

FLOWER MORPHOLOGY AND POLLINATOR DYNAMICS IN *SOLANUM CAROLINENSE* (SOLANACEAE): IMPLICATIONS FOR THE EVOLUTION OF ANDROMONOECY¹

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Flower morphology and pollinator dynamics play an important role in the evolution and maintenance of many breeding systems, including andromonoecy. We used a series of field experiments to test the functional relationship between flower morphology and pollination dynamics (e.g., pollen receipt and export) in *Solanum carolinense*. We find that long-styled flowers serve primarily as pollen recipients and short-styled flowers as pollen donors, making this the first study to support the male–female interference hypothesis for the evolution of andromonoecy. However, this difference in the primary male or female function of the flowers depends on the pollinator identity. In flowers visited by *Bombus impatiens*, style length has a positive relationship with pollen deposition and a negative relationship with pollen removal. In contrast, neither morphological nor behavioral traits determine pollen deposition or removal by small halictid bees. We demonstrate that different pollinators could select for different floral morphologies, and thus, our research suggests that pollinator-specific interactions with flower morphology play an important role in the evolution and maintenance of andromonoecy.

Key words: andromonoecy; male–female interference; style length; Pennsylvania; pollen deposition; pollen removal; pollinators; Solanaceae; *Solanum carolinense*.

Flower morphology influences which pollinators can visit a flower and how efficient they are as pollen vectors (Fukuda et al., 2001; Fenster et al., 2004). In particular, changes in floral morphology that occur in primary sex organs (androecium and gynoecium) or in secondary sexual characters (e.g., inflorescence characters, corolla width) can alter the plant–pollinator relationship and influence the dynamics of pollen removal and deposition (Cresswell, 2000; Fetscher, 2001; Fukuda et al., 2001; Cesaro et al., 2004). Because these traits directly affect plant fitness, it is not surprising that strong selection on floral morphological traits that increase the efficiency of pollination has been found (Cresswell, 2000). The breeding system of such plant populations could change if these morphological responses are accompanied by changes in the gender functions of the flowers.

It is likely that the relationship between flower morphology and pollinator dynamics plays an important role in the evolution of andromonoecy, a breeding system where individuals bear hermaphrodite and male flowers. Traditionally, flowers

with long styles have been assumed to function primarily as hermaphrodites and flowers with reduced styles have been assumed to function primarily as males (Solomon, 1986; Anderson and Symon, 1989; Emms, 1993; Elle, 1998; Connolly and Anderson, 2003; Huang, 2003; Cuevas and Polito, 2004). Hypotheses to explain the evolution of andromonoecy have focused on the advantages of having male flowers, and include: (1) the resource allocation hypothesis (Bertin, 1982; Spalik, 1991; Elle, 1999), (2) the pollen donation hypothesis (Bertin, 1982; Anderson and Symon, 1989; Podolsky, 1992, 1993; Elle and Meagher, 2000; Huang, 2003), (3) increased pollen receipt hypothesis (Podolsky, 1993; Vallejo-Marin and Rausher, 2007b), and (4) the outcrossing hypothesis (Heithaus et al., 1974).

The resource allocation hypothesis proposes that the production of energetically cheaper male flowers saves resources that can then be allocated to seed production. Hypotheses two and three propose contrasting, but not mutually exclusive functions for male flowers to increase the effectiveness of the pollen removal and deposition. In the pollen donation hypothesis, male flowers can increase male fitness as a result of an increase in pollen removal, as compared to plants with only hermaphrodite flowers. The increased pollen receipt hypothesis proposes that male flowers increase female fitness through more efficient pollen deposition compared to plants with only hermaphrodite flowers. Last, the outcrossing hypothesis proposes that male flowers enhance outcrossing because they cannot be self-pollinated.

Solanum species, especially *S. carolinense* (Solanaceae) have served as model organisms to test all these hypotheses (Elle, 1999; Elle and Meagher, 2000; Connolly and Anderson, 2003; Vallejo-Marin and Rausher, 2007b). To date, results of studies reject the outcrossing hypothesis (Dulberger et al., 1981, Whelan and Costich, 1986), find no support for the resource allocation hypothesis (Solomon, 1986; Elle, 1999; Vallejo-Marin and Rausher, 2007b) and find variable support for both the pollen donation (Elle and Meagher, 2000; Vallejo-Marin

¹ Manuscript received 24 March 2008; revision accepted 23 May 2008.

The authors thank E. Elle for germination information, A. Randle for identifying the bees, T. Pendergast for help in the field and revision of the manuscript, E. York for assistance in the greenhouse, Ernst Seed Conservation for land use, L. Aguilar for providing the digital camera, T. Harper for help editing the pollinator movie, A. Stephenson and M. Vallejo-Marin for comments and discussion, C. Majetic and the Kalisz and Ashman laboratories for discussion, and Greg Anderson and two anonymous reviewers for comments on a previous version of the manuscript. This research was supported by a Fabiola Aguirre Fellowship from the Center for Latin American Studies (CLAS) of the University of Pittsburgh and by the McKinley-Darbaker grant from the Pymatuning Laboratory of Ecology to A.Q.-A. Additional logistical support from NSF DEB 0108099 to T.L.A. and from NSF DEB 0324764 to S.K. This is contribution 221 to the Pymatuning Laboratory of Ecology.

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and Rausher, 2007b) and increased pollen receipt hypotheses (i.e., in one of two populations studied by Vallejo-Marin and Rausher, 2007a). The lack of clear support for these hypotheses in *Solanum* species led several authors to propose that a full understanding of the evolution of andromonoecy would require researchers to focus on flower differences and specifically determine how the style length differences between male and hermaphrodite flower types affect pollinator efficiency (pollen removal and deposition) (Solomon, 1986; Elle and Meagher, 2000; Diggle and Miller, 2004).

These authors proposed that style length differences could evolve to reduce male–female interference, which arises because of the close juxtaposition of sexual structures within hermaphrodite flowers (Lloyd and Webb, 1986). When pollinators visit flowers, these structures can interfere with either pollen removal or deposition (Fetscher, 2001; Barrett, 2002; Cesaro et al., 2004). In particular, it has been suggested that the style of *S. carolinense* interferes with pollen removal as buzz-pollinating bees attempt to grasp the anthers to extract pollen (Solomon, 1986; Elle and Meagher, 2000; Diggle and Miller, 2004). Female function can also be affected: anthers in close proximity with the stigma could result in higher levels of self-pollination and/or stigma clogging (Lloyd and Webb, 1986; Anderson and Symon, 1989; Fetscher, 2001). However, no direct tests of this hypothesis have been conducted.

Solanum carolinense presents an ideal system to determine the functional relationship between style length and pollination dynamics because populations have wide and continuous variation in style lengths (Connolly and Anderson, 2003) and individuals vary in the proportions of flowers with given style lengths. We predict that reduced style length allows bees to remove pollen more easily. Thus, short-styled flowers are expected to have greater amounts of pollen removed per visit and potentially export more pollen to other plants. We also predict that long-styled flowers will receive more outcross pollen because their styles will interfere with a pollinator's ability to grasp the anthers, reducing the likelihood of collecting self-pollen and depositing it on the stigma. Thus, long-styled flowers are expected to have greater female function in the form of greater outcross pollen receipt per visit. However, in some species the relationship between pollen deposition and style length is not linear. For example, Cresswell (2000) found that flowers with long styles receive less pollen than those with intermediate style lengths and proposed that there may be stabilizing selection that maintains architectural invariability. Thus, we are also interested in determining whether there is an optimum style length for pollen receipt in *S. carolinense*. Given the wide, continuous variation in style length in *S. carolinense*, here we focus on style length as the target of selection, although we acknowledge other features may covary with style length (Connolly and Anderson, 2003).

Studies that have proposed exploration of the male–female interference hypothesis in *Solanum* species base most of their predictions on buzz-pollinating visitors and do not specify if differences in pollinator's body sizes or behaviors might affect the predictions (but see Anderson and Symon, 1989). Focusing only on the most abundant or effective pollinator is common in floral evolution studies but neglecting to address the diversity of pollinators could lead to variability in or misinterpretation of results, when asking evolutionary questions in plant species that are visited by multiple effective pollinator species. Different pollinators have different pollen deposition and removal

rates, and if the abundance and visitation rates of the pollinator taxa varies among populations, then a variety of morphological phenotypes could occur due to different selective pressures (Glover and Barrett, 1986; Arroyo and Dafni, 1995; Alves Dos Santos, 2002; Arroyo et al., 2002; Javorek et al., 2002; Cariveau et al., 2004; Pérez-Barrales et al., 2007). Thus, quantifying the effect that varying pollinator assemblages have on floral function is critical to understanding the evolution of floral morphology and plant sexual systems (reviewed in Aigner, 2006). *Solanum carolinense* and its pollinators are an excellent system to assess whether pollination dynamics vary with pollinator taxon because this plant species grows in a diverse range of environments that vary in their pollinator faunal composition (A. Quesada-Aguilar, unpublished data).

Natural variation in floral traits, the ability of floral traits to respond to selection via pollinators, the diversity of pollinators, and the pollinator-specific relationship between morphology and pollinator effectiveness are key elements that could affect and define the evolution and maintenance of andromonoecy. Here we explore these issues and present the first test of the male–female interference. We sought to answer the following specific questions: (1) What is the level of phenotypic and genotypic variation in floral traits affecting pollen receipt or removal (stamen and style length, and stigma–anther distance) in *S. carolinense*? (2) What is the composition of the pollinator pool and visitation rate of individual pollinators of *S. carolinense* populations in northwestern Pennsylvania? (3) What is the relationship between style length and pollination dynamics (pollen deposition and removal), and does the relationship vary among pollinator taxa?

MATERIALS AND METHODS

Study species—*Solanum carolinense* (Solanaceae) is a rhizomatous, weedy, perennial plant native to the eastern United States that commonly grows in fields, roadsides, and sandy stream banks (Rhoads and Block, 2000). Flowers of *S. carolinense* are white, lilac, or purple and star-shaped with five yellow poricidal anthers (Rhoads and Block, 2000). They are generally considered self-incompatible, but this can break down as flowers age (Travers et al., 2004). Flowers mature acropetally, and in the greenhouse an inflorescence may contain up to 20 flowers (Travers et al., 2004). In natural populations, however, inflorescences tend to have only one flower open at a time (Connolly and Anderson, 2003; A. Quesada-Aguilar, unpublished data). We conducted weekly surveys in three populations in northwestern Pennsylvania (PA) in 2004 and 2005. In total, we surveyed 278 plants of which 60% had only one flower open at a given time. Like other nonhermaphroditic species of the genus *Solanum*, basal flowers of *S. carolinense* are larger than distal flowers (Symons, 1981; Anderson and Symon, 1989; Diggle and Miller, 2004). Flowering occurs from June to September, followed by the production of round orange to yellow berries that mature after the first frost and contain on average 160 seeds (Elle, 1999).

Individual plants of *S. carolinense* bear either hermaphrodite flowers or male flowers or both (Solomon, 1986; Elle, 1998, 1999; Elle and Meagher, 2000; Quesada-Aguilar, 2007; Vallejo-Marin and Rausher, 2007b). Such a sexual system has been variously described as andromonoecious, when all individuals possess both male and hermaphrodite flowers (Connolly and Anderson, 2003). However, some individuals can have only male or only hermaphrodite flowers, and it has been argued that when unisexual morphs are present, the species or population should be characterized as androdioecious or subdioecious (Sakai and Weller, 1999). In *S. carolinense*, the main morphological difference between hermaphrodite and male flowers is the length of the style (Solomon, 1986; Emms, 1993; Elle, 1998; Huang, 2003; Cuevas and Polito, 2004), although Connolly and Anderson (2003) noted differences in the stigmata. Short-styled flowers in *S. carolinense* are reported to lack female function (Solomon, 1985; Connolly and Anderson, 2003), but in northwestern PA populations, short-styled flowers can produce fruit bearing seeds after pollination with compatible pollen (Quesada-Aguilar, 2007), suggesting that this trait and

gametophytic incompatibility system are labile (Travers et al., 2004). Moreover, some hermaphrodite flowers are functionally male because they do not produce fruits (Solomon, 1985; Connolly and Anderson, 2003; Quesada-Aguilar, 2007). Clearly, style length does not predict female or male function in this species; thus here we focus on style length variation. We refer to flowers with styles that are longer than the staminal column as long-styled flowers and flowers with styles that are shorter than the anthers as short-styled flowers.

Pollinators—*Solanum carolinense* is visited by pollen-gathering bees, many of which vibrate or buzz the flowers to remove pollen from anthers (Hardin et al., 1972). Bumblebees are described as the main pollinator of this species (Travers et al., 2004; Vallejo-Marin and Rausher, 2007b), but Connolly and Anderson (2003) also observed *Lassioglossum* spp. visiting the flowers in Connecticut. In our study populations, we observed three bee taxa visiting *S. carolinense* flowers during the summers of 2004 and 2005: *Lassioglossum* spp. (Halictidae), *Augochloropsis metallica* (Halictidae), and *Bombus impatiens* (Apidae).

Source of plant material for experiments—To produce flowers for use in our field experiments and to estimate broad-sense heritability of floral traits, we collected three mature fruits from open pollinated flowers from 107 *S. carolinense* plants from one population, EC, (41°58' 93.98" N, 80°15' 36.52" W) to form sibships. Each sibship was considered to be related as half sibs. In spring 2005, seeds were germinated in the laboratory and then potted seedlings were placed in a pollinator-free hoop house at the Pymatuning Laboratory of Ecology, Crawford County, PA. These plants were used to estimate broad-sense heritability and individual flowers from these were used in the pollinator experiments (described later).

In the spring of 2006, we planted the 286 surviving plants directly in the ground in the pollinator-free hoop house. In both years, the plants started flowering in late June, in synchrony with the local wild populations (A. Quesada-Aguilar, personal observation). Each year, flowering plants were randomly assigned to different experiments as described next.

Experiment 1: What is the level of phenotypic and genotypic variation in floral traits affecting pollen receipt or removal (stamen and style length, and stigma-anther distance) in *S. carolinense*?—To characterize the level of phenotypic variation in floral traits, we measured 1671 flowers produced by a subset of the 107 sibships (300 siblings total with a mean of six flowers per sibling). Specifically, for each flower we measured style length (from the base of style to the stigma), stamen length (base of the filament to the tip of the tallest anther) to the nearest 1 mm using digital calipers. Because some individuals had curved styles, the measurement was taken from the base of the style to the stigma without straightening the style. Thus, our measure reflects the functional style length rather than the total linear length. Stigma-anther distance was calculated by subtracting the stamen length from the style length. Additionally, we measured corolla width as a surrogate for flower size. We made all floral measurements either on the first or second day after the flower had opened to control for flower age.

Because only 65 siblings produced short-styled flowers, we calculated the broad-sense heritability, h_b^2 , for style length, stigma-anther distance and anther length in long-styled flowers only. To do so, we measured these traits on at least two flowers for all siblings that flowered in either 2005 or 2006. We obtained data from 1349 flowers on 259 plants belonging to 86 sibships (range 2–5 siblings/sibship). Assuming that seeds were half-sibs, we used average flower measurements per sibling to calculate the mean squares from ANOVA [model: Trait = 3.0082 × (Variance in sibship + Variance in error)] using PROC GLM of SAS. We calculated variance estimates, h_b^2 and the standard error of h_b^2 for half-sibs using the formulas for unbalanced experimental design in Lynch and Walsh (1998). We tested whether the h_b^2 estimates were significantly different from zero using the formula for a two-tailed *t*-test (Zar, 1996).

Experiment 2: What is the composition of the pollinator pool and visitation rate of individual pollinators of *S. carolinense* populations in northwestern Pennsylvania?—We conducted weekly pollinator observations on sunny days from 0900 to 1700 hours in three natural populations (EC, BR, and FA) of *S. carolinense* in Crawford County, PA over the following time periods: EC, 11–28 July 2004; BR, 29 July–9 August 2004 and 20 July–2 August 2005; FA, 7–25 August 2005. We selected areas in each population with groups of flowering *S. carolinense* plants and recorded the number and the floral morph of all open flowers per plant in the sampling area. Each flower was uniquely numbered. We observed the area for 15 min (2004) or 30 min (2005) and recorded

the identity of every bee that visited a flower. We defined a visit as a bee landing on a flower and a contact to occur each time a bee's body contacted the anthers. We recorded the number of contact events/visit. We observed 87 total visits in 17.25 h of observation.

We calculated the proportional bee abundance for each population as the total number of visits per bee species/total number of bees visits. To determine if the abundance varied spatially, we compared the bee abundances between EC and BR in 2004 and between BR and FA in 2005 using a χ^2 test (PROC FREQ in SAS). To determine if the abundance varied temporally, we compared BR in 2004 and 2005 using a χ^2 test (PROC FREQ in SAS).

We calculated the visitation rate for each pollinator taxon as (no. contact events per pollinator species)/(no. flowers observed)/(time observed). We then used ANOVA (PROC GLM) to determine whether visitation rate varied among the pollinator taxa. We considered each population-year combination (e.g., EC 2004, BR 2004, BR 2005, and FA 2005) as replicates to avoid confounding results spatially or temporally. We also assessed whether there was an interaction between replicate and bee species (model: (Visitation rate)_{ij} = μ + replicate_i + bee species_j + (replicate × bee species)_{ij} + ϵ_{ij}).

Experiment 3: What is the relationship between style length and pollination dynamics (pollen deposition and removal) and does the relationship vary among pollinator taxa?—**Style length and pollen deposition and removal**—To determine whether style length affects the amount of pollen deposited on the stigma or removed from the anthers, we collected paired flowers of equal style length and age from the same plant. In our study populations, stigmas are receptive, and pollen is available regardless of floral age (Quesada-Aguilar, 2007). In total, 142 pairs belonging to 69 sibships were used (51 in 2005; 91 in 2006). Individual flowers were placed in Aquapics and nondestructive floral measurements were performed (see Question 1 methods). We placed the flowers in Aquapics in a plastic box and took them to the BR site. Once in the field, one of the flowers of a pair was placed in an area where pollinators had access to the flower (Open treatment). The other flower served as a control to account for any self-pollination that occurs during handling, (i.e., it received the same handling as the Open treatment flowers but was not exposed to pollinators).

Observations were conducted from 22 June–18 August 2005 and from 29 June–1 August 2006. Observations started at 0900 hours and ended at 1800 hours. After a bee visited the Open treatment flower, we returned it to the box. At the end of each day, both flowers (Open and Control) were separately preserved in ethanol.

Preserved flowers were taken to the laboratory where we counted the number of pollen grains deposited on the stigma and number of pollen grains remaining in the anthers. For all flowers (Open and Control), the stigma and one of the anthers were digested separately using a modified version of the acetolysis technique described by Kearns and Inouye (1993). After digestion, the stigma and anther samples were centrifuged, and the pellets were suspended in 100 μ L or 300 μ L of distilled water, respectively. We estimated the number of pollen grains deposited on stigmas or remaining in the anthers in 10 μ L subsamples of each sample in a haemocytometer; grains in all squares in the grid were counted (9 μ L³) for the stigma samples, while grains in the four corner squares of the grid were counted for anthers samples (4 μ L³). We counted 4–6 subsamples for each stigma or anther sample. Because not all the pollen in the anther samples precipitated after centrifugation, we also counted 4–6 subsamples of the 570 μ L of remaining supernatant. We counted grains in all squares of the grid for the anther supernatant (9 μ L). The lowest number of pollen grains that we found using this technique was 27 grains/flower.

We used Control flowers to correct for handling as a source of pollen deposition in Open flowers. We corrected our estimates of the pollen deposited on an Open flower by subtraction: Bee-mediated pollen deposition on Open flowers = (number of pollen grains on the Open flower stigma) – (number of pollen grains on the Control flower stigma). A total of 141 Control-Open flower pairs were used to calculate pollen deposition and removal (in 2005: 46 pairs were long-styled, and four were short-styled; in 2006: 40 pairs were long-styled, and 51 were short-styled). We lost one pair due to handling. Most pairs represent different siblings, however, in 18 siblings two pairs were analyzed, and in four siblings three pairs were analyzed.

For pollen removal, Control flowers provide an estimate of the average number of pollen grains per anther. The proportion of pollen removed from the Open flower anthers was calculated as: [(number of pollen grains in Control anther) – (number of pollen grains counted in Open anther)]/(number of pollen grains in Control anther). Data for pollen removal was obtained for 112 pairs of flowers collected from 92 siblings that belonged to 61 families. We had to discard 30 pairs in this experiment due to experimental problems. Forty-one observations were made in 2005 and 69 in 2006. In 2005, 39 pairs were long-styled

flowers, and two were short-styled. In 2006, 32 observations were in long-styled flowers and 37 in short-styled. Most pairs were collected from different siblings; however, in 13 siblings two pairs of flowers were collected, and in three siblings three pairs were collected. In many of these cases, multiple pairs of flowers were collected from the sibling because they produced both flower morphs.

Pollinator-taxa specific behavior at flowers—To determine whether pollinator taxa interacted with the flowers differently, pollinators that visited the Open flowers were recorded using a digital video camera (Sony Handycam DCR DVD 101, Tokyo, Japan). Only one pollinator was allowed to visit each Open flower, and the recording started when the pollinator contacted the flower in the Aquapic. Once the pollinator left, the Open flower was collected as detailed. We then analyzed the videotapes in the laboratory. For each video, we recorded the bee taxon, the number of contact events per visit and the length of each contact event in seconds. We calculated the total contact time per visit as the sum of all the contact events per visit and the mean contact time as total contact time per visit per number of contact events. In the case of bumblebee visitors, we also recorded if the stigma touched the bee's corbiculae.

Data analysis—All data were analyzed using SAS/STAT software, version 8 (SAS, 2001).

Style length and pollen received or removed—We used a two-step approach to determine the relationship between style length and pollen deposition. First, we used logistic regression (PROC LOGISTIC) to determine if style length affects whether a flower receives pollen after a single visit by any bee species (i.e., yes = 1, no = 0). Second, flowers that received pollen were used to determine if the style length (mm) affects the quantity of pollen received. Number of pollen grains deposited was \log_{10} -transformed to increase normality. To determine the relationship between the quantity of pollen deposited and style length, a multiple regression with style length, style length squared, stigma width (mm), and number of contact events was analyzed using PROC REG. We included style length squared to determine if there is an optimum style length, stigma width to account for variation in the surface area for pollen receipt, and contact events because pollen is deposited only when bees contact the flower. Six flowers were excluded from the analysis because we did not measure their stigmas. Model: \log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length² + β_3 stigma width + β_4 number of contact events + ϵ_{ij} .

To determine the relationship between the proportion of pollen grains removed and style length, we used PROC REG to run a multiple regression with style length and number of contact events as the dependent variables (Model: Proportion of pollen removed = $\alpha + \beta_1$ style length + β_2 number of contact events + ϵ_{ij}).

Pollinator-taxa-specific behavior at flowers—We recorded three genera of bees visiting the flowers placed in Aquapics in 2005 and 2006: *A. metallica*, *Lassioglossum* spp., and *Bombus impatiens*. Pollinators varied temporally, with *A. metallica* and *Lassioglossum* spp. being more abundant in early (June) and late (August) in the flowering season, while *B. impatiens* visited flowers mainly during July. In 2005, we videorecorded 52 bees: 1 *A. metallica*, 13 *Lassioglossum* spp., and 38 *B. impatiens*. In 2006, we recorded 98 bees: 22 *A. metallica*, 19 *Lassioglossum* spp., and 57 *B. impatiens*.

We determined whether pollinator taxa differed in the number of contact events (contact events = $\mu_{\text{bee spp}} + \epsilon_{ij}$), mean time per contact event (mean time = $\mu_{\text{bee spp}} + \epsilon_{ij}$), and the total contact time per visit (total contact time = $\mu_{\text{bee spp}} + \epsilon_{ij}$) with ANOVA using PROC GLM. Post hoc Tukey's tests were used to determine if the three species differed significantly. We also compared the number of contact events on short-styled flowers and the number of contact events on long-styled flowers for each pollinator taxon via *t* test (PROC TTEST).

Style length and pollination dynamics by taxa—We compared the number pollen grains deposited and the proportion of pollen removed by each bee taxa using ANOVA (PROC GLM). To determine whether the functional relationship between style length and pollen deposition varied with pollinator taxa, we analyzed the data separately for each pollinator taxon that visited the flowers using PROC REG [Model: \log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length², β_3 stigma width + β_4 number of contact events + ϵ_{ij}]. In the case of bumblebees, we conducted a logistic regression to determine the relationship between the style length and the probability of the stigma touching the corbiculae using PROC LOGISTIC. We determined whether the mean number of pollen grains deposited on stigmas that did or did not touch the

corbiculae differed using PROC TTEST. We also analyzed pollen removal data separately for each pollinator species using multiple regression (PROC REG Model: Proportion of pollen removed = $\alpha + \beta_1$ style length + β_2 number of contact events + ϵ_{ij}).

Preliminary statistical analysis suggested that the small bees (*A. metallica* and *Lassioglossum* spp.) had similar effects on pollen deposition ($P > 0.05$) and removal ($P > 0.05$). Because of this and the low frequency of each of these small bees in the data set, we pooled data from *A. metallica* and *Lassioglossum* spp. We compared the slopes for style length and pollen deposition between *Bombus impatiens* and the small bees (halictids) via ANCOVA (PROC GLM Model: \log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length² + β_3 stigma width + β_4 number of contact events + β_5 pollinator + β_6 (pollinator \times style length) + β_7 (pollinator \times style length²) + β_8 (pollinator \times stigma width) + β_9 (pollinator \times contact events) + ϵ_{ij}). We also used ANCOVA to compare the slopes for pollen removal (PROC GLM Model: Proportion of pollen removed) = $\alpha + \beta_1$ style length + β_2 number of contact events + β_3 pollinator + β_4 (pollinator \times style length) + β_5 (pollinator \times contact events) + ϵ_{ij}).

RESULTS

Phenotypic and genetic variation in floral traits—Flower size was normally distributed with an average corolla width of 31.44 ± 5.03 mm (Fig. 1A). Likewise, stamen length was normally distributed (Fig. 1B), with average stamen length of 8.92 ± 1.01 mm. While the distribution of style lengths was continuous, it was also bimodally distributed (Fig. 1C). Because of this, stigma-anther distance was also bimodally distributed (Fig. 1D). Hereafter, we refer to flowers with style lengths greater than 8 mm as long-styled flowers (the style protrudes above the anthers) and those with style lengths smaller than 8 mm as short-styled flowers (the style is below the top of the anthers). This 8-mm breakpoint was chosen based on our phenotypic distribution (Fig. 1D). (Note: our definition of long and short-styled flowers differs from that used by Connolly and Anderson [2003].)

The h_b^2 for both style length and stigma-anther distance for long-styled flowers was large and significant for both traits. The h_b^2 for style length (0.63 ± 0.28) and stigma-anther distance (0.89 ± 0.28) were both significantly different from zero ($t = 2.30$, $df = 85$, $P < 0.05$ and $t = 3.19$, $df = 85$, $P < 0.005$, respectively). The h_b^2 for stamen length was 0.49 ± 0.27 and was not significantly different from zero ($t = 1.80$, $df = 85$, $0.05 < P < 0.10$).

Pollinator composition and visitation of *S. carolinense*—Of the three genera of bees we observed visiting flowers of *S. carolinense* (*Lassioglossum* spp., *Augochloropsis metallica*, and *Bombus impatiens*), only *A. metallica* had not previously been described as a pollinator of *S. carolinense*. We observed the following bee visits: 21 (EC 2004); 9 (BR 2004), 40 (BR 2005), and 17 (FA 2005). Due to the small sample size in BR 2004 and FA 2005, we used Fisher's exact test. The bee species abundances differed between the populations in 2004 (EC vs. BR: $\chi^2 = 10.83$, $df = 2$, $P < 0.005$). In EC, the most abundant bee was *A. metallica* (71% of visits; Fig. 2), whereas 24% visits were from *Lassioglossum* spp. We observed only one *B. impatiens* visiting the flowers at EC, while 56% of all visits were from *B. impatiens* at BR (Fig. 2). We observed equal numbers of *A. metallica* and *Lassioglossum* spp. (2 of each). In 2005, the abundance of each bee species was similar in BR and in FA ($\chi^2 = 0.080$, $df = 2$, $P < 1.0$). In both populations, 82% of the visits were from *B. impatiens*, and 1% was from *Lassioglossum* spp. We observed few *A. metallica* in 2005, only recording one individual in FA and three in BR. There was no significant temporal difference

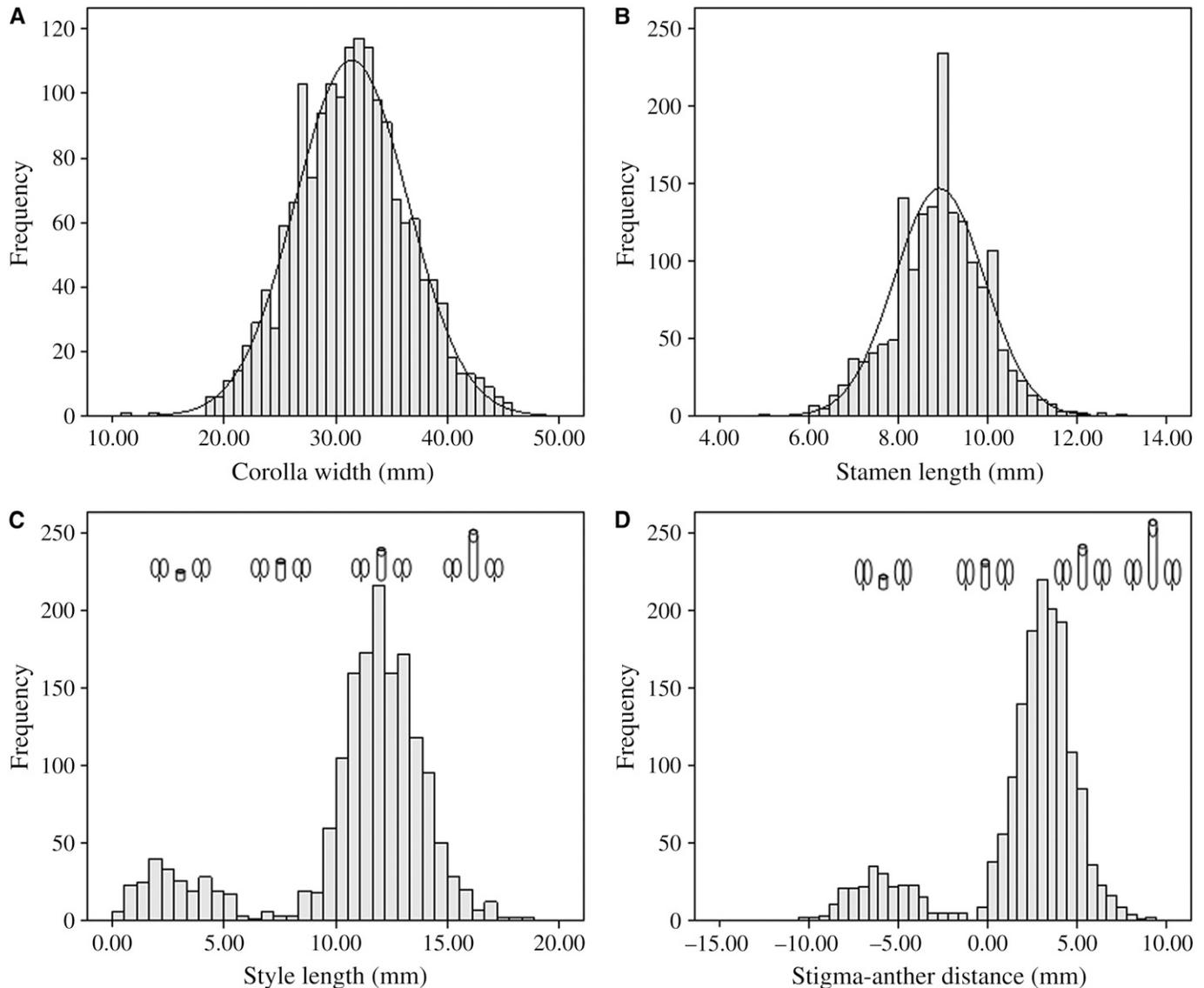


Fig. 1. Frequency distribution of (A) corolla width (mm), (B) stamen length (mm), (C) style length (mm), and (D) stigma–anther distance (mm) of *Solanum carolinense* flowers. Top inserts in (C) and (D) are stylized representations of the flower morphology. Stamen length is kept constant to demonstrate the effect of variation in style length on the stigma–anther distance.

(2004 vs. 2005) in the bee composition of BR ($\chi^2 = 3.14$, $df = 2$, $P > 0.14$).

In general, the visitation rates for the three bee species were low with high levels of variation (Fig. 3). Visitation rates were significantly different among the different bee taxa (Table 1). We also found that there was a significant interaction between the replicate (population-year) and pollinator taxon (Table 1). For instance, *B. impatiens* had a higher visitation rate than *A. metallica* and *Lassioglossum* spp. in all replicates except the EC 2004 (Fig. 3). *Augochloropsis metallica* had a higher visitation rate than *Lassioglossum* spp. in 2004, but in 2005 the visitation rate for these two species was similar.

Pollen deposition and receipt is pollinator specific—The probability of receiving pollen significantly increases with style length when considering all the pollinator taxa (pollen = $0.30 \times$

(style length) – 1.72, $N = 141$, Wald $\chi^2 = 38.76$, $P < 0.0001$). Long-styled flowers have a 35% greater chance of receiving pollen than short-styled flowers. Of the 141 flowers, 93 received pollen; of these, 79 were long-styled. Interestingly, most of the long-styled flowers that did not receive pollen were visited by either *Lassioglossum* spp. or *A. metallica*. In contrast, of the 48 flowers that did not receive pollen, 74% were short-styled flowers. These flowers did not receive pollen regardless of the pollinator identity.

When considering only those flowers that received pollen (across all pollinator taxa), the number of pollen grains deposited significantly increased with style length, style length squared, and the number of contact events, but not with stigma width (Table 2A). On average, flowers with styles that are 10–15 mm long received 10 times more pollen than flowers with either very short or very long styles (Fig. 4). Flowers with styles

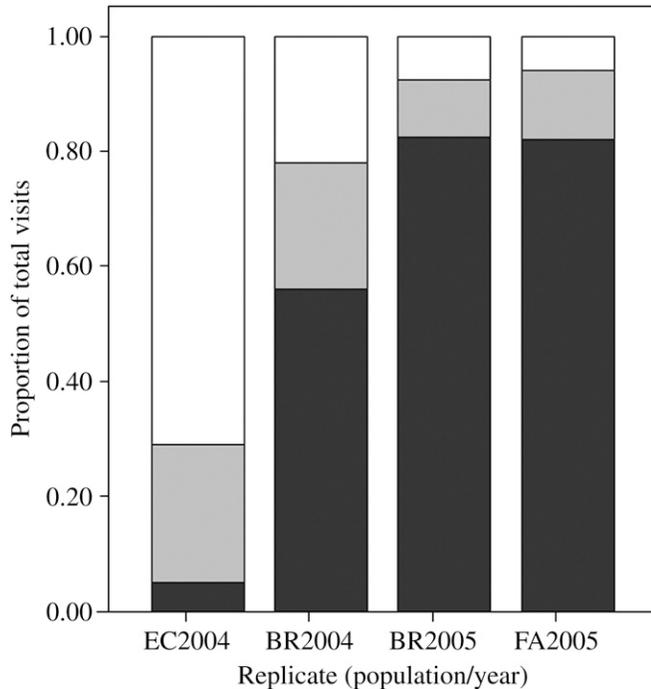


Fig. 2. Proportional composition of the pollinator fauna for three *Solanum carolinense* populations (EC, BR, FA) in Crawford County, Pennsylvania over two years (2004, 2005). Pollinator taxa are *Augochloropsis metallica* (white), *Lassioglossum* spp. (gray) or *Bombus impatiens* (black) bars.

shorter than 8 mm received 116 ± 34 pollen grains, flowers with styles that are 10–15 mm received 1496 ± 267 pollen grains, and flowers whose styles are >15 mm received 128 ± 64 pollen grains. These results show that there is both a linear and a quadratic relationship between style length and number of pollen grains deposited (Table 2A; Fig. 4), suggesting that there is an optimal style length between 10 and 15 mm for pollen receipt.

When considering all bees together, pollen removal is strongly influenced by the bee's behavior. The proportion of pollen removed increases positively with the number of contact events (Table 2A). After we account for contact events, style length is significantly negatively correlated with the proportion of pollen removed (Table 2A). The general pattern is that more pollen is removed from short-styled flowers than from long-styled flowers.

All three bee species interacted significantly differently with *S. carolinense* flowers with respect to number of contact events, average time per contact event, and total contact time (all $P < 0.001$) (Appendix S1, see video in Supplemental Data with online version of this article). On average, *Lassioglossum* spp. contacts the flower once, *A. metallica* contacts the flower twice, and *B. impatiens* contacts the flower four times during a foraging bout (Fig. 5A). Total time in contact with a flower is negatively related to the number of contact events: on average, *Lassioglossum* spp. spent the longest time per contact event (~ 45 s), whereas *A. metallica* spent 41 s per contact event, *B. impatiens* spent only 7 s per contact event (Fig. 5B).

Despite their small size, *Augochloropsis metallica* bees were able to buzz the anthers. However, they then had to groom the tip of the anthers to collect the pollen, spending a total of ~ 91 s in contact with the flower in each visit (Fig. 5C). *Lassioglossum*

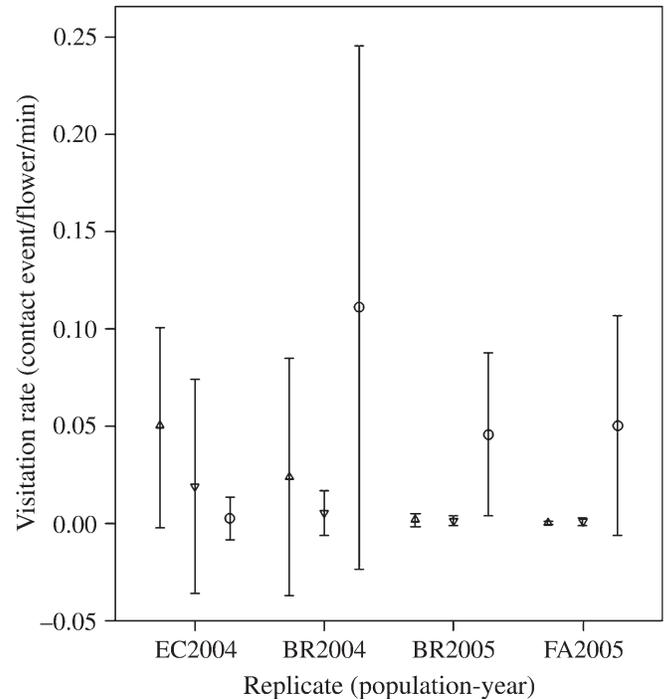


Fig. 3. Visitation rate (contact event per flower per time) to *Solanum carolinense* in three populations over two years (see Fig. 2). Bees are denoted as *Augochloropsis metallica* (upward triangle), *Lassioglossum* spp. (downward triangle), and *Bombus impatiens* (circle). Means ± 1.0 SE are shown.

species usually contacted a flower once and left after collecting pollen. Interestingly, their average contact time was higher than that of *A. metallica*, but the total time spent on the flowers was lower due to fewer contact events. *Bombus impatiens* spent the least amount of time in contact with a flower (~ 30 s, Fig. 5C). This species had typical Apidae behavior: the bee contacted the flower (contact event), vigorously vibrated the anthers (buzz pollination), then hovered in front of the flower while grooming, collecting, and compressing the pollen into corbiculae.

The number of contact events for short- vs. long-styled flowers did not differ significantly when they were visited by either *A. metallica* ($t = 0.32$, $df = 20$, $P = 0.75$) or *Lassioglossum* spp. ($t = -0.47$, $df = 29$, $P = 0.64$). In contrast, the number of contact events did differ significantly between the short- v. long-styled flowers when they were visited by *B. impatiens* ($t = 2.14$, $df = 89$, $P = 0.04$). On average, *B. impatiens* contacts short-styled flowers three times and long-styled flowers five times.

All bee species visiting *S. carolinense* appeared to be effective pollinators because they removed and deposited pollen, although the source of the pollen deposited cannot be determined from the present experiment. However, the magnitude of pollen deposition varied among the bee species ($F = 6.84$, $df = 2$, $P < 0.001$). On average in a single visit, *A. metallica*, *Lassioglossum* spp., and *B. impatiens* deposited 97 ± 41 , 87 ± 28 , or 1153 ± 218 pollen grains, respectively, on the stigma. The magnitude of pollen removal also varied among the bee species ($F = 6.6$, $df = 2$, $P = 0.002$). On average in a single visit, *A. metallica*, *Lassioglossum* spp., and *B. impatiens* removed 17454 ± 3972 ; 26027 ± 8199 ; or 41766 ± 3084 pollen grains, respectively, from the anthers.

TABLE 1. ANOVA of visitation rate (contact event/flower/minute) for three pollinators (*Bombus impatiens*, *Augochloropsis metallica*, and *Lassioglossum* spp.) to *Solanum carolinense* plants. Replicate represents four population-year combinations (EC2004, BR2004, BR2005, FA2005). Model: $F = 4.74$, $N = 144$, $P < 0.0001$, $R^2 = 0.28$.

Source	df	F	P
Visitation rate	3	2.29	0.0815
Replicate	2	10.59	<0.0001
Pollinator	2	10.59	<0.0001
Replicate × pollinator	6	5.82	<0.0001

The ANCOVA model for pollen deposition was highly significant and explained 62% of the observed variance ($F = 15.91$, $N = 87$, $P < 0.0001$, $R^2 = 0.62$). Again across all bee species, we found a positive relationship between style length and pollen deposition ($F = 23.88$, $df = 1$, $P < 0.0001$), and a negative quadratic relationship between style length and pollen deposition ($F = 18.35$, $df = 1$, $P < 0.0001$). We also found a significant difference in the amount of pollen deposited by each bee taxon ($F = 3.88$, $df = 1$, $P = 0.05$). Here, there is a significant difference between the slopes for style length and pollen deposition of the small vs. large bees ($F = 7.09$, $df = 1$, $P = 0.01$) and between the slopes for style length squared and pollen deposition ($F = 5.06$, $df = 1$, $P = 0.03$). Additionally, there was a significant interaction between bee species and the number of contact events ($F = 4.68$, $df = 1$, $P = 0.03$).

The ANCOVA model for pollen removal was also highly significant and explains 37% of the variance observed ($F = 12.57$, $N = 112$, $P < 0.0001$, $R^2 = 0.37$). Across all bee species, the only variable that was significant was the number of contact events ($F = 6.33$, $df = 1$, $P = 0.013$). The slopes for style and pollen removal were not significantly different between the two small bees and *B. impatiens* ($F = 0.94$, $df = 1$, $P = 0.33$).

None of the morphological or behavioral traits measured in this experiment seemed to influence the amount of pollen deposited by *A. metallica* or *Lassioglossum* spp. (Table 2C). The positive relationship between style length and the amount of pollen deposited was significant only for *B. impatiens* (Table 2B). This relationship is strongly influenced by the number of contact events that the bumblebee has with the flower (Table 2B). The significant positive relationship between style length and pollen deposition (pollen = 0.35 style length – 3.84, $N = 87$, Wald $\chi^2 = 19.39$, $P < 0.0001$) is due to the higher probability the stigma of long-styled flowers touching *B. impatiens*' corbiculae (Appendix S1, see video in Supplementary Data with

online version of this article). The odds of the stigmas touching the bees' corbiculae are 41% greater for long- vs. short-styled flowers (Fig. 6). Extremely long styles (>15 mm) usually did not make contact with either the bee's body or the corbiculae (Fig. 6). Because the corbiculae carry large amounts of pollen, a stigma will receive ~8 times more pollen when it contacts the corbiculae rather than other parts of the bee (mean 2500 ± 500 pollen grains vs. 400 ± 100 pollen grains, respectively; $t = -4.98$, $df = 85$, $P < 0.0001$).

The negative relationship between style length and pollen removal was significant only for *B. impatiens* (Table 2B). Neither the number of contact events nor the style length determined the proportion of pollen removed when *Lassioglossum* or *A. metallica* visited the flowers (Table 2C).

DISCUSSION

We found that both style length and stigma-anther distance in *S. carolinense* flowers have high levels of phenotypic variation within the EC population. If the high broad-sense heritability here reflects additive genetic variation, then these traits may evolve in response to selection. We also found that style length determines the amount of pollen deposited and removed in *S. carolinense*. While others have suggested this relationship (see introduction), this study is the first to show that the floral morphs of *S. carolinense* contribute differently to the male and female components of fitness. Long-styled flowers serve primarily as pollen recipients and short-styled flowers serve primarily as pollen donors. These data support the male-female interference hypothesis because (1) short-styled flowers receive less pollen but more is removed from them and (2) long-styled flowers receive more pollen because their stigmas more frequently touch the bee's body, but less pollen is removed per visit. However, the quality of pollen received during these interactions might differ. For instance, short-styled flowers may primarily receive within-flower self-pollen, and the longest-styled flowers may receive mostly outcross pollen, while the intermediate-styled flowers may receive both self- and outcross pollen. Further, if multiple flowers are open simultaneously on a plant, the pollen transferred by bees may be dominated by geitonogamous self pollen.

Our pollinator observations show that both large and small bees visit *S. carolinense* and that the primary function of the flowers (as a pollen donor or pollen recipient) varies depending on the pollinator that visits the flower. The relationship between style length and pollen dynamics is significant only when

TABLE 2. Multiple regression analyses of the pollen deposited on stigmas and of the proportion of pollen removed from the anthers of *Solanum carolinense* with morphological variables (style length, stigma width) and bee behavior (contact events) for all bees, the bumblebee *Bombus impatiens*, and the halictids *Augochloropsis metallica* and *Lassioglossum* spp.

Source of variation	A) All bees		B) <i>Bombus impatiens</i>		C) Halictids	
	Pollen deposited	Pollen removed	Pollen deposited	Pollen removed	Pollen deposited	Pollen removed
Style length (mm)	0.35 ± 0.068***	-0.0085 ± 0.0035*	1.00 ± 0.18***	-0.0091 ± 0.0046*	-0.37 ± 0.22	-0.0021 ± 0.0045
Style length ² (mm)	-0.016 ± 0.0036***	NA	-0.046 ± 0.0092***	NA	-0.018 ± 0.011	NA
Stigma width (mm)	-0.024 ± 0.063	NA	0.58 ± 1.05	NA	0.032 ± 0.12	NA
No. contact events	0.11 ± 0.019***	0.032 ± 0.005***	0.19 ± 0.044***	0.029 ± 0.001***	0.077 ± 0.20	0.0077 ± 0.011
Model						
F	14.34***	23.24***	16.98 ***	12.51***	0.78	0.36
N	87.00	112.00	56.00	73.00	30.00	38.00
R ²	0.41	0.30	0.57	0.26	0.11	0.02

Notes: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

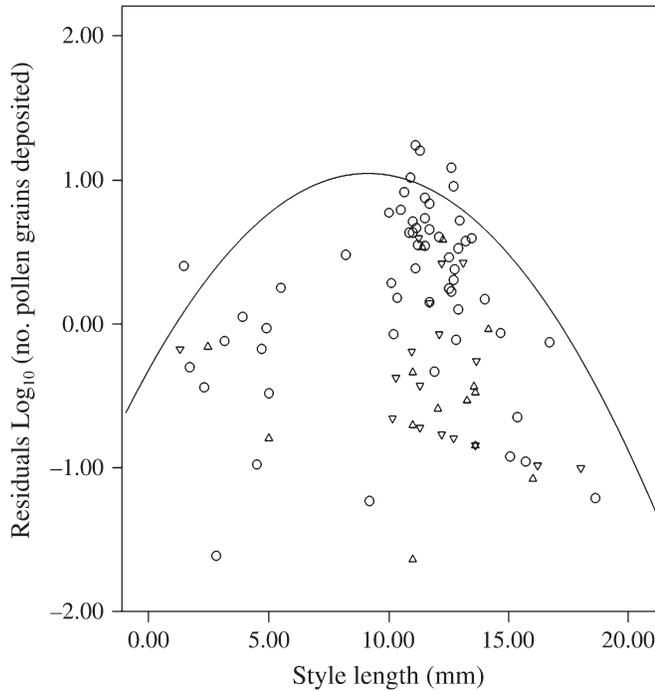


Fig. 4. Relationship between the style length (mm) and the residuals of the number of pollen grains deposited (\log_{10}) when visited by three bee species. *Augochloropsis metallica* (upward triangle), *Lassioglossum* spp. (downward triangle), and *Bombus impatiens* (circle). See text and Table 2 for full regression results.

B. impatiens (large bee) is the pollinator. Thus, if a population of *S. carolinense* was visited exclusively by *B. impatiens*, then differences in floral morph and andromonoecy could be favored by selection, as long as there are no countervailing selective pressures. In contrast, if a population of *S. carolinense* was visited mainly by either *A. metallica* or *Lassioglossum* spp. (small bees), then there may be no selection for the dimorphism (or any particular style length). Because different pollinators can potentially exert different selective pressures, the variation observed for style length among individuals and in the proportional production of short-styled flowers within and among natural populations of *S. carolinense* could be due to variation in abundance and visitation rate of the pollinators.

Style length and flower function—As we predicted, style length affected both pollen deposition and removal. We found a positive relationship between pollen deposition and style length (Table 2A) and a negative relationship between pollen removal and style length (Table 2A). Our data support the interference hypothesis proposed by several studies (Solomon, 1986; Elle and Meagher, 2000; Fetscher, 2001; Diggle and Miller, 2004) because style length (1) determines whether a flower receives any pollen, (2) determines the amount of pollen received (Table 2A), and (3) appears to interfere with the pollen removal process (Table 2A). These results suggest that the primary contributions of two flower morphs to plant fitness could be different because long-styled flowers serve as better pollen recipients and short-styled flowers as better pollen donors.

Interestingly, there seems to be an optimal style length that maximizes pollen deposition. We observed a significant quadratic relationship between style length and pollen deposition in

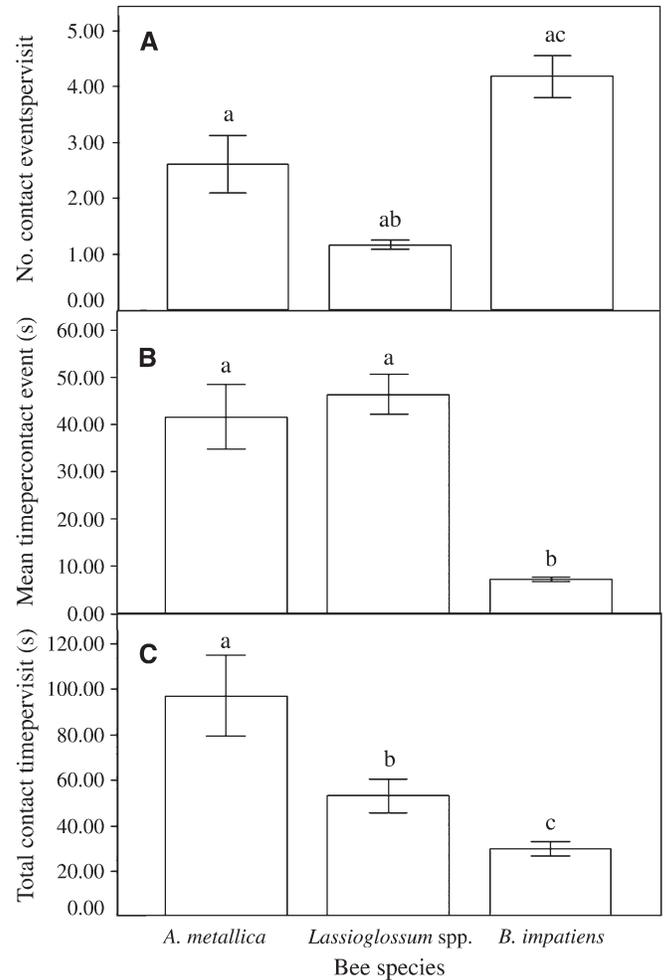


Fig. 5. Behavioral differences among bee species that visit *Solanum carolinense*. (A) Number of contact events with flower per visit ($F = 12.63$, $df = 144$, $R^2 = 0.15$, $P < 0.0001$). (B) Mean time on the flower ($F = 79.86$, $df = 144$, $R^2 = 0.53$, $P < 0.0001$). (C) Total time on the flower ($F = 20.47$, $df = 144$, $R^2 = 0.22$, $P < 0.0001$). Means ± 1.0 SE are shown and were compared via Tukey tests. Different letters represent significant differences among the means.

which the longest styles (>15 mm) actually received little pollen (Fig. 4). These results are similar to those found by Cresswell (2000) in *Brassica napus*; flowers with intermediate style lengths received the most pollen on their stigmas. Cresswell proposed that there might be stabilizing selection that maintains architectural invariability. Two lines of evidence suggest that there is an optimal style length for long-styled flowers in *S. carolinense*. First, most of the long-styled flowers in our study and the study of Connolly and Andersen (2003) had style lengths between 9–13 mm. Our data shows that styles in this range received the most pollen grains on their stigmas (Fig. 4). Second, in a separate study of the two natural populations described here, flowers with style lengths around 9 mm were more likely to set fruit (Quesada-Aguilar, 2007).

Style length and pollination dynamics by taxa—Because pollinator taxa interacted differently with the flowers (Fig. 5), the effects of floral morphology on pollination dynamics

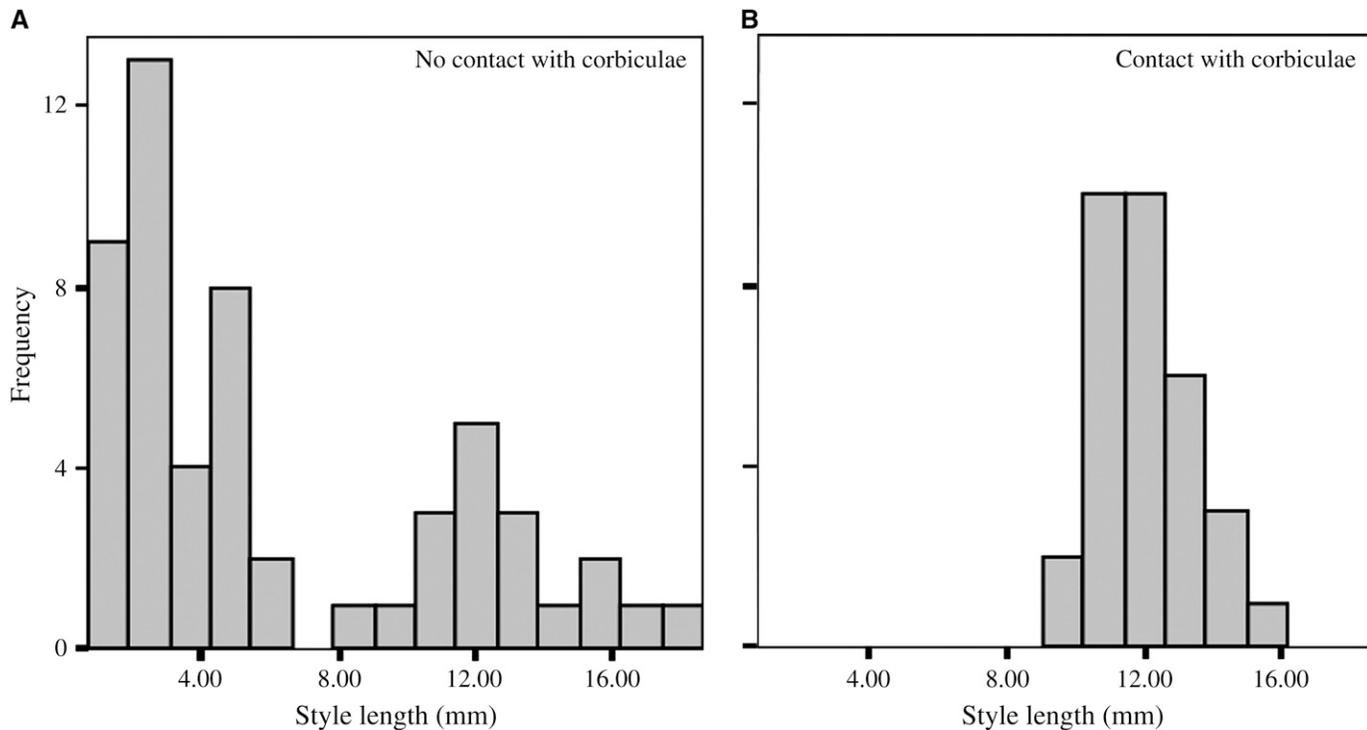


Fig. 6. Style length histograms for flowers in which the stigma did (A) or did not (B) touch the corbiculae of *Bombus impatiens*. (A) Mean = 6.28, SD = 5.00, $N = 54$. (B) Mean = 12.64, SD = 1.32, $N = 32$. Logistic regression model: pollen deposited = $0.35 \times (\text{style length}) - 3.84$, $N = 87$, Wald $\chi^2 = 19.39$, $P < 0.0001$.

differed among taxa. When plants are visited either by *A. metallica* or *Lassioglossum* spp. (small bees), no floral morphological or bee behavioral traits influenced the amount of pollen deposited or removed (Table 2C). These bees usually land on the corolla, move upward to the tips of the anthers, and manipulate one anther at a time; thus, the presence of a style of any length does not interfere with their behavior and thus we do not see that any of the morphological traits affect pollen removal or deposition.

In contrast, both the positive relationship between style length and the amount pollen deposited and the negative relationship between style length and pollen removal are significant for *B. impatiens* (Table 2B). The positive relationship is explained by the fact that longest-styled flowers have a higher probability of touching the corbiculae of *B. impatiens*, while the short-styled flowers may only receive self-pollen that falls onto the stigma when *B. impatiens* buzzes a flower (Fig. 6). The negative relationship could result from the style interfering with the bee's ability to maintain a firm grasp of the anthers. The behavior observed in our videos supports this idea (Appendix S1, see Supplemental Data with online version of this article); in long-styled flowers, the bee contacts the anthers, separates from the anthers, and then recontacts the flower repeatedly. Short-styled flowers do not have this physical barrier to pollen removal, and more pollen is removed even though bumblebees have fewer contact events with these flowers. Our results show that variation in style length significantly affects the interaction with *B. impatiens*, allowing the two morphs of *S. carolinense* to increase the pollinator efficiency of this visitor.

Our data support the idea that variation in pollinator abundance, behavior, and diversity can result in a diverse array of selective pressures and thus different morphological composi-

tions of the plant populations (Anderson and Symon, 1988; Campbell et al., 1991; Cresswell and Galen, 1991; Herrera, 1995; Sanchez-Lafuente et al., 2005). *Bombus impatiens* is the most frequent pollinator in three of the four populations of *S. carolinense* we studied (Figs. 2, 3). However, *S. carolinense* grows in a diverse array of environments, within which the three bee species vary in their abundance and rate of visitation (Figs. 2, 3). Small halictid bees forage more locally than *Bombus* and could be important selective agents via their influence on the rate of compatible pollen deposition (Anderson and Symon, 1988, 1989). Small halictid bees could be important selective agents in populations where *B. impatiens* has a low population sizes or simply do not visit the flowers. For example, within our study populations, *S. carolinense* in the EC population cooccurs and coflowers with many other flowering species. Here, *B. impatiens* rarely visits *S. carolinense* (Fig. 2), but was observed visiting the other flowering species (A. Quesada-Aguilar, personal observation). Thus, the flowering community could affect which pollinators visit *S. carolinense*. In addition, temporal variation within the season in pollinator fauna can result in small bees playing an important role in pollinating *S. carolinense*. For example, halictid bees could be the main pollinators of plants that flower at the beginning (mid June) or the end of the flowering season (August) when *B. impatiens* visitation rates are low (A. Quesada-Aguilar, personal observation). The variation observed for style length (Fig. 1C) and production of short-styled flowers observed in natural populations of *S. carolinense* (Elle, 1998), could be due to variation in abundance and visitation rates of these pollinators (Fig. 1C). However, broad scale studies that determine if variation in style length and in short-styled flowers is due to variation in the pollinator assemblage are needed to test this idea.

Evolution of andromonoecy—The leading hypotheses for the evolution of andromonoecy focus on the male vs. female fitness advantages of having two morphs. Here we tested and found support for the male–female interference hypothesis (Solomon, 1986; Elle and Meagher, 2000; Diggle and Miller, 2004). We conclude that long-styled flowers serve mainly as pollen recipient flowers and short-styled flowers serve mainly as pollen donors when *B. impatiens* visit *S. carolinense* flowers. Thus, the presence of two morphs increases the efficiency of *B. impatiens* as a pollinator. Further, if we extrapolate these results to the whole plant level, then when *B. impatiens* is the primary pollinator our data also support both the pollen donation hypothesis (Bertin, 1982; Podolsky, 1992, 1993; Elle and Meagher, 2000; Huang, 2003) and the increased pollen receipt hypothesis (Podolsky, 1993; Vallejo-Marín and Rausher, 2007b). The pollen donation hypothesis is supported because short-styled flowers increase the proportion of pollen removed, which the bees can potentially transport to other flowers. The increased pollen receipt hypothesis may also be supported because short-styled flowers do not remove pollen from pollinators, so less of the pollen deposited on bees during visits to long-styled flowers is removed during subsequent geitonogamous visits to short-styled flowers. Moreover, our study provides additional support for the increased pollen receipt hypothesis: long styles optimize outcross pollen receipt, allowing the flower to contact the bee's corbiculae (Fig. 2) and potentially decrease the chance for self-pollination because less pollen is removed from their anthers (Table 2A).

In addition, we found that the relationship between flower morphology and visitation is pollinator-specific in *S. carolinense*. Acknowledging this difference is crucial when testing such hypotheses, because the evolution and maintenance of andromonoecy at the morphological level is likely to depend on the pollinator assemblage and visitation rates. One could predict, for example, that when *B. impatiens* is the main pollinator, *S. carolinense* will experience selection that favors floral dimorphism and andromonoecy might evolve as a strategy to increase both male and female components of fitness. In contrast, when *S. carolinense* is visited primarily by *A. metallica* or *Lasiosiglossum* species, there will be no selection for floral dimorphism (or any particular style length).

Variation in the selective pressures on dimorphic flowers resulting from pollination variation could explain why Elle and Meagher (2000) found that plants with higher proportions of short-styled flowers had higher male success but Vallejo-Marín and Rausher (2007b) found that arrays of hermaphrodite flowers sired just as many seeds per flower as did arrays of short-styled flowers. These conflicting results could be due to differences in the pollinator composition and abundance of their study populations because the advantage of male flowers will be determined by the identity of pollinators that visit the plants.

In conclusion, our findings show that different pollinator taxa impose different selective pressures on *S. carolinense* plants. These results underscore the importance of studying pollinators to understand the evolution of sexual systems. Interestingly, only two of the seven studies that have tested the hypotheses regarding the evolution of andromonoecy in *S. carolinense* have conducted pollinator observations (Connolly and Anderson, 2003; Vallejo-Marín and Rausher, 2007b). This lack of observation might be why results on the evolution of this breeding system have been inconclusive so far. Future studies that include detailed information on pollinator identity and how they interact with the flower's morphology will increase our understanding of the evolution of andromonoecy and other breeding systems.

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