

## AN INVASIVE PLANT ALTERS POLLINATOR-MEDIATED PHENOTYPIC SELECTION ON A NATIVE CONGENER<sup>1</sup>

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- *Premise of study:* Recent studies suggest that invasive plants compete reproductively with native plants by reducing the quantity or quality of pollinator visits. Although these studies have revealed ecological consequences of pollinator-mediated competition between invasive and native plants, the evolutionary outcomes of these interactions remain largely unexplored.
- *Methods:* We studied the ecological and evolutionary impact of pollinator-mediated competition with an invasive jewelweed, *Impatiens glandulifera*, on a co-occurring native congener, *I. capensis*. Using a pollinator choice experiment, a hand pollination experiment, and a selection analysis, we addressed the following questions: (1) Do native pollinators show preference for the invasive or native jewelweed, and do they move between the two species? (2) Does invasive jewelweed pollen inhibit seed production in the native plant? (3) Does the invasive jewelweed alter phenotypic selection on the native plant's floral traits?
- *Key results:* The pollinator choice experiment showed that pollinators strongly preferred the invasive jewelweed. The hand pollination experiment demonstrated that invasive pollen inhibited seed production in the native plant. The selection analysis showed that the presence of the invasive jewelweed altered phenotypic selection on corolla height in the native plant.
- *Conclusions:* Invasive plants have the potential to alter phenotypic selection on floral traits in native plant populations. If native plants can evolve in response to this altered selection pressure, the evolution of floral traits may play an important role in permitting long-term coexistence of native and invasive plants.

**Key words:** *Impatiens capensis*; *Impatiens glandulifera*; plant invasions; pollen interference; pollinator constancy; pollinator-mediated selection.

Invasive plants can reduce the fitness of their native neighbors through competition for pollinator services (Chittka and Schürkens, 2001; Brown et al., 2002; Flanagan et al., 2009; Matsumoto et al., 2010). Whether native plants may evolve in response to this competition for pollination has yet to be fully explored (Mitchell et al., 2009). By measuring selection on a native plant in the presence and absence of an invasive competitor, we can gain insight into how an invader might alter the evolutionary trajectory of a native plant's floral traits. If native plant populations can evolve in response to pollinator-mediated competition with invasive species, they may be able to coexist alongside invasives instead of risking competitive exclusion.

Invasive plants may alter native plant communities through the evolution of native floral traits in response to pollinator-mediated

competition (Beans, 2014). For example, to reduce competition for pollinators with co-occurring invasive species, native plant populations have diverged in corolla length (Caruso, 2000), display size (Wassink and Caruso, 2013), mating system (Fishman and Wyatt, 1999), flower color (Levin, 1985; Hopkins and Rausher, 2012), and the degree that reproductive parts are extended beyond the corolla (Muchhala and Potts, 2007). Selection on floral traits may thus be similar to the rapid evolution that has been found in vegetative traits of some native plants in response to invasive competitors (Rowe and Leger, 2011; Lankau, 2013; Oduor, 2013).

Pollinator competition with invasive plants is often intense and, therefore, could act as a strong selective force in two different ways. Invasive plants may draw pollinators away from native plants, and subsequently reduce seed set in pollen-limited populations of native plants (Chittka and Schürkens, 2001). This would then result in selection for increased pollinator-attraction traits in the presence of a competitor. Invasive plants may also reduce the quality of pollinator visits to native plants through heterospecific pollen deposition (Waser, 1978a, 1978b; Brown and Mitchell, 2001; Mitchell et al., 2009; Matsumoto et al., 2010). Pollen from interspecific pollinator movements may inhibit seed production by reducing the stigmatic space available for conspecific pollen or by hindering conspecific pollen germination through allelopathy (Waser, 1978b; Feinsinger, 1987). This could then result in stronger selection for pollination efficiency traits.

Strong competition for pollination may also result from floral similarities between congeners because pollinator movement is most common between plants that share similar floral shapes or colors (Morales and Traveset, 2009; Gibson et al., 2012). Invasive pollen is especially likely to negatively affect seed production in closely related native plants because similarities in

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stigma and style morphology and chemistry may encourage heterospecific pollen germination on native stigmas (Ashman and Arceo-Gomez, 2013). At the extreme, the fitness of native plants may be reduced if heterospecific pollination results in the production of sterile hybrids (Mitchell et al., 2009). Selection may favor a divergence in flower form if pollinator sharing results in reduced seed set in the native plant, or a convergence in flower form if pollinators prefer the invasive plant and invasive pollen has no effect on the native plant's seed set.

We studied the ecological and evolutionary consequences of pollinator-mediated competition with an invasive jewelweed, *Impatiens glandulifera* Royle, on a native congener, *I. capensis* Meerb., in the northeastern United States. Using a pollinator choice experiment, a hand pollination experiment, and a selection analysis, we addressed the following questions: (1) Do native pollinators show preference for the invasive or native jewelweed, and do they move between the two species? (2) Does invasive jewelweed pollen inhibit seed production in the native plant? (3) Does the invasive jewelweed alter phenotypic selection on the native plant's floral traits? The results of our study suggest that invasive competitors for pollination have the potential to alter phenotypic selection on floral traits in native plants.

## MATERIALS AND METHODS

**Study species**—*Impatiens capensis* (Balsaminaceae), spotted jewelweed, is an annual plant native to the United States and Canada. It germinates in early May and often reaches 1.5 m in height by August. Flowers descend on pedicels from leaf axils and typically occur from late June through first frost. *Impatiens capensis* produces chasmogamous, zygomorphic flowers that are composed of three sepals and one upper and two lower petals. The posterior sepal is modified into a conical structure leading to a nectar-filled spur (Rust, 1979). The flowers are orange with red spots on the lower petals. Each flower opens in the male stage and then progresses to the female stage when the androecium falls off and exposes the mature stigma. Typically, the male stage lasts about 2 days and the female stage lasts <1 day (Bell et al., 1984). Although self-compatible, fertilization of these flowers depends on pollinator visitation, as protandry prevents self-fertilization within each flower. Fertilization typically occurs when a pollinator's back (in the case of bumblebees) or head (in the case of hummingbirds) brushes the androecium or stigma as the pollinator enters the open flower to reach the nectar-filled spur (Rust, 1979; Travers et al., 2003). *Impatiens capensis* also produces cleistogamous flowers that are highly reduced in size and result in selfed, less fit seed (Mitchell-Olds and Waller, 1985).

*Impatiens glandulifera*, showy jewelweed, is native to the Himalayas, and in the late 1800s it began spreading in the northeastern United States, where it now grows intermixed with *I. capensis* along roadsides and stream banks (Tabak and von Wettberg, 2008). It germinates synchronously with *I. capensis* and can reach a height of nearly 3 m. *Impatiens glandulifera* produces only chasmogamous flowers, which range in color from pale pink to deep magenta. Although slightly larger, these flowers are shaped similarly to those of the native jewelweed.

Both species are visited by a diversity of pollinators, including hummingbirds, sweat bees, honeybees, bumblebees, and hover flies. Pollinator assemblages, however, vary greatly by population (Travers et al., 2003). Bumblebees (*Bombus* spp.), which are especially efficient pollinators of *Impatiens* species (Rust, 1977), were the primary pollinators at our study sites. *Bombus vagans* was the most commonly observed species (C. M. Beans, University of Virginia, personal observation).

**Pollinator choice experiment**—*Experimental design*—We designed a pollinator choice experiment to estimate pollinator preference and constancy when offered flowers of the two *Impatiens* species. Pollinator preference is the preferential visitation of one plant species over another, while pollinator constancy is the preferential movement between flowers of the same species over movement across species (Waser, 1986; Flanagan et al., 2009). Pollinator preference may reduce seed production in the less desirable species when pollen is limited.

When pollinators are capable of transitioning between floral species, pollinator constancy may increase seed production by minimizing heterospecific pollen deposition.

In 2011, we conducted pollinator observations at the Hitchcock Center for the Environment in Amherst, Massachusetts, USA, in a 20 × 10 m open patch of *I. capensis* bordered on one side by trees and on three sides by mown lawn. The patch also contained sparse occurrences of *Verbena urticifolia* and *Solidago juncea*. There was an additional patch of *I. capensis* in a wooded setting ~50 m from the study area. There were no naturalized *I. glandulifera* plants at this site.

The *I. glandulifera* plants used in this experiment were grown from seed collected in September 2010 from an invaded community in Petersham, Massachusetts. Starting in March 2011, the seeds were cold stratified at 4°C on moistened filter paper in Petri dishes for 3 mo. They were then planted in Metro Mix 200 potting medium in a greenhouse at the University of Virginia. In early June 2011, the seedlings were transplanted into 3-gallon pots filled with Fafard 3B potting medium.

In late July 2011, we placed potted *I. glandulifera* plants into three 1 × 1 m plots of naturally occurring *I. capensis*. Plots were spaced 3–9 m apart and contained 8–10 *Impatiens* individuals, with an equal number of each species. We placed plants directly adjacent to one another with branches overlapping to mimic natural density. Over the course of the experiment, we continuously manipulated flower number within each plot to offer pollinators an equal number of open flowers from each species. We controlled flower number by either removing flowers from one species or rotating in different *I. glandulifera* individuals. Over the course of the experiment, the total number of open flowers across both species within a plot ranged from 8 to 32 (mean ± SE = 15.57 ± 0.59).

**Pollinator observations**—We observed pollinator visits for 7 consecutive days in July 2011. Each day, weather permitting, there were five observation periods between 0950 and 1820 hours EDT. Within each period, we observed each plot for 20 min. The order of plot observations was randomized. We logged a total of 23 hr of pollinator observation.

During observation of a plot, two observers worked together, each tracking one pollinator at a time. Each observer recorded the order of all flower visits made by a pollinator until it left the plot, and then began following the next pollinator to enter. Movement from one open flower to another on the same plant was counted as a new visit to that species. If the same pollinator returned to a flower previously visited after visiting a different flower, this was also counted as a new visit to that species.

**Data analysis**—We used a replicated *G*-test of goodness-of-fit to test for pollinator preference (Ippolito et al., 2004). This analysis compares the actual number of visits to flowers of each species to the expected number of visits under the null hypothesis of equal visitations.

We tested for pollinator constancy in pollinators that we knew to be capable of transitioning between floral species (those that visited both species at frequencies >0.1 within a single foraging bout) using a replicated *G*-test of independence (Aldridge and Campbell, 2007; Flanagan et al., 2009). This test compares the observed to the expected number of heterospecific and conspecific transitions between flowers (Ippolito et al., 2004). The expected number for a given transition (either invasive to invasive, invasive to native, native to native, or native to invasive) is calculated by the following equation:

$$\frac{(\text{sum of all transitions observed}) \times (\text{frequency of visits to first flower type visited})}{(\text{frequency of visits to second flower type visited})}$$

Even if there were more interspecific than intraspecific transitions observed, pollinators might still be considered to exhibit pollinator constancy if the observed number of intraspecific transitions is greater than expected given the frequencies of visits to each species.

We calculated *G* statistics separately for each plot, as well as pooled across plots. This method allowed us to test for pollinator preference and constancy across all plots, as well as for differences in pollinator preference and constancy among plots (Flanagan et al., 2010). *G*-statistics were calculated in Excel (McDonald, 2009).

**Hand pollination experiment**—*Experimental design*—We designed a hand pollination experiment to estimate how the heterospecific transitions observed in our choice experiment may influence seed production. We tested both the potential for pollen interference and for hybridization. In May 2012,

we collected *I. capensis* seedlings from a natural population at Hampshire College Farm Center in Amherst, Massachusetts. *Impatiens glandulifera* does not grow at this site. We grew seedlings of *I. glandulifera* from seed collected in Petersham, Massachusetts, the previous summer. We treated this seed following the same protocol described in the pollinator choice experiment. We allowed seedlings of both species to grow to adult size in a mown field in 3-gallon pots filled with Fafard 3B potting medium at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, Massachusetts.

In September 2012, while *Impatiens* plants were at the peak of their flowering period, we conducted two rounds of hand pollinations on 30 of the potted *I. capensis* individuals; 36 additional potted *I. capensis* plants and 36 potted *I. glandulifera* plants served as pollen donors. For each round of the experiment, we haphazardly selected four male-stage flowers per pollen recipient and secured a bridal-veil bag over each flower with a fine wire. We then haphazardly assigned one bagged flower per plant to each of the following treatments by marking the wire with colored paint: (1) conspecific pollen, (2) mixed pollen, (3) heterospecific pollen, or (4) no pollen. We surveyed flowers once a day for the following 3 days and performed hand pollinations on all flowers that had advanced to female stage. Because the stigma is exposed only after the androecium falls off, we did not need to emasculate flowers before hand pollinating.

We performed pollinations using “bee sticks”—commercially available freeze-dried honeybees (*Apis mellifera*) that we glued onto toothpicks. We used these bee sticks to best mimic the fitness effect of the pollinator transitions observed during our pollinator choice experiment. Although bumblebees were the primary pollinators of our *I. capensis* plants, honeybees foraging for nectar are equally efficient pollinators of this species (Young et al., 2007). We randomly assigned pollen donors to pollen recipients. We pollinated flowers in the conspecific pollination treatment with a bee stick that had visited a male-stage *I. capensis* flower on each of two donor plants. We pollinated flowers in the mixed pollination treatment with bee sticks that had visited one *I. capensis* donor and one *I. glandulifera* donor. For each round of this treatment, half of the flowers were pollinated with a bee stick that visited *I. capensis* first, while the other half were pollinated with a bee stick that visited *I. glandulifera* first, with the order of visitation for each plant alternating between rounds. Finally, we pollinated flowers in the heterospecific pollen treatment with bee sticks that had visited flowers on two separate *I. glandulifera* donor plants. Following pollination, we replaced the bridal-veil bag to prevent further pollinator visits. For the no-pollen control treatment, we removed and then replaced the bridal-veil bag. After all flowers were pollinated in the first round, we began the second round of the experiment. After the bagged fruits ripened, we collected them, counted their seeds, and averaged the number of seeds produced per treatment across the two rounds for each plant.

To estimate the number of pollen grains deposited during hand pollinations, we performed 4 or 5 test pollinations for each treatment and then immediately deposited the stigmas in microcentrifuge tubes and dried them at a constant temperature for 48 hr. We then estimated the pollen deposited on each of the stigmas with a hemacytometer. As expected in natural populations, the amount of pollen deposited by each bee stick varied. However, the average number of pollen grains ( $\pm$  SE) deposited in the conspecific ( $2659 \pm 510$ ), mixed ( $2404 \pm 1213$ ), and heterospecific ( $3644 \pm 1940$ ) treatments far outnumbered the four to seven ovules available in an *I. capensis* ovary (Young, 2008). Even in the mixed pollination treatments, therefore, there should have been sufficient conspecific pollen for maximum seed production.

**Data Analysis**—To test for heterospecific pollen interference and the potential for hybridization, we compared treatment effects using a mixed-model analysis of variance (ANOVA) with average seed set as the dependent variable, hand pollination treatment as the independent variable, and plant as a random effect. We tested all pairwise comparisons and adjusted *P* values using a Tukey

adjustment for multiple comparisons. Additionally, we tested whether the order of bee-stick visits in the mixed pollination treatment affected seed production, using a mixed-model ANOVA with seed set as the dependent variable, the species the bee stick visited first as the independent variable, and plant as a random effect. We performed analyses using PROC GLM in SAS version 9.3 (SAS Institute, 2013).

**Selection Analysis—Experimental design**—We designed an experiment to test whether the invasive jewelweed affects its native relative by reducing pollinator visits and seed production, and by altering phenotypic selection on floral traits. The plants used in this experiment were grown from the same populations as described in the hand pollination experiment.

We conducted the experiment in late August and early September 2011 in a mown field at the University of Massachusetts Crop and Animal Research and Education Center in South Deerfield, Massachusetts. We randomly assigned *Impatiens capensis* and *I. glandulifera* individuals in 3 gallon pots to  $2 \times 2$  m plots representing two treatments: (1) *I. capensis* in intraspecific competition and (2) *I. capensis* in mixed competition with conspecifics and *I. glandulifera* in a 50:50 mixture. Each plot contained 10 plants. The treatments were arranged in a grid formation with each treatment replicated once across six spatial blocks. There was 5 m between plots within a block and 5 m between blocks. We repeated the experiment with random reassignment of plants to plots before the second trial. Both trials were initiated within a single week.

We haphazardly selected two flowers per plant for floral measurements. Using calipers, we measured corolla height (the tip of the upper petal to the tip of the lower lip), corolla width (the widest horizontal point on the corolla tube), and corolla depth in millimeters. On these same flowers we also measured spur angle using a protractor (following Travers et al., 2003). We averaged floral trait values for each individual to represent each plant. Previous studies have shown that spur angle is highly repeatable within *I. capensis* plants (Travers et al., 2003; Young, 2008). A repeatability analysis following Travers et al. (2003) showed that, for the plants included in our selection analysis, spur angle and all other floral traits were repeatable within plants (repeatability estimates with 95% confidence intervals [CIs]: corolla depth =  $0.34 \leq 0.52 \leq 0.66$ ; corolla height =  $0.42 \leq 0.59 \leq 0.71$ ; corolla width =  $0.27 \leq 0.47 \leq 0.61$ ; spur angle =  $0.53 \leq 0.69 \leq 0.77$ ).

We removed two plants from the analysis that were outliers for corolla height because we did not want these plants to, by chance, end up in the same treatment and allow a greater range for selection to act upon in one treatment than in the other. The mean floral trait values were similar across treatments (Table 1). The floral traits measured were not strongly correlated with one another (all *r* values  $< 0.55$ ). Intraplot variance in floral traits was similar across treatments (data not shown) and was large enough for selection to act upon (all coefficients of variation within plots  $> 1$ ). We also measured floral traits on all *I. glandulifera* individuals included in the experiment. Although *I. glandulifera* flowers are typically larger than those of *I. capensis*, the size distributions of each species for all traits measured were close enough to overlap or touch (Fig. 1).

We estimated female fitness during each trial by the average number of seeds per fruit on each individual. At the start of a trial, we placed a thin wire around the pedicel of up to 3 male-phase flowers on each plant. After the flowers transitioned from male to female to fruit, we secured bridal-veil bags over the fruits for seed capture. This method ensured that the entire female phase passed within the trial period.

We estimated male fitness during the first trial by recording pollinator visitation to all individual *I. capensis* plants. We also recorded visits to all individual *I. glandulifera* plants during this time. Each plant received 40 min of pollinator observation spread out in 10-min increments over a single day between 1030 and 1740 hours EDT. We recorded the total number of pollinator visits to each individual plant during an observation period. All observations were made within 1 wk.

TABLE 1. Mean ( $\pm$  SE) floral trait measurements by treatment of plants in selection analysis.

Fitness measure	Treatment	Corolla depth (cm)	Corolla height (cm)	Corolla width (cm)	Spur angle
Average seed set	Intraspecific	1.63 $\pm$ 0.01	1.65 $\pm$ 0.02	0.78 $\pm$ 0.01	152.58 $\pm$ 2.71
	Mixed	1.63 $\pm$ 0.02	1.69 $\pm$ 0.03	0.79 $\pm$ 0.02	141.41 $\pm$ 3.72
Number of pollinator visits	Intraspecific	1.61 $\pm$ 0.02	1.63 $\pm$ 0.02	0.76 $\pm$ 0.01	154.60 $\pm$ 3.73
	Mixed	1.65 $\pm$ 0.02	1.72 $\pm$ 0.04	0.80 $\pm$ 0.02	139.81 $\pm$ 4.77

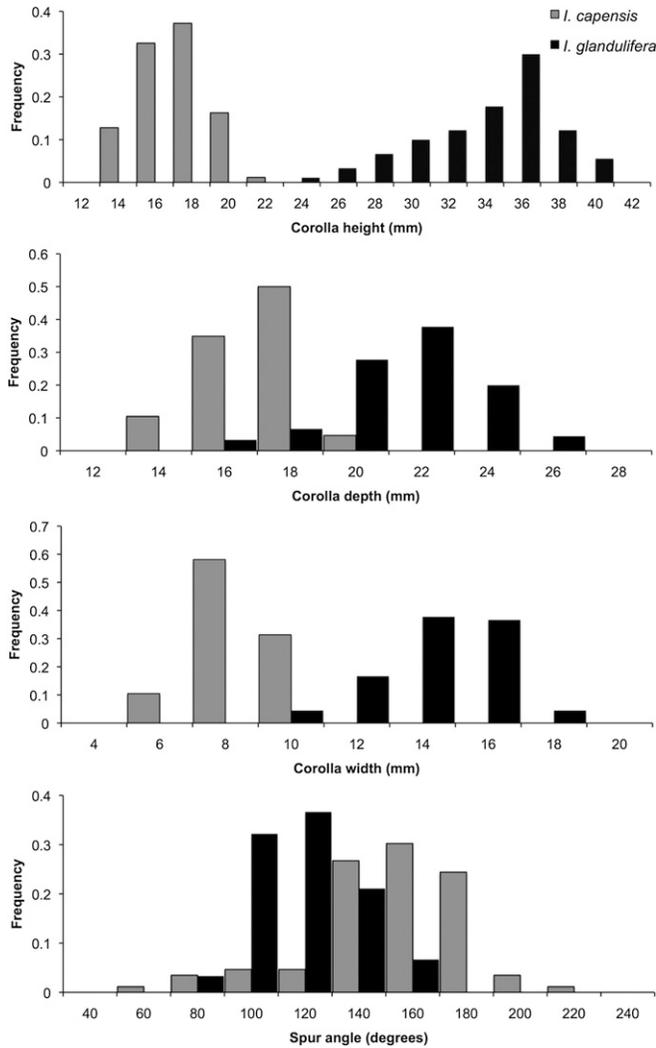


Fig. 1. Floral trait distributions by species for *Impatiens* plants included in floral selection analysis (*I. capensis*,  $n = 86$ ; *I. glandulifera*,  $n = 90$ ).

**Data Analysis**—We tested for the effects of competition treatment on seed production and on pollinator visits using mixed-model ANOVAs with PROC GLM (SAS Institute, 2013). We included block and treatment  $\times$  block interactions as random effects, and designated the treatment  $\times$  block interaction as the error term for testing the significance of the treatment effect. We square-root transformed pollinator visits to meet ANOVA assumptions.

We also tested for differences in pollinator visitation between *I. capensis* and *I. glandulifera* individuals in the mixed competition treatment. For this analysis, we again used mixed-model ANOVAs with PROC GLM. We included block and *Impatiens* species  $\times$  block interactions as random effects, and designated the *Impatiens* species  $\times$  block interaction as the error term for significance testing. We again square-root transformed pollinator visits to meet ANOVA assumptions.

We estimated standardized linear selection gradients ( $\beta$ ) for floral traits in each treatment using two separate fitness components, average seeds per fruit and pollinator visits (Lande and Arnold, 1983). Within each treatment, we transformed fitness components to relative fitness and trait values to units of variance from a mean of zero. We then regressed each fitness component over the trait values in each treatment in a multiple regression. We included block in all regression models. Because pollinator visits were not normally distributed, we tested the significance of selection gradients for this fitness component using untransformed data in a generalized linear model that assumed a Poisson error distribution (Formica et al., 2011). The standard errors for selection

gradients generated by this model were calculated using the formula outlined in McGlothlin et al. (2010).

We used analysis of covariance (ANCOVA) to test whether there were significant differences in selection between treatments for each fitness component. The models included treatment, all floral traits, block, all treatment  $\times$  trait interactions, and the treatment  $\times$  block interaction as independent variables. The significance of the treatment  $\times$  trait interaction term indicates whether selection differs between treatments. For the pollinator visits fitness component, we again used a model that assumed a Poisson error distribution. We performed all selection analyses in PROC GLIMMIX (SAS Institute, 2013).

## RESULTS

**Pollinator choice experiment**—Both *Impatiens* species were visited by a diversity of pollinators, including bumblebees, sweat bees, and hover flies. For both species, however, bumblebees made up the vast majority of visits (76% of *I. glandulifera* and 74% of *I. capensis* visits). More critically for plant fitness, bumblebees accounted for 85% of all pollinator transitions between individual flowers. Because bumblebees were overwhelmingly the primary pollinators, and because the other pollinators included in the dataset are considered less efficient pollinators of *Impatiens* species (Rust, 1977), we present here pollinator preference and constancy analyses specifically for bumblebee visits. Including all other pollinators, however, does not greatly influence the results (data not shown).

Bumblebees preferred *I. glandulifera* at a ratio of more than 4 to 1, with *I. glandulifera* flowers receiving 1568 visits and *I. capensis* flowers receiving only 364 ( $G_P = 808.52$ ,  $df = 1$ ,  $P < 0.0001$ ). There was no significant difference in preference across plots ( $G_H = 5.26$ ,  $df = 2$ ,  $P = 0.07$ ). We commonly observed pollinator movement between species. Out of 294 bumblebees that made one or more transition, 142 visited both species. Bumblebees did, however, exhibit constancy. Individual bumblebees were more likely to transition between flowers within species than across species ( $G_P = 18.21$ ,  $df = 3$ ,  $P < 0.001$ ). There were 469 transitions between *I. glandulifera* flowers, compared with an expectation of 443.1. There were 101 transitions between *I. capensis* flowers, compared with an expectation of 74.2. Interestingly, when bumblebees did move between species, they made the exact same number of transitions from *I. capensis* to *I. glandulifera* as vice versa, with each transition type receiving 155 visits compared to an expectation of 181.3. There was no difference in pollinator constancy across plots ( $G_H = 0.27$ ,  $df = 6$ ,  $P > 0.99$ ).

**Hand pollination experiment**—Heterospecific pollen interfered with *I. capensis* seed production. Flowers that received mixed pollen produced, on average, 42% fewer seeds than flowers that received conspecific pollen (Tukey adjusted  $P = 0.001$ ; Fig. 2). The heterospecific pollen treatment produced the same number of seeds as the no-pollen treatment, so there was no evidence for hybridization (Tukey adjusted  $P = 0.99$ ; Fig. 2). For the mixed pollination treatment, seed production was not affected by whether the bee stick visited the invasive or native plant first (mean  $\pm$  SE for bee-stick visits to native first:  $1.37 \pm 0.29$ , and invasive first:  $1.13 \pm 0.27$ ;  $F_{1,29} = 0.33$ ,  $P = 0.57$ ).

**Selection analysis**—As in the pollinator choice experiment, bumblebees were the primary pollinators, making up 93% of visits to *I. capensis* in the intraspecific competition treatment and 94%

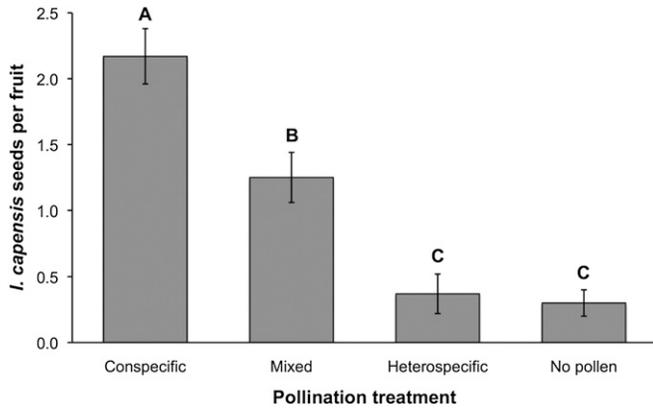


Fig. 2. Mean ( $\pm$  SE) *I. capensis* seeds per fruit resulting from hand pollination treatments. Treatments with different letters are significantly different (Tukey adjustment for multiple comparisons,  $P < 0.05$ ).

of visits to *I. capensis* in the mixed competition treatment. We therefore present results for bumblebee visits specifically. Including other pollinator types in the analysis, however, does not greatly influence the results (data not shown).

There was no difference in *I. capensis* seed production or pollinator visitation between treatments. *Impatiens capensis* plants in intraspecific and mixed competition treatments averaged 2.90 (95% CI: 2.60–3.21,  $n = 92$ ) and 2.91 (95% CI: 2.47–3.34,  $n = 48$ ) seeds per fruit, respectively ( $F_{1,11} < 0.001$ ,  $P = 0.97$ ). Bumblebee visits for these treatments averaged 8.98 (95% CI: 7.56–10.55,  $n = 49$ ) and 7.11 (95% CI: 5.46–8.98,  $n = 30$ ) per individual *I. capensis* plant, respectively ( $F_{1,5} = 0.88$ ,  $P = 0.39$ ). As in our pollinator choice experiment, bumblebees preferred the invasive over the native jewelweed. In the mixed competition treatment, invasive jewelweed individuals received, on average, 15.07 visits (95% CI: 11.72–18.84,  $n = 30$ ), over twice as many as native jewelweed individuals received ( $F_{1,5} = 7.03$ ,  $P < 0.05$ ).

There were significant differences in phenotypic selection on *I. capensis* floral traits between treatments (Table 2). With average seed set per fruit as the fitness measure, there was no selection on floral traits in the intraspecific competition treatment. There was, however, selection for shorter corolla height in the mixed competition treatment, and selection on this trait differed significantly between treatments. With pollinator visitation as the fitness measure, there was selection for greater corolla depth

and taller corolla height in the intraspecific competition treatment. In the mixed competition treatment, there was again selection for shorter corolla height, and this selection gradient differed significantly between treatments. There was also selection for greater corolla depth, but selection on this trait did not differ between treatments.

## DISCUSSION

The presence of the invasive jewelweed significantly altered selection on corolla height in the native congener. In the intraspecific competition treatment, there was no selection on the floral traits measured via female fitness. There was, however, selection for taller corollas via bumblebee visits. In the presence of the invasive congener, *I. capensis* plants experienced selection for shorter corollas via both seed set and bumblebee visits. The fact that these two fitness measures resulted in similar selection estimates strongly suggests that bumblebees are the mechanism behind altered selection in this system. These results also demonstrate that when *I. glandulifera* is present, there is selection for *I. capensis* to diverge in floral form from the invasive jewelweed, as has been found in studies of character displacement of floral traits when there is a cost to sharing pollinators with a neighboring species (Caruso, 2000; Smith and Rausher, 2008).

The altered selection experienced by the native jewelweed in the presence of the invasive plant may stem from two non-mutually exclusive forms of competition. Competition with the invasive plant may have resulted in changes in the quantity of pollinator visits by specific bumblebee species, changes in the efficiency of those visits, or both. For example, it is possible that some bumblebee species moved frequently between the invasive plant and native plant individuals with tall corollas, whereas other bumblebee species continued to specialize on the native jewelweed even in the presence of the invasive. This scenario would have the effect of reducing the quantity of visits to *I. capensis* plants with taller corollas in relation to those with shorter corollas. It would also result in heterospecific pollen deposition on flowers with taller corollas, which could reduce seed. Further studies that track the behavior of individual bumblebee species are needed to more fully understand which form of competition results in altered selection in this system.

Although we found differences in selection, we found no difference in mean seed production or pollinator visits between *I. capensis* plants in intraspecific versus mixed competition

TABLE 2. Directional selection gradients ( $\beta \pm$  SE) on *Impatiens capensis* floral traits when in intraspecific and mixed-species competition treatments. The interaction column demonstrates the significance of the difference between selection gradients in the two treatments. Significant selection gradients and interactions are shown in bold.

Fitness measure	Floral trait	Competition treatment		Interaction		
		Intraspecific	Mixed	<i>F</i>	df	<i>P</i>
Average seed set	Corolla depth	-0.01 $\pm$ 0.06	0.10 $\pm$ 0.10	0.73	1, 108	0.40
	Corolla height	0.03 $\pm$ 0.07	<b>-0.28 <math>\pm</math> 0.11*</b>	<b>5.69</b>	<b>1, 108</b>	<b>0.02</b>
	Corolla width	0.07 $\pm$ 0.06	0.12 $\pm$ 0.10	0.19	1, 108	0.67
	Spur angle	0.04 $\pm$ 0.06	0.11 $\pm$ 0.09	0.38	1, 108	0.54
Number of pollinator visits	Corolla depth	<b>0.11 <math>\pm</math> 0.05*</b>	<b>0.15 <math>\pm</math> 0.06*</b>	0.16	1, 59	0.70
	Corolla height	<b>0.17 <math>\pm</math> 0.06*</b>	<b>-0.12 <math>\pm</math> 0.03*</b>	<b>5.50</b>	<b>1, 59</b>	<b>0.02</b>
	Corolla width	-0.04 $\pm$ 0.03	0.15 $\pm$ 0.11	3.73	1, 59	0.06
	Spur angle	0.08 $\pm$ 0.04	0.23 $\pm$ 0.12	1.15	1, 59	0.29

\* $P < 0.05$ .

treatments. This result matches other floral selection studies that have found that a selective agent can change the slope of the relationship between fitness and a trait value without altering mean fitness (Caruso, 2001; Strauss et al., 2005; Parachnowitsch and Kessler, 2010; Wassink and Caruso, 2013). In our system, as discussed above, additional studies that track the behavior of specific bumblebee species are needed to more fully understand the cause of this shift in preference.

One possible explanation for the similar seed production and pollinator visits between treatments is that the distance of 5 m apart was not sufficient for isolating treatments and, therefore, *I. capensis* plants across both treatments experienced the same degree of pollinator competition with the invasive species. We think this explanation is unlikely, however. Pollinators tended to complete long foraging bouts within plots rather than making frequent transitions between plots (C. M. Beans, personal observation). Furthermore, other studies examining competition for pollination have found significant differences in pollinator visitation and seed production between treatments even when those treatments were spaced <5 m apart (Brown et al., 2002; Caruso, 2002).

For our selection analysis, we chose to keep the total number of plants in our competition treatments constant to present pollinators with similar display sizes across treatments, as well as to mimic natural populations where total jewelweed abundance appears relatively constant as *I. glandulifera* displaces the native plant (C. M. Beans, personal observation). Because of this experimental design, however, we cannot be certain that differences in selection between treatments are the direct result of *I. glandulifera* presence, rather than the result of a change in intraspecific competitor abundance (Snaydon, 1991).

The ecological and evolutionary consequences of reproductive interactions between invasive and native plants depend first on the extent that the species share the same pollinators (Gibson et al., 2012). Our pollinator choice experiment demonstrated that the native and invasive jewelweed species share bumblebee visitors. Given that bumblebees at our study site had never encountered *I. glandulifera* before, we might have expected them to prefer the familiar native plant. Our results, however, showed that native bumblebees strongly prefer the invasive jewelweed. This result agrees with studies in Europe that showed that generalist native pollinators readily visited introduced *I. glandulifera* plants (Chittka and Schürkens, 2001; Lopezaraiza-Mikel et al., 2007; Thijs et al., 2012). This result also lends support to other studies that have found that invasive plants are often readily incorporated into native pollinator networks (Olesen et al., 2002; Morales and Aizen, 2006; Bartomeus et al., 2008).

Our hand pollination experiment suggests that when pollinators move between jewelweed species, there can be fitness consequences for the native plant. It is possible that *I. glandulifera* pollen may reduce seed set in the native jewelweed through clogging of the stigmatic surface. *Impatiens glandulifera* pollen germinates readily on many surfaces, sometimes even within the androecium or on the bodies of pollinators (Titze, 2000). These foreign pollen tubes, therefore, may result in less space available for conspecific pollen germination. In another hand pollination study, heterospecific pollen from *Impatiens pallida*, a native co-occurring congener, was shown to inhibit seed production in *I. capensis* by germinating and clogging the stigmatic surface and style (Randall and Hilu, 1990). As in our study, there was no evidence for hybridization between jewelweed species (Randall and Hilu, 1990).

*Impatiens capensis* populations have repeatedly been shown to evolve, at a microenvironmental scale, to a variety of environment stimuli, including light and water availability (Dudley and Schmitt, 1995; Heschel et al., 2002). It seems possible, then, that they may also evolve in response to the altered selection pressure imposed by *I. glandulifera*. For this evolutionary response to occur, however, the selected floral traits must be heritable. A repeatability analysis, which offers an upper-limit estimate of trait heritability (Falconer and Mackay, 1996), showed that corolla height, depth, width, and spur angle were all highly repeatable (repeatability score >0.5) in a natural population in Maine, USA (C. M. Beans and B. Bailey, University of Virginia, unpublished data). The repeatability analysis of floral traits for plants included in the selection analysis study also showed floral traits to be consistent within plants. Investigations of narrow-sense heritability in other taxa have commonly found corolla size dimensions to be heritable (Mitchell and Shaw, 1993; Campbell, 1996; Kulbaba and Worley, 2008; Gomez et al., 2009). Our results, combined with the results of these studies in other taxa, suggest that *I. capensis* corolla height is likely to be heritable and, thus, may have the potential to evolve in response to the introduced jewelweed.

Our study suggests that invasive plants may alter phenotypic selection on floral traits in native plant populations. More studies are needed, however, before we can determine whether invasive plants commonly alter floral selection on their neighbors, and under what conditions altered selection is likely to arise. For example, it is unknown whether our results would remain the same in a more complex plant community where multiple plant competitors may impose conflicting selection on *I. capensis* floral traits (Connell, 1980). Additionally, more studies are needed to determine whether native-plant floral traits commonly evolve in response to the altered pollinator-mediated selection imposed by invasive plant species. The further exploration of these topics can provide a unique window into plant community assembly (Strauss et al., 2006; Sargent and Ackerly, 2008; Thorpe et al., 2011). By studying native plants as they respond to invasive competitors, we can learn which factors lead to competitive exclusion, and which factors permit the adaptive floral-trait evolution that enables long-term coexistence. Our study is a first step toward understanding the evolutionary consequences of pollinator-mediated interactions between invasive and native plants.

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