reproductive interference (RI+ treatment), selection was significant for six of the seven traits examined (table 2). Moreover, comparison of slopes across treatments by ANCOVA indicates that reproductive interference from *I. purpurea* in the RI+ treatment significantly altered the pattern of selection on four *I. hederacea* floral traits: corolla tube length, stigma position, and both measures of anther position (table 2). Selection acted in opposite directions on short and tall anthers, favoring an increase in the height of the shortest anthers (for anther position<sub>short</sub>,  $\beta = 0.17$ ) and a decrease in the height of the tallest anthers (anther position<sub>tall</sub>;  $\beta = -0.18$ ; table 2). There was also weak selection for increased stigma height ( $\beta = 0.05$ ) and shorter corolla tubes ( $\beta = -0.11$ ).

### Discussion

#### *Functional Significance and Alternate Explanations*

In our experiment, the presence of *Ipomoea purpurea* flowers altered the pattern of selection on floral traits in *Ipomoea hederacea*. In the absence of *I. purpurea* flowers, we did not detect directional selection on any of the floral traits measured. By contrast, in the presence of *I. purpurea* flowers, directional selection was detected on six traits. Of these, four traits were shown by ANCOVA to have significantly altered patterns of selection in the RI+ treatment: corolla tube length, stigma position, and both measures of anther position. Most important, tall anthers were selected to be shorter, while short anthers were selected to be taller. Because in *I. hederacea* the tallest anther is

**Table 1:** ANOVA on floral traits showing significant genetic and spatial variation

Source	Corolla diameter				Corolla tube length				Corolla tube width				Stigma position			
	df	SS	F	P	df	SS	F	P	df	SS	F	P	df	SS	F	P
Model	63	2,621.2	3.9	<.0001	63	293.9	5.5	<.0001	63	46.3	3.8	<.0001	63	311.9	12.2	<.0001
Pop.	5	436.5	2.4	.061	5	107.9	5.0	.0015	5	5.9	1.2	.32	5	38.6	1.1	.36
Family	34	1,260.9	3.5	<.0001	34	145.8	5.1	<.0001	34	32.5	4.9	<.0001	34	231.0	16.8	<.0001
Block	24	948.3	3.8	<.0001	24	28.0	1.4	.109	24	9.7	2.0	.0021	24	32.1	3.3	<.0001
Error	512	5,387.8			514	435.1			512	99.7			513	207.5		
Source	Anther position <sub>short</sub>				Anther position <sub>tall</sub>				Flower number							
	df	SS	F	P	df	SS	F	P	df	SS	F	P				
Model	63	404.99	9.65	<.0001	63	596.07	10.72	<.0001	63	491.45	3.08	<.0001				
Pop.	5	162.29	5.41	.0009	5	282.40	7.8	<.0001	5	48.52	2.62	.042				
Family	34	204.14	9.01	<.0001	34	246.07	8.2	<.0001	34	126.16	1.47	.046				
Block	24	36.75	2.30	.0005	24	54.40	2.57	<.0001	24	306.94	5.05	<.0001				
Error	507	337.80			510	450.19			514	1,301.65						

Note: "Pop." refers to the source population from which each inbred line was originally collected in central North Carolina. We report results of analyses using the appropriate mean squares as error terms (Sokal and Rohlf 1995).

**Table 2:** Directional selection gradients ( $\pm 1$  SE) for seven *Ipomoea hederacea* floral traits in the presence and absence of reproductive interference from *Ipomoea purpurea*

Trait of interest	RI–	RI+	$P^a$
Corolla diameter	.019 $\pm$ .027 NS	.085 $\pm$ .027**	.081
Corolla tube length	–.038 $\pm$ .026 NS	–.114 $\pm$ .026**	<b>.045*</b>
Corolla tube width	.028 $\pm$ .029 NS	.004 $\pm$ .029 NS	.569
Stigma position	–.014 $\pm$ .02 NS	.053 $\pm$ .02*	<b>.019*</b>
Anther position <sub>tall</sub>	–.026 $\pm$ .056 NS	–.18 $\pm$ .056**	<b>.055</b>
Anther position <sub>short</sub>	–.011 $\pm$ .059 NS	.169 $\pm$ .059**	<b>.034*</b>
Flower number	–.03 $\pm$ .017 NS	–.039 $\pm$ .017*	.749

Note: Selection gradients are standardized partial-regression coefficients from a multiple regression of relative fitness on seven floral traits. Anther position<sub>tall</sub> refers to the distance between the base of the corolla and the top of the tallest anther; anther position<sub>short</sub> refers to the distance between the base of the corolla and the top of the shortest anther. The last column indicates whether the presence/absence of *I. purpurea* flowers in the RI+ treatment significantly altered the selection gradient for a particular trait. Significant and marginally significant effects in the last column are shown in boldface. NS = not significant.

<sup>a</sup> Significance of treatment  $\times$  trait interaction term in ANCOVA.

\*  $P < .05$ .

\*\*  $P < .01$ .

typically positioned above the stigma and the shortest anther is typically positioned below the stigma (see fig. 2), the combined effect of such selection is expected to be both reduced separation between short and tall anthers and tighter clustering of anthers about the stigma.

Two explanations, not mutually exclusive, can be offered for the different patterns of selection we observed between our two treatments. The first hypothesis is that character displacement was favored in *I. hederacea* in the RI+ treatment as a way of avoiding heterospecific pollen flow from *I. purpurea*. This hypothesis is consistent with the results of a previous experiment (Smith and Rausher 2007), in which close clustering of anthers around the stigma in *I. hederacea* was found to enhance prezygotic isolation from *I. purpurea*. Although the exact mechanism by which this trait contributes to reproductive isolation in this system is unknown, studies in other taxa show that close clustering of anthers and stigma mechanically prevents pollinators from coming into direct contact with the stigma and maximizes the number of self pollen grains deposited on the stigma, both limiting contact with heterospecific pollen and enhancing deposition of conspecific pollen (Webb and Lloyd 1986). Because pollen from *I. purpurea* decreases seed set in *I. hederacea* by producing inviable, hollow seeds (Guries 1978; Stucky 1985), either of these effects is expected to increase *I. hederacea* seed set. If this explanation is correct, then the selection we detected constitutes selection for increased reproductive isolation.

The second hypothesis is that selection favored tighter clustering of anthers and stigma in the RI+ treatment as a way of promoting self-fertilization in the absence of pollinator visits. Because all *I. purpurea* plants in the RI–

treatment were covered with mesh bags, the treatments differed in the density of floral display accessible to pollinators ( $\sim 1.0$  m between adjacent uncovered plants in the RI+ treatment vs.  $\sim 2.0$  m between adjacent plants in the RI– treatment). Bumblebees were observed visiting uncovered flowers of both species throughout the course of the experiment, but because we did not measure visitation rates, we have no data on the effects of mesh bags on the relative attractiveness of each treatment as a whole. Thus, it is possible that the different patterns of selection we observed were mediated not by the presence or absence of heterospecific pollen flow from *I. purpurea* but instead by unmeasured differences in pollinator behavior or abundance between the treatments. If pollinator visitation to *I. hederacea* was reduced in the RI+ treatment, for example (either because the presence of *I. purpurea* flowers drew pollinators away from *I. hederacea* or because, in this higher-density treatment, pollinators visited individual *I. hederacea* plants less frequently), then tighter clustering of anthers around the stigma—a trait generally thought to promote self-fertilization (Webb and Lloyd 1986)—may reflect selection for increased selfing when pollinator visitation is limiting. If this explanation is correct, then the selection we detected may reflect selection for reproductive assurance under pollen limitation caused by competition for pollination.

Because we do not have pollinator visitation data from this experiment, we cannot distinguish unambiguously between these two explanations. Several lines of evidence indicate that differences in pollinator visitation are unlikely to explain our results, however. First, because *I. hederacea* is already predominantly selfing, it is highly unlikely to be

pollen limited in its seed set. Selfing rates in the field (in the presence of abundant pollinators) have been estimated to be 93% (Ennos 1981), and seed set in a pollinator-free greenhouse is universally high (R. A. Smith, personal observation). In a previous experiment in a separate mixed experimental population (Smith and Rausher 2007), we calculated the relative importance of two consequences of anther-stigma clustering: reproductive assurance in the absence of pollinator visits and mechanical protection from *I. purpurea* pollen. We found that the effects of anther-stigma clustering as mechanical protection from *I. purpurea* pollen were substantially larger (30% greater increase in *I. hederacea* seed set) than the reproductive-assurance effects of this trait. In that experiment (Smith and Rausher 2007), we either completely removed anthers from *I. hederacea* flowers or left them intact. In our study, we would expect the relative magnitude of the reproductive-assurance effect to be even smaller because anthers were intact in all plants, making all plants capable of high degrees of selfing. Taken together, these results support the view that the altered patterns of selection we measured in the RI+ treatment are unlikely to be due to insufficient pollination but are instead more consistent with selection for increased reproductive isolation.

Regardless of the relative importance of selection for enhanced reproductive isolation and selection for reproductive assurance, it is clear that the presence of *I. purpurea* flowers alters the pattern of selection on reproductive characteristics of *I. hederacea*. To the extent that this result is general, it lends support to the claim that distributional patterns interpreted as ecological and reproductive character displacement in other plant species (see the introduction to this article) have been caused by natural selection generated by interactions among plant species.

Although our study shows that interactions between *I. hederacea* and *I. purpurea* have the potential to drive floral evolution in *I. hederacea*, we do not have definitive evidence indicating they have done so. In particular, because *I. hederacea* and *I. purpurea* occur sympatrically throughout the southeastern United States (USDA NRCS 2007 [PLANTS Database]), we do not have data from allopatric populations to indicate whether a pattern of divergence exists in nature. However, it is interesting to note that in the congener *Ipomoea nil*, close clustering of anthers does not occur (Jaramillo 1995). Although recognized by some as distinct species, *I. nil* and *I. hederacea* are often considered to be conspecific (Austin 1986; R. Miller, personal communication). The two taxa have disjunct, though nearly abutting, distributions, and *I. nil* co-occurs only infrequently with *I. purpurea*. This pattern is consistent with the interpretation that close clustering of anthers in *I. hederacea* is a derived state resulting from frequent in-

teractions between *I. hederacea* and *I. purpurea* in the southeastern United States.

Any evolutionary response to the selection documented here may also be constrained by various factors, including the amount of genetic variation in the traits and their genetic correlation structure (Lande and Arnold 1983). Future reports will examine the genetic architecture of *I. hederacea* floral traits to assess the importance of genetic constraint in the evolution of anther-stigma separation and other floral traits in this species.

### Acknowledgments

We thank J. Bowsher, J. Rapp, and two anonymous reviewers for helpful comments on earlier versions of this article. We also thank A. Case for her advice on data analysis. K. Ford, P. Holland, and C. King provided invaluable assistance with the installation of and maintenance of the field site. J. Stinchcombe shared his *Ipomoea hederacea* inbred lines, and A. Wilson kindly sewed the mesh bags used to exclude pollinators. This work was made possible with financial support from National Science Foundation (NSF) grant DEB-0308923 to R.A.S. and M.D.R. and an NSF predoctoral fellowship to R.A.S. (DGE-9818618).

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