FLOWER MORPHOLOGY, GENDER FUNCTIONALITY, AND POLLINATOR DYNAMICS IN *SOLANUM CAROLINENSE*: IMPLICATIONS FOR THE EVOLUTION OF ANDROMONOECY

by

Andrea Quesada-Aguilar

Bach. Biologia, Universidad de Costa Rica, 2001

Submitted to the Graduate Faculty of

The School of Arts and Sciences in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2007

UNIVERSITY OF PITTSBURGH

SCHOOL OF ARTS AND SCIENCES

This thesis was presented

by

Andrea Quesada-Aguilar

It was defended on

April 24, 2007

and approved by

Cynthia Morton, Ph. D, Adjunt Professor

Stephen Tonsor, Ph.D, Associate Professor

Thesis Co-Director: Susan Kalisz, Ph. D, Professor

Thesis Director: Tia-Lynn Ashman, Ph.D, Associate Professor

FLOWER MORPHOLOGY, GENDER FUNCTIONALITY, AND POLLINATOR DYNAMICS IN *SOLANUM CAROLINENSE*: IMPLICATIONS FOR THE EVOLUTION OF ANDROMONOECY

Andrea Quesada-Aguilar, M.S.

Morphological differences in flowers have important evolutionary consequences; they influence the plant's relationship with pollinators and are strongly correlated with sexual function in some breeding systems. Here, I explore the functional relationship between flower morphology and pollination dynamics (e.g. pollen receipt / export) in *Solanum carolinense* (Solanaceae) and evaluate whether this relationship varies with pollinator taxa. I also investigate if flower morphology determines fruit setting ability of flowers under different pollination regimes. *Solanum carolinense* has been characterized as having an andromonoecious sexual system where individual plants bear both hermaphroditic and male flowers. This species presents an ideal system to study the relationship between floral morphology, functionality and pollinators because flowers in natural populations vary in their style length and grow in diverse array of environments that vary in their pollinator fauna composition. I conducted a series of greenhouse experiments, pollinator observations and natural population surveys to test these relationships. My results demonstrate that long styled flowers serve as pollen recipients and short styled flowers as pollen donors. However, only bumblebees when (*Bombus impatiens*) are the pollinators I observe a positive relationship between style length and pollen deposition and a negative relationship with pollen removal. These findings support the female/male interference hypothesis and suggest that when plants are visited by species of species of *Bombus*, the differences in fitness could favor the evolution of andromonoecy. In contrast, when plants are

visited either by *Augochloropsis metallica* or *Lassioglossum* spp. there is no selection for the dimorphism (or any particular style length). I also found that flower morphology, in particular style length, determines the fruit setting ability of the flowers in *S. carolinense* under different pollination regimes. However, in some flowers sexual functionality varies and does not accord with traditional classification of the flowers. The variation observed for style length, functionality and production of staminate flowers among individuals in natural populations of *S. carolinense* could be due to variation in abundance and visitation rate of pollinator taxa. Future studies should not neglect taxa-specific plant-pollinator interactions because the evolution of plant breeding systems can be determined by taxa specific interactions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGMENTS

I would like to thank my advisor Tia-Lynn Ashman for all the help she provided during my studies. In particular, I would like to thank her for giving me the opportunity to join the department because studying here at Pitt has been an invaluable journey from which I gained not only scientific knowledge but personal knowledge. The lessons learned here at Pitt will accompany me throughout life.

I would also like to thank my co-advisor Susan Kalisz who opened the doors of her lab to me nine months ago. Sue's contribution to my thesis has been invaluable. She improved my project with insightful comments, long discussions which organized my ideas and improved my communication skills (which sometimes seemed an impossible task), and provided me with key resources like greenhouse and lab space. Thanks to her good disposition, my scientific skills have improved greatly and I have gained knowledge that will continue to influence me as a scientist. At a personal level, I would like to thank Sue for her enduring support, patience, positive energy, motivation and understanding. She was a key figure that enabled me to complete this process. Sue has changed my life and I hope she knows that I have become a better scientist and a better person thanks to her.

Many thanks also to the two other members of my committee: Cynthia Morton and Steve Tonsor for all the great insights. Cynthia was always a pillar of support and Steve selflessly spent many hours helping me organize my ideas and teaching me statistics and quantitative genetics. It was always a joy to talk to Steve because our discussions of science were combined with many invaluable life lessons; thanks to Steve I know that one could be a great scientist and also a great fisherman.

I am a very fortunate person because I was able to interact with the members of both the Ashman and the Kalisz labs. In the Ashman lab I would like to thank Carrie Evenoski, Cassie Majetic and Jannette Steets who helped me endure my first years at Pitt. In the Kalisz lab I would like to thank Jessica Dunn, Chris Heckel, Ting-Shuang Yi, and April Randle for helping me finish this long process and for all of their invaluable contributions. The members of both labs have contributed greatly to my research with insightful comments, editorial reviews and help in the field and in the lab. Special thanks to April Randle who not only identified my bees, but spent numerous hours helping me organize my ideas and improve my science. Special thanks to Tom Pendergast for four years of invaluable help in the field and with my project, without his help this thesis would not be finished and the process would have been less fun. Many thanks also to the PeER group and all the other graduate students whose comments contributed to the development of my ideas. Special thanks also to Dr. Mario Vallejo-Marin, Dr. Elizabeth Elle, and Dr. Andrew Stephenson for invaluable information on *Solanum carolinense* and helpful impute to my work.

I am also a very lucky person because at Pitt I was able to meet my great friends: Tom Pendergast, April Randle, John Paul, Erika Zolotow, Cecilia Egan and Hugo Losada who have opened their houses, feed me well, motivated me, supported me, and filled my life with

warmness and joy. I will always be grateful to them and I hope that no matter where we end up our friendship will endure.

During these past four years I have also had immense support from ALL of my family. One of the most valuable lessons I have learned is that no matter where you go you will always have a home to go back to. Their calls, emails, skype and good energy kept me going and helped me to overcome many hard tasks. I would like to thank my mother in particular, because she has not only supported me emotionally and financially, but she kept fighting even when the battle seemed lost. She reminded me that I have her genes. I would also like to thanks Daniel, you have made me see the world with different eyes and have shown me that I am a wonderful woman that knows how to fly.

This is the end of an amazing journey through immense fields of flowers where I had to find the stars of my life. As I remember all of the experiences of this journey, I think of what the Costa Rican poet, Isaac Felipe Azofeifa said: “nunca se pone más oscuro que cuando va a amanecer”. Thru the darkness I have been lucky enough to have my family and my friends, but in particular I was able to enjoy the sunrise thanks to two very special ladies: my mother and Susan. To them I dedicate my work.

INTRODUCTION

Since Darwinian times, there has been great interest in determining the evolutionary effects of pollinators on plants and vice versa (Sakai and Weller, 1999). Consequently, pollinators have been identified as noteworthy selective agents on plant reproductive systems (Klinkhamer and de Jong, 1993; Harder and Barrett, 1996; Sato, 2002; Barrett, 2003; Kudo and Kasagi, 2004). Pollinators can determine the direction and magnitude of adaptive evolutionary change in the floral morphology (Neal, Dafni, and Giurfa, 1998; Schemske and Bradshaw Jr., 1999; Mazer and Meade, 2000; Boyd, 2004; Dohzono, Suzuki, and Murata, 2004; Fenster et al., 2004a; Irwin and Strauss, 2005; Kaczorowski, Gadener, and Holtsford, 2005). Fewer studies have identified pollinators as the main selective agent in the evolution and maintenance of a breeding system (Glover and Barrett, 1986; Harder, Barrett, and Cole, 2000; de Jong and Geritz, 2001; Williams et al., 2001; Dorken, Friedman, and Barrett, 2002; Pannell, 2002; Sato, 2002).

Andromonoecy is a breeding system where individual plants bear both hermaphrodite and male flowers (Bertin, 1982) and where pollinators could play an important role in the maintenance and evolution of the breeding system. In many andromonoecious species the main difference between the two flower morphs is the length of the style, with male flowers bearing reduced styles (Solomon, 1986; Emms, 1993b; Elle, 1998; Huang, 2003; Cuevas and Polito, 2004). Hypotheses to explain the evolution of this breeding system have focused on the advantages of having short-styled male flowers, and these include: 1) The Resource Allocation

Hypothesis (Bertin, 1982; Spalik, 1991; Elle, 1999); 2) The Pollen Donation Hypothesis (Bertin, 1982; Podolsky, 1992, 1993; Elle and Meagher, 2000; Huang, 2003) 3) Increased Pollen Receipt Hypothesis (Podolsky, 1993; Vallejo-Marin and Rausher, 2007b). The lack of clear support for any of these hypotheses in *S. carolinense* and other *Solanum* species, lead Diggle and Miller (2004) to propose that to understand the evolution of andromonoecy it was critical to determine how the style length differences between male and hermaphrodite flowers types affects pollinator efficiency (pollen removal and deposition).

Andromonoecy presents an ideal scenario to resolve if the expression and maintenance of this breeding system at the morphological and functional level is determined by the interaction with pollinators. The two main goals of my thesis are to: 1) to determine the functional relationship between flower morphology and pollination dynamics in *Solanum carolinense* and to assess whether this relationship varies with pollinator taxa and 2) to assess whether flower morphology is correlated with fruit setting ability different pollination treatments.

CHAPTER 1: FUNCTIONAL RELATIONSHIP BETWEEN FLOWER MORPHOLOGY AND POLLINATION DYNAMICS IN *SOLANUM CAROLINENSE*: THE IMPORTANCE OF DIFFERENT POLLINATORS

INTRODUCTION

There is a strong relationship between floral morphology and pollinators. The relationship arises because flowers are structures that have evolved to promote the transfer of plant gametes by animals (Dilcher, 2000). This relationship has been studied ever since Darwin and even today there is a great interest in understanding the effects of flower morphology on the pollinators behavior and vice versa (Sakai and Weller, 1999). Flower morphology dictates which pollinators can visit a flower and how efficient they are as pollen vectors (Fukuda, Susuki, and Murata, 2001; Fenster et al., 2004b). Conversely, pollinators have been identified as noteworthy selective agents that determine the direction and magnitude of adaptive evolutionary change in the floral morphology (Neal, Dafni, and Giurfa, 1998; Schemske and Bradshaw Jr., 1999; Mazer and Meade, 2000; Boyd, 2004; Dohzono, Suzuki, and Murata, 2004; Fenster et al., 2004a; Irwin and Strauss, 2005; Kaczorowski, Gadener, and Holtsford, 2005). I will explore these two ideas in the following paragraphs.

Changes in floral morphology that occur in primary sex organs (androecium and gynoecium) or in secondary sexual characters (inflorescence characters, corolla width, etc) can

directly influence the relationship with the pollinators and determine the dynamics of pollen removal and deposition (Cresswell, 2000; Fetscher, 2001; Fukuda, Susuki, and Murata, 2001; Cesaro et al., 2004). Because these traits directly affect plant fitness, some studies show there is strong selection on morphological traits to increase the efficiency of pollination (Harder and Barrett, 1995, 1996; Cresswell, 2000; Motten and Stone, 2000). More studies that determine the relationship between morphology and pollinator dynamics are needed because many species exhibit variation in their floral morphology and in most of them the evolutionary consequences of the variation are unknown.

It has been shown that the floral morphology of a species is determined by the traits that optimize the relationship with the most abundant or efficient pollinator (Armbruster, 1988; Schemske and Horvitz, 1989; Gomez, 2000; Sanchez-Lafuente et al., 2005). However, if pollinator species vary behaviorally and these behaviors result in different pollen deposition and removal (Glover and Barrett, 1986; Alves Dos Santos, 2002; Javorek, Mackenzie, and Vander Kloet, 2002; Cariveau et al., 2004), then variation could have important evolutionary consequences. In particular, the array of morphological phenotypes that exist may depend on the abundance and visitation rate of the pollinator taxa.

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 plants bear both male and hermaphrodite flowers. In many andromonoecious species the main difference between the two flower morphs is the length of the style, with male flowers bearing reduced styles (Solomon, 1986; Emms, 1993b; Elle, 1998; Huang, 2003; Cuevas and Polito, 2004). Hypotheses to explain the evolution of this breeding system have focused on the advantages of having male flowers, and these include: 1) The Resource Allocation Hypothesis (Bertin, 1982;

Spalik, 1991; Elle, 1999); 2) The Pollen Donation Hypothesis (Bertin, 1982; Podolsky, 1992, 1993; Elle and Meagher, 2000; Huang, 2003) 3) Increased Pollen Receipt Hypothesis (Podolsky, 1993; Vallejo-Marin and Rausher, 2007a). The resource allocation hypothesis proposes that the production of male flowers saves resources that can then be allocated to seed production. Interestingly, the last two hypotheses propose contrasting functions for male flowers. Specifically, the presence of male flowers can increase male fitness or female fitness because the interaction with the pollinators becomes more efficient for pollen deposition or receipt.

Many studies have used a weedy native species from the northeastern United States, *Solanum carolinense* horsenettle (Solanaceae) as the model organism to test these hypotheses (Elle, 1999; Elle and Meagher, 2000; Connolly and Anderson, 2003; Vallejo-Marin and Rausher, 2007a). To date, results of these studies find no support for the resource allocation hypothesis (Vallejo-Marin and Rausher, 2007a) and conflicting support for both the pollen donation hypothesis (Elle and Meagher, 2000; Vallejo-Marin and Rausher, 2007a), and the increased pollen receipt (i.e. one of two populations studied) (Vallejo-Marin and Rausher, 2007b). The lack of clear support for any of these hypotheses in *S. carolinense* and other *Solanum* species, lead Diggle and Miller (2004) to propose that to understand the evolution of andromonoecy it was critical to determine how the style length differences between male and hermaphrodite flowers types affects pollinator efficiency (pollen removal and deposition).

Style length differences could evolve to reduce female-male interference (Diggle and Miller, 2004). Female-male interference arises because hermaphrodite flowers have their sexual structures close together and when pollinators visit the flowers these structures can interfere with the pollen removal or deposition (Fetscher, 2001; Barrett, 2002a; Cesaro et al., 2004). In particular, Diggle and Miller (2004) suggest that the style interferes with pollen removal when

large bees that pollinate *Solanum* species grasp the anthers and vibrate them to extract the pollen. They did not, however, consider how this relationship might differ for small bees.

Here I present the first test of the female-male interference hypothesis in *S. carolinense*. I predict that reduced style length in male flowers allows bees to more easily remove pollen from these flowers. Thus, short-styled flowers are expected to exhibit greater male function. I also predict that long styled flowers will receive more outcross pollen because their styles will interfere with a pollinator's ability to extract pollen and reduce the likelihood of self-pollen deposition. Thus, long-styled flowers are expected to exhibit greater female function in the form of pollen receipt.

S. carolinense presents an ideal system to determine the functional relationship between flower morphology and pollination dynamics (i.e., pollen receipt/export) because natural populations of *S. carolinense* vary in their style length and stigma-anther distance. Also, *S. carolinense* and its pollinators are an excellent system to assess whether pollination dynamics varies with pollinator taxa since the species grows in diverse array of environments that vary in their pollinator fauna composition (A. Quesada unpublished data). Thus, the variation in style length and the expression of andromonoecy might be strongly related to the pollinator communities that visit *S. carolinense* and how they vary.

My main objective here is to determine the functional relationship between flower morphology and pollination dynamics in *Solanum carolinense* and to assess whether this relationship varies with pollinator taxa. My experiments focused on the following questions: 1) What is the level of phenotypic variation in floral traits (e.g., anther and style length, and stigma-anther distance) in *S. carolinense*? 2) Is there heritable variation in style length, anther length and stigma-anther distance in a wild population of *S. carolinense*? 3) What is the composition of the

pollinator pool in populations of *S. carolinense* in north-western Pennsylvania? Does the composition vary spatially and temporally? 4) Do bee taxa vary in their visitation rate? 5) Does style length affect the amount of pollen received or removed? 6) Do different bee taxa interact differently with the flowers? 7) Does the relationship between style length and pollination dynamics vary with taxa visiting the flower?

MATERIAL AND METHODS

Study plant *Solanum carolinense* (Solanaceae) is a rhizomatous perennial plant that is native to the northeastern United States and commonly grows in fields, roadsides and sandy stream banks (Rhoads and Block, 2000). *Solanum. carolinense* has white, lilac or purple star-shaped flowers with five yellow poricidal anthers (Rhoads and Block, 2000). Flowers mature acropetally, and in the greenhouse an inflorescence may contain up to 20 flowers (Travers, Mena-Ali, and Stephenson, 2004). In natural populations, however, inflorescences tend to have only one open flower at a time (A Quesada unpublished data). Like other species of the genus *Solanum,* basal flowers of *S.carolinense* tend to be larger than distal flowers (Diggle and Miller, 2004). In North America *S. carolinense* flowers from June to September and produces round orange to yellow berries that mature after the first frost (around October) and contain on average 160 seeds (Elle, 1999).

Solanum carolinense has been characterized as having an andromonoecious sexual system where individual plants bear both hermaphrodite and male flowers (Bertin 1982). In *S. carolinense* (Solomon, 1986; Elle, 1998, 1999; Elle and Meagher, 2000; Vallejo-Marin and Rausher, 2007a) and many other andromonoecious species the main difference between the

hermaphrodite and male flowers is the length of the style (long vs. short respectively) (Solomon, 1986; Emms, 1993a; Elle, 1998; Huang, 2003; Cuevas and Polito, 2004). Male flowers are assumed to lack female function (Solomon, 1985), but in northwestern PA populations, some short styled flowers are able to produce fruit (see Chapter 2). Moreover, some hermaphrodite flowers are functionally male (Connolly and Anderson, 2003; see Chapter 2). At the plant level, not all individuals produce both floral morphs (Elle, 1998; Elle and Meagher, 2000). In natural populations in northwestern PA, 67% of the inflorescence survey had only hermaphrodite flowers, 23% had only male flowers and 9% had both hermaphrodite and male flowers.

Pollinators *Solanum carolinense* is visited by pollen-gathering bees, many of which vibrate the flowers to remove pollen from anthers (Hardin et al., 1972). Bumblebees are described as the main pollinator of this species (Travers, Mena-Ali, and Stephenson, 2004; Vallejo-Marin and Rausher, 2007a), but Connolly and Anderson (2003) also observed *Lassioglosum* spp. visiting the flowers in Connecticut. In northwestern Pennsylvania populations, I observed three bee taxa visiting flowers of *S. carolinense* 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 my experiments and to estimate heritability of flowering traits, three mature fruits from open pollinated flowers were collected from one hundred and seven *S. carolinense* plants in a population located on property owned by the Ernst Seed Company in Crawford County PA (N 41° 58”9398’ W 80° 15”3652’). Plants were growing in an open rocky area surrounded by native species. To ensure that plants were distinct genets, all plants chosen were separated by at least four meters. The fruits were

taken to the laboratory; seeds were removed from the pulp, and rinsed with 2% bleach to prevent fungal growth. Dry seeds from the three fruits per plant were combined. One hundred and seven half-sibling families were used in this study. Halfsib families of seeds were then stored in manila envelopes at room temperature for three months. Thirty randomly selected seeds from each family were placed in individual Petri dishes on moistened filter paper. Seeds were germinated in a growth chamber at the University of Pittsburgh through January 2005. Chamber conditions were 30°C 16 hour days/ 20° C 8 hour nights. Petri dishes were watered as needed. Once 80% of the seeds had germinated (2-3 weeks), five siblings per family were randomly selected, transplanted into 48 well flats filled with Fafard # 4 soil (Conrad Fafard Inc, Agwan, MA), and kept in the growth chamber until March 2005. Plants were watered every two days or as needed. In mid-March seedlings were transplanted into 11.4 cm black press fit pots (Nursery Supplies) filled with Fafard #4 soil and moved into the greenhouse. In May 2005 these plants were transported to the Pymatuning Laboratory of Ecology, Crawford County PA and placed in a pollinator-free hoop house covered with white 50% light shade-cloth. In June the plants were transplanted into 3.8 liter pots (Nursery Supplies Classic 300) in Farfard # 4 soil. Plants were water once or twice a day as needed. During the summer, these plants were fertilized each week with 100 ppm (1.26 g/L) of high phosphorus fertilizer (i.e., 15 N/ 30 P/ 15 K) to promote flowering.

Plants remained outdoors during the winter of 2005 under a layer of straw. In the spring of 2006, I planted the 286 surviving plants directly in the ground at a randomly chosen location in a grid 30 cm apart and enclosed by the pollinator-free hoop house. These plants were fertilized every week from June to August. In both 2005 and 2006 plants from the 107 open pollinated sibships started flowering in late June. The flowering time of the plants in the greenhouse was

synchronous with the flowering time of the plants in wild population in northwestern Pennsylvania (A Quesada-Aguilar pers. obs). Flowering plants were randomly assigned to different experiments described below.

Experiments

1) What is the level of phenotypic variation in floral traits (anther and style length, and stigma-anther distance) in S. carolinense?

To characterize the level of variation in floral traits, I measured 1,671 flowers produced by the 107 sibships. Specifically, I measured style length (from the base of style to the stigma), anther length (base of the anthers to the tip of the tallest anther), and corolla width 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, my measure reflects the functional style length rather than the actual style length. Stigma-anther distance was calculated for each flower by subtracting the anther length from the style length for each flower. I made all floral measurements either on the first or second day after the flower had opened to control for the age of the flower. In cases when the exact date that the flower had opened was unknown, light colored flowers were used because flower color is a good indicator of flower age. In 2005 I recorded flower color and age for 148 flowers and determined that flower color becomes progressively darker with age (r = 0.21; n = 148, p=0.01); flowers progress in color from either from white flowers to lilac to purple or from lilac to purple to dark purple with time.

2) Is there heritable variation in the style length, anther length and stigma-anther distance in a wild population of S. carolinense?

To estimate the broad sense heritability of style length, anther length and stigma-anther distance for long-styled flowers, I measured these traits on at least two flowers for all siblings that flowered either in 2005 or 2006. I obtained data from 1349 flowers on 259 plants belonging to 86 sibships (range 2-5 siblings/sibship). I used average flower measurements per sibling to calculate the mean squares using PROC GLM (SAS Institute Inc 1999-2001). Mean square was determined by ANOVA (Model: Var (error) + 3.0082 (Var (sibship)). I calculated variance estimates, broad sense heritabilities h_b^2 and standard error of h_b^2 using the formulas for unbalanced experimental design in Lynch and Walsh (1998). I determined if the broad sense heritability was different than zero using the formula for two tailed t-test in Zar (1996). Because only 65 siblings produced male flowers I only calculated h_b^2 for style length, stigma-anther distance and anther length in hermaphroditic flowers.

3) What is the composition of the pollinator pool in populations of S. carolinense in northwestern Pennsylvania? Does the composition vary spatially and temporally?

In 2004 and 2005 I conducted pollinator observations in two natural populations of *S. carolinense*. In 2004, I observed pollinators once a week from the 11 July to 28 July at the Ernst Seed Conservation (EC) property and from 29 July to 9 August in a population located at the Beagle road property (BR) of the Pymatuning Laboratory of Ecology (PLE) Crawford County, PA. In 2005 I re-visited the BR population and conducted observation from 20 July to 2 August

and a population growing on the Livingston Road Farm property (FA) of PLE from 7 August to 25 August.

In 2004 and 2005, on sunny days, I selected areas in each population where there were groups of flowering *S. carolinense* plants. I then recorded the number and the morph of all open flowers per plant in the sampling area. Each flower was numbered uniquely. I observed the area for 15 min. (2004) or 30 min. (2005) and recorded the identity of every bee that contacted a flower. For each bee visit I recorded the flower number and the number of bee contact events. I define a contact event occurring when a bee contacts the anthers and extracts pollen. In total, I conducted 17.25 hours of pollinator observations.

I calculated the proportional bee abundance for each population as: (total number of visits of each bee species)/ (total number of visits by all bees observed). To determine if the abundance varied spatially I compared the bee abundances between EC 2004 and BR 2004 and between BR 2005 and FA 2005 using a chi-square (PROC FREQ in SAS). To determine of the abundance varied temporally I compared BR 2004 and BR 2005 using a χ^2 (PROC FREQ in SAS).

4) Do bee taxa vary in their visitation rate?

I calculated the visitation rate for each pollinator taxa in a given observation time as: ((# contact events per pollinator species/ # flowers observed)/ time observed)). I then used ANOVA (PROC GLM) to determine whether visitation rate varied among the pollinator taxa. I considered each population: EC 2004, BR 2004, BR 2005 and FA 2005 as replicates to avoid confounding results spatially or temporally. I also assessed whether there was an interaction between replicate

and bee species (Model: $(\text{Visitation rate})_{ij} = \mu + \text{replicate}_i$ bee species$_j$ + (replicate x bee species)$_{ij}$).

5) Does style length affect the amount of pollen received or removed? 6) Do bee taxa interact differently with the flowers? 7) Does the relationship between style length and pollination dynamics vary with taxa visiting the flower?

To answer these questions, I used the general approach of presenting individual flowers of similar age and development stage but different style lengths to pollinators in a natural population during the summers of 2005 and 2006. To control for flowerl age and developmental stage, I used only flowers that were one or two days post anthesis. To assess the relationship of flower age with stigma receptivity or pollen availability I conducted two experiments. First, I hand outcross pollinated flowers that were one, two, three or four days post anthesis (sample sizes= 56, 56, 59, and 48 flowers respectively). Flower age did not affect fruit set ($\chi^2 = 5.17$, $n = 115$, $df = 3$, $p = 0.16$). Second, I determined the timing of pollen availability by vibrating the anthers and extracting pollen from flowers that were one, two, three or four days post anthesis (sample sizes= 31, 32, 30, and 16 flowers respectively). Only one first day flower did not release pollen. Thus my results show that stigmas are receptive and pollen is available regardless of the flower age and are expected to be attractive to pollinators.

Style length and pollen deposition and removal

To determine if style length affects the amount of pollen deposited on the stigma or removed from the anthers, paired flowers of equal style length were collected from the same plant. In total 142 observations where made on 51 pairs in 2005 and 91 pairs in 2006. These 142 pairs came from 112 siblings belonging to 69 sibships. Individual flowers were placed in Aquapics^{TM} and non-destructive floral measurements were performed. I placed the flowers in Aquapics, stored them in a cooler and took them to the BR field where pollinator observations where made. This old-field has a natural population of *S. carolinense* of ~100 plants (A. Quesada, pers. obs). In the field, one of the flowers of a pair was placed in an area where pollinators had access (Open). The other flower was kept as an unpollinated control in the cooler, but received the same manipulations as the Open treatment to account for any self-pollination that might occur during handling.

Observations were conducted from the 22 June to the 18 of August 2005 and on from the 29 of June to 1 of August 6. On most days, observations started at 9 a.m. and ended at 6 p.m. After the Open treatment flower was visited by a bee I returned it to the cooler. At the end of each day, I collected both flowers (Open and Control) and preserved them in ethanol.

Flowers were then taken to the laboratory where I 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 acetolysis technique of Kearns and Inouye (1993). After digestion, the samples were collected by centrifugation. I suspended the stigma pellets and anther pellets in 100 μL or 300 μL of distilled water, respectively. I determined the number of pollen grains deposited on stigmas or remaining

in the anthers by counting 10 μL sub-samples of each sample in a haemocytometer. All of the squares in the grid were counted (9 μL^3) for the stigma samples while the four corner squares in the grid were counted for anthers samples (4 μL^3). I counted four to six subsamples of each stigma or anther sample. Because not all the pollen precipitated after centrifugation in the anther samples, I counted four to six sub-samples of the 570 μL of remaining supernant. I counted all of the squares in the grid for the anther supernatant (9 μL). The lowest number of pollen grains that I could count using this technique was 27 pollen grains.

I used stigmas from Control flowers to quantify the amount of self-pollen that was deposited on the stigma due to handling and to correct for this as a source of pollen deposition in Open flowers. I corrected my estimates of the pollen deposited on open flower by subtraction: Bee mediated pollen deposition on Open flowers = (# pollen grains on Open flower stigma) - (# pollen grains on the Control flower stigma).

In total of the 142 flower pairs were used, in 2005 46 pairs were long styled and 4 were short styled. In 2006 40 pairs were long styled and 51 were short styled. Most of the pairs were collected from different siblings, however in 18 siblings two pairs were analyzed and in 4 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: (#pollen grains in Control anther) - (# pollen grains counted in Open anther)/ (#pollen grains in Control anther). Data for anther removal was obtained for 112 pairs of flowers collected from 92 siblings that belonged to 61 families. I had to discard 30 pairs of this experiment because the controls were not appropriate. Out of these 112 observations 41 were done in 2005 and 69 were done in 2006. In 2005 39 pairs were long styled flowers and 4 were

short styled. In 2006 32 observations were in long styled flowers and 37 in short styled. Most of the pairs were collected from different siblings; however in 13 siblings two pairs of flowers were collected and in three siblings 3 pairs were collected. In many of these cases, multiple pairs of flowers were collected from the sibling because they produced both flower morphs.

Interaction of pollinator taxa with the flower

To determine how the pollinator taxa interacted with the flowers, pollinators that visited the Open flowers were recorded using a digital video camera (Sony Handycam DCR DVD 101). Only one pollinator was allowed to visit each Open flower, and the recording started when the pollinator contacted the flower on the Aquapic. Once the pollinator left, Open flowers were collected as mentioned above. I then analyzed the videotapes in the laboratory. For each video I recorded the bee taxa, the number of contact events per visit and the length of each contact event in seconds. I define total contact time per visit as the sum of all the contact events per visit. I define the mean contact time as (total contact time per visit)/ (number of contact events). In the case of bumblebee visitors, I also recorded if the stigma touched the bee's corbiculae. The corbiculae is a basket like structure in the hind legs of bees from the Apidae family (Roubik, 1992).

Data analysis

Style length and pollen received or removed

I used a two step approach to determine the relationship between style length and pollen deposition. First, I used logistic regression (PROC LOGISTIC) to determine if style length affects the probability of receiving pollen (i.e,. yes=1, no=0) after a single visit by any bee species. Afterwards, 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 amount of pollen deposited and style length a multiple regression with style length, style length^2, stigma width (mm), anther length (mm), corolla length (mm), number of contact events, and total contact time was performed using PROC REG. Six flowers were excluded from the analysis because I did not have stigma measurements. Preliminary analyses revealed that anther length (mm), corolla length (mm) and total contact time were not significant and were eliminated from the final analysis. The final model tested was: Log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length^2, β_3 stigma width + β_4 # contact events.

To determine the relationship between proportion of pollen removed and style length, I analyzed the data using a multiple regression with style length^2, number of contact events, anther length, corolla length, stigma width and total contact as the dependent variables using PROC REG. Preliminary analysis, similar to those conducted for pollen deposition, revealed that style length^2, anther length, corolla length, stigma width and total contact time were not significant

and I removed them from the model. The final model tested was: Proportion of pollen removed = $\alpha + \beta_1$ style length + β_2 # contact events.

Interaction of pollinator taxa with the flower

I recorded three genera of bees visiting the flowers placed in Aquapics^{TM} in 2005 and 2006: *A. metallica*, *Lassioglossum* spp, and *B. impatiens*. Pollinators varied temporally, with *A. metallica* and *Lassioglossum* spp. being more abundant early (June) and late (August) in the flowering season, while *B. impatiens* visited flowers mainly during July (A.Quesada per obs). In 2005, I recorded 52 bees on video: 1 *A. metallica*, 13 *Lassioglossum spp.* and 38 *B. impatines*. In 2006, I recorded 98 bees on video: 22 *A. metallica*, 19 *Lassioglossum* spp., and 57 *B. impatiens*. The most number of visits recorded in one single day were 5 in 2005 and 17 in 2006.

I determined differences between the pollinator taxa in the number of contact events (contact events= $\mu_{\text{bee spp}} + \varepsilon_{ij}$), mean time per contact event (mean time= $\mu_{\text{bee spp}} + \varepsilon_{ij}$), and the total contact time per visit total contact time = $\mu_{\text{bee spp}} + \varepsilon_{ij}$) via ANOVA using PROC GLM. Post hoc Bonferroni test was used to determine if the averages were different among the three species. I also compared the number of contact events on short styled flowers and the number of contact event on long styled flowers for each pollinator taxa via t-test (PROC TTEST).

Style length and pollination dynamics by taxa

I calculated the average number of pollen grains deposited and removed by bee and compared them via ANOVA (PROC GLM). To determine whether the functional relationship

between style length and pollination varied with pollinator taxa, I analyzed the data separately for each pollinator taxa that visited the flowers using PROC REG (Model: Log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length^2, β_3 stigma width + β_4 # contact events). In the case of bumblebees, I conducted a logistic regression to determine the relationship between the style length and the probability of touching the corbiculae using PROC LOGISTIC. I determined the difference in the mean number of pollen grains deposited between styles that did or did not touch the corbiculae using PROC TTEST. I also analyzed data for pollen removal separately for each pollinator species that visited the flowers via multiple regression (PROC REG Model: Proportion of pollen removed = $\alpha + \beta_1$ style length + β_2 # contact events).

Preliminary statistical analysis suggests that the small bees (*A..metallica* and *Lassioglossum* spp) have similar effects on pollen deposition ($p = 0.99$) and removal ($p = 0.83$). Because of this and due to the low frequency of these small bees in the data set, I pooled data from *A. metallica* and *Lassioglossum* spp.. I compared the slopes for style length and pollen deposition of these two groups via ANCOVA (PROC GLM Model: Log_{10} (number of pollen grains deposited) = $\alpha + \beta_1$ style length + β_2 style length^2 + β_3 stigma width + β_4 # contact events + β_5 pollinator + β_6 (pollinator x style length) + β_7 (pollinator x contact events)). I also used ANCOVA to compare the slopes for pollen removal (PROC GLM Model: Proportion of pollen removed) = $\alpha + \beta_1$ style length + β_2 # contact events + β_3 pollinator + β_3 (pollinator x style length) + β_5 (pollinator x contact events)).

RESULTS

1) What is the level of phenotypic variation in floral traits (e.g., anther and style length, and stigma-anther distance) in S. carolinense? 2) Is there heritable variation in the style length, anther length and stigma-anther distance in a wild population of S. carolinense?

In 2005 and 2006 siblings from the 107 sibships started flowering in late June. Flowers in these plants had corolla widths and anther lengths that are monomorphic (Fig 1A and Fig 1B). The average anther length was 8.92 ± 1.01 mm and the average corolla was 31.44 ± 5.03 mm. Style length in these flowers has a continuous distribution with two peaks (Fig 1C). These peaks indicate that two flowers morphs can be identified: long styled flowers and short styled flowers. The main difference between the two flower morphs is in the size of the gynoecium, thus variation in style length translates into a variation in stigma-anther distance (Fig 1D). The pattern observed for stigma-anther distance is similar to style length; two distinct types can be identified. However, there are some flowers where the style and the anthers have similar lengths and cannot be easily classified in either type.

The broad sense heritabilities for style length and stigma anther distance for long styled flowers were large and significant for both traits. The broad sense heritability for style length was 0.63 ± 0.28 and was different from zero (*t-test*= 2.30, *d.f.* = 85, $0.02 < p < 0.05$). The broad sense heritability for stigma anther distance was 0.89 ± 0.28 and was different from zero (*t-test*= 3.19, *d.f.*= 85, $0.002 < p < 0.005$). The broad sense heritability for anther length was 0.49 ± 0.27 and was not different form zero (*t-test*= 1.80, *d.f.* = 85, $0.05 < p < 0.10$).

3) What is the composition of the pollinator pool in populations of S. carolinense in north western Pennsylvania? Does the composition vary spatially and temporally?

I observed three genera of bees visiting flowers of *S. carolinense* during the summers of 2004 and 2005: *Lassioglossum* spp Halictidae (sweat bees), *Augochloropsis metallica* Halictidae (sweat bees) and *Bombus impatiens* Apidae (bumblebee). *A. metallica* has not been previously described as pollinator of *S. carolinense*. I observed 21 bees in the EC 2004 population, 9 bees in the BR 2004 population, 40 bees in the BR 2005 population and 17 bees in the FA 2005 population. Due to the small sample size in BR 2004 and FA 2005, chi-square tests should be evaluated with respect to the qualitative aspects instead of the quantitative aspects; and p values should be taken with caution.

The abundance of each bee species differed between the populations studied in 2004, i.e. EC vs BR ($\chi^2 = 10.83$, $d.f. = 2$, $p < 0.005$) In 2004, in EC the most abundant bee was *A. metallica* and 71% of the visits were from this species (Fig 2); 24% of all visits were from *Lassioglossum* spp. I only observed one *B. impatiens* visiting the flowers in the EC population. In contrast, in the BR population 56% of all visits were from *B. impatiens* (Fig. 2). I 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 ($\chi^2 = 0.080$, $d.f. = 2$, $p > 0.9$). In both populations 82% of the visits were from *B. impatiens* and 1% were from *Lassioglossum* spp. I observed very few *A. metallica* in 2005, only recording 1 individual in the FA population and 3 in the BR population. There was no significance temporal difference (2004 vs. 2005) in the bee composition of BR ($\chi^2 = 3.14$, $d.f. = 2$, $p > 0.2$).

4) Do bee taxa vary in their visitation rate?

In general, the visitation rate for the three bee species was low and showed high levels of variation (Fig 3). Visitation rate was significantly different among the different bee species (Table 1). I also found that there is a significant interaction between the replicate and pollinator (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). *A. metallica* had a higher visitation rate than *Lassioglossum* spp. in 2004 but in 2005 the visitation rate for these two species was similar.

5) Does style length affect the amount of pollen received or removed?

The probability of receiving pollen significantly increases as the style length increases when considering all the pollinator taxa (pollen= 0.30 style length – 1.72, n= 141, Wald χ^2 = 38.76, $p < 0.0001$; Fig. 4). The odds that long styled flowers receive pollen are 35% greater than short styled flowers. Of the 141 flowers I used in the analysis, 93 flowers received pollen and of these 90% were classified as long styled flowers. Interestingly, most of the long styled flowers that did not receive pollen were visited by either *Lassioglossum* spp. or *A. metallica* (Fig. 4). In contrast, of the 48 flowers that did not receive pollen 74% were short styled flowers. These flowers that did not receive pollen were not visited by any particular pollinator taxa.

When considering all bees together, in flowers that do receive pollen the number of pollen grains deposited significantly increases with the style length, the style^2 length and the number of contact event, but not with stigma width (Table 2A). On average flowers with styles

that are 10 to 15 mm receive 10 times more pollen than flowers that have either very short or very long styles (Fig. 5). Flowers with styles shorter than 9 mm receive 116 ± 34 pollen grains, flowers with styles that are 10 to 15 mm receive 1496 ± 267 pollen grains and flowers whose styles are above 15 mm receive 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.

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 I account for contact events, style length is significantly negatively correlated with the proportion of pollen removed (Table 2A). In flowers whose styles are shorter than 8 mm the average amount of pollen removed was 39, 268 ± 4,419. A smaller amount was removed from flowers with styles longer than 8 mm. On average 32, 923 ± 3,576 pollen grains were removed from these flowers. The general pattern for pollen removal seems to be that more pollen is removed from short styled flowers than from long styled flowers.

6) Do different bee taxa interact differently with the flowers?

These three bee species interact significantly differently (all $p < 0.001$) with the flowers of *S. carolinense* with respect to number of contact events, average time per contact event, and total contact time. *A. metallica* usually contacts 2 or 3 times the flower, *Lassioglossum* spp. have the lowest number of contact events, and *B. impatiens* contact on average 4 times the flower (Fig 6A). *A. metallica* spend on average 41 seconds per contact event, *Lassioglossum* spp. spend the longest time per contact event (~ 45 sec.), *B. impatiens* spend only 7 sec per contact event (Fig. 6B).

A. metallica bees are able to buzz the anthers but have to groom the tip of the anthers to collect the pollen and spend ~ 91 seconds in contact with the flower in each visit (Fig. 6C). *Lassioglossum* spp usually visit the flower once and leave after collecting the pollen. Interestingly, their average contact time is higher *Lassioglossum* spp. than for *A. metallica* but the total time spent on the flowers is less due to fewer contact events. *B. impatiens* are the visitors that spend the least amount of time in contact with the flower (~ 30 sec.) (Fig. 6C). *B. impatiens* vigorously vibrate the anthers (buzz pollination) and exhibit typical Apidae behavior where the bee contacts the flower (contact event), then separates and grooms the pollen to determine the quality of the flowers and then contacts the flower again (Pellmyr, 1986). Pollen is collected and compressed into the bee's corbiculae.

There is no significant difference between the number of contact events between short styled flowers and long styled flowers when the flowers were visited by either *A. metallica* (*t-test* = 0.32, *d.f.* = 20, $p = 0.75$) or *Lassioglossum* spp. (*t-test* = -0.47, *d.f.* = 29, $p = 0.64$). There is a significant difference in the number of contact events between the short styled flowers and long styled flowers (*t-test* = 2.14, *d.f.* = 89, $p = 0.04$). On average *B. impatiens* contacts short style flowers three times and long styled flowers five times.

7) Does the relationship between style length and pollination dynamics vary with taxa visiting the flower?

All bee species visiting *S. carolinense* are effective pollinators because they both deposit and remove pollen. However, the magnitude of pollen deposition varies among the bees species ($F = 6.84$, *d.f.*= 2, $p < 0.001$). On average in a single visit, *A. metallica* deposit 97 ± 41,

Lassioglossum spp. deposit 87 ± 28, and *B. impatiens* deposit 1,153 ± 218 pollen grains on the stigma The magnitude of pollen removal also varies among the bees species (F= 6.6, $d.f.$= 2, p =0.002). On average in a single visit, *A. metallica* removed 17,454 ± 3,972 *Lassioglossum* spp. 26,027 ± 8,199, and *B. impatiens* removed 41,766 ± 3,084 pollen grains from the anthers.

The ANCOVA model for pollen deposition was highly significant and it explains 62% of the variance observed (F= 15.91, n= 87, $p < 0.0001$, $R^2 = 0.62$). Again across all bee species I found a positive relationship between style length and pollen deposition (F= 23.88, $d.f.$= 1, $p <$ 0.0001), and negative quadratic relationship between style length and pollen deposition (F= 18.35, $d.f.$= 1, $p < 0.0001$). I find a difference in the amount deposited by each bee taxa (F= 3.88, $d.f.$= 1, $p = 0.05$). In this model there is a significant difference between the slopes for style length and pollen deposition of the two bee types (F= 7.09, $d.f.$= 1, $p = 0.01$) and between the slopes for style length^2 and pollen deposition (F= 5.06, $d.f.$= 1, $p = 0.03$). I also found that the was a significant interaction between the bee species and the number of contact events (F= 4.68, $d.f.$= 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 parameter that was significant was the number of contact events (F= 6.33, $d.f.$ = 1, $p = 0.013$). There was not significant interaction between the bee species and the style length, which means that the slopes for style and pollen removal are not different between the two bee types (F= 0.94, $d.f.$= 1, $p = 0.33$).

No morphological or behavioral traits seem to influence the amount of pollen deposited by *A. metalllica* or *Lassioglossum* spp bees (Table 2C). The positive relationship between style length and the amount pollen deposited is significant only for *B. impatiens* (Table 2B, Fig. 7).

This relationship is also strongly influenced by the number of contact events that the bumblebee has with the flower (Table 2B). The positive relationship between style length and pollen deposition is due to long styles having a higher probability of touching the *B. impatiens* corbiculae (pollen= 0.35 style length – 3.84, n= 87, Wald χ^2 = 19.39, $p < 0.0001$). The odds of touching the bees' corbiculae are 41% greater for long styled flowers than for short styled flowers. Flowers with extremely long styles (longer than 15 mm) usually do not contact either the bee's body or the corbiculae. These basket-like structures carry a large amount of pollen and when a stigma contacts this structure it will receive around eight times more pollen, whereas, when stigmas do not touch the corbiculae the stigma receives ~400 pollen grains and when they contact the stigma the number increases to more than 2500 pollen grains (*t-test*= -4.98, *d.f.* = 85, $p < 0.0001$; Fig. 9).

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

TABLES

Table 1. ANOVA of visitation rate (contact event/flower/minute) for three pollinators (*B. impatiens, A. metallica,* and *Lassioglossum* spp.). Replicate represents four population-year combinations (EC2004, BR2004, BR2005, FA2005). Model: $F = 4.74$, $n = 144$, $p < 0.0001$, $R^2 = 0.28$.

Source	D.F.	F	P
Visitation rate			
Replicate	3	2.29	0.0815
Pollinator	2	10.59	< 0.0001
Replicate x Pollinator	6	5.82	< 0.0001

Table 2. Multiple regression analyses of the pollen deposited on stigmas and of the proportion of pollen removed from the anthers with morphological variables (Style length, stigma width) and bee behavior (contact events) for A) all bees, B) *B. impatiens,* and C) *A. metallica* and *Lassioglossum* spp.

	A) All Bees		B) *Bombus impatiens*		C) Halictids (*A. metallica* and *Lassioglossum* spp)	
Source of variation	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
# Contact events (mm)	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 *** n= 87, R^2 = 0.41	F= 23.24 *** n= 112 R^2 = 0.30	F=16.98*** n= 56 R^2 = 0.57	F= 12.51*** n= 73 R^2 = 0.26	F= 0.78 n= 30 R^2 = 0.11	F= 0.36 n= 38 R^2 = 0.02

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

FIGURES

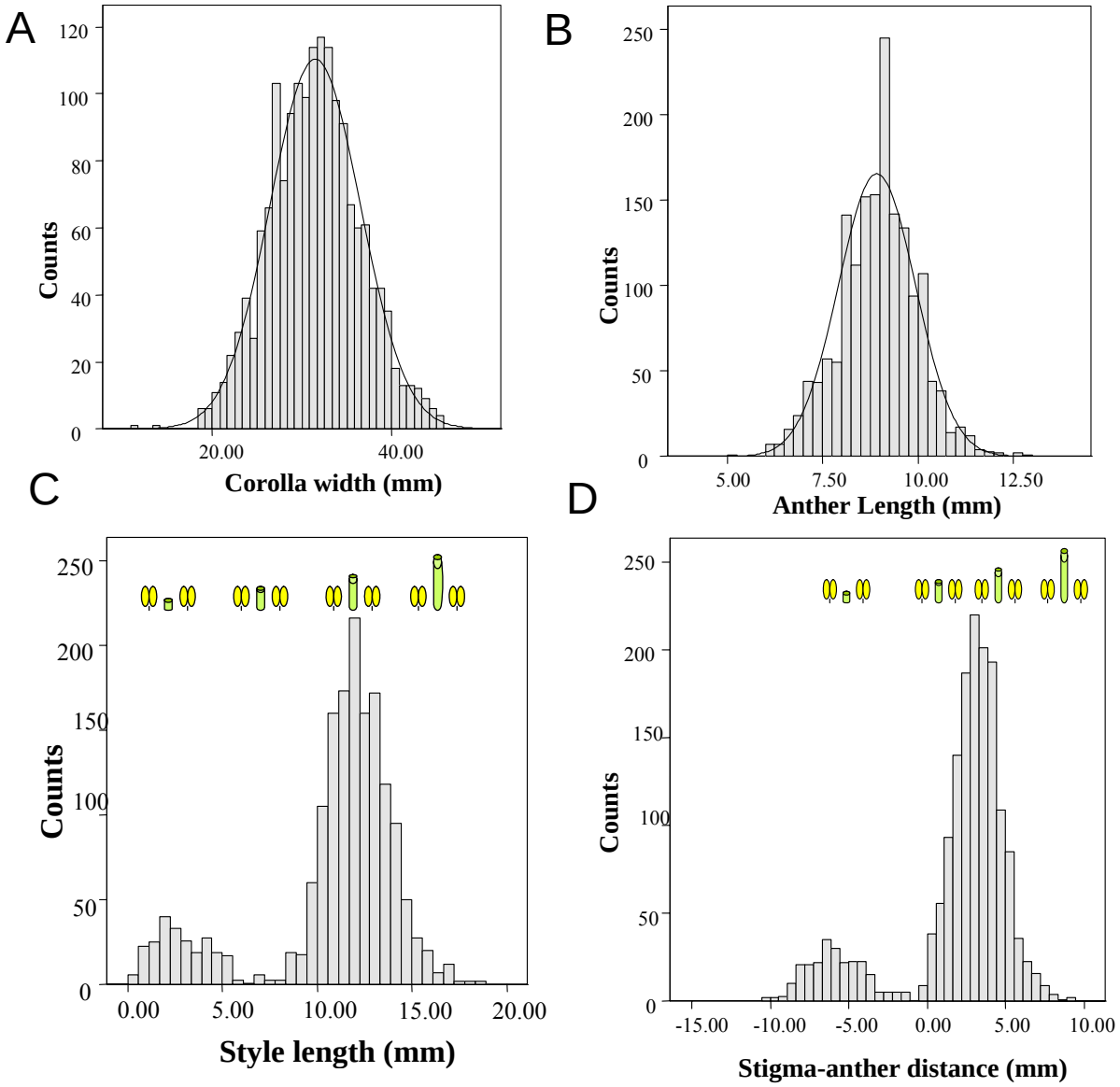

Figure 1. Histogram of: A. corolla width (mm), B. anther length (mm), C. style length (mm) and D. stigma-anther distance (mm) of 1 671 flowers measured from 107 families grown during 2005 and 2006 in the greenhouse at Pymatuning Laboratory of Ecology. Top inserted in graphs C and D are a stylized representation of the flower morphology. Anther length is kept constant to demonstrate how variation in style length affects stigma-anther distance.

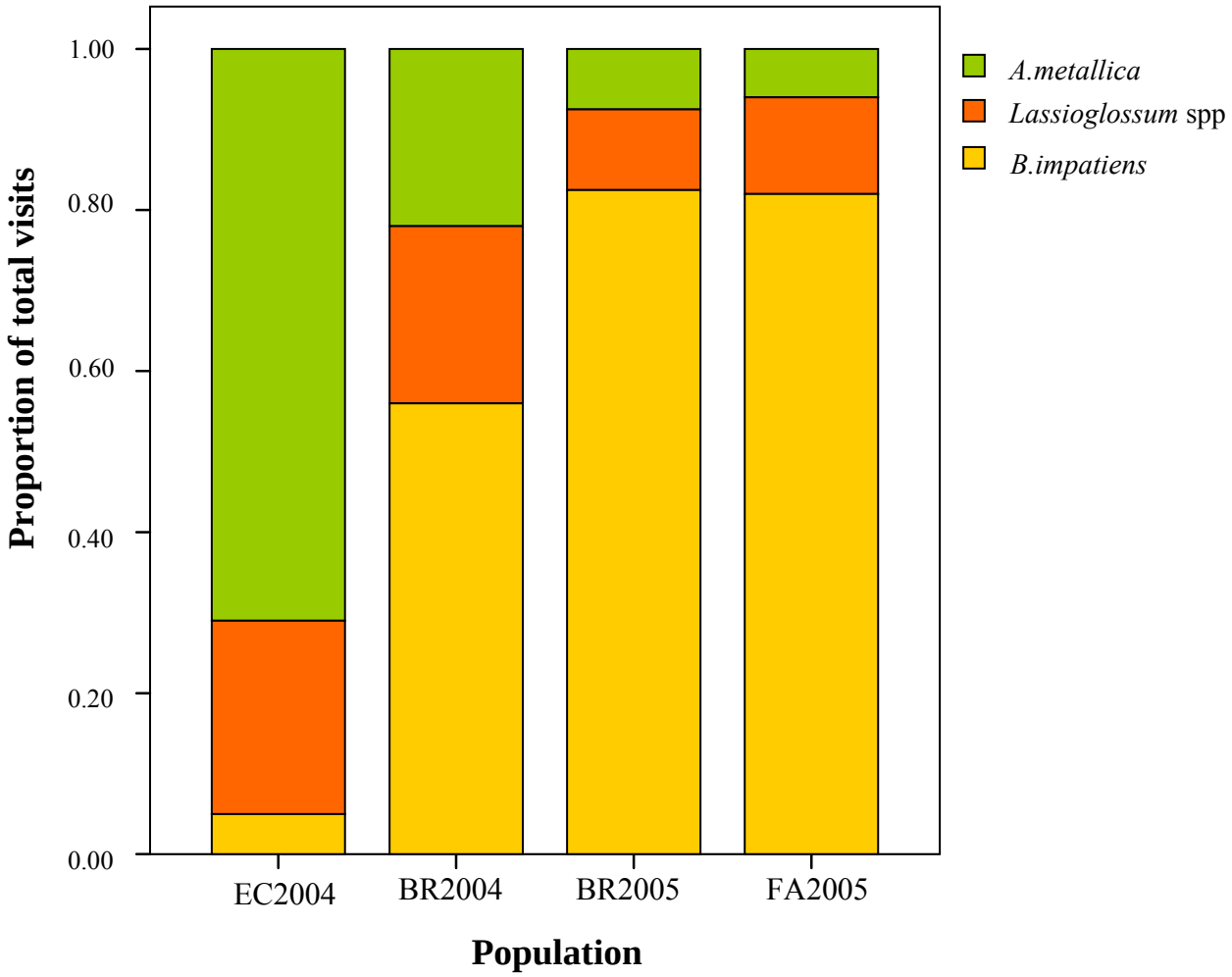

Figure 2. Proportion of total pollinator visits done by *A. metallica, Lassioglossum,* or *B. impatiens* in three populations in Crawford County, PA: Ernst Seed Conservation (2004), Beagle Road Property (2004, 2005) and Livingston Road Farm (2005).

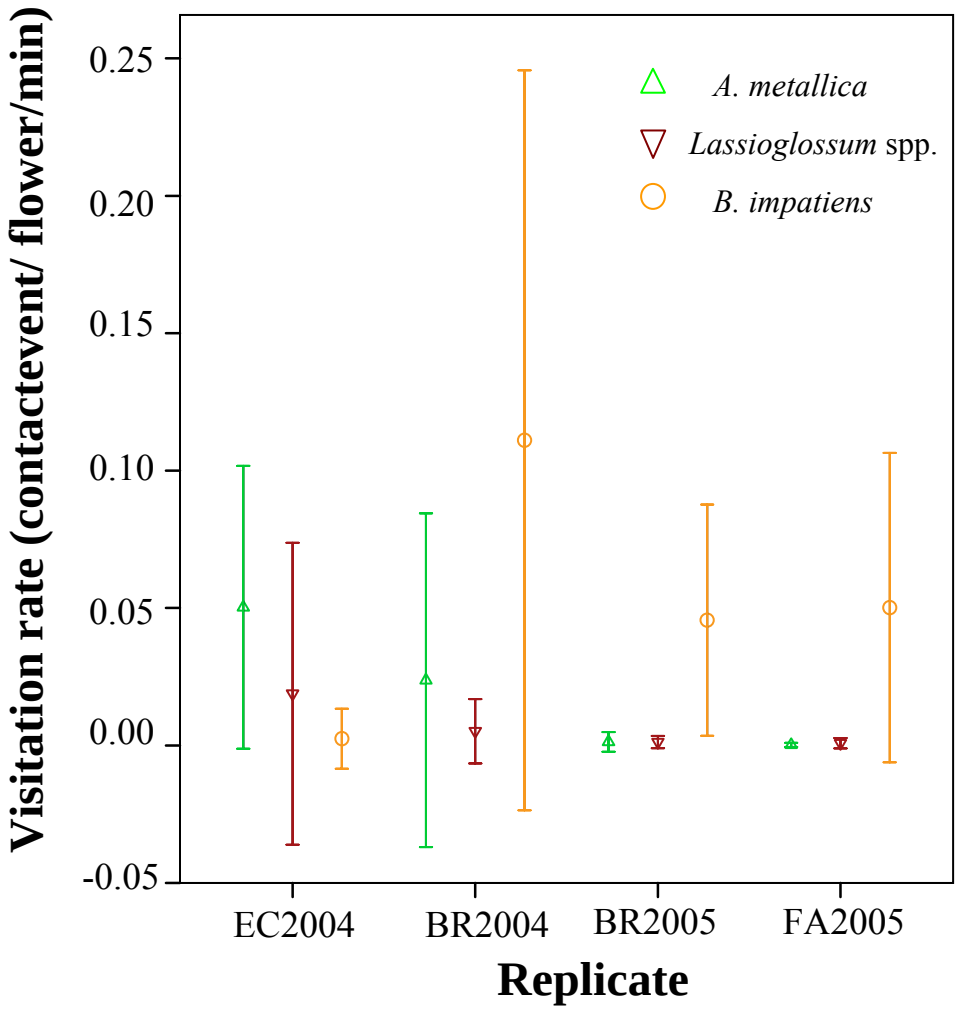

Figure 3. Visitation rate (contact event/flowers/time) per bee species for *A. metallica* (▲) *Lassioglossum* spp. (▼), and *B. impatiens* (●) in three populations in Crawford County, PA: Ernst Seed Conservation (EC 2004), Beagle Road Property (BR 2004, BR 2005) and Livingston Road Farm (FA 2005). Means ± 1.0 SE.

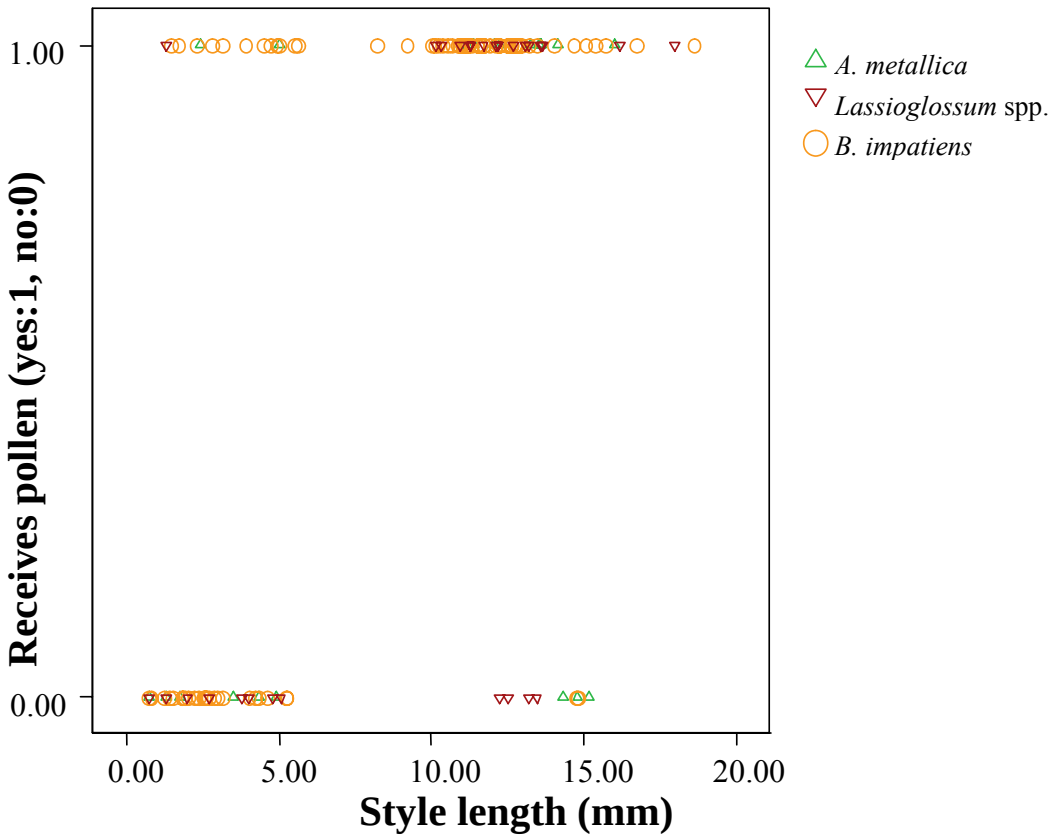

Figure 4. Relationship between the style length (mm) and probability of receiving pollen when visited by three bee species: *A. metallica* (▲) *Lassioglossum* spp. (▼), and *B. impatiens* (●). Logistic regression model: pollen= 0.30 style length – 1.72, n= 141, Wald x^2 = 38.76, p < 0.0001.

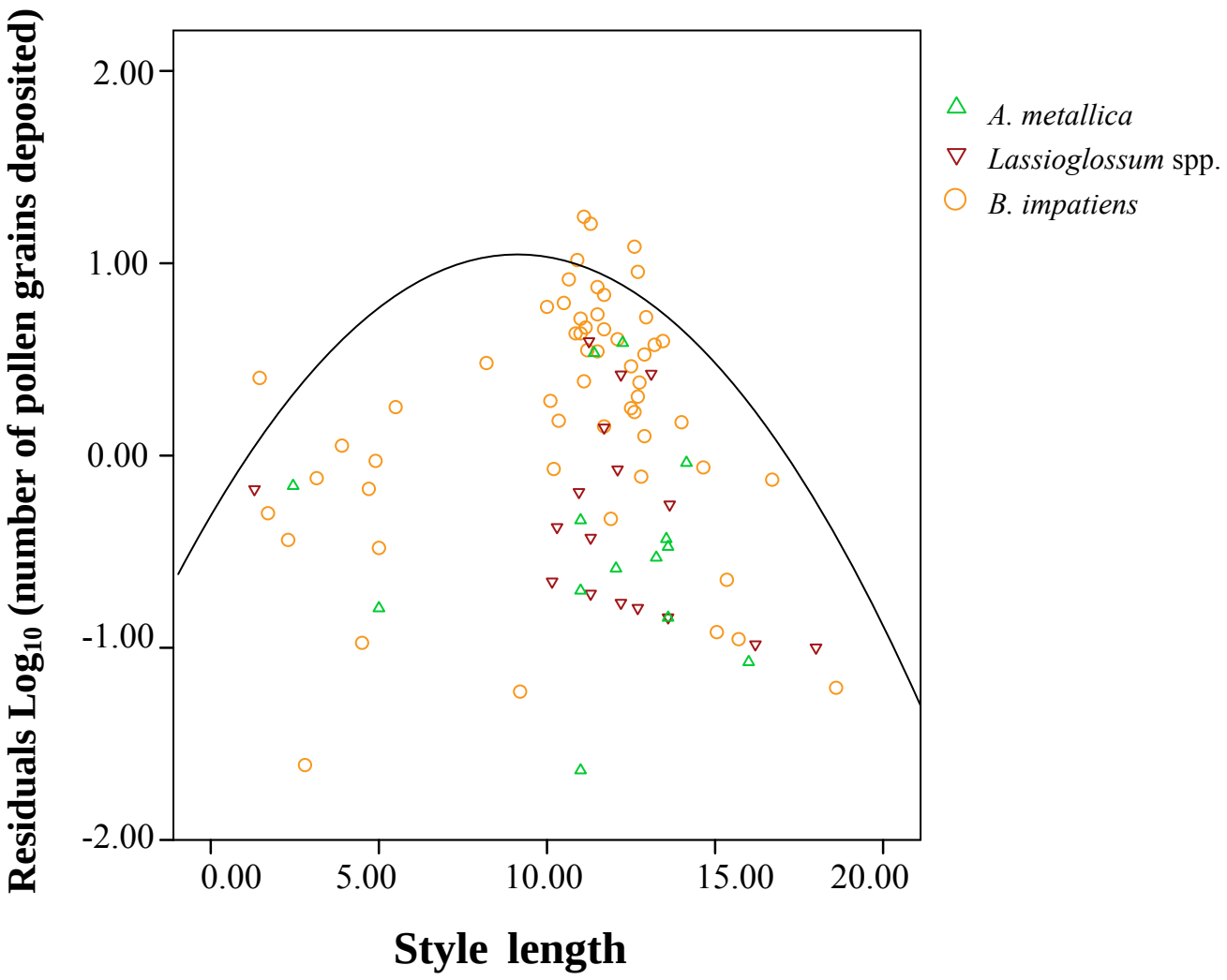

Figure 5. Relationship between the style length (mm) and the number of pollen grains deposited (residuals) when visited by three bee species: *A. metallica* (▲) *Lassioglossum* spp. (▼), and *B. impatiens* (●).

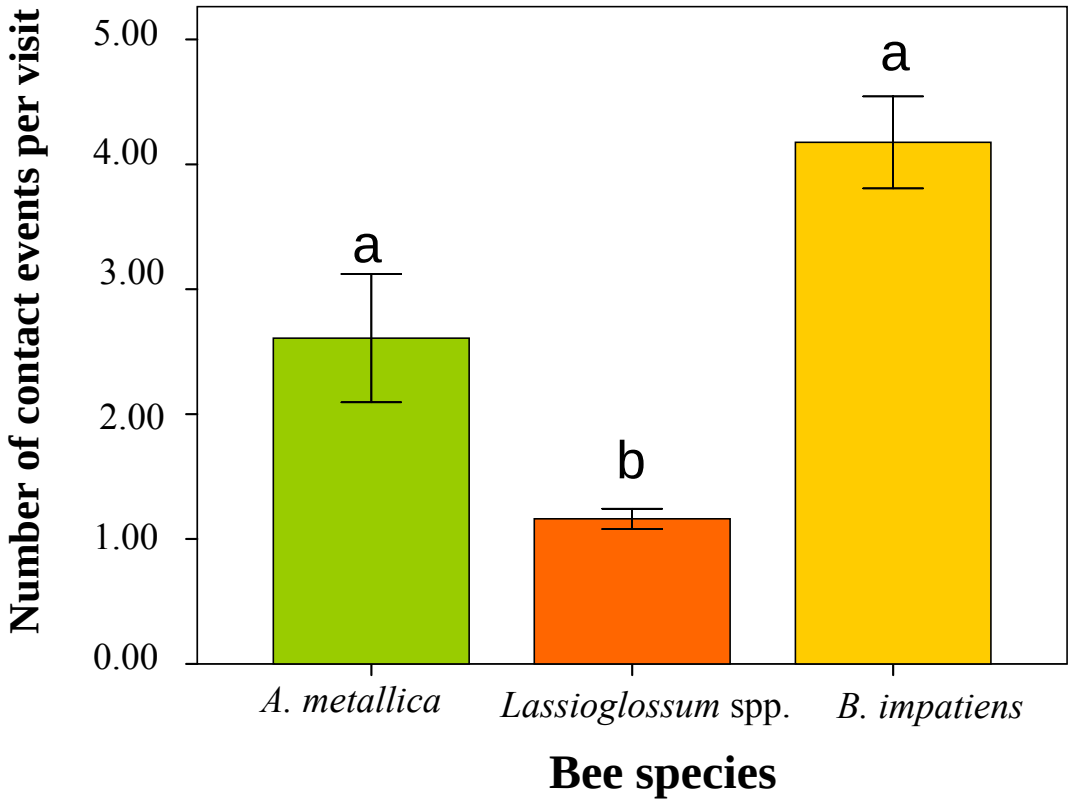

Figure 6A. Number of contact events for each bee species (F= 12.63, $d.f.$= 144, R^2= 0.15, p <0.0001). Error Bars show mean ± 1.0 SE. Letter represent differences among the means determined via Bonferroni.

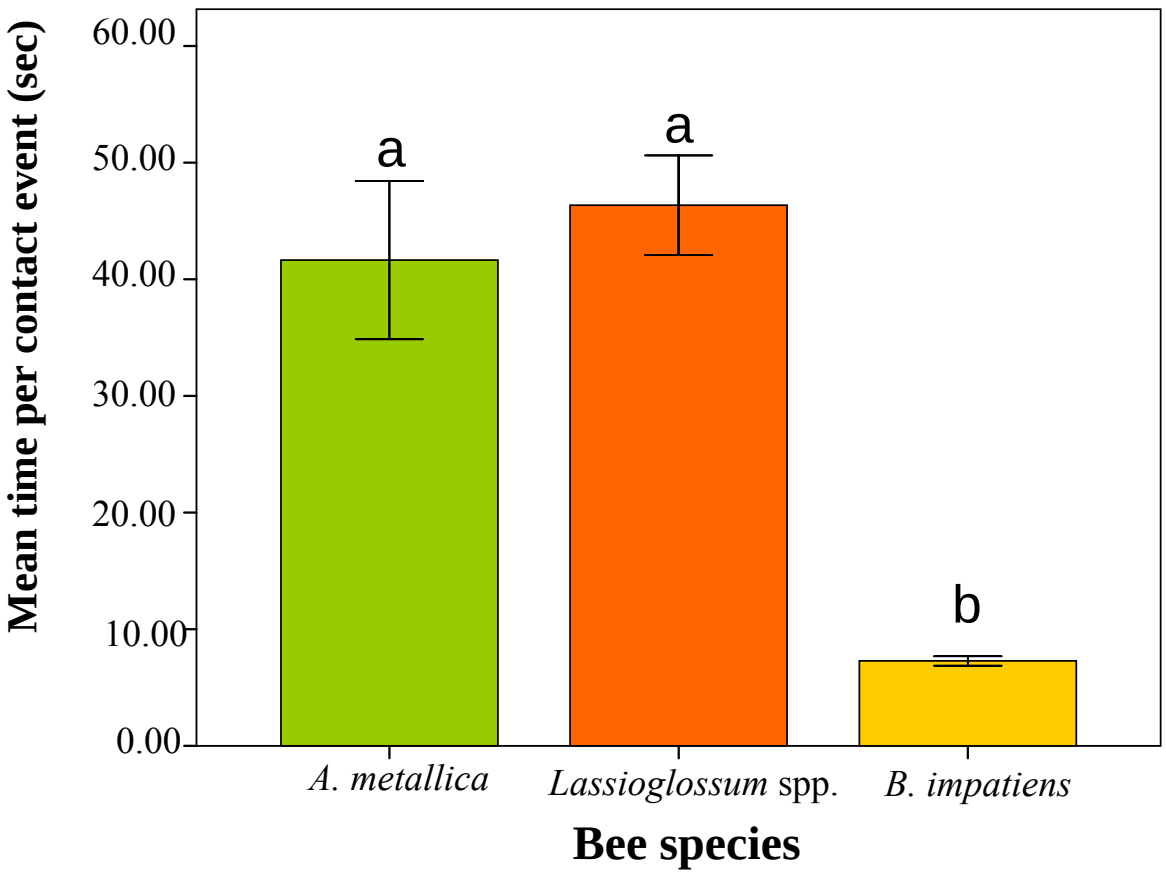

Figure 6B. Average time spent on the flower for each bee species (F= 79.86, $d.f.$= 144, R^2= 0.53, p <0.0001). Error Bars show mean ± 1.0 SE. Letter represent differences among the means determined via Bonferroni.

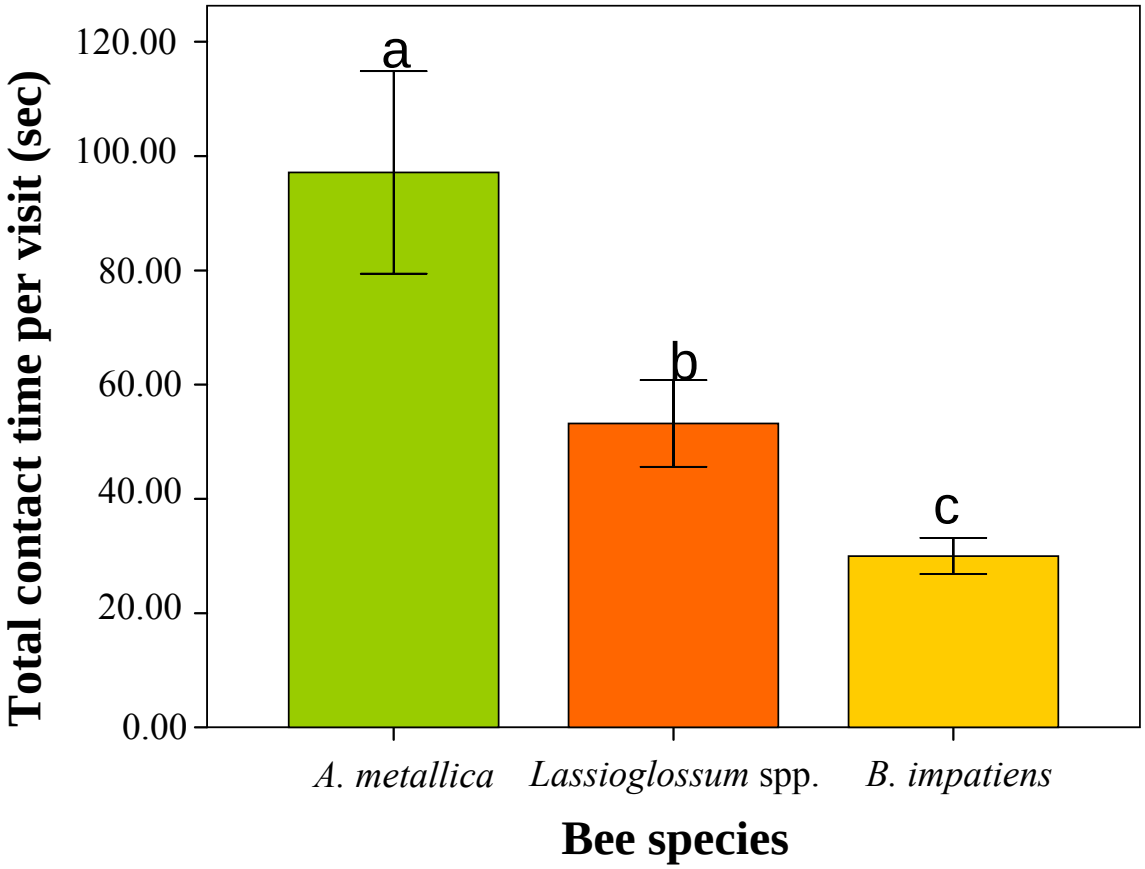

Figure 6C Total time spent on the flower for each bee species (F= 20.47, $d.f.$= 144, R^2= 0.22, p <0.0001). Error Bars show mean ± 1.0 SE. Letter represent differences among the means determined via Bonferroni.

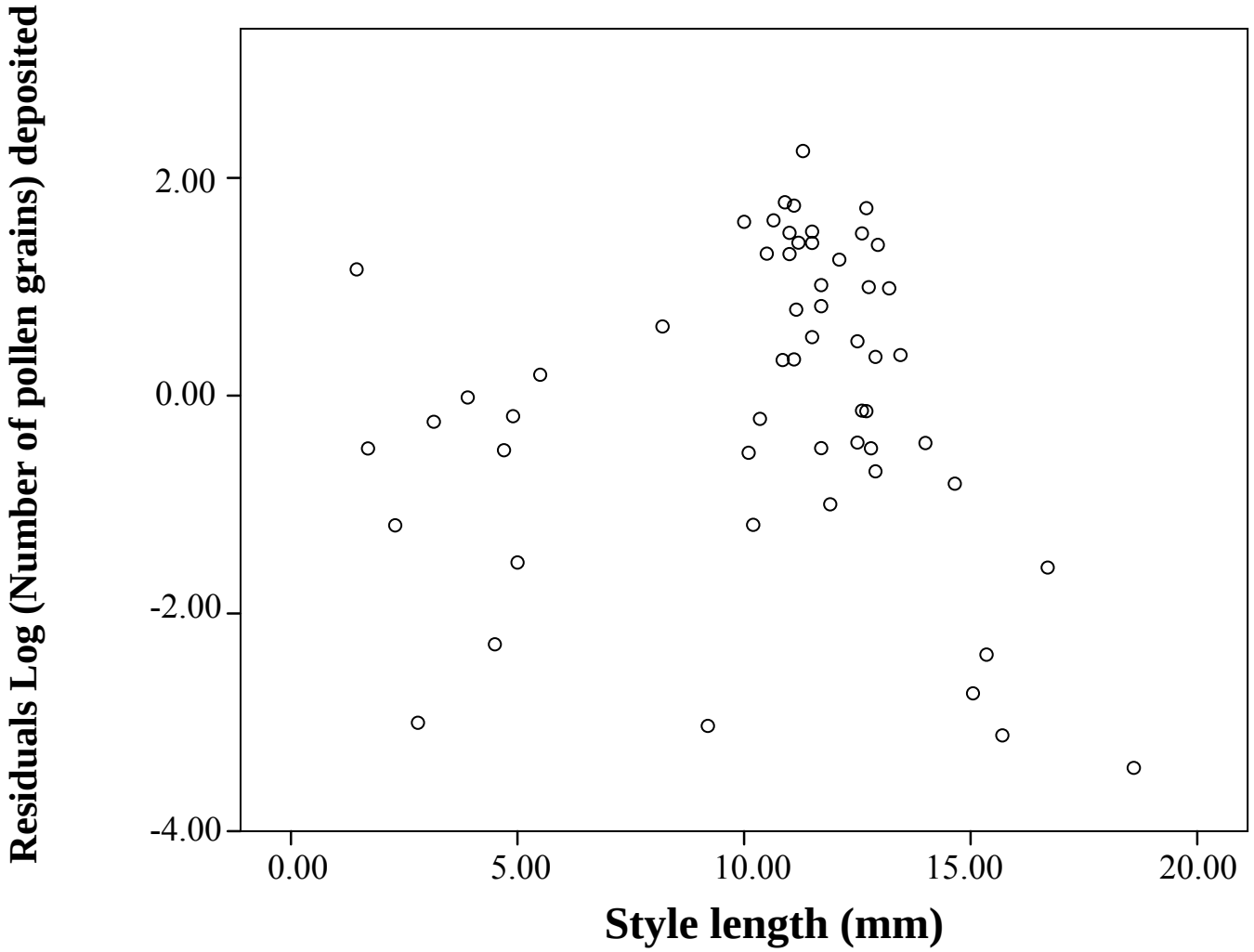

Figure 7. Relationship between the style length (mm) and the number of pollen grains (residuals) that were deposited on the stigma of open flowers visited by *Bombus impatiens.*

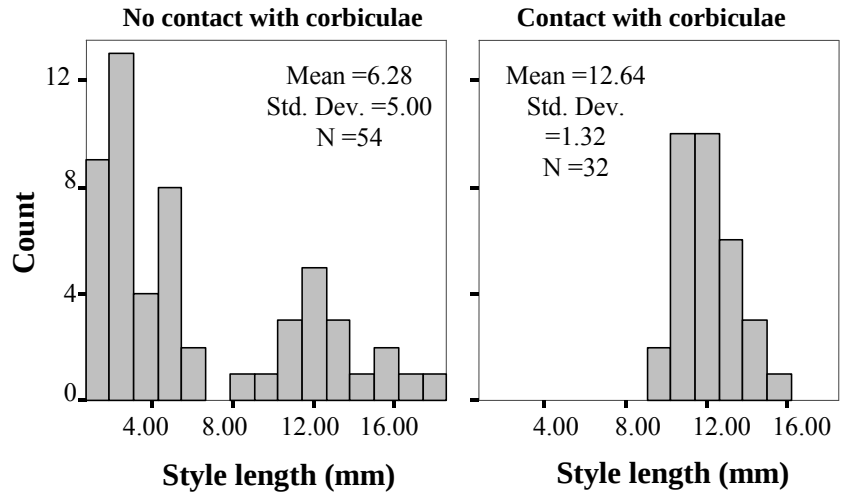

Figure 8. Histograms of the style length of flowers that did not touch the corbiculae (A) and those where the stigma touched the bee's corbiculae (B) of *Bombus impatiens.* Logistic regression model: pollen= 0.35 style length – 3.84, n= 87, Wald χ^2 = 19.39, $p < 0.0001$.

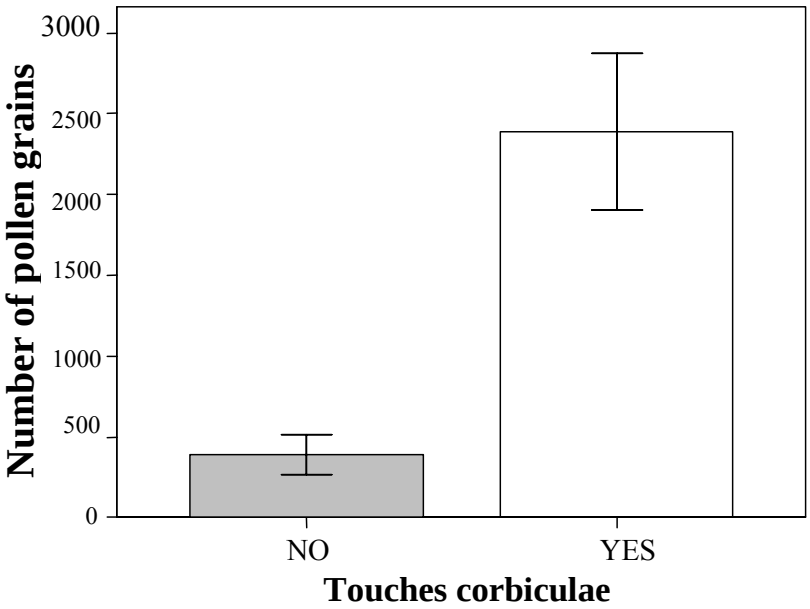

Figure 9. Average number of pollen grains when stigma touched the bee's corbiculae in flowers visited by *Bombus impatiens.* (*t-test*= -4.98, *d.f.*= 85, $p < 0.0001$)

DISCUSSION

The results show that style length determines the amount of pollen deposited and removed in *S. carolinense.* This is the first study to show that the floral morphs of andromonoecious *S. carolinense* will contribute differently to the female and male fitness: long styled flowers serve as pollen recipient flowers and short styled flowers as pollen donors. This data supports the female/male interference hypothesis because the two morphs largely serve different functions. Both large and small bees are pollinators of *S. carolinense*. However, the relationship between style length and pollen dynamics is significant only when *B. impatiens* is the pollinator. When plants are visited by *Bombus* spp., the differences in floral morph would be favored and thus this select for andromonoecy. When plants are visited either by *A. metallica* or *Lassioglossum* spp. there is no selection for the dimorphism (or any particular style length). Because the different pollinators have different selective pressures the mosaic of variation observed for style length and in production of staminate 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 predicted, style length affected both pollen deposition and removal. I found a positive relationship between pollen deposition and style length (Table 2A) and a negative relationship between pollen removal and style length (Table 2A). My data support the interference hypothesis proposed by Fetscher (2001) and Diggle and Miller (1994) because style length determines whether a flower receives pollen or not (Fig. 4). In addition, when a flower receives pollen, style length also determines the amount of pollen the stigma receives (Fig. 5). Further, style length

appears to interfere with the pollen removal process. On average, 16% fewer pollen grains were removed from flowers with long style than flowers whose styles are shorter than 8 mm. These results strongly suggest that the two flower morphs contribute differently to plant fitness because long styled flowers serve as pollen recipient flowers and short styled flowers as pollen donors.

In *S. carolinense* style length not only determines the amount of pollen but also the quality of the pollen received. The chance of self pollination in long styled flowers is significantly reduced because less pollen is removed their anthers and as a result bees likely carry less self pollen (Table 2A). In *S. carolinense* differences in the stigma-anther distance among flowers is caused exclusively by style length, which can reduce autonomous pollination (Fig 1D). My study supports the proposed positive association between stigma anther distance and the amount of outcrossed pollen received reported in a variety of species (Glover and Barrett, 1986; Belaoussoff and Shore, 1995; Motten and Stone, 2000).

Interestingly there seems to be a threshold in the relationship between pollen deposition and style length because the longest styles (>15 mm) actually receive little pollen. These results are similar to those found by Cresswell (2000) in *Brassica napus* were flowers with intermediate style lengths received the most pollen on their stigmas. Cresswell (2000) proposed that there may be stabilizing selection that maintains architectural invariability. Two pieces of evidence suggest that this could be the case for long styled flowers in *S. carolinense.* First, most of the long styled flowers in my study and the study of Connolly and Andersen (2003) had style lengths (9-13 mm) that corresponded to those that received the most number of pollen grains on their stigma (Fig 5). Second, in the two natural populations I studied in 2005 and 2006, flowers with style lengths around 9 mm were more likely to set fruit (see Chapter 2).

Style length and pollination dynamics by taxa

Since pollinator taxa interacted differently with the flowers (Fig. 6), I analyzed the relationship between style length, pollen deposition, and pollen removal for each bee species. 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. The presence of a style does not interfere with their behavior and thus I do not see that any of the morphological traits affect pollen removal or deposition.

Both the positive relationship between style length and the amount pollen deposited and the negative relation between style length and pollen removal are significant only for *B. impatiens* (Table 2B, Fig. 7). The positive relationship is explained by the fact that long styled flowers have a higher probability of touching the *B. impatiens* corbiculae (Fig 8). The presence of a style interferes with the bee's ability to remain on the flower and thus it separates and re-contacts the flower more times as it tries to remove pollen from the anthers. The short-styled male flowers do not present this barrier to pollen removal. Even though the bumblebees have fewer contacts events when they visit short styled flowers more pollen is removed from these flowers. My results show that variation in style length significantly affects the interaction with *B. impatiens* and the two morphs of *S. carolinense* increase the pollinator efficiency when *B. impatiens* visits.

Evolution of andromonoecy

From my results I can conclude that long styled flowers serve as pollen recipient flowers and short styled flowers as pollen donors when *Bombus* visit the flowers. At the flower level, having two morphs reduces male-female interference and increases the pollinator efficiency when *B. impatiens* visits. Sixty percent of the plants surveyed in natural populations in northwestern Pennsylvania had only one open flower per inflorescence, which suggests that my data collected at the individual flower level is applicable to the plant level.

If my results are extrapolated to the plant level in a *Bombus* environment the data supports both the pollen donation hypothesis (Bertin, 1982; Podolsky, 1992, 1993; Elle and Meagher, 2000; Huang, 2003) and the increase pollen receipt hypothesis (Podolsky, 1993; Vallejo-Marin and Rausher, 2007a). The pollen donation hypothesis is supported because having short styled flowers increases in the amount of pollen removed that can potentially be transported by the bees. The increase pollen receipt hypothesis is also supported because short styled flowers do not remove pollen from pollinators. Moreover, long styled flowers' morphology could optimize receiving outcross pollen because long styles both allow the flower to contact the bees' corbiculae (Fig. 8) and reduces the chance for self-pollination (Table 2A).

Moreover, both style length and stigma-anther distance have high levels of phenotypic variation within natural populations on which selection can operate and the high broad sense heritability suggests that these traits are likely heritable. One could then predict that populations where *Bombus* are the main pollinators will experience selection that favors the floral dimorphism and andromonoecy might evolve as a strategy to increase both female and male fitness. In contrast, when plants are in populations that are visited primarily by either by *A.*

metallica or *Lassioglossum* spp. there is no selection for the dimorphism (or any particular style length).

This variation in the selective pressures to produce dimorphic flowers caused by the variation in pollinator that visit the flower, could help clarify why results presents by Elle & Meagher (2000) and Vallejo-Marin and Rausher (2007) are conflicting. Elle & Meagher (2000) found that plants with higher proportions of staminate flowers had higher male success and Vallejo-Marin and Rausher (2007) found that arrays of hermaphrodite flowers sired just as many seeds per flower as arrays of staminate flowers. It is likely that pollinator composition of the populations studied by Elle & Meagher (2000) and Vallejo-Marin and Rausher (2007) were different; if the pollinators are different the advantage of male flowers will be different in the two populations studied. Future studies that test if male flowers increase siring success should include detailed pollinator observations as a part of their experimental design.

My data then supports the idea that variation in pollinator abundance and diversity can result in a diverse array of selective pressures and thus different morphological compositions of the plant populations (Campbell et al., 1991; Cresswell and Galen, 1991; Herrera, 1995; Sanchez-Lafuente et al., 2005). *B. impatiens* is the most important and frequent pollinator in three of the four populations of *S. carolinense* I studied (Fig. 2 and Fig. 3). However, *S. carolinense* grows in diverse array of environments and the three bee species vary in their abundance and their rate of visitation (Fig 2 and Fig 3). Small halictid bees could play an important role in populations where *Bombus* has low population sizes or simply do not visit the flowers. For example, within my study populations, *S. carolinense* in the EC population co-occurs and co-flowers with many other flowering species. Here, *Bombus* rarely visit *S. carolinense* (Fig 2), but were observed visiting the other flowering species (Quesada-Aguilar

pers. obs). Thus the flowering community could affect which pollinators visit *S. carolinense*. Also, temporal variation within the season in pollinator fauna can result in small bees playing an important role 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 rate is low (Quesada-Aguilar per obs.). The variation observed for style length (Fig 1C) and production of staminate flower observed in natural population of *S. carolinense* (Elle and Meagher, 2000), could be due to variation in abundance and visit rate of these pollinators (Fig 1C).

In conclusion, my findings show that different pollinators have different selective pressures on *S. carolinense* plants. The advantage of producing two flower morphs changes depending on the pollinator that visits the flowers. These results suggest that studying pollinators is crucial because differences in the pollinator composition and abundance can result in different evolutionary outcomes. Interestingly, only two of the seven studies that have tested the hypotheses regarding the evolution of andromonoecy in *S. carolinense* have done pollinator observations. This lack of observation might be the reason why results on the evolution of this breeding system have been inconclusive so far. Future studies should then study in detail the pollinators and how interact with the plants before they test or develop hypotheses for the evolution of andromonoecy or any other breeding system.

CHAPTER 2: FLOWER MORPHOLOGY AND FRUIT SETTING ABILITY IN *SOLANUM CAROLINENSE*.

INTRODUCTION

Studying the evolution and maintenance of a breeding system involves recognizing the selective pressures and agents that might lead to the appearance of a new strategy (Webb, 1999). Evolutionary biologists have identified several key selective agents that drive the evolution and maintenance of different breeding systems. The magnitude and direction of the effect that some factors have, such as pollinations, vary from one breeding system to the other (Bell, 1985; Herrera, 1993; Barrett, 2002b). It is crucial to study the extent of the effects and the evolutionary implications that selective agents like pollinators, have on the evolution and maintenance of different breeding systems.

In many breeding systems morphological differences are strongly correlated with the sexual function of the flowers (Campbell, 1992; Ramsey, 1993; Wolfe and Shmida, 1997; Akimoto, Fukuhara, and Kikuzawa, 1999; Dawson and Geber, 1999; Widen and Widen, 1999; Wolfe, 2001). However, in some breeding systems, functionally male or female plants retain well developed sex organs for the opposite sex function that have not lost their sexual functionality (Geber, 1999). Several examples have demonstrated that pollinators are the selective agents that determine the direction and magnitude of this change in floral morphology

and functionality (Neal, Dafni, and Giurfa, 1998; Schemske and Bradshaw Jr., 1999; Mazer and Meade, 2000; Boyd, 2004; Dohzono, Suzuki, and Murata, 2004; Fenster et al., 2004a; Irwin and Strauss, 2005; Kaczorowski, Gadener, and Holtsford, 2005). Because pollinators are such strong selective agents, they could play a key role in determining the evolution and maintenance of breeding systems.

Solanum carolinense has been characterized as andromonoecious species with two distinct flower morphs, hermaphrodite and male flowers (Solomon, 1985, 1986; Elle, 1998, 1999; Elle and Meagher, 2000). The main difference between the flower morphs is in its style length. However, functionality seems to vary within the traditional morphs (Solomon, 1986; Quesada-Aguilar unpublished data). I determined that *S. carolinense* is visited by different pollinators that have different selective pressures on flower morphology (see Chapter 1). Perhaps the variation in sexual expression is also product of these different selective pressures.

Understanding the relationship between flower morphology and the variation in fruit production under different pollination regimes is crucial if one plans to use *S. carolinense* as a model system to test the hypotheses about the evolution of andromonoecy. The main objective of this chapter is to determine if flower morphology correlates with the fruit setting ability of flowers under different pollination treatments. In particular I am interested in answering the following questions: 1) Is fruit production related to style length in natural populations? 2) Is fruit production related to style length when flowers received outcross pollen? 3) Does style length and/or stigma-anther distance affect the plant's ability to autonomously self-pollinate?

MATERIAL AND METHODS

1) Is fruit production related to style length in natural populations?

To determine the relationship between style length and fruiting success in the wild, I selected flowering plants growing in two natural populations. I haphazardly selected and tagged flowering plants on July 23, 2006, and visited the populations weekly through August 06, 2006. In total, I measured fifty flowers on 20 plants growing in the Beagle Road Property of the University of Pittsburgh near Linesville, PA and 50 flowers on 26 plants in the Livingston Road property of the University of Pittsburgh. Of these, 27 were short styled and 73 were long styled. For all 100 flowers, non-destructive floral measurements were taken and were marked with acrylic paint at the base of the peduncle. In October 2006, I scored fruit set of the marked flowers. Six of the 100 flowers were lost to herbivory.

Data Analysis

To analyze the data from natural populations and establish if there was a relationship between the style length (mm) and the probability of producing a fruit, I used a logistic regression (PROC LOGISTIC). Data from the two populations were pooled because I found that there was no difference between average style length of the flowers that produced a fruit between the populations (*t-test* = 0.67, *d.f* .= 15, $p = 0.51$).

2) Is fruit production related to style length when flowers received outcross pollen?

In 2005, I conducted outcross pollinations to determine if fruit setting ability is related to style length on 115 flowers of different style lengths (100 long-styled and 15 short styled) on 32 siblings growing in a hoop greenhouse at Pymatuning Laboratory of Ecology (PLE). These flowers had their anther pores glued on the day they opened to avoid self-pollination. For each flower I recorded the day it opened, conducted non-destructive flower measurements and randomly assigned the date of the pollination. I did 39 pollinations on first day flowers, 31 pollinations on second day flowers, 30 pollinations third day flowers and 15 pollinations on fourth day flowers. I collected pollen from 5 non-related plants to obtain outcross pollen for the pollinations. I marked the flowers with acrylic paint after each outcross-pollination. Plants remained in the hoop house until October, when I collected and recorded any fruits that were produced. Fruits were then taken into the laboratory where I washed and counted the seeds.

Data Analysis

To determine if style length (mm) is related with fruit setting ability when a plant receives outcross pollen, I used a logistic regression (PROC LOGISTIC). I pooled the data from the four pollination treatments because I found that flower age does not affect fruit set ($\chi^2 = 5.17$, $n = 115$, $df = 3$, $p = 0.16$). In those flowers that produced a fruit, I then regressed the number of seeds against the style length (mm) using a repeated measured approach (PROC MIXED).

3) Does style length and/or stigma-anther distance affect the plant's ability to autonomously self?

To determine if plants can self-pollinate in a no-pollinator environment and if floral morphology affects this process, I isolated 46 siblings from 46 sibships. I kept the plants in a pollinator free screen house in the Beagle Road property of University of Pittsburgh and watered and fertilized them as previously described. From the June 26, 2005 to August 20, 2005, I maintained a daily census of the plants and newly open flowers were measured and marked with a dot of acrylic paint. I measured the flowers without touching the anther or the style and manipulated the plants carefully to avoid promoting self pollination. I measured a total of 510 flowers. Plants remained in the screen house until October, when I collected any fruits that were produced. Fruits were then taken into the laboratory where I washed the seeds as described above. In cases where seeds were produced, I counted the number per fruit.

Data Analysis

I used data from the autonomous selfing experiment to determine the relationship between morphological traits and the induction of fruit. Previous studies have identified that reduced stigma-anther distances might be a strategy to increase the amount of self-pollen deposited and obtain reproductive assurance (Glover and Barrett, 1986; Barrett, Morgan, and Husband, 1989; Stace, 1995; Dorken, Friedman, and Barrett, 2002; Cesaro et al., 2004; Eckert and Herlihy, 2004). I regressed style length and stigma anther distance on fruit set using a

logistic regression in a repeated measures approach (PROC GENMODE) to account for the fact that some flowers came from the same sibling.

RESULTS

Is fruit production related to style length in natural populations?

Fruit set in the two populations was low with only 18.1% of the flowers measured setting fruit. All of the flowers that produced a fruit had long styles (Fig 10). The average style length for those flowers that produced a fruit in the Beagle Road population was 10.66 +/- 1.20 and in the Farm Population was 10.32 +/- 0.85 (*t-test*= 0.67, *d.f.*= 15, $p = 0.51$). In these populations the odds of setting a fruit are 48% higher for long styled flowers fruit= 0.30 style length – 5.17, *d.f.* =1, *n*= 94, Wald $\chi^2 = 7.28$, $p = 0.007$).

Is fruit production related to style length when flowers received outcross pollen?

In flowers that receive outrcross pollen, I found a positive relationship between style length and setting a fruit (Model fruit= 0.45 style length – 3.14, *d.f.* =1, *n*= 115, Wald $\chi^2 = 22.99$, $p < 0.0001$ (Fig 11). I categorized 100 flowers as long styled flowers. These flowers had styles that ranged from 8.5 mm to 14.60 mm. Of these 100 flowers, 88% produced a fruit. The 15 flowers categorized as short styled and had styles that ranged from 1.10 mm to 5.00 mm. Two of these flowers produced a fruit (13% of all short styled flowers). Fruits from these two short styled flowers had 13 and 161 seeds. I found no relationship between the style length and the

number of seeds per fruit ($y = 2.02x + 73.76$; $F = 0.80$, $n = 90$, $p = 0.3742$). On average these fruit had 97 ± 39 seeds.

Does style length and/or stigma-anther distance affect the plant's ability to autonomously self?

Fruits produced via autonomous self-pollination are approximately 1/4 of the size of fruits produced via outcross pollination (Fig 12). Although 179 of the 501 flowers measured produced a fruit, only 15 of these had seeds. Most of these fruits had a single seed and the maximum seed number counted per fruit was 15. These seeded fruits were produced in 11 siblings.

Both style length and stigma-anther distance influence the probability of producing an autogamous fruit (Table 3). There is a positive relationship between style length and fruit production (Fig 13 A,B) where 96% of flowers that produced a fruit had long styles and only 8 flowers that had short styles produced a fruit. Fruit induction seems to be more common in flowers that fall in the 7 mm to 14 mm range. Stigma-anther distance has a negative relationship with fruit production (Table 2, Fig 13 C, D). Most of the flowers that produced a fruit had stigma-anther distances around 2.40 m, which means that the stigmas were close to the anthers. The range for stigma-anther distance of long styled flowers that produced a fruit is 0 to 4 mm (Fig 13D).

TABLES

Table 3. Logistic regression model for the relationship between the style length (mm) stigma anther distance (mm) and induction of fruit when plants are isolated from pollinators.

Parameter	Estimate	Standard Error	Chi-Square	P
Intercept	-3.96	1.14		
Style length (mm)	0.40	0.12	6.53	**0.0106**
Stigma anther distance (mm)	-0.28	0.13	3.99	**0.0457**

FIGURES

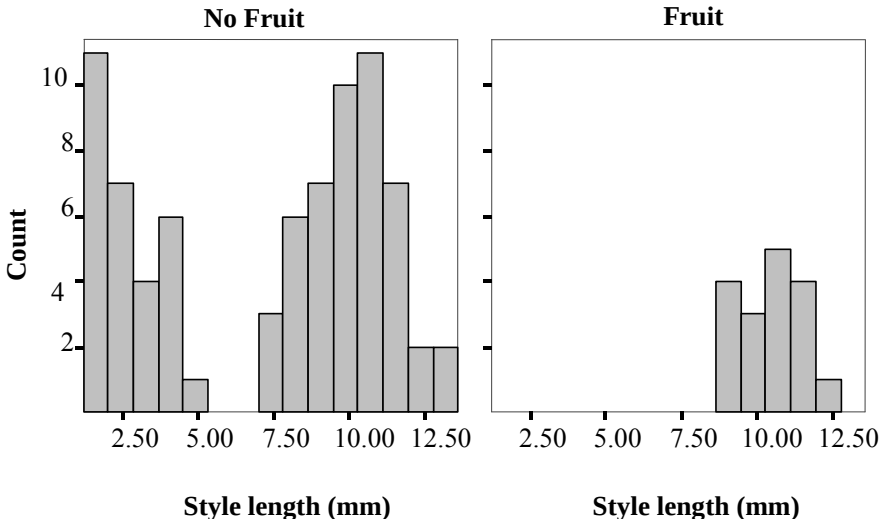

Figure 10. Relationship between the style length (mm) and probability of producing a fruit in Natural Populations.

Logistic regression model: fruit= 0.30 style length – 5.17, *d.f.* =1, *n*= 94, Wald x^2 = 7.28, *p* =0.007.

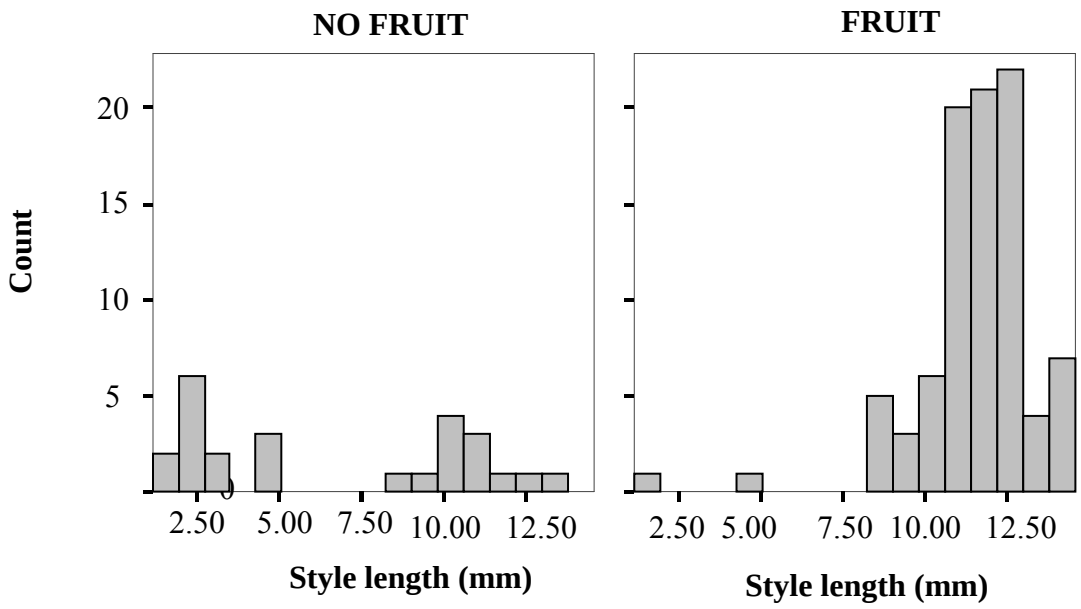

Figure 11. Treatment Outcross Pollination A) Histogram of the style length (mm) of flowers that did not produce a fruit B) Histogram of the style length (mm) of flowers that produced a fruit. Logistic regression model: fruit= 0.45 style length – 3.14, *d.f.* =1, *n*= 115, Wald $x^2 = 22.99$, $p < 0.0001$.

Figure 12. Infructescence of *Solanum carolinens*e produced via outcross pollination (large fruit) and via autonomous selfing (small fruit).

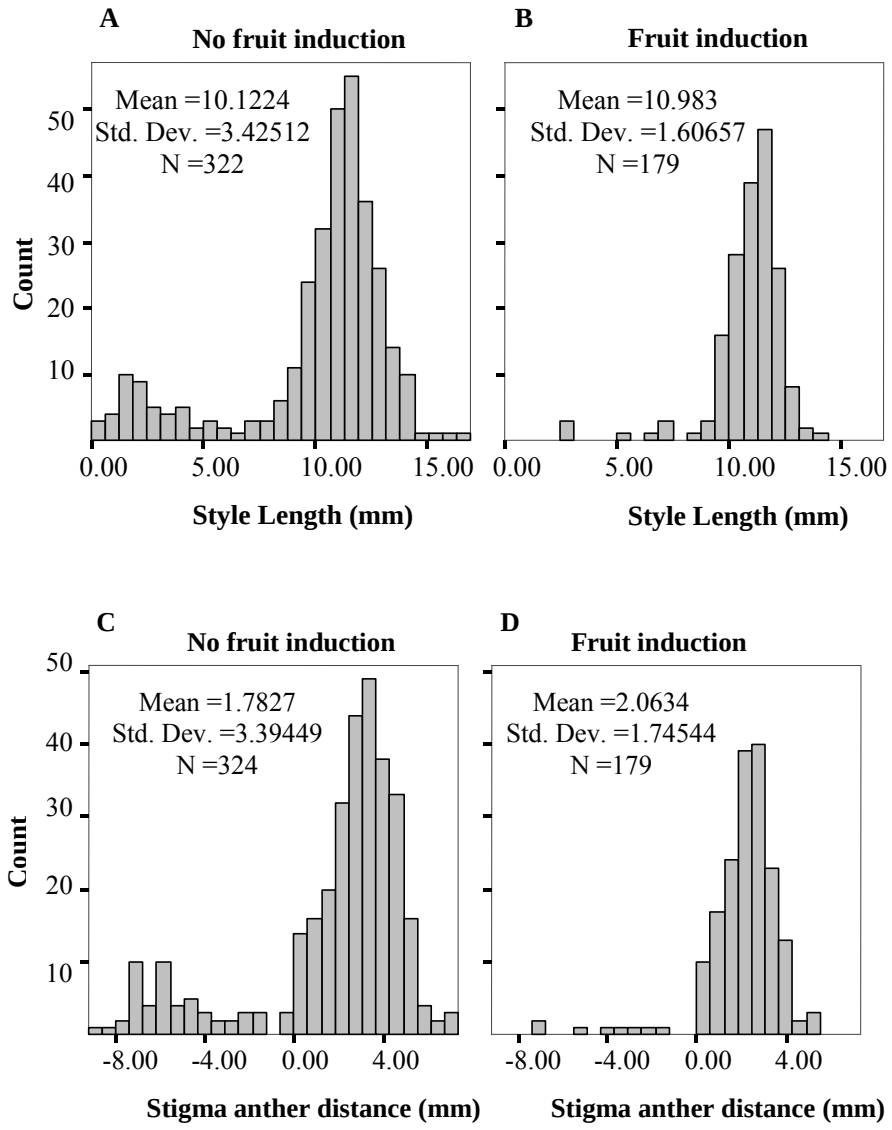

Figure 13. Treatment Pollinator Isolation A) Histogram of the style length (mm) of flowers that did not have a fruit induced B) Histogram of the style length (mm) of flowers that induced a fruit C) Histogram of the style length (mm) of flowers that did not have a fruit induced D) Histogram of the style length (mm) of flowers that induced a fruit.

DISCUSSION

Flower morphology, in particular style length, determines the fruit setting ability of the flowers in *S. carolinense* under different pollination regimes. However, my data shows that sexual functionality seems to vary in *S. carolinense*. Interestingly, 13% of the short styled flowers produced a fruit when they receive outcross pollen and eight short styled flowers produced a fruit via autonoumous pollination. This is the first study that has found that some short styled flowers have not lost their female function and are capable of producing a fruit with viable seeds. Moreover, 90% of the long styled flowers produced fruit when they receive outcross pollen. The fact that some long styled flowers did not produce fruits supports the idea some hermaphrodites are acting as pollen donors (Solomon, 1986). More studies that determine if short styled flowers can produce fruits and if some hermaphrodites are pollen donors in other populations are needed.

Not only functionality varies in this species because even though *S. carolinense* has been recognized as a self-incompatible species, previous studies have determined that compatibility can break down (Travers, Mena-Ali, and Stephenson, 2004). I found that *S. carolinense* can produce fruits (and in some cases seeds) via autonomous self-pollination. Interestingly, morphology determines which flowers can self-pollinate. My results concord with previous studies that have determined that flowers can evolve mechanisms that facilitate the deposition of self-pollen and the production of self-seeds when pollinators are unreliable or scarce (Kalisz and Vogler, 2003).

My results suggest that understanding the relationship between flower morphology, female functionality, and self compatibility are crucial if one wants to understand the evolution of andromonoecy in this species. My data previous data strongly suggests that pollinators cause the variation in floral morphology in *S.carolinense*; which suggests that pollinators might also

cause the variation in sexual function and self-incompatibility. For example, my previous data shows that when *Bombus* visit the flowers there is a positive relationship between style length and pollen deposition and a negative relationship between style length and pollen removal. Due to these differences in the gender functionality of the flowers, in a *Bombus* environment there could then be selection for the loss of male function in long styled flowers and loss of female function in short styled flowers in order to optimize the pollination. As a result, plants visited by *B*. *impatiens* will experience selection that favors the floral dimorphism and the evolution of andromonoecy. Selection for strong incompatibility will then be relaxed because flowers will have other mechanisms that avoid self fertilization.

In contrast, when plants are in populations that are visited primarily by either by *A*. *metallica* or *Lassioglossum* spp. there is no selection for the dimorphism (or any particular style length). Halictid bees differ greatly in their behavior from bumblebees (see Chapter 1). Their visitation rate is low; they usually visit only one flower per plant and tend to roam on the tops on the anthers (see Chapter 1). Since the probability of receiving a visit is low in a halictid environment, the optimal strategy in this environment is to produce flowers that retain both their female and male function. In the scenario here will be strong selection for self-incompatibility as the mechanism to reduce self-pollination.

When pollinators are absent individuals that are self-compatible will have the highest fitness because these flowers can self-pollinate and thus enjoy reproductive assurance (Vogler, Das, and Stephenson, 1998; Stephenson, Good, and Vogler, 2000; Good-Avila, Frey, and Stephenson, 2001; Dorken, Friedman, and Barrett, 2002; Moller and Geber, 2005). Morphologically, intermediate styled hermaphrodites are optimal because their stigma-anther distance is shorter and they could receive more self-pollen (Cresswell, 2000; Fetscher, 2001;

Cesaro et al., 2004). In a no pollinator environment the andromonoecious phenotype will be lost, all flowers will retain their male and female functions, and plants will be self-compatible.

APPENDIX A

VARIANCE ESTIMATES, BROAD SENSE HERITABILITY AND STANDARD ERROR FOR LONG STYLED FLOWERS

	Style length	Stigma anther distance	Anther length
Genetic Variance	0.31	0.37	0.086
Environmental Variance	1.64	1.29	0.62
Phenotypic Variance	1.95	1.66	0.71
Heritability	0.63	0.89	0.49
Standard error	0.28	0.28	0.27

APPENDIX B

MOVIE OF POLLINATOR TAXA VISITING FLOWERS OF *SOLANUM CAROLINENSE*. CLIPS 1 AND 2: *LASSIOGLOSSUM* SPP. CLIPS 3 AND 4: *AUGOCHLOROPSIS METALLICA* AND CLIPS 5 AND 6: *BOMBUS IMPATIENS*

BIBLIOGRAPHY

AKIMOTO, J., T. FUKUHARA, AND K. KIKUZAWA. 1999. Sex ratios and genetic variation in functionally androdioecious species *Schizopepon bryoniaefolius* (Cucurbitaceae). *American Journal of Botany* 86: 880-886.

ALVES DOS SANTOS, I. 2002. Flower-visiting bees and the breakdown of the tristylous breeding system of *Eichhornia azurea* (Swartz) Kunth (Ponteriaceae). *Biological Journal of the Linnean Society* 77: 499-507.

ARMBRUSTER, W. S. 1988. Multilevel comparative analysis of the morphology, function, and evolution of *Dalechampia* blossoms. *Ecology* 69: 1746-1761.

BARRETT, S. C. 2002a. Sexual interference of the floral kind. *Heredity* 88: 154-159.

BARRETT, S. C. H. 2002b. The evolution of plant diversity. *Nature Reviews/Genetics* 3: 274-284.

_____. 2003. Mating strategies in flowering plants: the outcrossing-selfing paradigm and beyond. *Philosophical Transactions of the Royal Society of London* 358: 991-1004.

BARRETT, S. C. H., M. T. MORGAN, AND B. C. HUSBAND. 1989. The Dissolution of a Complex Genetic Polymorphism: The Evolution of Self-Fertilization in Tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 43: 1398-1416.

BELAOUSSOFF, S., AND J. S. SHORE. 1995. Floral correlates and fitness consequences of mating-system variation in *Turnera ulmifolia*. *Evolution* 49: 545-556.

BELL, G. 1985. On the function of flowers. *Procedings of the Royal Society of London* 224: 223-265.

BERTIN, R. I. 1982. The evolution and maintenance of andromonoecy. *Evolutionary Theory* 6: 25-32.

BOYD, A. E. 2004. Breeding system of *Macromeria viridiflora* (Boraginaceae) and geographic variation in pollinator assemblages. *American Journal of Botany* 91: 1809-1813.

CAMPBELL, D. R. 1992. Variation in sex allocation and floral morphology in *Ipomopsis aggregata* (Polemoniaceae). *American Journal of Botany* 79: 516-521.

CAMPBELL, D. R., N. M. WASER, M. V. PRICE, E. LYNCH, AND R. MITCHELL. 1991. Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution* 45: 1458-1467.

CARIVEAU, D., R. E. IRWIN, A. K. BRODY, L. SEVILLANO GARCIA-MAYEYA, AND A. VON DER OHE. 2004. Direct and indirect effects of pollinators and seed predators to selection on plant and floral traits. *Oikos* 104: 15-26.

CESARO, A. C., S. C. H. BARRETT, S. MAURICE, B. E. VAISSIERE§, AND J. D. THOMPSON. 2004. An experimental evaluationof self-interferance in *Narcissus assoanus*: functional and evolutionary implications. *Journal of Evolutionary Biology* 17: 1367-1376.

CONNOLLY, B. A., AND G. J. ANDERSON. 2003. Functional significance of the androecium in staminate and hermaphroditic flowers of *Solanum carolinense* (Solanaceae). *Plant Systematics and Evolution* 240: 235-243.

CRESSWELL, J. E. 2000. Manipulation of female architecture in flowers reveals a narrow optimum for pollen deposition. *Ecology* 81: 3244-3249.

CRESSWELL, J. E., AND C. GALEN. 1991. Frequency-dependent selection and adaptive surfaces for floral character combinations: the pollination of Polemonium viscosum. *American Naturalist* 138.

CUEVAS, J., AND V. S. POLITO. 2004. The Role of Staminate Flowers in the Breeding System of *Olea europaea* (Oleaceae): an Andromonoecious, Wind-pollinated Taxon. *Annals of Botany* 93: 547-553.

DAWSON, T. E., AND M. A. GEBER. 1999. Sexual Dimorphism in Physiology and Morphology. *In* T. E. D. M.A Geber, and L.F. Delph [ed.], Gender and sexual dimorphism in flowering plants, 176-215. Springer, Germany.

DE JONG, T. J., AND S. A. M. GERITZ. 2001. The role of geitonogamy in the gradual evolution towards Dioecy in Cosexual Plants. *Selection 2* 1-2: 133-146.

DIGGLE, P. K., AND J. S. MILLER. 2004. Architectual effects mimic floral sexual dimorphism in *Solanum* (Solanaceae). *American Journal of Botany* 91: 2030-2040.

DILCHER, D. 2000. Toward a new synthesis: major evolutionary trends in the angiosperm fossil record. *Proceedings of the National Academy of Sciences* 97: 7030-7036.

DOHZONO, I., K. SUZUKI, AND J. MURATA. 2004. Temporal changes in calyx tube length of *Clemantis stans* (Ranunculaceae): a strategy for pollination by two bumble bee species with different poboscis lenghts. *American Journal of Botany* 91: 2051-2059.

DORKEN, M. E., J. FRIEDMAN, AND S. C. H. BARRETT. 2002. The Evolution and Maintenance of Monoecy and Dioecy in *Sagittaria latifolia* (Alistamtaceae). *Evolution* 56: 31-41.

ECKERT, C. G., AND C. R. HERLIHY. 2004. Using a cost-benefit approach to understand the evolution of self-fertilization in plants: the perplexing case of *Aquilegia canadensis* (Ranunculaceae). *Plant Species Biology* 19: 159-173.

ELLE, E. 1998. The quantitative genetics of sex allocation in the andromonoecious perennial, *Solanum carolinense* (L.). *Heredity* 80: 481-488.

_____. 1999. Sex allocation and reproductive success in the andromonoecious perennial Solanum carolinense (Solanaceae) I. Female success. *American Journal of Botany* 86: 278-286.

ELLE, E., AND T. R. MEAGHER. 2000. Sex allocation and reproductive success in the andromonoecious perennial *Solanum carolinense* (Solanaceae) II. Paternity and Functional Gender. *American Naturalist* 156: 622-636.

EMMS, S. K. 1993a. Andromonoecy in *Zigadenus paniculatus* (Liliaceae):spatial and temporal patterns of sex allocation. *American Journal of Botany* 80: 914-923.

_____. 1993b. Andromonoecy in *Zigadenus paniculatus* (Liliaceae): Spatial and temporal patterns of sex allocation. *American Journal of Botany* 80: 914-923.

FENSTER, C. B., W. S. ARMBRUSTER, P. WILSON, M. R. DUDASH, AND J. D. THOMPSON. 2004a. Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolution and Systematics* 35: 375-403.

_____. 2004b. Pollination Syndromes and Floral Specialization. *Annual Review of Ecology, Evolotion and Systematics* 35: 375-403.

FETSCHER, A. E. 2001. Resolution of male-female conflict in a hermaphrodite flower. *Procedings of the Royal Society of London* 268.

FUKUDA, Y., K. SUSUKI, AND J. MURATA. 2001. The function of each sepal in pollinator behavior and effective pollination in *Aconitum japonicum* var. *montanum*. *Plant Species Biology* 16: 151-157.

GEBER, M. A. 1999. Theories of the evolution of sexual dimorphism. *In* T. E. D. M.A Geber, and L.F. Delph [ed.], Gender and Sexual Dimorphism in Flowering Plants, 97-122. Springer-Verlag, Berlin.

GLOVER, D. E., AND S. C. H. BARRETT. 1986. Variation in the Mating System of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Evolution* 40: 1122-1131.

GOMEZ, J. 2000. Phenotypic selection and response to selection in *Lobularia maritima*: importance of direct and correlational components of natural selection. *Journal of Evolutionary Biology* 13: 689-699.

GOOD-AVILA, S. V., F. FREY, AND A. G. STEPHENSON. 2001. The effect of partial self-icompatibility on the breeding system of *Campanula rapunculoides* L. (Campanulaceae) under conditions of natural pollination. *Internaciona Journal of Plant Sciences* 162: 1081-1087.

HARDER, L. D., AND S. C. H. BARRETT. 1995. Mating cost of large floral displays in hermaphrodites plants. *Nature* 373: 512-515.

_____. 1996. Pollen Dispersal and Mating Patterns in Animal-Pollinated Plants. *In* D. G. Lloyd and S. C. H. Barrett [eds.], Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants, 140-190. Chapman & Hall, New York.

HARDER, L. D., S. C. H. BARRETT, AND W. W. COLE. 2000. The mating consequences of sexual segregation within inflorescence of flowering plants. *Procedings of the Royal Society of London* 267: 315-320.

HARDIN, J., G. DOERKSEN, H. HERNDON, M. HOBSON, AND F. THOMAS. 1972. Pollination Ecology and floral biology of four weedy genera in southwestern Oklahoma. *Southwestern Naturalist* 16: 403-412.

HERRERA, C. M. 1993. Selection on Floral Morphology and Environmental Determinants of Fecundity in a Hawk Moth-Pollinated Violet. *Ecological Monographs* 63: 251-275.

_____. 1995. Microclimate and Individual Variation in Pollinators: Flowering Plants are More than Their Flowers. *Ecology* 76: 1516-1524.

HUANG, S.-Q. 2003. Flower dimorphism and the maintenance of andromonoecy in *Sagittaria guyanensis* ssp. *lappula* (Alismataceae). *New Phytologyst* 157: 357-364.

IRWIN, R. E., AND S. Y. STRAUSS. 2005. Flower color microevolution in Wild Radish:Evolutionary Response to Pollinator-Mediated Selection. *The American Midland Naturalist* 165: 225-237.

JAVOREK, S. K., K. E. MACKENZIE, AND S. P. VANDER KLOET. 2002. Comparative Pollination Effectiveness Among Bees (Hymenoptera:Apoidea) on Lowbush Blueberry. *Annals of the Entomological Society of America* 95: 345-351.

KACZOROWSKI, R. L., M. C. GADENER, AND T. P. HOLTSFORD. 2005. Nectraits in *Nicotiana* section *Alatae* (Solanaceae) in relation to floral traits, pollinator, and mating system. *American Journal of Botany* 92: 1270-1283.

KALISZ, S., AND D. VOGLER. 2003. Benefits of autonomous selfing under unpredictable pollinator environments. *Ecology* 84: 2928-2942.

KLINKHAMER, P. G. L., AND T. J. DE JONG. 1993. Attractiveness to pollinators: a plant's dilemma. *Oikos* 66: 180-184.

KUDO, G., AND T. KASAGI. 2004. Floral sex allocation in Corydalis ambigua populations visited by different pollinators. *Ecoscience* 11: 218-227.

LYNCH, M., AND B. WALSH. 1998. Genetics and analysis of quatitative traits. Sinauear Associates, Inc., Sunderland, MA.

MAZER, S. J., AND D. E. MEADE. 2000. Geographic variation in Flower size in the wild radish. *In* T. A. Mousseuu, B. Sinervo, and J. A. Endler [eds.], Adaptive Geographic Variation in the wild. Oxford University Press.

MOLLER, D. A., AND M. A. GEBER. 2005. Ecological Context of the evolution of self-pollination in *Clarkia xantiana:* population size, plant communities, and reproductive assurance. *Evolution* 59: 786-799.

MOTTEN, A. F., AND J. L. STONE. 2000. Heritability of stigma position and the effect of stigma-anther separation on outcrossing in a predominantly self-fertilizing weed, *Datura stramonium* (Solanaceae). *American Journal of Botany* 87: 339-347.

NEAL, P. R., A. DAFNI, AND M. GIURFA. 1998. Floral symmetry and its role in plant-pollinator systems:terminology,distribuition, and hypothesis. *Annual Review of Ecology, Evolution and Systematics* 29: 345-373.

PANNELL, J. R. 2002. The evolution and maintenance of androdioecy. *Annual Review of Ecology, Evolution and Systematics* 33: 397-425.

PELLMYR, O. 1986. Pollination ecology of two nectariferous *Cimicifuga* sp. (Ranunculaceae) and the evolution of andromonoecy. *Nordic Journal of Botany* 6: 129-138.

PODOLSKY, R. D. 1992. Strange Floral Attractors: pollinator attraction and the evolution of plant sexual systems. *Science* 258: 791-793.

_____. 1993. Evolution of a Flower Dimorphism: How effective is pollen dispersal by "male" flowers? *Ecology* 74(8): 2255-2260.

RAMSEY, M. 1993. Floral Morphology, biology, and sex allocation in disjunct populations of Christmas Bells (*Blandfordia grandiflora*, Liliaceae) with different breeding systems. *Australian Journal of Botany* 41: 749-762.

RHOADS, A. F., AND T. A. BLOCK. 2000. The Plants of Pennsylvania. University of Pennsylvania Press, Philadelphia, Pennsyvania.

ROUBIK, D. W. 1992. Ecology and Natural History of Tropical Bees Cambridge University Press, New York.

SAKAI, A. K., AND S. G. WELLER. 1999. Gender and Sexual Dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. *In* T. E. D. M.A Geber, and L.F. Delph [ed.], Gender and sexual dimorphism in flowering plants, 1-31. Spinger, Germany.

SANCHEZ-LAFUENTE, A. M., J. GUITIAN, M. MEDRANO, C. M. HERRERA, P. J. REY, AND X. CERDA. 2005. Plant traits, Environmental Factors, and Pollinator Visitation in winter-flowering *Helleborus foetidus* (Ranunculaceae). *Annals of Botany* 96: 845-852.

SATO, H. 2002. Invasion of Unisexuals in hermaphrodite populations of animal-pollinated plants: effects of pollination ecology and floral size-number trade-offs. *Evolution* 56: 2374-2382.

SCHEMSKE, D. W., AND C. HORVITZ. 1989. Temporal variation in selection on a floral character. *Evolution* 43: 461-465.

SCHEMSKE, D. W., AND H. D. BRADSHAW JR. 1999. Pollinator preference and the evolution of floral traits in monkeyflowers (Mimulus). *procedings of the National Academy of Science* 96.

SOLOMON, B. P. 1985. Environmentally influenced changes in sex expression in an andromonoecious plant. *Ecology* 66: 1321-1332.

_____. 1986. Sexual allocation and andromonoecy: resource investment in male and hermaphrodite flowers of *Solanum carolinense* (Solanaceae). *American Journal of Botany* 73: 1215-1221.

SPALIK, K. 1991. On evolution of andromonoecy and 'overproduction" of flowers: a resource allocation model. *Biological Journal of the Linnean Society* 42: 325-336.

STACE, H. M. 1995. Protogyny, self-incompatibility and pollination in *Anthocercis gracilis* (Solanaceae). *Australian Journal of Botany* 43: 451-459.

STEPHENSON, A. G., S. V. GOOD, AND D. VOGLER. 2000. Interrelationships Among Inbreeding Depression, Plasticity in the Self-incompatibility System, and the Breeding System of Campanula rapunculoides L. (Campanulaceae). *Annals of Botany* 85: 211-219.

TRAVERS, S. E., J. MENA-ALI, AND A. G. STEPHENSON. 2004. Plasticity in the self-incopatibility system of Solanum carolinense. *Plant Species Biology* 19: 127-135.

VALLEJO-MARIN, M., AND M. D. RAUSHER. 2007a. The role of male flowers in andromonoecious species: energetic costs and siring success in *Solanum carolinense* L. *Evolution*: 405-412.

_____. 2007b. Selection through female fitness helps to explain the maintenance of male flowers *The American Naturalist* 169: 000-000.

VOGLER, D., C. DAS, AND A. G. STEPHENSON. 1998. Phenotypic plasticity in the expression of self-incompatibility in Campanula rapunculoides. *Heredity* 81: 546-555.

WEBB, C. J. 1999. Gender and Sexual Dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. *In* T. E. D. M.A Geber, and L.F. Delph [ed.], Gender and sexual dimorphism in flowering plants. Springer, Germany.

WIDEN, M., AND B. WIDEN. 1999. Sex expression in the clonal gynodioecious herb *Glechoma hederacea* (Lamiaceae). *Canadian Journal of Botany* 77: 1689-1698.

WILLIAMS, C. F., J. RUVINSKY, P. E. SCOTT, AND D. K. HEWS. 2001. Pollination, breeding system,and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species. *American Journal of Botany* 88: 1623-1633.

WOLFE, L. M. 2001. Associations among multiple floral polymorphisms in *Linum pubescens* (Linaceae), a heterostylous plant. *International Journal of Plant Sciences* 162: 335-342.

WOLFE, L. M., AND A. SHMIDA. 1997. The ecology of sex expression in a gynodioecious israeli desert shrub (Ochradenus baccatus). *Ecology* 78: 101-110.

ZAR, J. H. 1996. Biostatistical Analysis. Prentice Hall, New Jersey.