FUNCTIONAL SIGNIFICANCE OF POLLEN SIZE VARIATION IN *IPOMOEA PURPUREA*, COMMON MORNING GLORY: SMALL POLLEN SIZE LEADS TO SIRING ADVANTAGE UNDER NATURAL POLLINATION

by

GREGORY ALLEN EVANS

(Under the Direction of Shu-Mei Chang)

ABSTRACT

Variation in pollen size is present in *Ipomoea purpurea*, common morning glory, and this study looks at its functional role. Based on the findings in previous studies, we hypothesize that large pollen grains have a siring advantage over small pollen grains in a pollination setting that is natural for the species. We found that small pollen grains sire more seeds than large pollen grains, suggesting that small pollen grains have a mechanism to overcome their disadvantage when in direct competition with large pollen grains. However, small pollen plants and large pollen plants did not significantly differ in terms of overall fitness, implying that large pollen plants produce more seeds than small pollen plants. Future work will need to focus on the mechanisms that small pollen plants utilize in order to sire more seeds than large pollen plants.

INDEX WORDS: Pollen competition, *Ipomoea purpurea*, Pollination, Angiosperm reproduction, Sexual selection

FUNCTIONAL SIGNIFICANCE OF POLLEN SIZE VARIATION IN *IPOMOEA PURPUREA*, COMMON MORNING GLORY: SMALL POLLEN SIZE LEADS TO SIRING ADVANTAGE UNDER NATURAL POLLINATION

by

GREGORY ALLEN EVANS

B.A., The University of Virginia, 2010

M.A., The University of Virginia, 2011

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

FUNCTIONAL SIGNIFICANCE OF POLLEN SIZE VARIATION IN *IPOMOEA PURPUREA*, COMMON MORNING GLORY: SMALL POLLEN SIZE LEADS TO SIRING ADVANTAGE UNDER NATURAL POLLINATION

by

GREGORY ALLEN EVANS

Major Professor:	Shu-Mei Chang
Committee:	Jill Anderson Lisa Donovan

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
December 2018

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Shu-Mei Chang, for her guidance and support during my time here at UGA. My scientific pursuits would not have proceeded as far without her. I would also like to thank my committee, Jill Anderson and Lisa Donovan, for their input and help during my research and writing.

I would also like to thank my lab mates Katie Putney, Dorothy Christopher, Rachel Perez-Udell, Angel Chen, and Grace Zheng for all of their support, ideas, conversations, and labor that helped make this research possible, as well as keep me sane. The Plant Biology Greenhouse, specifically Kevin Tarner and Michael Boyd, made my research possible with their constant attention to my plants, in addition to their many provided moments of levity and perspective. Thanks also goes to the Georgia Genomics and Bioinformatics Core for their fast turnarounds on plate genotyping.

Special thanks go to Susan Watkins for all of her amazing help throughout my time here in Plant Biology. Thank you to Brigitte Bruns and Kimberly Brown for providing me with a wonderful teaching experience. In addition, I'd like to thank the rest of my friends and colleagues here at UGA for an exceptional graduate experience.

TABLE OF CONTENTS

Page

ACKNOWLEDGEMENTS……………………………………………………………………iv

LIST OF TABLES…………………………………………………………………………..vi

LIST OF FIGURES……………………………………………………………….……...vii

CHAPTER

1 INTRODUCTION ………………………...……………………......………………...1

2 SMALL POLLEN SIZE LEADS TO SIRING ADVANTAGE UNDER NATURAL POLLINATION…………………………………………………………….………..3

3 CONCLUSION...35

LITERATURE CITED 38

LIST OF TABLES

Page

Table 2.1: Paternity assignments and patterns for the Athens array…………………..…………..29

Table 2.2: Paternity assignments and patterns for the Bogart array………………….………..30

LIST OF FIGURES

Page

Figure 2.1: Experimental array setup……………………………………………….…………………27

Figure 2.2: Average flower numbers (least square means) across experimental sites and flowering day types..28

Figure 2.3: Proportion of seeds sired by the pollen plant types across experimental sites and flowering-day types...……………………....………..……………........…………...31

Figure 2.4: Average fruit numbers (least square means) across experimental sites and flowering day types..32

Figure 2.5: Average seed numbers (least square means) across experimental sites and flowering day types..33

Figure 2.6. Average plant fitness (least square means) and maternal and paternal contributions across experimental sites and flowering day types...34

CHAPTER 1

INTRODUCTION

In plants, male-male competition can occur before pollination via floral displays and attractiveness, or after pollination when a plethora of pollen grains race to reach a limited number of ovules (Winsor et al. 2000, Bernasconi et al. 2004). This latter phenomenon, termed pollen competition, is defined as competition among pollen grains to reach ovules when the number of pollen grains that are deposited on a stigma exceeds the number of ovules in the ovary (Johnston 1993, Delph et al. 1998, Erbar 2003, Bernasconi et al. 2004). In natural populations, this is a common occurrence as insect-deposited pollen loads often exceed the number of ovules available to be fertilized, especially if a flower is visited several times (Mulcahy 1979, Winsor et al. 2000). Pollen competition can be an important population-level process, as it allows for the selection of traits that can directly increase pollen donor fitness through differential siring success (Krauss 2000, Lankinen and Skogsmyr 2001) or indirectly through traits that are correlated with pollen traits. There has been a strong focus on understanding how floral morphology and attractiveness influence the male component of fitness in flowering plants (Rathcke 1983, Mitchell et al. 2009 and references therein); however, much less is known about post-pollination processes and pollen competition.

Characteristics of the pollen could potentially influence the outcome of pollen competition (Epperson and Clegg 1987, Spira et al. 1992, Johnston 1993, Mazer et al. 2010, Lankinen and Madjidian 2011, Lankinen et al. 2017). In particular, pollen tube growth rate has been shown to consistently correlate with siring success: faster growing pollen tubes have a higher siring success rate than their slower-growing counterparts (Snow and Spira 1991, 1996, Delph et al. 1998, Lankinen and Skogsmyr 2002, Stephenson et al. 2003). In addition, overall pollen grain size has been proposed as an important trait, as larger pollen size is correlated with increased energy stores (Baker and Baker 1979, Cruden and Miller-Ward 1981), as well as higher germination and higher pollen tube growth rates (Baker and Baker 1979, Lord and Eckard 1984). Considering the competitive superiority of large pollen grains, the question remains as to why there is standing variation in pollen size within a species. In other words, why does small pollen still exist in natural populations?

Small pollen plants may numerically overwhelm large pollen plants by producing more pollen, either on a per-flower or per-plant basis (Cruden and Miller-Ward 1981). Small pollen plants may also arrive earlier than large pollen plants, leading to pollen primacy (Epperson and Clegg 1987). On the other hand, small and large pollen grains may represent a trade-off between male and female reproductive functions (Lankinen et al. 2017). For instance, small pollen plants may produce more and/or better quality seeds than large pollen plants, as they may have moved resources away from male function to female function (Charlesworth 2006, Shuster 2009). Small pollen plants may also better control stigma receptivity (Hiscock and Allen 2008), allowing pollen competition to occur more regularly, which benefits pollen parents with improved male function (Galen et al. 1986).

This thesis compromises a study that investigates whether large pollen plants maintain their competitive advantage under natural pollination conditions and if there are trade-offs between pollen size and seed production. We will investigate these questions using lines of *Ipomoea purpurea* that have been artificially selected for large and small pollen grains at two field sites near the University of Georgia.

CHAPTER 2

SMALL POLLEN SIZE LEADS TO SIRING ADVANTAGE UNDER NATURAL POLLINATION[1]

---

[1] Evans, GA and SM Chang. To be submitted to *American Journal of Botany*.

Abstract.— Pollen competition is competition among pollen grains to reach ovules when the number of pollen grains that are deposited on a stigma exceeds the number of ovules in the ovary. Faster pollen tube growth rates and larger pollen sizes are traits that have been shown to be correlated with siring success in pollen competition. Here we use lines of *Ipomoea purpurea* that have been selected for divergence in pollen size to investigate if larger pollen sizes maintain their competitive advantage under a natural pollination setting. We measure competitive success by the proportion of seeds sired by large and small pollen plants. Specifically, we looked at 1) Does the siring advantage of large pollen remain in a natural pollination setting? 2) Does seed production on a per-flower and whole-plant basis differ between large-pollen and small-pollen plants? and 3) Is there a negative correlation between male (siring success) and female (seed production) fitness? Our study does not support the idea of large pollen grains having an advantage, as small pollen plants significantly sired more seeds. However, small pollen plants did not have greater overall fitness, implying that large pollen plants produce more seeds.

INTRODUCTION

Male-male competition, the intrasexual component of sexual selection, has demonstrated to be a widespread phenomenon. While originally proposed for animals by Darwin, it has since been shown to occur in numerous types of organisms, including plants (Skogsmyr and Lankinen 2002, Bernasconi et al. 2004, Shuster 2009). In plants, male-male competition can occur before pollination via floral displays and attractiveness to pollinators, or after pollination when a plethora of pollen grains race to reach a limited number of ovules (i.e. pollen competition) (Winsor et al. 2000, Bernasconi et al. 2004). There has been a strong focus on understanding how floral morphology and attractiveness influence the male component of fitness in flowering plants (Rathcke 1983, Mitchell et al. 2009 and references therein); however, much less is known about post-pollination processes and pollen competition. Yet, pollen competition can be an important population-level process, as it allows for the selection of traits that can directly increase pollen donor fitness through differential siring success (Krauss 2000, Lankinen and Skogsmyr 2001) or indirectly through traits that are correlated with pollen traits.

Pollen competition is defined as competition among pollen grains to reach ovules when the number of pollen grains that are deposited on a stigma exceeds the number of ovules in the ovary (Johnston 1993, Delph et al. 1998, Erbar 2003, Bernasconi et al. 2004). In natural populations, this is a common occurrence as insect-deposited pollen loads often exceed the number of ovules available to be fertilized, especially if a flower is visited several times (Mulcahy 1979, Winsor et al. 2000). Characteristics of the pollen – germination rate, pollen tube growth rate, interaction with the stigma, interaction with the other pollen grains, deposition timing, and overall pollen quality – could potentially influence the outcome of pollen competition (Epperson and Clegg 1987, Spira et al. 1992, Johnston 1993, Mazer et al. 2010, Lankinen and Madjidian 2011, Lankinen et al. 2017). In particular, pollen tube growth rate has been shown to consistently correlate with siring success: faster growing pollen tubes have a higher siring success rate than their slower-growing counterparts (Snow and Spira 1991, 1996, Delph et al. 1998, Lankinen and Skogsmyr 2002, Stephenson et al. 2003). However, pollen tube growth rate is a complex trait that likely is a composite of the genetic identity of the pollen donors (Mulcahy 1979, Walsh and Charlesworth 1992, Lankinen and Skogsmyr 2002, Stephenson et al. 2003), the haploid genotype of the pollen grain (Mulcahy 1979, Spira et al. 1992, Walsh and Charlesworth 1992, Arthur et al. 2003), interactions between the pollen and style environment (i.e. style attrition), and the ecological conditions of the pollen and ovule parents (Stephenson et al. 1992, Lankinen and Skogsmyr 2001, Tejaswini 2002, Marshall and Evans 2016).

In addition to pollen tube growth rate, overall pollen grain size has been proposed as an important trait, as larger pollen size is correlated with increased energy stores (Baker and Baker 1979, Cruden and Miller-Ward 1981). Pollen size varies across species, even closely related ones (Cruden and Lyon 1985, Tejaswini 2002, Jürgens et al. 2012), and has been shown to correlate with style length (Baker and Baker 1979, Lord and Eckard 1984, Jürgens et al. 2012) and stigma depth (Cruden and Lyon 1985). This correlation may be explained by larger pollen grains having higher germination and higher pollen tube growth rates, thereby allowing longer styles and deeper stigmas to select pollen with these positive attributes (Baker and Baker 1979, Lord and Eckard 1984). For example, between interfertile species *Mimulus guttatus* and *M. nasutus*, *M. guttatus* has larger pollen grains that confer a faster pollen tube growth rate. Pollen competition studies between the two species found that *M. guttatus* outcompeted *M. nasutus* in *M. guttatus* styles (Diaz and Macnair 1999). However, similar studies within a single species have produced more mixed results. Larger pollen grains in *Brassica rapa* (Sarkissian and Harder 2001) and maize (Kumar and Sarkar 1980) were shown to have a faster pollen tube growth rate. Conversely, pollen grain size and pollen tube growth rate correlated only sometimes in *Dianthus caryophyllus* (Tejaswini 2002) and not at all in *Erythronium grandiflorum* (Cruzan 1990).

These conflicting results may be due to confounding effects between genetically-based and environmentally-based pollen size variation. It is intuitive that pollen size differences among species is based in genetic differences, but variation within a species is likely a mixture of genetic and environmental differences. Significant heritability of pollen size has been found in

Phaseolus vulgaris (Montes-R and White 1996), *Brassica rapa* (Sarkissian and Harder 2001), and *Mimulus guttatus* (Lamborn et al. 2005). However, how this genetic variation in pollen size may affect the outcome of pollen competition is largely uncertain, as results from within-species studies on pollen performance often involve ecological treatments (Stephenson et al. 1992, Lau and Stephenson 1993, Delph et al. 1997, Hersch 2006, Distefano et al. 2012), resource allocation/paternal provisioning (Cruzan 1990, Delph et al. 1997, Stephenson et al. 2003), or even unspecified differences (Snow and Spira 1991, 1996, Spira et al. 1992). In addition, studies that used distinct genetic lines for pollen competition and showed consistent siring success variation among pollen donors did not examine the effect of pollen size (Marshall and Folsom 1991, Snow and Spira 1991, 1996, Spira et al. 1992, Delph et al. 1998, Marshall 1998, Marshall and Diggle 2001, Marshall and Oliveras 2001).

To date, only a few studies have focused on the intra-specific genetic variation of pollen size and how selection may be acting on the trait (Harder 1998, Diaz and Macnair 1999, Sarkissian and Harder 2001, McCallum and Chang 2016). In the McCallum and Chang study (2016), distinct small and large pollen lines of *Ipomoea purpurea* were artificially selected for in the greenhouse, and pollen from these lines were then placed in roughly equal numbers on a recipient stigma. Post-pollination processes and fertilization were then allowed to occur normally. Large pollen was found to outcompete small pollen the majority of the time and to produce larger seeds (Foltz 2013), though small pollen also dominated in some competitions, supporting the idea that the interplay between pollen and style is also important. A follow-up study explored the reason for the competitive superiority of large pollen by looking at pollen tube growth rates (Chen and Chang, unpublished data). Large pollen grains were found to produce pollen tubes that travel through a style faster than those of small pollen grains.

Considering the competitive superiority of large pollen grains, the question remains as to why there is standing variation in pollen size within a species. In other words, why does small pollen still exist in natural populations? The previous study focused on pollen competition within a limited scope: equal numbers of pollen placed onto a single stigma. However, this does not consider factors that may lead to differential numbers of pollen reaching a stigma. Small pollen plants may numerically overwhelm large pollen plants by producing more pollen, either on a per-flower or per-plant basis (Cruden and Miller-Ward 1981). Small pollen plants may also arrive earlier than large pollen plants, leading to pollen primacy (Epperson and Clegg 1987).

On the other hand, small and large pollen grains may represent a trade-off between male and female reproductive functions, that is sexually antagonistic evolution (Lankinen et al. 2017). For instance, small pollen plants may produce more and/or better quality seeds than large pollen plants, as they may have moved resources away from male function to female function (Charlesworth 2006, Shuster 2009). Small pollen plants may also better control stigma receptivity (Hiscock and Allen 2008), allowing pollen competition to occur more regularly, which benefits pollen parents with improved male function (Galen et al. 1986).

If any of these factors are present, then we should see more equal reproductive output from large- and small-pollen plants than is suggested by the previous pollen competition study (McCallum and Chang 2016). In this study, we investigate whole-plant flower and seed production between large and small pollen *Ipomoea purpurea* lines in a common garden where pollinations were accomplished by natural pollinators. Paternity analyses are done to investigate whether the competitive advantage of large pollen grains remain when plants were exposed to natural pollination. We investigated three main questions: 1) Does the siring advantage of large pollen remain in a natural pollination setting? 2) Does seed production on a per-flower and whole-plant basis differ between large-pollen and small-pollen plants? and 3) Is there a negative correlation between male (siring success) and female (seed production) fitness?

MATERIALS AND METHODS

Study Species

Ipomoea purpurea (Convolvulaceae), or common morning glory, is a climbing, self-compatible, weedy annual vine. Native to Mexico and Central America, it now occurs in most parts of the U.S and is commonly found in fields and roadside ditches. It is considered a major agricultural pest (Webster 2013). The corollas have a funnelform shape and can be white, pink, or purple (Radford et al. 1968), often with five pigmented rays. Flowers last one day: they usually open just after sunrise and senesce by late morning or early afternoon. The androecium has five stamens with filaments of different lengths; the gynoecium has an ovary with three locules, with two ovules per locule. The fruits are dry, dehiscent capsules (Radford et al. 1968, Zomlefer and Knapp 1994). Bumblebees (*Bombus spp.*) are the predominant pollinators, but other bees (*Apis spp., Xylocopa spp.*), hummingbirds, and butterflies occasionally visit (Radford et al. 1968, Clegg and Durbin 2000, McCallum and Chang 2016).

Pollen Phenotype Lines

The creation of small pollen (SP) and large pollen (LP) plant lines are described in McCallum and Chang (2016). Overall, a selection experiment selected for increased (LP line) or decreased (SP line) average pollen grain size over six generations, beginning in 2007. The parent individuals for both lines were collected from two populations in Watkinsville, Oconee County, Georgia. Two replicates were created for both LP and SP lines and maintained separate from each other. Two replicate control lines were also maintained by randomly selecting plants in each generation to cross and generate seeds. By the fifth generation, pollen size between the LP and SP lines had significantly diverged: LP lines had average pollen diameters of 114.40 and 113.79 µm and SP lines had average pollen diameters of 105.52 and 107.04 µm. Crosses between the two replicate lines were carried out to generate the seeds used for the current study.

Pollen diameter, and the subsequent faster pollen tube growth rate and improved siring success, is the major floral difference between LP and SP lines (McCallum and Chang 2016). LP plants produce more pollen grains per flower than SP plants (370.80 ± 17.05 vs. 298.24 ± 17.05), though a previous natural pollination study showed that both types of plants transferred similar numbers of pollen onto recipient stigmas when only one type of donor parent was present (Foltz 2013). Corolla width is marginally significantly different, with LP plants having slightly larger widths than SP plants (59.36mm ± 1.01 vs. 57.24mm ± 0.97). Also, style length is significantly longer in LP plants than in SP plants (32.02mm ± 0.19 vs. 30.46mm ± 0.27). All other floral traits are not significantly different between the two plant types (McCallum and Chang 2016).

Experimental Arrays Setup

In the summer of 2014, we germinated seeds at the University of Georgia (UGA) Botany Greenhouses in Athens, Georgia. We scarified seeds using a razor blade and planted them in 1-gallon plastic pots. We used the pine-bark soil mixture typically used for greenhouse cultivation of this species: 125 gallons of fine grade pine bark, eight cubic feet of coarse vermiculite, eight cups of dolomitic limestone, two cups of superphosphate, one cup of calcium nitrate, one cup of potassium nitrate, one cup of gypsum, and one cup of Micromax. We watered the plants daily or when needed throughout the entire experiment. Upon reaching the flowering stage, we moved 30 plants (10 LP, 10 SP, and 10 Control plants) to an outdoor array in Bogart, Georgia and another 36 plants (12 LP, 12 SP, and 12 Control plants) to an outdoor array just outside the Botany Greenhouses in Athens, Georgia. Array plants were arranged in 6x5 and 6x6 grid-formations. We designated the C, LP, and SP groups in repeated, sequential positions in the array such that each plant will be neighboring the other two plant types (Figure 2.1). Each position in the array was assigned a randomly selected plant from its designated group. We kept the plants in the pots, which were anchored in position by long stakes driven into soil, to limit belowground competition with each other and other interspecifics, as well as allow easy whole-plant extraction later. We erected deer fencing to prevent large-animal herbivory, though insects (including pollinators) had free access to the array plants/flowers.

Pollination Study and Seed Collections

The pollen competition study lasted twelve days, from July 3 to July 14, 2014. During the first six days of the study (July 3-8), each plant was kept at five flowers per day by mechanically removing excess flowers before they opened. We chose the excess flowers randomly. If a plant produced fewer than five flowers, we recorded the flower number. In the Athens array, on average, slightly fewer than nine plants produced fewer than five flowers each day, with those plants having produced 3.1 flowers on average. In the Bogart array, on average, nine plants produced fewer than five flowers each day, with those plants having produced 3.25 flowers on average. We kept all the flowers that each plant produced during the second six days of the study (July 9-14). Comparisons between the two experimental periods (equal- and free-flowering) allow us to contrast estimates at per-flower versus per-plant reproductive success. Using a paint marker, we marked each flower at its base with a specific color combination to denote the flowering date. In addition, we recorded total numbers of flowers produced, even those that were later mechanically removed.

At the end of the study, we moved the plants from both arrays into the UGA Botany Greenhouses to allow the fertilized flowers to mature into seed pods. As the seed pods matured, we collected them and moved them to the lab for data collection. We counted total numbers of fruits and total seed counts for each plant.

Genotyping and Paternity Analysis

In April – October 2017, we planted a subset of seeds that were produced from the pollen competition study. For the Athens array, we planted seeds produced on July 3, 4, 5, 8 (equal flowering days), 9, 10, 11, and 12 (free flowering days); for the Bogart array, we planted seeds produced on July 5, 6, 7, 8 (equal flowering day), 9, 12, and 13 (free flowering days). For each experimental date, we planted 96 seeds, sampling from array plants proportional to their relative seed production that day. In total, we planted and germinated 1440 seeds. We scarified seeds with a razor blade and planted them in 96-plug trays at the UGA Botany Greenhouses. After the first true leaves emerged (typically two to three weeks after planting), we collected leaf samples into 1.5-mL polypropylene tubes. The samples were immediately placed on ice and transported back to the lab for DNA extraction. Upon reaching the lab, we ground the samples with liquid nitrogen and extracted genomic DNA using a modified CTAB method (Doyle and Doyle 1987, Cullings 1992). We stored DNA samples in a -20°C freezer until they were used for PCR.

We used a touchdown PCR program to amplify six polymorphic microsatellite loci (Roux 1995, Korbie and Mattick 2008). This type of PCR program initially begins with a DNA annealing temperature higher than optimal, and then gradually lowers it to optimal over successive cycles. This promotes the accumulation of the desired amplicon, as well as the prevention of primer-dimers. We developed three of the loci and the other three were developed by Kuester and colleagues (Aksoy et al. 2013). We analyzed microsatellite fragment sizes using Peak Scanner Software v2.0 (Applied Biosystems).

We assigned paternity using Cervus Software v3.0 (Kalinowski et al. 2007). The most-likely candidate father was assigned with a 60% level of confidence. The experimental plant with the highest log of the odds ratio (LOD) score was considered as the most likely pollen parent. In the event when the LOD score was equal between multiple fathers, the paternity assignment was split equally between those plants. Paternity assignment analyses were completed by combining plants produced at a particular study site across all days with the same flowering-type (i.e. equal- vs free-flowering).

Statistical Analysis

Observed frequencies of paternity assignments were compared to expected frequencies using chi-squared tests in R (R Development Core Team 2017). Expected frequencies for paternity assignments were based on the proportion of parents of a given pollen phenotype within a study site, or the proportion of flowers produced by parents of a pollen phenotype within a study site. That is, two types of chi-squared tests were completed: once investigating whole-plant differences and the other investigating differences on a flower number level.

To calculate total fitness numbers for each plant pollen type by array and flowering-day type, total seed numbers were multiplied by the proportion of seeds sired by a particular plant. These numbers were then added to seed numbers produced by the plants and divided by two to equally represent maternal and paternal contributions.

Total fitness and flower, fruit, and seed numbers for each parent plant were pooled by pollen phenotype (i.e. small, large, or control), across all days of a particular type (i.e. equal- or free-flowering), and from each study site. Separate ANOVAs with total fitness, flower numbers, fruit numbers, and seed numbers as dependent variables were run utilizing pollen phenotype, flowering day type, study site, and all possible interactions between these factors as independent variables in R (R Development Core Team 2017). Study site interacted with at least flowering day type in each ANOVA, so separate statistical analyses were run for each study site.

RESULTS

Flower Production

Athens array – Total flower numbers for equal-flowering days (i.e. four days) in the Athens array were 233 for control pollen plants, 223 for small plants, and 209 for large plants. On an average plant basis (reported as least square means) control plants produced 19.417 ±

1.498 flowers, small plants produced 18.583 ± 1.498, and large plants produced 17.417 ± 1.498 (Figure 2.2). Total flower numbers for free-flowering days (i.e. four days) were 298 for control plants, 354 for small plants, and 280 for large plants. On an average plant basis (reported as least square means) control plants produced 24.833 ± 1.498 flowers, small plants produced 29.5 ± 1.498, and large plants produced 23.333 ± 1.498.

The interaction between pollen plant type and flowering day type was not significant (F(2,66) = 2.062, $p = 0.135$) for total flower numbers. It was nearly significantly different between the three pollen plant types (F(2,66) = 3.000, $p = 0.057$), and contrasts between large and small pollen plant flower numbers showed that they significantly differed (F(1,66) = 5.995, $p = 0.017$). Flowering day type was also significant. (F(1,66) = 36.793, $p < 0.001$).

Bogart array – Total flower numbers for equal-flowering days (i.e. four days) in the Bogart array were 168 for control pollen plants, 184 for small plants, and 187 for large plants. On an average plant basis (reported as least square means) control plants produced 16.8 ± 1.445 flowers, small plants produced 18.4 ± 1.445, and large plants produced 18.7 ± 1.445 (Figure 2.2). Total flower numbers for free-flowering days (i.e. three days) were 188 for control plants, 218 for small plants, and 201 for large plants. On an average plant basis (reported as least square means) control plants produced 18.8 ± 1.445 flowers, small plants produced 21.8 ± 1.445, and large plants produced 20.1 ± 1.445.

The interaction between pollen plant type and flowering day type was not significant (F(2,54) = 0.252, $p = 0.778$) for total flower numbers. It also did not significantly differ between the three pollen plant types (F(2,54) = 1.331, $p = 0.273$). Flowering day type was nearly significant (F(1,54) = 3.689, $p = 0.060$).

Paternity Assignments and Siring Success

Most of the genotyped offspring were assigned to one or more paternal plants, though the numbers that reached the 60% level of confidence were few (Tables 2.1 and 2.2). Paternity exclusion probabilities calculated in the GenAlEx program (Peakall and P.E. 2006, 2012) showed exclusion probabilities only reached 68% for the Athens genotypes and 79% for the Bogart genotypes. Future work will require the use of more markers to reach higher exclusion probabilities. Split paternities were primarily between paternal plants with the same genotype, and most had either few total assignments or were of the same pollen phenotype. So, the effects of split paternities should be minimal on the overall patterns between pollen groups (LP vs. SP).

Athens array – For equal-flowering days in the Athens array, control pollen plants sired more than half (proportion 0.508) of the seeds sampled (Figure 2.3). This was largely driven by the outsized success of one parental plant, which had over one-third (67.5 of 187.9) of that group's assignments. Small pollen plants sired almost one-third of the seeds produced (proportion 0.314), while large pollen plants sired only slightly more than half of what small pollen plants did (proportion 0.178). On average, small pollen plants sired 1.77 seeds for every one by large pollen plants. These results were significantly different from expectations (see Methods for definitions of expectations), both on a per-plant basis, X^2 (N=2) = 61.127, $p < 0.001$, and on a per-flower basis, X^2 (N=2) = 47.978, $p < 0.001$.

For free-flowering days in the Athens array, control plants sired slightly less than half (proportion 0.461) of the seeds produced. Again, this was largely driven by the success of the same parental plant, which had almost half (79.0 of 170.2) of that group's assignments. Small pollen plants sired over one-third of the seeds produced (proportion 0.380), while large pollen plants sired only fewer than half of what small pollen plants did (proportion 0.160). On average, small pollen plants sired 2.38 seeds for every one by large pollen plants. These results were significantly different from expectations, both on a per-plant basis, X^2 (N=2) = 53.686, $p < 0.001$, and on a per-flower basis, X^2 (N=2) = 47.006, $p < 0.001$.

Bogart array – For equal-flowering days in the Bogart array, control plants sired only a tenth (proportion 0.100) of the seeds produced (Figure 2.3). This was much lower than the pattern seen in the Athens array. Small pollen plants sired over a half of the seeds produced (proportion 0.555), while large pollen plants sired slightly more than a third of the seeds produced (proportion 0.345). The ratio of small pollen to large pollen siring success is consistent with that found in the Athens array: 1.61 seeds sired by small pollen plants for every one by large pollen plants. These results were significantly different from expectations, both on a per-plant basis, X^2 (N=2) = 117.48, $p < 0.001$, and on a per-flower basis, X^2 (N=2) = 105.09, $p < 0.001$.

For free-flowering days in the Bogart array, control plants again sired much fewer seeds than that of control plants in the Athens array (proportion 0.123). Small pollen plants sired over a half of the seeds produced (proportion 0.564), while large pollen plants sired slightly fewer than a third of the seeds produced (proportion 0.313). The ratio of small pollen to large pollen siring success is again consistent with that found in the Athens array: 1.81 seeds sired by small pollen plants for every one by large pollen plants. These results were significantly different from expectations, both on a per-plant basis, X^2 (N=2) = 82.255, $p < 0.001$, and on a per-flower basis, X^2 (N=2) = 74.064, $p < 0.001$.

Fruit and Seed Data

Athens array – For equal-flowering days in the Athens array, control pollen plants produced 190 fruits and 787 seeds, small plants produced 128 fruits and 459 seeds, and large plants produced 142 fruits and 553 seeds. On an average plant basis (reported as least square means) control plants produced 15.833 ± 1.595 fruits and 65.583 ± 8.281 seeds, small plants produced 10.667 ± 1.595 fruits and 38.25 ± 8.281 seeds, and large plants produced 11.833 ± 1.595 fruits and 46.083 ± 8.281 seeds (Figures 2.4 and 2.5). For free-flowering days, control pollen plants produced 225 fruits and 928 seeds, small plants produced 258 fruits and 975 seeds, and large plants produced 208 fruits and 806 seeds. On an average plant basis (reported as least square means) control plants produced 18.75 ± 1.595 fruits and 77.333 ± 8.281 seeds, small plants produced 21.5 ± 1.595 fruits and 81.25 ± 8.281 seeds, and large plants produced 17.333 ± 1.595 fruits and 67.167 ± 8.281 seeds.

The interaction between pollen plant type and flowering day type was significant for total fruit numbers ($F(2,66) = 3.202$, $p = 0.047$). This is likely because of the drastically lower fruit numbers for small and large pollen plants, in comparison to control plants, seen during the equal flowering days in Athens. This was the only array and flowering-day type where this pattern was seen. Total seed numbers also followed this pattern but did not rise to the level of statistical significance ($F(2,66) = 1.876$, $p = 0.161$). Neither fruit ($F(2,66) = 1.446$, $p = 0.243$) nor seed numbers ($F(2,66) = 1.783$, $p = 0.176$) were significantly different between the three pollen plant types. Flowering day type was significant for both fruit ($F(1,66) = 24.264$, $p < 0.001$) and seed numbers ($F(1,66) = 13.977$, $p < 0.001$).

Bogart array – For equal-flowering days in the Bogart array, control plants produced 150 fruits and 797 seeds, small plants produced 170 fruits and 881 seeds, and large plants produced 177 fruits and 976 seeds. On an average plant basis (reported as least square means) control plants produced 15.0 ± 1.539 fruits and 79.7 ± 8.370 seeds, small plants produced 17.0 ± 1.539 fruits and 88.1 ± 8.370 seeds, and large plants produced 17.7 ± 1.539 fruits and 97.6 ± 8.370 seeds (Figures 2.4 and 2.5). For free-flowering days, control plants produced 157 fruits and 759 seeds, small plants produced 174 fruits and 730 seeds, and large plants produced 170 fruits and 850 seeds. On an average plant basis (reported as least square means) control plants produced 15.7 ± 1.539 fruits and 75.9 ± 8.370 seeds, small plants produced 17.4 ± 1.539 fruits and 73.0 ± 8.370 seeds, and large plants produced 17.0 ± 1.539 fruits and 85.0 ± 8.370 seeds.

The interaction between pollen plant type and flowering day type was not significant for total fruit numbers (F(2,54) = 0.115, p = 0.892) nor seed numbers (F(2,54) = 0.251, p = 0.779). Neither fruit (F(2,54) = 1.048, p = 0.358) nor seed numbers (F(2,54) = 1.453, p = 0.243) were significantly different between the three pollen plant types. Also, flowering day type was not significant for neither fruit (F(1,54) = 0.011, p = 0.916) and seed numbers (F(1,54) = 2.360, p = 0.130).

Total Fitness

Athens array – For the equal-flowering days in the Athens array, control pollen plants had an average fitness (reported as least square means) of 70.87 ± 14.79, small plants had a fitness of 42.69 ± 14.79, and large plants had a fitness of 36.36 ± 14.79 (Figure 2.6). For free-flowering days, control pollen plants had an average fitness of 90.65 ± 14.79, small plants had a fitness of 83.48 ± 14.79, and large plants had a fitness of 51.62 ± 14.79.

The interaction between pollen plant type and flowering day type was not significant (F(2,66) = 0.424, p = 0.656) for total fitness. It was nearly significantly different between the three pollen plant types (F(2,66) = 3.091, p = 0.052), but contrasts between large and small pollen plant fitness values showed that they did not significantly differ (F(1,66) = 1.666, p = 0.201). Flowering day type was significant. (F(1,66) = 4.381, p = 0.040).

For the equal-flowering days in the Bogart array, control pollen plants had an average fitness of 53.11 ± 13.81, small plants had a fitness of 117.73 ± 13.81, and large plants had a fitness of 94.56 ± 13.81 (Figure 2.6). For free-flowering days, control pollen plants had an average fitness of 52.36 ± 13.81, small plants had a fitness of 102.49 ± 13.81, and large plants had a fitness of 79.05 ± 13.81.

The interaction between pollen plant type and flowering day type was not significant (F(2,54) = 0.187, p = 0.830) for total fitness. It was significantly different between the three pollen plant types (F(2,54) = 8.737, p = 0.001), but contrasts between large and small pollen plant fitness values showed that they did not significantly differ (F(1,54) = 2.851, p = 0.097). Flowering day type was also not significant. (F(1,54) = 0.868, p = 0.356).

DISCUSSION

Large pollen plants have been previously demonstrated to outperform small pollen plants when placed in equal numbers on recipient stigmas (McCallum and Chang 2016); a follow-up study showed that this was due to faster pollen tube growth of large pollen grains (Chen and

Chang, unpublished data). In addition, while large pollen plants produce more pollen than small pollen plants, pollen transfer by pollinators under natural conditions from the donor flower to the recipient flower is largely the same between plant types (Foltz 2013). Combining these findings, our hypothesis for the current study was that large pollen plants would have greater siring success than small pollen plants.

Contrasting to the predictions, we, in fact, found that when comparing the two selected groups, small pollen plants tended to sire more offspring than large pollen plants. On the other hand, there were no significant total fitness differences between small and large pollen plants, even though large pollen plants had more seeds than small pollen plants. These results show a possible mechanism for the maintenance of pollen size variation in *I. purpurea*, namely a trade-off between siring ability and seed production. In addition, the siring success of control pollen plants in the Athens array, particularly that of one maternal genotype, and lack of success in the Bogart array demonstrates that there are possibly other unexplored factors that contribute to success.

Pollen Size and Fitness

Contrary to predictions, small pollen plants tended to sire more offspring than large pollen plants. This held true in both arrays and across day types. The different flowering-type days were created to investigate differences at the flower and whole-plant levels. For instance, while large pollen plants were previously found to produce more pollen than small pollen plants (McCallum and Chang 2016), it would be possible for small pollen plants to still produce more pollen on a whole-plant level by producing more flowers. In fact, small pollen plants did significantly produce more flowers the large pollen plants in the Athens array. Therefore, small pollen plants in the Athens array may be deriving their siring advantage by producing more pollen on a whole-flower basis (Cruden and Miller-Ward 1981). However, small pollen plants did not significantly outproduce large pollen plants in the Bogart array, yet the siring advantage was still present. Therefore, the genotypes in each study site are deriving this advantage in different ways, or small pollen plants are deriving their advantage in some other manner.

As previously discussed, one potential reason for this siring advantage was pollen transfer by bumblebee pollinators (Foltz 2013), as bumblebees may be better transporters of small pollen (Harder 1998). Using an array experiment similar to this one, Foltz (2013) found no differences between the transfer efficiency of small or large pollen grains. Another potential mechanism of the siring advantage is showier flowers being more likely to attract pollinators, allowing more of their pollen to be collected and transferred to potential mates (Delph and Ashman 2006). Again, however, previous work showed that small and large pollen plants are visited with similar frequency and duration by pollinators (Foltz 2013).

It is worth noting that in the previous competition study by McCallum and Chang (2016) small and large pollen grains were not mixed before being applied to recipient stigmas. They were, instead, applied to different, though somewhat overlapping, portions of the stigma. Such segregation in pollen deposition seems unlikely to occur under natural systems, as *Bombus spp.* individuals can visit multiple flowers within a visiting bout (Couvillon et al. 2015), allowing them to collect a genetically-varied pollen load. Though the dynamic of pollen movement on a bumblebee's body is still not well understood, it seems likely that some level of mixing exists. In addition, studies have shown that pollen germination and pollen tube growth may change when pollen grains are mixed with those of different genotypes (Lankinen and Skogsmyr 2002, Kron and Husband 2006, Zhang et al. 2010). If this phenomenon also exists in *I. purpurea,* the competitive ability, and hence siring success, of small pollen grains may differ during natural pollination than that found in the previous, more controlled, study by McCallum and Chang (2016). This hypothesis will need to be investigated in future research.

Another uninvestigated mechanism in the current system is pollen primacy, or the timing of pollen arrival. While differences in competitive abilities dominate in interactions where pollen types arrive on stigmas at the same time (Snow and Spira 1991, 1996, Spira et al. 1992, Delph et al. 1998, Bernasconi et al. 2004, McCallum and Chang 2016)*,* it was shown in *I. purpurea* that pollen that arrives first enjoys a fertilization advantage that often overrides the competitive advantage, even if competing pollen reaches the stigma immediately after the first pollen grains (Epperson and Clegg 1987). If small pollen grains were somehow reaching potential mates before large pollen plants, small pollen plants' siring success advantage could be reflecting such pollen primacy, and not necessarily the direct competitive interactions inside the styles. However, it is unclear what this mechanism may be and will need to be investigated in future work.

Seed Production and Fitness

While small pollen plants sired more seeds than large pollen plants, they did not significantly differ from large pollen plants in terms of overall fitness. Counterintuitively, large pollen plants did not significantly produce more seeds to counteract the siring advantage of small pollen plants, which would lead to similar overall fitness between the pollen types. While there were no significant differences in seed production, large pollen plants did produce more seeds than small pollen plants for both arrays and flowering-day types, save for Athens free-flowering days. Therefore, taken together, there may be a trade-off between siring ability and seed production.

Antagonistic evolution between male and female traits in plants may seem counterintuitive (Skogsmyr and Lankinen 2002), as traits that benefit one sex often benefit the other (e.g. showy flowers to attract pollinators). However, trade-offs between male and female functions have been observed. In one study, the hermaphroditic plant *Collinsia heterophylla* underwent artificial selection to create lines with improved pollen performance and to investigate whether selection for this led to subsequent costs in female fitness (Lankinen et al. 2017). It was found that lines that had evolved improved pollen performance, most likely linked to faster pollen tube growth rates, also exhibited reduced seed production.

Fruit and seed numbers were generally comparable across arrays and flowering-day types, save for the depressed fruit and seed numbers seen for both large and small pollen plants in Athens equal-flowering days. As there were no substantial differences between the Athens and Bogart free-flowering days, it seems unlikely that experimental site was having an effect in the equal-flowering days for Athens. It is unclear what may be driving this result.

Individual Genotype Successes

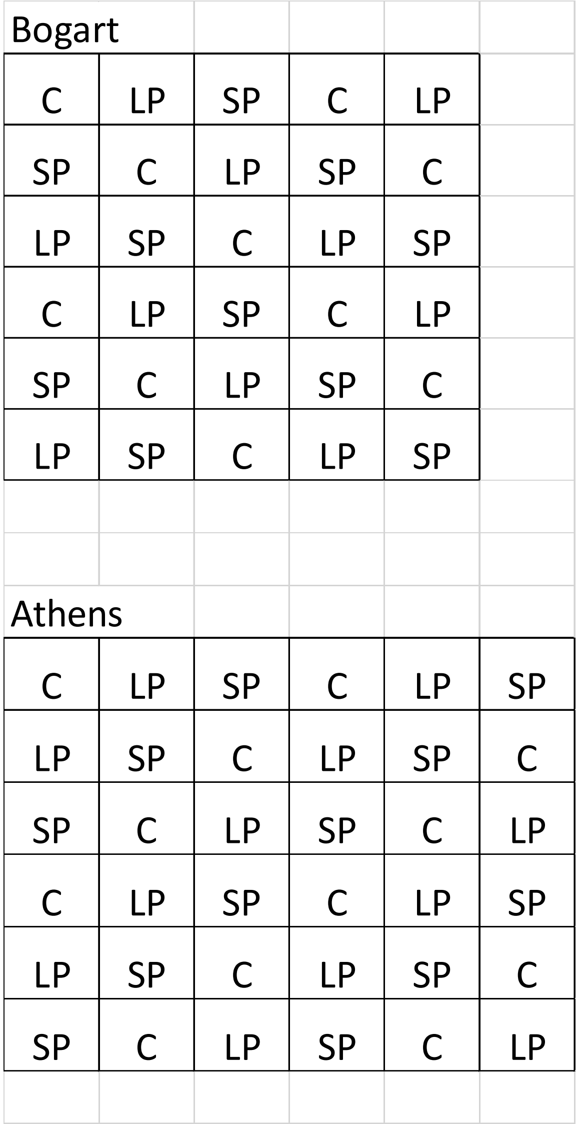
Our results also indicate that factors or traits other than pollen size are contributing to siring success. One control pollen plant genotype in the Athens array sired over one-third of the seeds on the equal-flowering days and almost half of the seeds on the free-flowering days. While this result was the most extreme, it was found that siring success in each pollen type group was dominated by a small number of the parental genotypes. The previous study found a similar phenomenon, where certain genotypes dominated in pollen competition races between pollen of the same size type (McCallum and Chang 2016). Other studies have also shown that certain genotypes are just better competitors: in *Persoonia mollis* (Krauss 2000) and *Hibiscus moscheutos* (Snow and Spira 1996). In the present study, it is unclear what factors or traits may be driving these individual successes, though the results suggest a 'ranking' of genotypes that transcends pollen size, unlike the McCallum and Chang study (2016). In addition, the variation in siring success among genotypes demonstrates the importance of including siring success within any measurement of a plant's fitness (Skogsmyr and Lankinen 2002).

One potential reason for these rankings may be based on environmental conditions, as control pollen plants had the best siring success in the Athens array and the worst in the Bogart array. Results from within-species studies on pollen performance often involve environmental treatments and show increased or decreased performance depending on the treatment (Stephenson et al. 1992, Lau and Stephenson 1993, Delph et al. 1997, Hersch 2006, Distefano et al. 2012). However, it is unclear what these environmental conditions may be in the current study, as the arrays were similar and geographically near each other.

One caveat that should be noted is that the number of genetic markers used in this study were likely insufficient, which resulted in that many of the paternity assignments did not meet the 60% of confidence threshold in Cervus analysis. Paternity exclusion probabilities calculated in the GenAlEx program (Peakall and P.E. 2006, 2012) showed exclusion probabilities only reached 68% for the Athens genotypes and 79% for the Bogart genotypes. These values are significantly lower than typically needed for paternity studies (Queller et al. 1993, Selkoe and Toonen 2006). It is, therefore, possible that the pollen parents assigned may not be the true, despite being the most likely, parents. Adding more markers will be needed to confirm the results found in this study.

Conclusions

In conclusion, it was found that pollen size in *Ipomoea purpurea*, grown in a common garden where pollinations were accomplished by natural pollinators, is negatively correlated with siring success and perhaps correlated with seed production. Specifically, and contrary to predictions, small pollen plants tended to sire more seeds than large pollen plants, though large pollen plants held a slight advantage in seed production. This may represent antagonistic evolution between male and female fitness components. Beyond this study, more genetic info is needed on the parental genotypes in order to confirm these results. If confirmed, other traits than pollen size need to be explored to investigate what may be leading to the improved siring success of small pollen plants and the possible trade-off between male and female functions.



Bogart

C	LP	SP	C	LP
SP	C	LP	SP	C
LP	SP	C	LP	SP
C	LP	SP	C	LP
SP	C	LP	SP	C
LP	SP	C	LP	SP

Athens

C	LP	SP	C	LP	SP
LP	SP	C	LP	SP	C
SP	C	LP	SP	C	LP
C	LP	SP	C	LP	SP
LP	SP	C	LP	SP	C
SP	C	LP	SP	C	LP

Figure 2.1. Experimental array setup. Top – Diagram of the Bogart array and position of parental plants. Bottom – Diagram of the Athens array and position of parental plants. SP = small pollen plants, LP = large pollen plants, and C = control pollen plants.

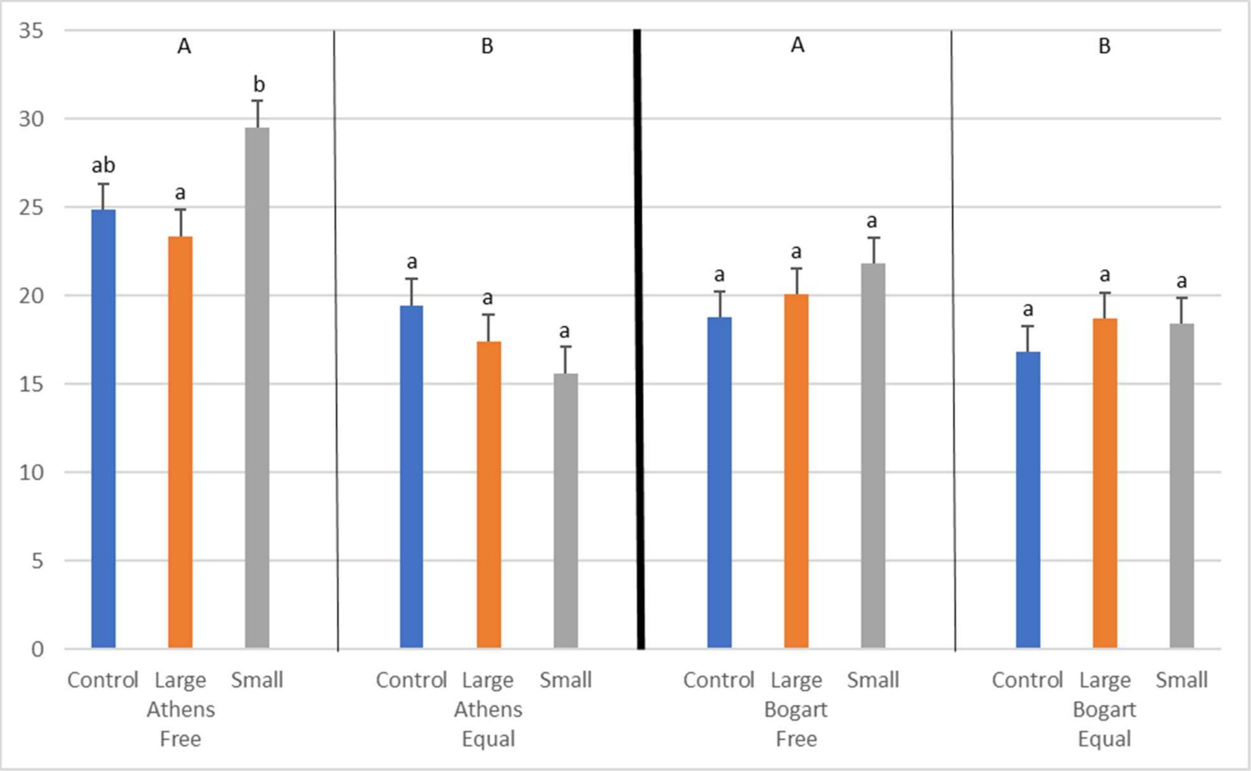


Figure 2.2. Average flower numbers (least square means) across experimental sites and flowering day types. Experimental site by flowering-day types are on the horizontal axis. Blue bars are control plants, orange bars are large pollen plants, and gray bars are small pollen plants. Letters above bars represent post-hoc Tukey tests: uppercase letters represent differences between flowering day types within a study site and lowercase letters represent different plant pollen types within a study site and flowering day type. Other levels of statistical significance are discussed in the text.

Table 2.1. Paternity assignments and patterns for the Athens array. ‘Seeds genotyped’ refers to the total number of offspring plants that were genotyped within the array on those flowering-day types. Other counts are subsets of that total number.

Athens Array Paternity Assignments			
Equal-Flowering Days		**Free-Flowering Days**	
Seeds genotyped	384	Seeds genotyped	384
Seeds assigned to paternal plant(s)	370	Seeds assigned to paternal plant(s)	369
Assignments with min. 60% confidence	14	Assignments with min. 60% confidence	8
Seeds with split paternities	79	Seeds with split paternities	63

Table 2.2. Paternity assignments and patterns for the Bogart array. ‘Seeds genotyped’ refers to the total number of offspring plants that were genotyped within the array on those flowering-day types. Other counts are subsets of that total number.

Bogart Array Paternity Assignments			
Equal-Flowering Days		**Free-Flowering Days**	
Seeds genotyped	384	Seeds genotyped	288
Seeds assigned to paternal plant(s)	377	Seeds assigned to paternal plant(s)	280
Assignments with min. 60% confidence	20	Assignments with min. 60% confidence	23
Seeds with split paternities	35	Seeds with split paternities	26

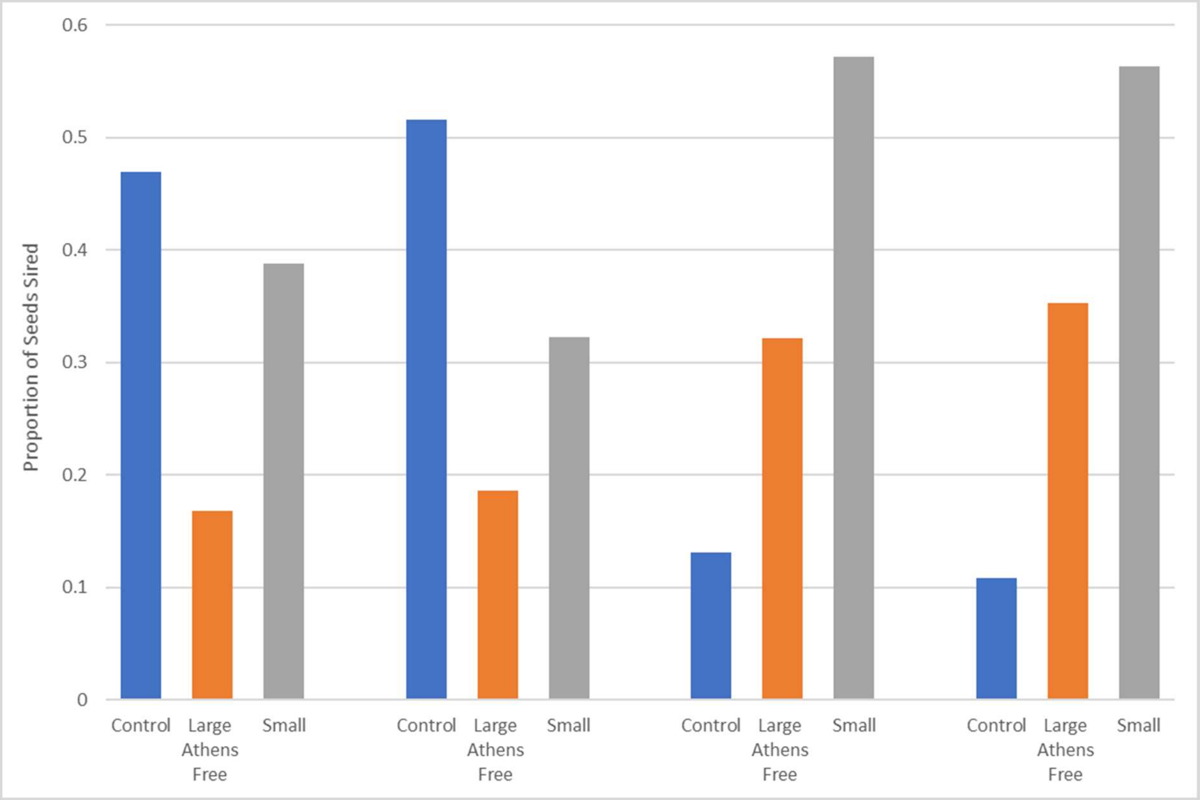


Figure 2.3. Proportion of seeds sired by the pollen plant types across experimental sites and flowering-day types. Experimental site by flowering-day types are on the horizontal axis. Blue bars are control plants, orange bars are large pollen plants, and gray bars are small pollen plants. Proportion of seeds sired significantly differed from expectations across all experimental site by flowering-day type combinations. Other levels of statistical significance are discussed in the text.

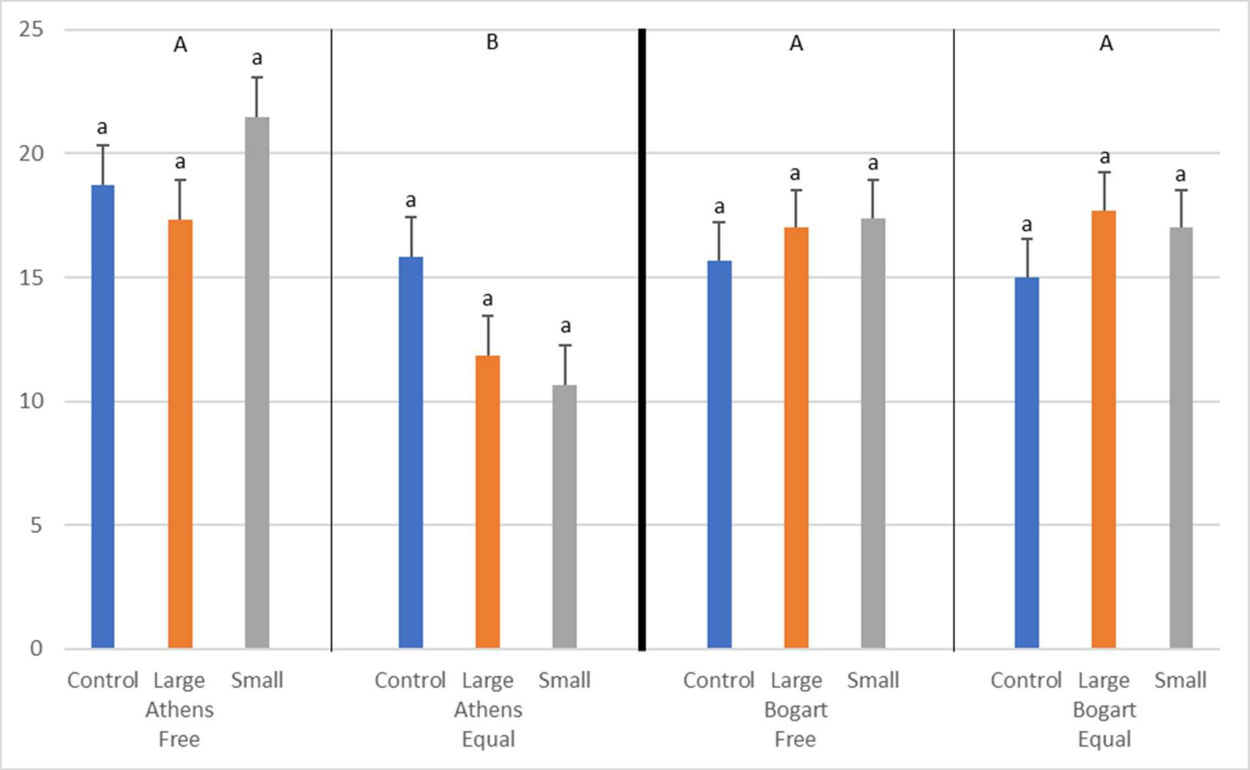


Figure 2.4. Average fruit numbers (least square means) across experimental sites and flowering day types. Experimental site by flowering-day types are on the horizontal axis. Blue bars are control plants, orange bars are large pollen plants, and gray bars are small pollen plants. Letters above bars represent post-hoc Tukey tests: uppercase letters represent differences between flowering day types within a study site and lowercase letters represent different plant pollen types within a study site and flowering day type. Other levels of statistical significance are discussed in the text.

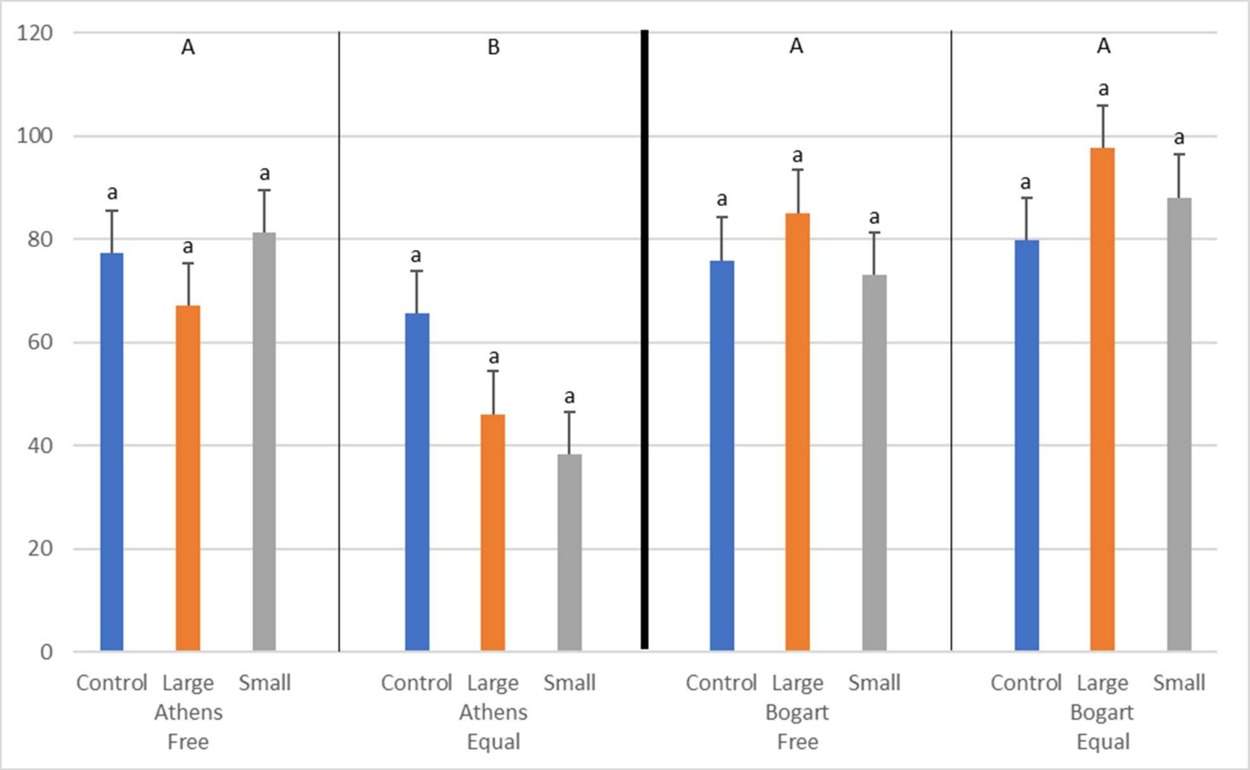


Figure 2.5. Average seed numbers (least square means) across experimental sites and flowering day types. Experimental site by flowering-day types are on the horizontal axis. Blue bars are control plants, orange bars are large pollen plants, and gray bars are small pollen plants. Letters above bars represent post-hoc Tukey tests: uppercase letters represent differences between flowering day types within a study site and lowercase letters represent different plant pollen types within a study site and flowering day type. Other levels of statistical significance are discussed in the text.

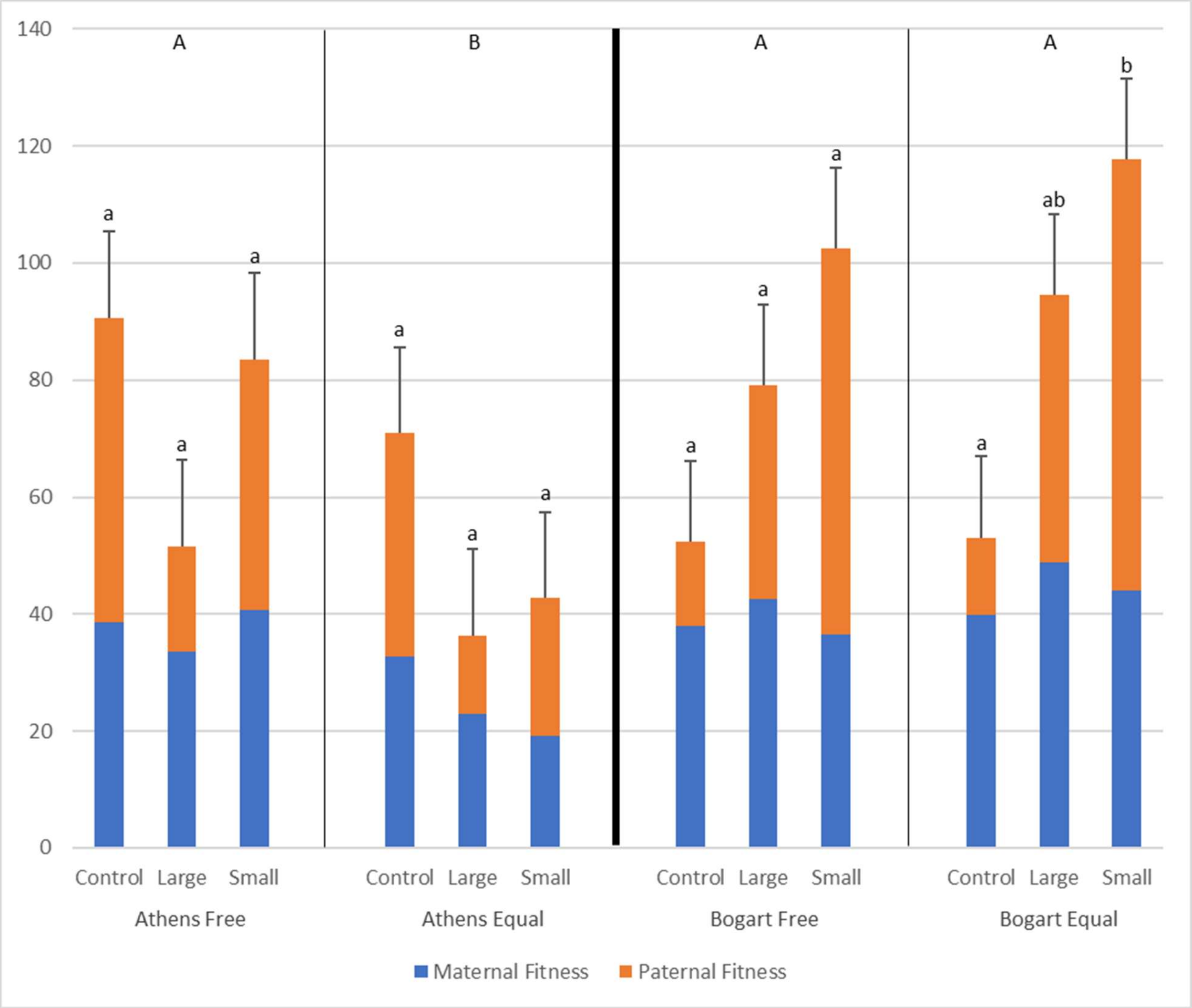


Figure 2.6. Average plant fitness (least square means) and maternal and paternal contributions across experimental sites and flowering day types. Vertical axis is total fitness for the average plant within that group. Blue bars represent maternal contributions and orange bars represent paternal contributions to the average number. Error bars are for that group's average fitness (i.e. maternal and paternal contributions combined). Letters above bars represent post-hoc Tukey tests: uppercase letters represent differences between flowering day types within a study site and lowercase letters represent different plant pollen types within a study site and flowering day type. Other levels of statistical significance are discussed in the text.

CHAPTER 3

CONCLUSIONS

This study found that when comparing the two selected groups, small pollen *Ipomoea purpurea* plants tended to sire more offspring than large pollen plants. This contrasts with our predictions that large pollen plants would have greater siring success than small pollen plants, due to their faster pollen tube growth rates (Chen and Chang, unpublished data). In addition, there were no significant total fitness differences between small and large pollen plants, even though large pollen plants produced more seeds than small pollen plants.

Specifically, plants with small pollen grains sired more seeds than plants with large pollen grains grown in a common garden where pollinations were accomplished by natural pollinators. However, a mechanism for this pattern is uncertain. One mechanism explored in this study was flower number differences, as greater flower numbers would allow a plant to outproduce a competitor in terms of whole-plant pollen production. That said, small pollen plants only significantly produced more flowers than large pollen plants in the Athens array, yet the siring advantage was always present. Therefore, the genotypes in each study site are deriving this advantage in different ways, or small pollen plants are deriving their advantage in some other manner.

It is worth noting that in the previous competition study by McCallum and Chang (2016) small and large pollen grains were not mixed before being applied to recipient stigmas. Studies have shown that pollen germination and pollen tube growth may change when pollen grains are mixed with those of different genotypes (Lankinen and Skogsmyr 2002, Kron and Husband

2006, Zhang et al. 2010). If this phenomenon also exists in *I. purpurea,* the competitive ability, and hence siring success, of small pollen grains may differ during natural pollination than that found in the previous, more controlled, study by McCallum and Chang (2016). This hypothesis will need to be investigated in future research.

Another uninvestigated mechanism in the current system is pollen primacy, or the timing of pollen arrival. It was shown in *I. purpurea* that pollen that arrives first enjoys a fertilization advantage that often overrides the competitive advantage (Epperson and Clegg 1987). If small pollen grains were somehow reaching potential mates before large pollen plants, small pollen plants' siring success advantage could be reflecting such pollen primacy. However, it is unclear what this mechanism may be and will need to be investigated in future work.

Though non-significant, large pollen plants produced more seeds than small pollen plants. These results show a possible mechanism for the maintenance of pollen size variation in *I. purpurea*, namely a trade-off between siring ability and seed production. Antagonistic evolution between male and female traits in plants may seem counterintuitive (Skogsmyr and Lankinen 2002), as traits that benefit one sex often benefit the other (e.g. showy flowers to attract pollinators). However, trade-offs between pollen performance and seed production have been observed (Lankinen et al. 2017).

Finally, our results indicate that factors or traits other than pollen size are contributing to siring success and seed production. It was found that siring success in each pollen type group was dominated by a small number of the parental genotypes. In the present study, it is unclear what factors or traits may be driving these individual successes, though the results suggest a 'ranking' of genotypes that transcends pollen size, unlike the McCallum and Chang study (2016).

One caveat that should be noted is that the number of genetic markers used in this study were likely insufficient, which resulted in that many of the paternity assignments did not meet the confidence threshold. It is, therefore, possible that the pollen parents assigned may not be the true, despite being the most likely, parents. Adding more markers will be needed to confirm the results found in this study.

LITERATURE CITED

Aksoy, R., V. M. F. Almeida-Val, V. C. R. Azevedo, R. Baucom, P. Bazaga, L. B. Beheregaray, J. L. Bennetzen, R. A. Brassaloti, T. I. Burgess, A. Caccone, S.-M. Chang, A. Y. Ciampi, S. Ciancaleoni, G. T. Clímaco, C. Clouet, M. R. M. Coimbra, L. L. Coutinho, H. L. Dantas, C. De Vega, R. Echodu, J. Enyaru, A. Figueira, M. A. G. Filho, B. Foltz, L. Fressigné, M. Gadomski, N. Gauthier, C. M. Herrera, C. Hyseni, E. C. Jorge, D. Kaczmarczyk, E. Knott, A. Kuester, A. P. S. Lima, M. A. Lima, M. P. Lima, A. L. B. Longo, G. Lor, R. Maggioni, T. S. Marques, A. R. Martins, D. A. Matoso, M. Medrano, M. A. C. Mendonça, R. Mettler, P. R. M. Nascimento, V. Negri, K. K. C. Oliveira, L. O. Oliveira, I. Ovcarenko, M. N. Paula-Silva, L. Raggi, J. Sandoval-Castillo, C. H. D. A. Santos, H. M. Schaefer, G. Segelbacher, M. M. Seino, M. Sistrom, M. M. Taole, P. R. Teske, A. Tsagkarakou, L. M. Verdade, P. M. S. Villela, C. C. Vinson, B. D. Wingfield, and M. J. Wingfield. 2013. Permanent Genetic Resources added to Molecular Ecology Resources Database 1 October 2012–30 November 2012. Molecular Ecology Resources 13:341–343.

Arthur, K. M., Z. Vejlupkova, R. B. Meeley, and J. E. Fowler. 2003. Maize ROP2 GTPase Provides a Competitive Advantage to the Male Gametophyte. Genetics 165:2137–2151.

Baker, H. G., and I. Baker. 1979. Starch in Angiosperm Pollen Grains and Its Evolutionary Significance. American Journal of Botany 66:591–600.

Bernasconi, G., T. L. Ashman, T. R. Birkhead, J. D. D. Bishop, U. Grossniklaus, E. Kubli, D. L. Marshall, B. Schmid, I. Skogsmyr, R. R. Snook, D. Taylor, I. Till-Bottraud, P. I. Ward, D.

W. Zeh, and B. Hellriegel. 2004. Evolutionary Ecology of the Prezygotic Stage. Science 303:971–975.

Charlesworth, D. 2006. Evolution of Plant Breeding Systems. Current Biology 16:726–735.

Clegg, M. T., and M. L. Durbin. 2000. Flower color variation: a model for the experimental study of evolution. Proc. Natl. Acad. Sci. U.S.A. 97:7016–7023.

Couvillon, M. J., C. M. Walter, E. M. Blows, T. J. Czaczkes, K. L. Alton, and F. L. W. Ratnieks. 2015. Busy Bees: Variation in Insect Flower-Visiting Rates across Multiple Plant Species. Psyche 2015.

Cruden, R. W., and D. L. Lyon. 1985. Correlations Among Stigma Depth , Style Length , and Pollen Grain Size : Do They Reflect Function or Phylogeny? Botanical Gazette 146:143–149.

Cruden, R. W., and S. Miller-Ward. 1981. Pollen-Ovule Ratio , Pollen Size , and the Ratio of Stigmatic Area to the Pollen-Bearing Area of the Pollinator : An Hypothesis. Society for the Study of Evolution 35:964–974.

Cruzan, M. B. 1990. Variation in Pollen Size , Fertilization Ability , and Postfertilization Siring Ability in Erythronium grandiflorum. Evolution 44:843–856.

Cullings, K. 1992. Design and Testing of a Plant-Specific PCR Primer for Ecological and Evolutionary Studies. Molecular Ecology 1:233–240.

Delph, L. F., and T. Ashman. 2006. Trait selection in flowering plants : how does sexual selection contribute ? Integrative and Comparative Biology 46:465–472.

Delph, L. F., M. H. Johannsson, and A. G. Stephenson. 1997. How Environmental Factors Affect Pollen Performance : Ecological and Evolutionary Perspectives. Ecology 78:1632–1639.

Delph, L. F., C. Weinig, and K. Sullivan. 1998. Why fast-growing pollen tubes give rise to vigorous progeny: the test of a new mechanism. Proceedings of the Royal Society B: Biological Sciences 265:935–939.

Diaz, A., and M. R. Macnair. 1999. Pollen tube competition as a mechanism of prezygotic reproductive isolation between Mimulus nasutus and its presumed progenitor M. guttatus. New Phytologist 144:471–478.

Distefano, G., A. Hedhly, G. Las Casas, S. La Malfa, M. Herrero, and A. Gentile. 2012. Male-female interaction and temperature variation affect pollen performance in Citrus. Scientia Horticulturae 140:1–7.

Doyle, J., and J. Doyle. 1987. A Rapid DNA Isolation Procedure for Small Quantities of Fresh Leaf Tissue. Phytochemical Bulletin 19:11–15.

Epperson, B. K., and M. T. Clegg. 1987. First-pollination primacy and pollen selection in the morning glory, ipomoea purpurea. Heredity 58:5–14.

Erbar, C. 2003. Pollen tube transmitting tissue: Place of competition of male gametophytes. International Journal of Plant Sciences 164:S265–S277.

Foltz, B. 2013. Functional Significance of Pollen Size ariation in Ipomoea Purpurea, Common Morning Glory. University of Georgia.

Galen, C., J. A. Shykoff, and R. C. Plowright. 1986. Consequences of stigma receptivity schedules for sexual selection in flowering plants. The American Naturalist 127:462–476.

Harder, L. D. 1998. Pollinated angiosperms with different pollination characteristics. Biological Journal of the Linnean Society:513–525.

Hersch, E. I. 2006. Foliar Damage to Parental Plants Interacts to Influence Mating Success of Ipomoea purpurea. Ecology 87:2026–2036.

Hiscock, S., and A. M. Allen. 2008. Diverse cell signalling pathways regulate pollen – stigma interactions : the search for consensus. New Phytologist 179:286–317.

Johnston, M. 1993. Tests of Two Hypotheses Concerning Pollen Competition in a Self-Compatible, Long-Styled Species (Lobelia cardinalis : Lobeliaceae). American Journal of Botany 80:1400–1406.

Jürgens, A., T. Witt, and G. Gottsberger. 2012. Pollen grain size variation in Caryophylloideae: A mixed strategy for pollen deposition along styles with long stigmatic areas? Plant Systematics and Evolution 298:9–24.

Kalinowski, S. T., M. L. Taper, and T. C. Marshall. 2007. Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. Molecular Ecology 16:1099–1106.

Korbie, D. J., and J. S. Mattick. 2008. Touchdown PCR for increased specificity and sensitivity in PCR amplification. Nature Protocols 3:1452–1456.

Krauss, S. L. 2000. The realized effect of postpollination sexual selection in a natural plant population. Proceedings of the Royal Society of London. Series B: Biological Sciences 267:1925–1929.

Kron, P., and B. C. Husband. 2006. The effects of pollen diversity on plant reproduction: Insights from apple. Sexual Plant Reproduction 19:125–131.

Kumar, D., and K. R. Sarkar. 1980. Correlations between pollen diameter and rate of pollen tube growth in maize (Zea mays L.). Indian Journal of Experimental Biology 18:1242–1244.

Lamborn, E., J. E. Cresswell, and M. R. Macnair. 2005. The potential for adaptive evolution of pollen grain size in Mimulus guttatus. New Phytologist 167:289–296.

Lankinen, Å., S. Hydbom, and M. Strandh. 2017. Sexually antagonistic evolution caused by male–male competition in the pistil. Evolution 71:2359–2369.

Lankinen, Å., and J. A. Madjidian. 2011. ENHANCING POLLEN COMPETITION BY DELAYING STIGMA RECEPTIVITY: POLLEN DEPOSITION SCHEDULES AFFECT SIRING ABILITY , PATERNAL DIVERSITY , AND SEED PRODUCTION IN C OLLINSIA HETEROPHYLLA (PLANTAGINACEAE). American Journal of Botany 98:1191–1200.

Lankinen, A., and I. Skogsmyr. 2001. The Effect of Pollen Competition on Maintenance of Variation in Fertilisation Ability. Oikos 93:459–469.

Lankinen, A., and I. Skogsmyr. 2002. Pollen competitive ability: the effect of proportion in two-donor crosses. Evolutionary Ecology Research 4:687–700.

Lau, T.-C., and A. G. Stephenson. 1993. Effects of Soil Nitrogen on Pollen Production, Pollen Grain Size, and Pollen Performance in Cucurbita pepo (Cucurbitaceae). American Journal of Botany 80:763–768.

Lord, A. E. M., and K. J. Eckard. 1984. Incompatibility between the Dimorphic Flowers of Collomia grandiflora, a Cleistogamous Species. Science 223:695–696.

Marshall, D. L. 1998. POLLEN DONOR PERFORMANCE CAN BE CONSISTENT ACROSS MATERNAL PLANTS IN WILD RADISH (RAPHANUS SATIVUS,BRASSICACEAE): A NECESSARY CONDITION FOR THE ACTION OF SEXUAL SELECTION. American Journal of Botany 85:1389–1397.

Marshall, D. L., and P. K. Diggle. 2001. MECHANISMS OF DIFFERENTIAL POLLEN DONOR PERFORMANCE IN WILD RADISH, RAPHANUS SATIVUS (BRASSICACEAE). American Journal of Botany 88:242–257.

Marshall, D. L., and A. S. Evans. 2016. Can selection on a male mating character result in evolutionary change? A selection experiment on California wild radish, Raphanus sativus.

American Journal of Botany 103:553–567.

Marshall, D. L., and M. W. Folsom. 1991. Mate Choice in Plants : An Anatomical to Population Perspective. Annual Review of Ecology and Systematics 22:37–63.

Marshall, D. L., and D. M. Oliveras. 2001. Does differential seed siring success change over time or with pollination history in wild radish, Raphanus sativus (Brassicaceae)? American Journal of Botany 88:2232–2242.

Mazer, S. J., A. A. Hove, B. S. Miller, and M. Barbet-Massin. 2010. The joint evolution of mating system and pollen performance: Predictions regarding male gametophytic evolution in selfers vs. outcrossers. Perspectives in Plant Ecology, Evolution and Systematics 12:31–41.

McCallum, B., and S. M. Chang. 2016. Pollen competition in style: Effects of pollen size on siring success in the hermaphroditic common morning glory, Ipomoea purpurea. American Journal of Botany 103:460–470.

Mitchell, R. J., R. J. Flanagan, B. J. Brown, N. M. Waser, and J. D. Karron. 2009. New frontiers in competition for pollination. Annals of Botany 103:1403–1413.

Montes-R, C., and J. W. White. 1996. Effect of Selection for Pollen Grain Size on Various Traits in Common Bean. Euphytica 90:59–63.

Mulcahy, D. L. 1979. The Rise of the Angiosperms : A Genecological Factor. Science 206:20–23.

Peakall, R., and S. P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288–295.

Peakall, R., and S. P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539.

Queller, D. C., J. E. Strassmann, and C. R. Hughes. 1993. Microsatellites and kinship. Trends in Ecology and Evolution 8:285–288.

Radford, A. E., H. E. Ahles, and C. R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. University of North Carolina Press, Chapel Hill.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pollination biology:305–329.

Roux, K. 1995. Optimization and Troubleshooting in PCR. Pcr Methods And Applications 4:185–194.

Sarkissian, T. S., and L. D. Harder. 2001. Direct and indirect responses to selection on pollen size in Brassica rapa L. Journal of Evolutionary Biology 14:456–468.

Selkoe, K. A., and R. J. Toonen. 2006. Microsatellites for ecologists: A practical guide to using and evaluating microsatellite markers. Ecology Letters 9:615–629.

Shuster, S. M. 2009. Sexual selection and mating systems. Proceedings of the National Academy of Sciences of the United States of America 106:10009–10016.

Skogsmyr, I., and Å. Lankinen. 2002. Sexual selection: An evolutionary force in plants? Biological Reviews of the Cambridge Philosophical Society 77:537–562.

Snow, A. A., and T. P. Spira. 1991. Pollen Vigor and the Potential for Sexual Selection in Plants. Nature 352:796–797.

Snow, A. A., and T. P. Spira. 1996. Pollen-Tube Competition and Male Fitness in Hibiscus moscheutos. Evolution 50:1866–1870.

Spira, T. P., A. A. Snow, D. F. Whigham, and J. E. N. Leak. 1992. Flower Visitation, Pollen Deposition, and Pollen-Tube Competition in Hibiscus moscheutos (Malvaceae). American Journal of Botany 79:428–433.

Stephenson, A. G., S. E. Travers, J. I. Mena-Ali, and J. A. Winsor. 2003. Pollen performance before and during the autotrophic-heterotrophic transition of pollen tube growth. Philosophical Transactions of the Royal Society B: Biological Sciences 358:1009–1018.

Stephenson, A., T.-C. Lau, M. Quesada, and J. Winsor. 1992. Factors that Affect Pollen Performance.

Tejaswini. 2002. Variability of pollen grain features: A plant strategy to maximize reproductive fitness in two species of Dianthus? Sexual Plant Reproduction 14:347–353.

Walsh, N. E., and D. Charlesworth. 1992. Evolutionary Interpretations of Differences in Pollen Tube Growth Rates. The Quarterly Review of Biology 67:19–37.

Webster, T. M. 2013. Weed Survey - Southern States: Broadleaf Crops Subsection. Proceedings of the Southern Weed Science Society 57:404–426.

Winsor, J. A., S. Peretz, and A. G. Stephenson. 2000. Pollen competition in a natural population of Cucurbita foetidissima (Cucurbitaceae). American Journal of Botany 87:527–532.

Zhang, C., N. Tateishi, and K. Tanabe. 2010. Pollen density on the stigma affects endogenous gibberellin metabolism, seed and fruit set, and fruit quality in Pyrus pyrifolia. Journal of Experimental Botany 61:4291–4302.

Zomlefer, W. B., and S. Knapp. 1994. Guide to Flowering Plant Families. University of North Carolina Press, Chapel Hill.