

Differential selection on floral traits of *Ipomopsis aggregata* growing in contrasting environments

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Interactions for pollination between co-flowering plant species have been hypothesized to shape the evolution of their floral traits, but this hypothesis has rarely been tested. I tested the prediction that the presence of a co-flowering plant species influences the strength and/or direction of pollinator-mediated selection on floral traits. I measured phenotypic selection via female fitness on four floral traits of *Ipomopsis aggregata* in five populations. Three contained only conspecifics (*I only*) and two also contained the co-flowering species *Penstemon barbatus* (*P + I*). Directional selection via fruits/plant on corolla length and width differed in both strength and direction between *P + I* and *I only* populations. On average, selection on corolla length and width (1) was stronger in *P + I* than *I only* populations and (2) was consistently negative in *P + I* populations, but consistently positive in *I only* populations. However, these differences in selection on *I. aggregata* between *P + I* and *I only* populations were not caused by interactions for pollination with *P. barbatus*. Although plants in *P + I* populations received approximately 31% less conspecific pollen/flower than plants in *I only* populations, this difference in pollination did not translate into differences in reproductive success, which indicates that *P. barbatus* and *I. aggregata* do not strongly compete for pollination. In addition, selection via fruits/plant and conspecific pollen deposited/flower was not congruent. For example, selection on corolla length via pollen/flower was uniformly positive and did not differ between *P + I* and *I only* populations. These data suggest that the presence of *P. barbatus* does influence selection on floral traits of *I. aggregata*, but not by influencing pollination. Instead, differences in selection between *P + I* and *I only* populations appear to be the result of post-pollination modification of selection by a factor correlated with the presence of *P. barbatus*.

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The prevalence of environment-specific selection in nature (e.g. Kalisz 1986, Conner 1989, Arnqvist 1992, Gilbert et al. 1996, O'Connell and Johnston 1998, Caruso 2000) suggests that measuring selection in multiple environments is necessary to predict accurately the rate of evolutionary change of traits under selection. For example, the response to selection on traits in one environment may be impeded by gene flow from another environment where selective pressures differ. Measuring selection in different biotic and abiotic environments may also illuminate how environmental vari-

ability influences adaptive evolution in natural populations (Wade and Kalisz 1990).

Plant-pollinator interactions have become an important model system for studying adaptive evolution (e.g. Campbell 1989, Johnston 1991, O'Connell and Johnston 1998). However, pollinator-mediated selection on floral traits is particularly likely to vary among environments. Any factor that influences the behavior, abundance, or taxonomic composition of pollinators may influence the strength or direction of selection by pollinators. Examples of such factors include population

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size, plant density, the presence of competitors for pollination (reviewed in Kunin 1997) and light availability (Herrera 1995). If these environmental factors vary within the scale of gene flow, then measuring selection in multiple environments is necessary to predict how floral traits will evolve.

The goal of this study was to determine if variation in one environmental factor – the presence of a co-flowering plant species – influences the strength or direction of selection on floral traits. The effect of a co-flowering plant species on floral evolution depends on whether the co-occurring species compete for or facilitate each other's pollination. Competition for pollination would be expected to select for the divergence of floral traits between co-occurring species. In contrast, facilitative interactions may select for the convergence of floral traits, as that would maximize the positive effects of co-occurring species on fitness (e.g. Rathcke 1983). If the divergence or convergence of floral traits is caused by the presence of a co-flowering species, then the strength and/or direction of pollinator-mediated selection on these traits should also be influenced by this species. The development in the last 15 years of methods to measure selection on continuously distributed traits in natural populations (e.g. Lande and Arnold 1983, Arnold and Wade 1984, Mitchell-Olds and Shaw 1987) makes a test of this prediction feasible.

As a first step towards testing the hypothesis that the divergence or convergence of floral traits is caused by the presence of a co-flowering species, I determined the effect of the presence of *Penstemon barbatus* on selection on floral traits of *Ipomopsis aggregata*. The reciprocal interaction (effect of *I. aggregata* on *P. barbatus*) would have to be examined to determine definitively if floral traits have reciprocally converged or diverged. When *I. aggregata* and *P. barbatus* co-occur in Arizona, their flowering phenologies overlap and they share hummingbird pollinators (Brown and Kodric-Brown 1979, Paige and Whitham 1985, C. M. Caruso pers. obs.), which indicates that they have the potential to interact for pollination. The direction of this interaction is unknown. Although it has been suggested that *P. barbatus* and *I. aggregata* facilitate each other's pollination (Brown and Kodric-Brown 1979), other species can compete with *I. aggregata* (Waser 1978, Caruso 1999). For example, *Castilleja linariaefolia* is known to compete with and influence selection on floral traits of *I. aggregata* in Colorado (Caruso 2000). By studying the *P. barbatus*–*I. aggregata* system, it will be possible to determine if different co-flowering species have contrasting effects on floral evolution in *I. aggregata*.

I measured phenotypic selection on floral traits, pollen deposition, and reproductive success in natural populations of *I. aggregata* growing with and without *P. barbatus* in order to answer the following questions:

1. Does *P. barbatus* influence the pollination and reproduction of *I. aggregata*?
2. Does phenotypic selection on floral traits of *I. aggregata* differ systematically between populations with and without *P. barbatus*?
3. If so, can differences in phenotypic selection between *I. aggregata* populations with and without *P. barbatus* be linked to differences in pollination?

Materials and methods

Species and study sites

Ipomopsis aggregata (Polemoniaceae) and *Penstemon barbatus* (Scrophulariaceae) are wildflowers native to western North America (e.g. Cronquist et al. 1984, Grant and Wilken 1986). The plants used in this study were located in meadow openings and the understory of *Pinus ponderosa* and *Populus tremuloides* stands in the San Francisco Peaks north of Flagstaff, Arizona (elevation 2400 m, 111°54'30" W, 35°17'30" N). *Ipomopsis aggregata* is monocarpic (Waser and Price 1989), while *P. barbatus* is a polycarpic perennial (Brown and Kodric-Brown 1979). Both *I. aggregata* (Waser and Price 1983, 1991) and *P. barbatus* (Lange et al. 2000) are self-incompatible. *Penstemon barbatus* flowers are red. In contrast, *I. aggregata* flower color varies from white to red at my study sites, presumably because of introgression with the white-flowered, hawkmoth-pollinated *Ipomopsis tenuituba* (Grant and Wilken 1986). *Penstemon barbatus* flowers are primarily pollinated by broad-tailed (*Selasphorus platycercus*) and rufous (*Selasphorus rufus*) hummingbirds (Brown and Kodric-Brown 1979, Paige and Whitham 1985, Lange et al. 2000, C. M. Caruso pers. obs.). At my study sites, *I. aggregata* is pollinated by hummingbirds and the hawkmoth *Hyles lineata* (Paige and Whitham 1985, Gronemeyer et al. 1997, C. M. Caruso pers. obs.). Both species flower from early July until early September, and where they co-occur, their flowering phenologies overlap extensively (Brown and Kodric-Brown 1979, Paige and Whitham 1985, C. M. Caruso pers. obs.). *Ipomopsis aggregata* flowers secrete an average of 3–4 µL nectar/24 h (Table 1). I did not measure nectar production of *P. barbatus* growing at my study sites, but flowers of plants in east-central Arizona produced 4–7 µL nectar/24 h (Brown and Kodric-Brown 1979).

I measured phenotypic selection on floral traits of *I. aggregata* in five populations during summer 1998. Three populations contained only *I. aggregata* (*I* only) and two also contained *P. barbatus* (*P* + *I*). Study populations were a minimum of 300 m from each other. They were separated by meadows and stands of *P. ponderosa* and *P. tremuloides* that contained other *I.*

aggregata and *P. barbatus* populations. The *I only* populations were all in meadow openings, whereas the *P + I* populations were in the understory of *P. tremuloides* and *P. ponderosa* stands. Plants in *I only* populations received little *P. barbatus* pollen compared to those in *P + I* populations (Table 1), suggesting that *I only* populations did not interact with heterospecifics. Population size varied from several hundred to several thousand plants (C. M. Caruso pers. obs.).

Data collection

Sixty *I. aggregata* plants per population were haphazardly selected before bud break. I measured four floral traits on each plant: corolla length, corolla width, total flower number, and per-flower nectar production. The first three floral traits were chosen because previous studies indicated that they could influence one or more components of *I. aggregata*'s female fitness (Campbell 1991, Mitchell 1994, Caruso 2000). Although nectar production does not significantly influence female fitness of *I. aggregata* (e.g. Mitchell 1994), in some populations it is correlated with traits that influence female fitness (e.g. Campbell 1996).

I measured the length and width of 10 flowers/plant, as described in Campbell (1989). *Ipomopsis aggregata* has radially symmetrical five-parted flowers, with the petals fused into a tube. Corolla length was measured from the base of the sepals to the top of one haphazardly chosen lobe of the tubular corolla. Corolla width was measured as the width of the opening of the corolla tube. To estimate nectar production, I placed a piece of plastic straw over one to three elongated *I. aggregata* buds per plant and measured nectar production 24 h later with a calibrated microcapillary tube (e.g. Mitchell

1994). I estimated total flower number by counting the number of buds. Bud number is strongly related to fruit production of *I. aggregata* (Caruso 2000), as is total flower number (Mitchell 1994), which suggests that bud number is a reasonable proxy for flower number.

Three measures of female fitness were estimated for each individual: fruits/plant, seeds/fruit, and conspecific pollen deposited/flower. Fruits/plant and seeds/fruit are multiplicative fitness components (Arnold and Wade 1984). The product of these variables is seeds/plant, which represents lifetime female reproductive success for the monogamous *I. aggregata*. I measured conspecific pollen deposition in order to determine if any differences in selection via fruit or seed set between *P + I* and *I only* populations were linked to differences in pollination. To estimate seeds/fruit, I counted the seeds in 10 haphazardly chosen fruits/plant. I collected 10 stigmas per plant in order to estimate the amount of conspecific pollen deposited/flower. I stained these stigmas with basic fuchsin (Kearns and Inouye 1993) and counted the number of *I. aggregata* and *P. barbatus* pollen grains deposited.

Statistical analysis

Fitness measures and floral traits

I used one-way analysis of variance (ANOVA), with population as the independent variable (as in Conner 1989, Dudley 1996, Caruso 2000), to determine if fitness measures or floral traits varied among populations. This ANOVA was followed by a 1-df contrast (Sokal and Rohlf 1995) to compare fitness measures or floral traits between *P + I* and *I only* populations. The assumptions of normality and homogeneity of variance for all ANOVAs and ANCOVAs presented in this

Table 1. Mean \pm 1 SE (*N*) for four floral traits, three fitness measures, and one heterospecific pollen transfer measure for *I. aggregata* in five populations. *I only* populations only contained *I. aggregata* plants, whereas *P + I* populations also contained *P. barbatus*. The last column indicates whether the trait or measure was significantly different between *P + I* and *I only* populations by a 1-df contrast following an ANOVA. When there was a significant difference between populations, the larger traits or measures are in bold.

	<i>P + I</i> # 1	<i>P + I</i> # 2	<i>I only</i> # 1	<i>I only</i> # 2	<i>I only</i> # 3	<i>P + I</i> vs <i>I only</i>
Corolla length (mm)	37.44 \pm 0.45 (59)	38.07 \pm 0.39 (60)	36.52 \pm 0.41 (60)	35.86 \pm 0.41 (60)	36.58 \pm 0.43 (60)	$F_{1,295} = 14.04$ $P = 0.000$
Corolla width (mm)	2.55 \pm 0.03 (59)	2.51 \pm 0.03 (60)	2.36 \pm 0.04 (60)	2.36 \pm 0.03 (60)	2.40 \pm 0.03 (60)	$F_{1,295} = 35.35$ $P = 0.000$
Flower number	79.61 \pm 5.14 (59)	48.17 \pm 3.42 (60)	66.03 \pm 4.96 (60)	50.37 \pm 2.76 (60)	87.58 \pm 6.53 (60)	$F_{1,295} = 1.18$ $P = 0.279$
Nectar production (μ L)	4.09 \pm 0.33 (56)	3.34 \pm 0.33 (51)	3.12 \pm 0.33 (50)	3.28 \pm 0.36 (36)	4.05 \pm 0.35 (51)	$F_{1,239} = 0.54$ $P = 0.463$
Fruits/plant	57.52 \pm 4.33 (48)	42.67 \pm 7.56 (24)	60.57 \pm 4.73 (51)	38.90 \pm 2.66 (49)	59.11 \pm 5.14 (47)	$F_{1,214} = 0.36$ $P = 0.551$
Seeds/fruit	6.61 \pm 0.45 (55)	6.63 \pm 0.46 (48)	5.57 \pm 0.45 (57)	4.53 \pm 0.43 (54)	7.39 \pm 0.57 (52)	$F_{1,261} = 3.29$ $P = 0.071$
Conspecific pollen/flower	52.37 \pm 3.09 (55)	84.31 \pm 5.83 (50)	98.82 \pm 5.40 (57)	92.80 \pm 5.09 (53)	106.63 \pm 5.38 (53)	$F_{1,263} = 45.52$ $P = 0.000$
Heterospecific pollen/flower	14.52 \pm 2.25 (55)	5.09 \pm 1.58 (50)	0.79 \pm 0.19 (57)	1.54 \pm 0.43 (53)	2.89 \pm 1.03 (53)	$F_{1,263} = 44.18$ $P = 0.000$

paper were tested using Lilliefors' (Wilkinson 1997) and Levene's (Underwood 1997) tests, respectively, and no significant deviations were detected.

Directional selection

I estimated phenotypic selection for each floral trait, fitness measure, and population. Sample size was reduced to approximately 50 plants in four populations and approximately 30 plants in one population because of late-season damage by deer. The sample size in the latter population ($P + I$ # 2) was inadequate to estimate selection gradients. I instead calculated standardized directional selection differentials for all floral traits using univariate regression (Conner 1988). Phenotypic correlations among floral traits of *I. aggregata* were weak ($r < 0.5$; data not shown) and did not differ significantly between populations (data not shown), suggesting that selection differentials and gradients should be similar. I relativized each fitness measure by dividing by mean fitness (Lande and Arnold 1983), and standardized each floral trait to a mean = 0 and variance = 1 (Sokal and Rohlf 1995). Fitness measures were relativized and floral traits standardized separately for each population.

I tested the assumption of normality of residual variance using Lilliefors' test (Wilkinson 1997). I tested the assumption of homogeneity of residual variance by calculating the Spearman rank correlation between the residuals and relative fitness (Neter et al. 1990). If the correlation was significant ($P < 0.05$), I rejected the assumption of homogeneity. When necessary, I log-transformed relative fitness to meet the assumptions of regression. A selection differential calculated using transformed relative fitness is no longer an unbiased point estimate (Lande and Arnold 1983). Therefore, when transformation was necessary, I present the selection differential from the untransformed model, but the P -value from the transformed model (Mitchell-Olds and Shaw 1987).

Comparing selection between populations

I used analysis of covariance (ANCOVA, as in Conner 1989, Dudley 1996, Gilbert et al. 1996, Caruso 2000) to determine if the strength of phenotypic selection varied among populations. ANCOVA is the standard method for testing differences in regression coefficients between groups (Sokal and Rohlf 1995, Zar 1999). Because it incorporates both population-specific selection estimates and the variation around these estimates, this analysis is also a powerful method for detecting differences in selection. Data from all populations were combined and analyzed with a model that included a continuous term for the floral trait, a categorical term coding for population, and a floral trait \times population term. Relative fitness was the dependent variable. I relativized fitness and standardized floral traits separately within each population. I compared the slope of

the fitness-floral trait relationship between $P + I$ and *I only* populations using a 1-df contrast on the floral trait \times population term.

Results

Fitness measures

Flowers of *I. aggregata* plants in $P + I$ populations received approximately 31% less conspecific pollen/flower than those in *I only* populations (Table 1). This difference in pollen deposition did not translate to differences in reproductive success. The number of seeds/fruit and fruits/plant did not differ significantly between $P + I$ and *I only* populations (Table 1).

Directional selection on corolla length and width

Directional selection on corolla length and width of *I. aggregata* via fruits/plant was significant in only one population, but it differed in both strength and direction between $P + I$ and *I only* populations (Table 2). On average, selection on corolla length and width was stronger in $P + I$ than *I only* populations (Table 2). Selection on these traits was also consistently negative in $P + I$ populations, but consistently positive in *I only* populations (Table 2). Despite this difference in the direction of selection via fruits/plant, *I. aggregata* flowers in $P + I$ populations were significantly longer (4%) and wider (6.8%) than those in *I only* populations (Table 1).

In contrast to selection via fruit set, selection on corolla length and width of *I. aggregata* via conspecific pollen deposited/flower did not differ significantly between $P + I$ and *I only* populations (Table 2). In addition, selection via fruits/flower and conspecific pollen deposited/flower was not congruent. For example, selection on corolla length via conspecific pollen deposited/flower was positive and did not differ between $P + I$ and *I only* populations (Table 2). In contrast, selection on this trait via fruits/plant was positive in *I only* populations, negative in $P + I$ populations, and did differ between $P + I$ and *I only* populations (Table 2).

Directional selection on flower and nectar production

Directional selection on flower number of *I. aggregata* via fruits/plant was uniformly significant, whereas selection via seeds/fruit and conspecific pollen deposited/flower were significant in one or a few populations (Table 2). Regardless of the fitness measure, selection on flower number did not differ significantly between

Table 2. Standardized directional selection differentials (S') for three fitness measures and four floral traits of *I. aggregata* in five populations. *I only* populations only contained *I. aggregata* plants, whereas *P+I* populations also contained *P. barbatus*. The last column indicates whether the strength of selection differed significantly between *P+I* and *I only* populations by a 1-df contrast following an ANCOVA. Sample size is in parentheses.

	<i>P+I</i> #1	<i>P+I</i> #2	<i>I only</i> #1	<i>I only</i> #2	<i>I only</i> #3	<i>P+I</i> vs <i>I only</i>
<i>Fruits/plant</i>						
Corolla length	-0.075 (47)	-0.190 (24)	0.043 (51)	0.068 (49)	0.067 (47)	$F_{1,208} = 4.60$ $P = 0.033$
Corolla width	-0.128 (47)	-0.247 (24)	0.058 (51)	0.151* (49)	0.097 (47)	$F_{1,208} = 10.83$ $P = 0.001$
Flower number	0.282** (47)	0.577** (24)	0.263** (51)	0.225** (49)	0.389** (47)	$F_{1,208} = 3.45$ $P = 0.065$
Nectar production	-0.037 (44)	0.169 (19)	-0.012 (42)	-0.013 (32)	0.025 (40)	$F_{1,167} = 0.41$ $P = 0.512$
<i>Seeds/fruit</i>						
Corolla length	0.191* (52)	-0.068 (48)	0.054 (57)	-0.042 (54)	-0.128 (52)	$F_{1,255} = 1.90$ $P = 0.170$
Corolla width	-0.014 (52)	0.081 (48)	0.075 (57)	0.021 (54)	-0.128 (52)	$F_{1,255} = 0.36$ $P = 0.549$
Flower number	-0.064 (52)	0.031 (48)	0.111 (57)	-0.005 (54)	0.033 (52)	$F_{1,255} = 0.73$ $P = 0.393$
Nectar production	0.077 (52)	-0.064 (42)	0.022 (47)	-0.227 (34)	0.074 (45)	$F_{1,209} = 0.57$ $P = 0.450$
<i>Conspecific pollen/flower</i>						
Corolla length	0.154* (55)	0.045 (50)	0.075 (55)	0.083 (53)	0.037 (53)	$F_{1,257} = 0.44$ $P = 0.508$
Corolla width	-0.059 (55)	-0.081 (50)	0.081 (55)	0.011 (53)	-0.014 (53)	$F_{1,257} = 3.19$ $P = 0.075$
Flower number	0.072 (55)	0.161* (50)	0.032 (55)	0.112* (53)	0.079 (53)	$F_{1,257} = 0.65$ $P = 0.420$
Nectar production	0.024 (52)	0.017 (44)	0.050 (45)	0.033 (34)	0.003 (46)	$F_{1,212} = 0.02$ $P = 0.890$

* $P < 0.05$, ** $P < 0.01$.

P+I and *I only* populations (Table 2). Consistent with this lack of significant differences in selection, flower number also did not differ between *P+I* and *I only* populations (Table 1).

Directional selection on nectar production of *I. aggregata* was uniformly non-significant and did not differ between *P+I* and *I only* populations (Table 2). Consistent with this lack of significant differences in selection, nectar production also did not differ between *P+I* and *I only* populations (Table 1).

Discussion

Environment-specific selection

Selection on corolla length and width of *I. aggregata* differed in strength and direction between *P+I* and *I only* populations (Table 2). But these differences in selection were not caused by differences in the pollination of *I. aggregata* in the presence and absence of *P. barbatus*. Patterns of selection via fruits/plant were not congruent with patterns via conspecific pollen deposited/flower (Table 2). In addition, plants in *P+I* populations received less conspecific pollen/flower than those in *I only* populations, but did not have

lower reproductive success (Table 1), suggesting that *I. aggregata* and *P. barbatus* do not strongly compete for pollination. Although selection on floral traits of *I. aggregata* in Colorado can be influenced by competition with another co-flowering species (Caruso 2000), selection on plants in Arizona does not appear to be influenced by interactions for pollination with *P. barbatus*.

Instead, differences in selection on corolla length and width of *I. aggregata* (Table 2) must be the result of post-pollination events. Post-pollination modification of pollinator-mediated selection on floral traits has been reported in other systems (Campbell 1991, Mitchell et al. 1998). However, in these studies, selection on floral traits was generally weaker via seed and fruit set than via pollination, suggesting that pollinator-mediated selection is attenuated later in the reproductive cycle (Campbell 1991, Mitchell et al. 1998). In contrast, I found differences in selection between *P+I* and *I only* populations via fruits/plant but not via conspecific pollen deposited/flower (Table 2). This result suggests that the strength and direction of pollinator-mediated selection on corolla length and width of *I. aggregata* was either reversed or amplified later in the reproductive cycle (Table 2).

Because selection via fruit set on corolla length and width of *I. aggregata* differed between *P+I* and *I only* populations (Table 2), the factor causing post-pollination modification of selection must be correlated with the presence of *P. barbatus*. I observed three aspects of the biotic or abiotic environment of these populations that were correlated with *P. barbatus*. First, abiotic resource availability likely varied between *P+I* and *I only* populations. Both *P+I* populations were growing in the understory of *P. ponderosa* and *P. tremuloides* stands, whereas all *I only* populations were growing in open meadows. Although I did not measure light, soil water availability, or atmospheric evaporative demand in my populations, they almost certainly differed between the understory and the open meadow (Bazzaz and Wayne 1994). Second, *Bombus* spp. robbed nectar from flowers on *I. aggregata* plants growing in *I only* but not *P+I* populations (C. M. Caruso pers. obs.). Third, the lepidopteran larvae *Heliothis phloxiphaga* fed on the buds, flowers, and fruits of *I. aggregata* plants growing in *I only* but not *P+I* populations (C. M. Caruso pers. obs.).

These factors are all likely to influence selection on floral traits. Variation in the abiotic resources such as water and light may constrain selection on floral traits by pollinators and herbivores (Galen et al. 1999). Nectar-robbing *Bombus* sp. may select for shorter *I. aggregata* flowers in Colorado populations (Irwin and Brody 1999). *Heliothis phloxiphaga* larvae significantly influence selection on at least one floral trait (date of first flower) of *I. aggregata* growing in Colorado (Juenger and Bergelson 1998). Although the effects of this floral herbivore on corolla length and width of *I. aggregata* have not been determined, work in another plant-herbivore system suggests that it would select for shorter and narrower flowers (Mothershead and Marquis 2000). Experimental manipulations of abiotic resources, nectar robbers, and lepidopteran herbivores could indicate whether any or all of these factors are responsible for differences in selection between *P+I* and *I only* populations.

The observed selection for longer and wider *I. aggregata* flowers (Table 2) in populations with shorter and narrower flowers (Table 1), and selection for shorter and narrower flowers (Table 2) in populations with longer and wider ones (Table 1), suggests that there may be an intermediate, optimum flower size common to all populations. It has long been hypothesized that floral morphology should be under stabilizing selection to produce an optimal fit between plant and pollinator (reviewed in Cresswell 1998). However, if there is selection via fruit set in my *I. aggregata* populations for optimal corolla length and width, the contradictory pattern of selection via pollen deposition (Table 2) suggest that pollinators cannot be the agent of this selection. Experimental studies using ar-

tificial populations of *I. aggregata* plants with a larger range of corolla length and widths than is found naturally in *P+I* or *I only* populations will be necessary to determine if there is selection for an intermediate, optimum flower size and to identify the agent of selection.

No response to selection?

Although the strength and direction of selection differed significantly between *P+I* and *I only* populations (Table 2), it apparently has not resulted in character displacement. There was selection for shorter and narrower corollas in *P+I* populations (Table 2), but the flowers in these populations were significantly longer and wider than those in *I only* populations (Table 1). This lack of a response to selection could be explained by weak selection, variable selection, or low heritability. Selection was weak within both *P+I* and *I only* populations (Table 2), and the response to such weak selection may be slow. Selection on floral traits via female function often varies between years (e.g. Conner et al. 1996), which will impede the response to selection. Although there is significant heritability for corolla length and width of *I. aggregata* growing in Colorado (Campbell 1996), heritabilities are environment-dependent (Falconer and Mackay 1996) and may be lower in the Arizona populations.

Given the lack of a response to selection on corolla length and width of *I. aggregata*, the existing differences in these traits (Table 1) may represent a plastic response to the abiotic environment. Water stress can reduce flower size (e.g. Carroll et al. 2001), primarily because the water cost of large flowers is high (Galen et al. 1999). The higher irradiance, temperature, and evaporative demand in open meadows (such as where the *I only* populations are) should result in a dry environment. In contrast, the lower irradiance, temperature, and evaporative demand in the forest understory (such as where the *P+I* populations are) should result in a relatively more mesic environment (Bazzaz and Wayne 1994). Thus, *I only* populations may have significantly shorter and narrower flowers than *P+I* populations (Table 1) only because they are growing in a more open environment.

Geographic variation in selection on *I. aggregata*

Selection on floral traits has also been measured for *I. aggregata* growing in the Front Range and Western Slope of the Rocky Mountains in Colorado (Campbell 1989, 1991, Campbell et al. 1991, 1996, 1997, Juenger and Bergelson 1997, 1998, Caruso 2000), which allows for geographic comparisons. Selection

on nectar and flower production of *I. aggregata* was very similar across regions. Consistent with the observation that nectar production primarily influences male fitness of *I. aggregata* (Mitchell 1993), selection on this trait via female fitness was non-significant in both Arizona (Table 2) and Colorado (Caruso 2000). As in other plant-pollinator systems (e.g. Johnston 1991, Conner et al. 1996, Maad 2000), selection on flower production of *I. aggregata*, at least via fruits/plant, was consistently positive and significant (Table 2; Caruso 2000). In contrast to nectar and flower production, selection on corolla length and width of *I. aggregata* varied as much among populations within a geographic region as between regions (Arizona vs Colorado, Table 2; Campbell 1989, 1991, Campbell et al. 1991, 1996, 1997, Caruso 2000). Factors that influence selection on corolla length and width of *I. aggregata*, including pollinator taxa (hummingbirds vs hawkmoths; Campbell et al. 1997) and the presence of a co-flowering species (Table 2; Caruso 2000), must also vary extensively among local populations.

Varying effects of co-flowering species on selection

Selection on corolla length and width of *I. aggregata* differs significantly in strength and direction between populations with and without the co-flowering plant species *P. barbatus* (Table 2). However, *P. barbatus* does not influence selection on floral traits of *I. aggregata* by influencing pollination (Table 2). Instead, differences in selection appear to be the result of post-pollination modification of selection by a factor correlated with the presence of *P. barbatus*. The effect of *P. barbatus* on selection on floral traits of *I. aggregata* growing in Arizona contrasts sharply with the effect of another co-flowering plant species (*C. linariaefolia*) in Colorado (Caruso 2000). *Castilleja linariaefolia* competes with *I. aggregata* for pollination, resulting in stronger pollen-limitation of reproduction and stronger selection on corolla length of *I. aggregata* (Caruso 2000). This contrast between the effects of *P. barbatus* and *C. linariaefolia* on the evolution of *I. aggregata*'s floral traits underscores the importance of measuring natural selection in multiple environments populated by different interacting species.

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