FITNESS, POLLEN DISPERSAL, AND FLORAL-DISPLAY SIZE EVOLUTION IN ANIMAL-POLLINATED PLANTS

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The great variation in floral-display size (the number of flowers per plant) (FDS) in the thousands of angiosperm species has intrigued biologists and others for centuries, eliciting investigations regarding the ecology, evolution, and development of this variation. Many studies have reported a strong relationship between FDS and reproductive success that indicates there can be considerable natural selection on FDS. My studies continued the exploration of FDS evolution by addressing how pollinators and their interactions with floral displays, from the inflorescence through the population level, affect plant reproduction and the consequent evolution of FDS. To address these questions I used computer simulations and the clonal eudicots *Asclepias syriaca* (Common Milkweed) and *Asclepias incarnata* (Swamp Milkweed) as model organisms.

I found that the insect pollinator taxa (*Apis mellifera*, *Bombus* spp., and a suite of butterflies and moths) of *Asclepias syriaca* can differ significantly in the proportions of self-pollen that they deposit on its flowers. Further, these rates differ with FDS. Using them, I estimated selection on FDS through plant male and female fitness and found that different pollinator taxa may exhibit differing selection pressures on inflorescence size.

The spatial arrangement of a plant’s flowers (e.g., inflorescence size versus total number of flowers on a plant) can also influence plant fitness. In *Asclepias incarnata*, I found that display size, from the patch level through the inflorescence level, affects the magnitude of fruit production and pollen dispersal and that there is a significant trade-off between the size of inflorescences and the number of inflorescences on a stem.
Angiosperm clone size varies from a few through thousands of ramets (stems). Clones can sport large floral displays that can increase their rates of geitonogamy (self-pollination). I used an individual-based simulation of pollen dispersal that accounted for the spatial arrangement of clonal plants and found that clone size, radius, and the distribution of ramets (stems) within clones all statistically interacted, and significantly influenced geitonogamy.

In conclusion, my studies add to the understanding of pollinators and their interactions with FDS and illustrate the complexities and interdependencies of many of the factors involved in floral-display evolution.
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Lots of love,
Aaron
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**Introduction**

There is an intriguingly large diversity of Angiosperms (flowering plants) on earth. Through agricultural, and artificial selection programs, humans have developed plants to provide the food and raw materials for the advancement and sustainment of civilization. Studying plant reproduction is extremely practical in terms of its importance to conservation, biotechnology, invasion ecology and many other areas of research (reviewed by Barrett 2010). The reproductive organs of angiospermous plants, flowers, have been a focal point of this research on plant reproduction, and one of the people to initially look at the flower critically, and more importantly, in an evolutionary framework was Charles R. Darwin (Barrett 2010). In his three books on plant reproduction (1862; 1876; 1877), he postulated many hypotheses about the adaptation and evolution of flowers that are still investigated today. One such aspect of plant mating that Darwin considered was the influence of self- and outcross pollen on reproductive success (1876), and since Darwin, theoretical and empirical advances, especially with the use of genetic markers, have shown a general, but sometimes questioned (Igic and Kohn 2006) dichotomy in mating patterns between selfing and outcrossing species (Lande and Schemske 1985). As a result, recent work has focused on the maintenance of mating patterns, and whether or not mixed mating is evolutionarily tractable or the non-adaptive result of self-pollen deposition among flowers on the same plant (Porcher and Lande 2005). In this context of plant mating systems and the movement of self-pollen among flowers, much of the investigation on the evolution of floral-display size began.

*Floral-display size evolution and plant reproduction*

Geitonogamy is the pollination of a flower with pollen from another flower of the same plant. Xenogamy is the pollination of a flower with pollen from a conspecific flower of another plant. Within
the context of these two types of pollination, the study of floral-display-size evolution is framed in the logic of trade-offs between the advantages and disadvantages of increased or decreased floral-display size. The central notion behind the advantage of increased floral-display is increased attraction of pollinators and, their consequent increase in visitation and outcross pollen deposition (Broyles and Wyatt 1995; Mitchell et al. 2004; Bell et al. 2005). Increased pollen deposition is beneficial to a plant particularly if pollen is limited, which means that its fruit production is limited by the number of male gametes available for ovule fertilization (Harder and Wilson 1998). The increase in pollen deposition, therefore, can result in increased fruit production, and higher quality fruit through selective maturation of fruits with more seeds, higher quality seeds, or both (Wyatt and Broyles 1994). For example, in Asclepias exaltata (Poke Milkweed), inflorescences that abort fruits contain more flowers, receive more pollen, and produce seeds with more fruit than inflorescences that did not abort any fruit (Broyles and Wyatt 1990). Numerous studies have quantified pollinator behavior in the context of floral-display size, and the general trend is that the frequency of pollinator visits increases as floral-display size increases, and more flowers are visited as the number of flowers on the plant increase (though usually at a decreasing rate as flower number increase). However, this is not always the case and it may depend on the context of floral patch size, density, distance between patches, rewards (pollen and nectar), and many other factors (Kunin 1993; Morris 1993; Bond and Maze 1999; Ishihama et al. 2003; Cresswell and Osborne 2004). In addition to increased pollen deposition and fruit production, there is also a correlative increase in pollen removal as a result of increased flower number (Willson and Rathcke 1974), and a requisite increase in seed siring (Broyles and Wyatt 1990). While estimating male reproductive success is more challenging, the literature shows a strong correlation between increased floral-display size and an increase in male reproductive success (Stanton 1991; Broyles and Wyatt 1990; Broyles and Wyatt 1995; Karron et al. 2004).

While there is a positive correlation between floral-display size, pollinator visitation and pollen
deposition, increasing visitation rate can have many detrimental effects on mating success as well. As mentioned above, with increased floral visitation within a plant, the potential for geitonogamy increases as well (Klinkhamer and de Jong 1993). Geitonogamy is considered likely to reduce reproductive output because it does not have the benefit of reproductive assurance, unlike intrafloral autogamy, and it may result in pollen-discounting, inbreeding depression, or both (Lloyd and Schoen 1992; Klinkhamer and de Jong 1993). Pollen-discounting, in this case, is defined as the reduction in available pollen for the siring of outcrossed fruits, and has the greatest effect on reproduction in self-incompatible plants. Inbreeding depression, and reduced reproductive assurance can influence fruit production and seed siring in both self-compatible and self-incompatible plants (Lipow and Wyatt 2000). Along with geitonogamy, increased floral-display size may seriously effect a plant's resource allocation and survival. For example, in the dioecious shrub, *Leucadendron xanthoconus* (Sickle-leaf Conebrush), Bond and Maze (1999) found that increasing the number of flowers on a male plant is correlated with thinner branches, smaller leaves and increased mortality, suggesting a trade-off between siring success and survival. They also found that female reproductive success and plant mortality did not increase with floral-display size. Bond and Maze (1999) is an example of the use of sexual selection theory, or selection acting differentially through male and female fitness, to explain the evolution of floral display, and sexual selection is where the study of floral-display size started in *Asclepias* (Milkweeds, Apocynaceae). The two empirical chapters (1 and 2) of my thesis investigate floral-display size in the North American *Asclepias incarnata* (Swamp Milkweed) and *A. syriaca* (Common Milkweed). So in the next section, I present background on *Asclepias* floral-display size.

*Floral-display size evolution in Asclepias*

The formalization of sexual-selection theory began with Bateman's principle, or more
appropriately Bateman's hypothesis, which postulates that female reproduction is resource limited and male reproduction is limited by access to females (Bateman 1948). More specifically for hermaphroditic (monoecious) plants it states, if female reproductive success is not pollen limited, then an abiotic resource or resources (e.g., light, water, and nitrogen) that limits fruit production should drive the evolution of reproductive characters (Queller 1983; Broyles and Wyatt 1990). Therefore, increasing the number of flowers on a plant’s inflorescence should not increase the plant’s fruit production due to lack of non-pollen resources. However, increasing floral-display size should increase the plant’s male reproductive success because pollinators should pick up and subsequently deposit more of the plant’s pollen on other plant's stigmas and increase its frequency of siring seeds (Queller 1997). In short, the expectation is that as floral-display size increases, male fitness increases, but female fitness does not.

Willson and Rathcke (1974) were the first to find support for this hypothesis when studying pollen deposition in Milkweeds, and many other researchers who also used indirect measurements of male fitness (e.g., pollen removal) have obtained supporting evidence for Bateman's principle in Asclepias by showing that there was an increased rate of pollen removal with increased floral-display size (Willson and Price 1977; Wilson et al.; 1979; Queller 1983). Nonetheless, Wyatt (1980) determined that the increased pollination rate found by Willson and Rathcke (1974) and others does not necessarily result in increased reproduction because of the possibility of high selfing rates in the fully or partially self-incompatible Asclepias spp. (Broyles and Wyatt 1994). This means that even though there are high rates of pollen deposition, an appreciable amount of the pollen is wasted, or discounted to the degree that there is inbreeding depression, because it is self-pollen. This conclusion led to the formalization of the hypothesis of trade-offs between increased attractiveness and self-pollination according to Klinkhamer and De Jong (1993) who state that the “evolution of larger inflorescences is driven by selection for greater pollinator attraction, but constrained by higher rates of geitonogamy
experienced by larger inflorescences” (Finer and Morgan 2003). Finer and Morgan (2003) found, in support of this hypothesis, that geitonogamy significantly reduced fruit production in *A. speciosa*.

In fact, the above hypotheses are not mutually exclusive, and the idea of trade-offs are a natural extension of sexual selection theory in that increased floral-display size, that is correlated with increased pollen deposition and removal, must at some point be limited either by the abiotic resources required to produce seeds and pollen, or the negative effects of geitonogamy, or both. The empirical evidence suggests that the processes in both hypotheses may be simultaneously occurring in *Asclepias* (Queller 1983; Broyles and Wyatt 1990; Finer and Morgan 2003) and selection on floral-display may vary greatly due to environmental and ecological conditions (Fishbein and Venables 1996a; Ivey at al. 2003).

*Overall Goal*

Up to this point, I have addressed the direct relationships between pollinator attractiveness, resources and self-pollination, which most studies, in *Asclepias* and other flowering plants, have investigated in relation to floral-display size and plant reproduction. However, many studies have gone beyond these direct relationships and found that factors including plant density, population and patch size, spatial arrangement of plants, clonality, variation in pollinator taxon behavior, and many others can influence plant reproductive success (*e.g.*, Sih and Baltus 1987; Kunin 1993; Fishbein and Venables 1996b; Thompson 2001; Ivey et al. 2003; Cresswell and Osborne 2004; Mori et al. 2009). Therefore, the general goal of my thesis is to further elucidate the interaction between some of these ecological factors and the evolution of floral-display size. Specifically, chapter one investigates the relationship between the self-pollination rate for various pollinator taxa and floral-display size, chapter two quantifies how multiple levels of floral-display size (*e.g.*, patch size, stem size) may effect female
reproductive success and pollen dispersal, and the chapter three examines how clonal growth form and spatial aggregation influences pollen dispersal and geitonogamy via modeling.

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Chapter 1:

Self-pollination rate and floral-display size evolution in *Asclepias syriaca* (Common Milkweed) with regard to various pollinator taxa

Introduction

In most flowering plants, reproduction depends on the movement and deposition of pollen. Plant immobility complicates reproduction because the transmission of pollen between individuals requires a vector. The interconnection of pollinating vectors and plants are the flowers, and floral characteristics (such as display architecture, shape, spatial arrangement, size, color, and phenology) can influence pollinator behavior, which in turn can impact the deposition of self- and outcross pollen (Broyes and Wyatt 1995).

The roles of pollinators in plant reproduction have been estimated in several ways including (1) the proportion of visitors bearing pollen (Beattie 1971), (2) the rate of pollen deposition (Herrera 1987), (3) amount of pollen removed from anthers (Armbruster 1990), (4) fruit set and seed set (Schemske and Horovitz 1984; Stanton et al. 1991), (5) potential for geitonogamy (pollen deposition among the flowers of one plant) (Ivey et al. 2003), and (6) pollinator-seed outcrossing rates (Brunet and Sweet 2006). However, previous studies that quantify pollen movement alone lack an estimate of genetic transmission, and the studies that measure seed and fruit production cannot differentiate between a possible effect of pollinator behavior and post-pollination processes (except see Karron et al. 2009).

Many of the above studies found a relationship not only between pollinators and plant reproduction, but also an interaction among pollinator behavior, reproduction, and floral-display size (*i.e.*, Brunet and Sweet 2006). Floral-display size is an important factor that may influence the rate of
self- or outcrossed pollen deposition. The rate of outcross-pollen deposition often increases as population density, size, or both increase (Karron et al. 1999; Herlihy and Eckert 2004). This may result from increased pollinator visitation that decreases pollen limitation and intrafloral autogamy (pollination of a flower with its own pollen), or a reduction in the number of flowers visited in succession on a plant which reduces geitonogamy (interfloral autogamy, pollination of a flower with pollen from another flower on the same plant) (Klinkhamer and de Jong 1993; Kunin 1993; Wyatt and Ivey 1999). However, geitonogamy may also increase with floral-display size, if pollinators visit more flowers per visit to individual plants (Klinkhamer and de Jong 1993; Karron 2004).

The pollination and floral-display size of *Asclepias* (Milkweeds) have been studied in great detail because they provide a model system for studying pollen movement, as their pollen is packaged in discrete structures termed pollinia that function as units of pollen dispersal (Wyatt 1976). In order for pollination to occur, a pollinium must be removed from a flower by a pollinator and inserted, in its correct orientation, into the stigmatic slit of another flower. There are 10 pollinia per flower and two pollinia are attached by a small dark organ called the corpusculum that is easily seen with the naked eye. This allows one to see discrete and easily quantifiable pollination events. Previous studies have shown that different pollinator types do insert pollinia at different rates (Fishbein and Venables 1996; Ivey et al. 2003), and that floral-display size does impact reproductive success (Willson 1974; Broyles and Wyatt 1990; Broyles and Wyatt 1995). However, the level of floral display (e.g., the inflorescence or whole plant) that most influences reproductive success and at which level selection on floral-display occurs is still in question (Queller 1983; Broyles and Wyatt 1995; Fishbein and Venables 1996; Broyles and Wyatt 1997).

The majority of *Asclepias* spp. may be partially through fully self-in incompatible (Wyatt and Broyles 1994). This is potentially a very serious problem for the reproductive success of *Asclepias* spp. because their flowers have only five stigmatic slits and there can be stigma clogging and competition
for ovules with outcrossed pollinia. *Asclepias* spp. have a late-acting self-incompatibility system, and self-pollen tubes germinate just as fast as outcross-pollen ones, and can reduce the number of compatible fertilizations (Lipow and Wyatt 2000). However, estimating *Asclepias* self-pollination rate is methodologically difficult with only a few studies having made population-wide estimates (Pleasants and Ng 1987, Pleasants 1991; Shore 1992), with Shore (1992) being the only study that used genetic markers. Additionally, while several studies have investigated the relative quantity of pollen deposited by different pollinators in *Asclepias* (Fishbein and Venables 1996; Ivey et al. 2003), none have estimated the source or quality of the pollen (e.g., the proportion of self- versus outcrossed pollen), which is critical when quantifying reproduction in self-incompatible plants.

In general, *Asclepias* plants are pollinated by a wide range of pollinators, including native bees, butterflies, moths, flies, beetles, and the introduced *Apis mellifera* (Western Honey Bee) (Wyatt 1976; Willson and Bertin 1979; Kephert 1983; Wyatt and Broyles 1994; Fishbein and Venables 1996; Ivey et al. 2003). *Apis mellifera* is an efficient pollinator for many plant species including several *Asclepias* species (Fishbein and Venables 1996; Goulson 2003; Ivey et al. 2003). However, a few studies have demonstrated the *A. mellifera* may detrimentally affect seed set (Roubik 1996; Gross and Mackay 1998), and these results are consistent with Paton (1990; 1993) who found that *A. mellifera* moved among plants less than the native pollinators, despite removing more pollen, resulting in decreased outcross pollen deposition and seed set. Therefore, even though it is clear that *A. mellifera* are efficient at removing and depositing the pollen of *Asclepias* plants it is unclear what their potential influence is on the reproductive success of these plants.

In this study, we took advantage of the evolutionarily derived pollination biology of *Asclepias* to examine how *A. mellifera* and the native pollinators of *A. syriaca* influence the rate of self-pollen (pollinia) deposition. Specifically, we tested the following hypotheses (1) that the various pollinators of *A. syriaca* behave differently when visiting flowers, and the difference in behavior is influenced by
floral-display size, (2) as a result of the differing behavior, the pollinator taxa have different self-pollination rates, and (3) that the contrasting self-pollination rates, in the context of floral-display size, may result in disparate natural selection on floral-display size between the pollinator taxa.

To test these hypotheses, we sampled two *A. syriaca* populations over 2 yr, each with thousands of stems, and we estimated pollen-deposition and the self-pollination rate, by counting and genotyping inserted pollinia using microsatellite genetic markers, for various pollinator taxa that visit *A. syriaca*. Then we examined those results with regard to pollinator behavior and select measures of floral-display size. Additionally, using the self-pollination data, we also quantified how different pollinators influence phenotypic selection on floral-display size.

**Methods**

**Pollinator types and pollen movement**

We obtained data from two populations of *A. syriaca*, each with thousands of stems. One population was located in the suburbs of Washington, D.C., on the grounds of the Audubon Naturalist Society in Chevy Chase, MD. The other population is located in the rural Blandy Experimental Farm in Boyce, VA. In June–July, 2008–2009, we bagged one inflorescence (an umbellate cyme in the genus *Asclepias* (Liede and Weberling 1995) per flowering stem. We bagged inflorescences with bridal veil because this kind of netting has the smallest effect on microenvironment, nectar production (Wyatt et al. 1992) and pollinator behavior (pers. obs.) compared to other netting materials. However, insects occasionally deposited pollinia through the bridal-veil bags, so we also used bagged inflorescences to account for these insertions. At peak flowering for each selected inflorescence, we
measured the following floral-display size and plant architecture characteristics: total number of inflorescences per stem, number of flowers per focal inflorescence (NFPFI, see Appendix A for an index of commonly used abbreviations), number of flowers per stem (NFPS), focal inflorescence position on the stem (starting with the lowest inflorescence as 1), stem height and stem density (distance to three nearest flowering stems).

First, immediately after collecting floral-display data for a stem, we removed the focal inflorescence’s bag and observed the inflorescence’s first insect visitor. Second, we attempted to collect each pollinator after its visiting bout, but that was often very challenging, especially with the lepidopterans. Therefore, we identified the visitor to lowest taxonomic level possible in the field and recorded its behavior (the number of flowers visited on the focal inflorescence (NFVOFI) and number of inflorescences it visited, and its visiting time (VT)). Third, we removed the inflorescence from its stem and quantified its number of pollinia inserted and removed. Fourth, we scored pollinium insertions and removals, under magnification of a dissecting microscope, by probing each flower’s stigmatic chambers with forceps (Fishbein and Venables 1996; Ivey and Wyatt 2003). Fifth, we removed inserted pollinia from the stigmatic chambers and immediately placed them on ice for subsequent genetic analysis. Last, we genotyped inserted pollinia using four polymorphic-microsatellite-locus primer sequences (O’Quinn and Fishbein 2009).

**Self-pollination rate**

From the genotypic data, we estimated self-pollination rate. Direct estimation of self-pollination rates is the most straightforward and powerful method for determining a plant’s fraction of self-pollen deposition. To estimate a self-pollination rate, the multilocus genotype of a pollinator-inserted pollinia in a flower are compared to the genotype of the plant bearing the flower. If any of the
pollinium alleles are different than the maternal plant’s alleles, the pollinium is classified as an outcrossed one. However, there is a finite probability that multiple plants have the same multilocus genotypes, through chance or relatedness, which could result in erroneously assigned insertions when the maternal plant and pollinium have the same genotype. Therefore, the direct-estimation method ($S_d$) results in an unbiased estimate of the self-pollination rate only if the probability of erroneously assigned insertions is very small. Since pollinia are aggregates of haploid pollen grains from the same sire, genotyping pollinia allows us to determine the paternal diploid genotype. Determining the diploid paternal genotype in pollinia increases the power of exclusion, and increases the power of self-pollination rate estimation.

To estimate the self-pollination rates, we used a modified method-of-moments estimator, that controls for incorrectly assigned outcross pollinations, based on Shaw (1981) and Shore (1992) and modified from Cruzan et al. (1994) that we designate as $S_m$. Cruzan (1994) estimated the probability of undetected outcross insertions ($\alpha$) using the paternal-pollen-pool allele frequencies, but here, we used the pollen-pool genotype frequencies (Shore 1992). Estimating the genotype frequencies in A. syriaca is potentially difficult because it is a clonal species with up to thousands of ramets (stems) per genet (entire plant) (Wilbur 1976). Considering each flowering ramet to be a separate genet will severely underestimate the genotypic diversity in the population, but conversely assuming that each genotype is an individual genet indiscriminately, may result in an overestimation of the frequency of rare alleles in the population. Both of these biases may result in inaccurate allele-frequency estimation and, therefore, inaccurate estimation of self-pollination rate. To overcome the overestimation of rare alleles, we used a round-robin method of allele frequency estimation described by Park and Werth (1993).

We were unable to estimate method-of-moments self-pollination rates with regard to the family level (defined as all pollinia inserted into flowers of the same plant) because the median-family insertion rate was 0, so we estimated $S_m$ for each pollinator taxon ($S_{mp}$). Observed $\alpha$ ($\alpha_d$) is
\[ \alpha_d = \sum_{i=1}^{N} w_i \prod_{k=1}^{n} P_{ik}, \] (1)

where \( P_{ik} \) is the diploid genotype frequency of multilocus paternal genotype \( i \) at locus \( k \), and \( w_i \) is the weighted frequency of inserted pollinia in inflorescences visited by a particular pollinator taxon (or inserted into flowers within a control bag) with multilocus paternal genotype \( i \). \( N \) is the number of unique maternal-family diploid genotypes visited by a particular pollinator taxon, and \( n \) is the number of loci. To control for potential bias in the self-pollination rate estimates due to insertions through the bags prior to pollinator visitation, we used weighted-means estimates of \( \alpha_d \) and the observed self-pollination rate \( (S_d) \) to attribute the portion of both variables to bagged- \( (\alpha_{dc} \) and \( S_{dc} \)) versus pollinator-inserted \( (\alpha_{dp} \) and \( S_{dp} \)) pollinia. The observed \( \alpha \) for pollinator-inserted pollinia \( (\alpha_{dp}) \) is

\[ \alpha_{dp} = \frac{\alpha_{dT} - (1-r)\alpha_{dc}}{r} \quad \text{for} \quad \alpha_{dT} > \alpha_s(1-r), \] (2)

where \( \alpha_{dT} \) is the estimate of observed \( \alpha \) for all insertions (including insertions through the bags and due to pollinators), and \( r \) is the proportion of insertions attributed to the pollinator. The observed self-pollination rate for pollinator-inserted pollinia \( (S_{dp}) \) is similarly calculated as

\[ S_{dp} = 1 - \frac{(t_{dT} - (1-r)t_{dc})}{r} \quad \text{for} \quad t_{dT} > t_{dc}(1-r), \] (3)

where \( t_{dT} \) is the total observed direct-outcrossing rate and \( t_{dc} \) is the observed outcrossing rate for pollinia inserted into the control inflorescences. We used the estimates of \( \alpha_{dp} \) and \( S_{dp} \) to estimate the self-
pollination rates ($S_{mp}$) attributed to each pollinator taxon using the equation described by Cruzan (1994). The estimates of mean family-level $\alpha_{mp}$ for the two more common diurnal pollinator taxa *A. mellifera* and *Bombus* spp. were $< 0.05$, so we just correlated the estimates of the family-level $S_{dp}$ with floral-display size. However, the lepidopteran $\alpha_{mp}$ was $> 0.05$, and was excluded from correlation analyses with floral-display size.

**Pollinator effectiveness and importance**

In order to evaluate the contribution of different pollinators to pollen deposition, and accurately quantify the role of pollinators in plant reproductive success it is important to incorporate other aspects of pollinator-specific pollen movement with self-pollination rate, including pollen-deposition rates and relative pollinator abundance (Olsen 1997). Therefore, we quantified pollinator effectiveness ($PE$), measured as the number of insertions per focal-inflorescence flower for each pollinator taxon, and pollinator importance ($PI$), measured as the pollinator effectiveness multiplied by the relative abundance of each pollinator taxon that deposited or removed pollinia. Relative abundance was estimated as the number of visits made by the focal pollinator taxon divided by the total number of insect visits observed during the study period (Olsen 1997). Additionally, *A. syriaca* is almost completely self-incompatible and only a small percentage ($< 5\%$) of self-polinia insertions contribute to reproductive success (Wyatt and Broyles 1994); therefore, we generated another metric of pollinator importance that includes only the expected proportion of self-insertions that lead to seed production that we term self-incompatibility controlled pollinator importance ($SICPI$)

$$SICPI = PI \left( 1 - (S_{mp} SI) \right),$$

(4)
where $SI$ is the rate of self-incompatibility. For this study, we used a conservative estimate of 5% for self-compatibility. While the ideal method to incorporate self-incompatibility would be to measure pair-wise self-incompatibility for each genotype in the population, which would also allow us to incorporate the variability of self-incompatibility into $SICPI$, the 5% value that we used has been found in many populations (Wyatt and Broyles 1994) including limited data for the populations in this study (pers. obs.).

*Selection on floral-display size*

To estimate the potential effect of self-pollination on floral-display evolution we estimated male and female selection gradients for each maternal family using insertion and removal rates for NFPFI and NFPS. We calculated fitness using the same logic as for quantify $SICPI$. Specifically, we used eq. 4 to calculate male and female fitness, but substituted the number of insertions and removals for $PI$, and $S_{dp}$ for $S_{sm}$. Also, for male fitness, we modified the equation further as

$$MF = R \left(1 - \left(\frac{I}{R}\right)\right)\left(1 - (S_{dp}SI)\right) \text{ for } R > I,$$

where $I$ is the number of insertions and $R$ is the number of removals, because the number of removed pollinia that are re-inserted through geitonogamy cannot be greater than the total number of inserted pollinia. Fitness values were relativized using the mean population fitness (Lande and Arnold 1983). While using insertions and removals as estimates of fitness are proximate, they are useful when combined with the self-pollination rate to determine how geitonogamous pollen deposition influences selection on floral-display size. We could not use male insertion rates to estimate male selection
gradients because we did not have enough of the paternal-plant floral-display size data for a meaningful analysis. Additionally, we used fitness estimates from a single inflorescence on each stem only, and not whole plant fitness, however, a subset of the floral display (in our study, an inflorescence) can accurately estimate pollinator-mediated fitness of the whole plant if pollinators are unable to differentiate between inflorescences on different plants, within a population (Bolstad 2010). While this indiscrimination is unlikely in *A. syriaca*, the inability to differentiate between stems of different plants seems likely in our population of *A. syriaca*, where stems of individual plants were interdigitated within a population. Therefore, to reduce bias in our fitness estimates, we included NFPS in our selection models to control for any correlated affect between NFPFI and NFPS on insertions or removals.

**Data analysis**

We used a negative-binomial generalized linear model (GLM) to quantify the possible statistical effects of pollinator taxa and floral-display size on pollinia insertion and removal because a large portion of the pollinator visits resulted in no pollinium insertions and overdispersion of the data relative to a normal model. We investigated the relationship between $S_{dp}$, pollinator taxa, and floral-display size using a quasi-binomial GLM. The number of inflorescences per stem and the position of the focal inflorescence were strongly correlated ($r = 0.89$) because inflorescences bloomed sequentially up their stems, and as a result, they were both removed from all analyses. Because stem height, stem density and population all had minimal explanatory power for all of the dependent variables, we did not include them in any of the models. Therefore, the results focus on pollinator taxon, NFPS, NFPFI, and pollinator behavior. The number of inflorescences visited on a stem was rarely > 1 and was not included in analyses of pollinator behavior. No corrections were used for post-hoc contrasts because no
more that three contrasts were made per data set, we calculated the probability of false-positives for the number of comparisons made within each data set using a binomial distribution, and all of the probabilities were still < 0.05.

To estimate the variance in $S_{mp}$, we performed 1,000 bootstraps with progeny array as the unit of re-sampling. We used the mean and SD of the 1,000 bootstraps as the mean and SE of $S_{mp}$ (Brunet and Sweet 2006). To compare the estimates of $S_{mp}$ for the three pollinator taxa, we used pairwise comparisons of the bootstrap estimates and two-tailed tests (e.g., *A. mellifera* and *Bombus* spp.). The $S_{mp}$ for two pollinator taxa were considered significantly different if 975, or greater, or 25, or fewer, of the differences between randomly selected bootstrap estimates from two pollinator taxa were greater than zero (Eckert and Barrett 1994; Eckert 2000; Brunet and Sweet 2006).

We estimated $PE$, by subtracting the mean insertion rate for the control bags from each of the pollinator insertion values. If the resulting value was negative, it was set at zero (Reynolds and Fenster 2008). We used a quasi-Poisson GLM to analyze $PE$ because the data were overdispersed and not categorical. In order to calculate the variance of $PI$ and $SICPI$, we had to incorporate the variance of the product of two random variables for $PI$ (i.e., $PE$ and relative abundance) and three random variables for $SICPI$ ($PE$, relative abundance and $S_{mp}$). To estimate these variances we used a Monte Carlo simulation method described by Reynolds and Fenster (2008) in which we bootstrapped the relative abundance and $PE$ data 1,000 times and multiplied the mean of each abundance and $PE$ bootstrap iteration to calculate $PI$. For $SICPI$, we took the abundance and $PE$-bootstrap iteration means and combined them as described in eq. (4) with 1,000 bootstraps of the $S_{mp}$ data. Then we took the means of the 1,000 $PI$ and $SICPI$ values and calculated the confidence intervals as the 25th and 975th values for both $PI$ and $SICPI$ when the 1,000 values were ranked in ascending order. We initially ran the bootstrapping with 10,000 iterations, but found that using only 1,000 bootstrap iterations gave essentially the same estimates as running 10,000 iterations. Our sampling effort for nocturnal
pollinators was significantly less than that for diurnal pollinators, so we controlled for unequal sampling effort by increasing the visitation frequency of nocturnal pollinators according to the ratio of the sampling effort of diurnal to nocturnal pollinators.

The multivariate selection gradients for NFPFI and NFPS were generated using the least-squares (LS) method (Lande and Arnold 1983). Our measures of fitness were overdispersed; therefore, we split the analysis of selection into two parts: the first was parameter estimation, and the second was significance testing (Mitchell-Olds and Shaw 1987; Kruuk et al. 2002). To test the significance of selection on floral display we estimated quasi-Poisson GLM selection gradients. For the results, we standardized both the LS and quasi-Poisson GLM estimates of the selection gradients using the trait (NFPFI and NFPS) standard deviations (Lande and Arnold 1983). We display standard errors of the LS estimates from 1,000 bootstrap iterations of the LS regressions. All statistics were estimated in R: A Language and Environment for Statistical Computing (R Development Core Team 2009).

Results

Floral visitors

We observed a total of 408 individual pollinator visits, with 183 in 2008 and 225 in 2009. We observed pollinator visitation across the flowering season for a total of 94 hr over 18 days in 2008, and 65 hr over 13 days in 2009. Insects from the orders Hymenoptera (Bees and kin), Lepidoptera (Butterflies and Moths), Diptera (Flies) and Coleoptera (Beetles) were observed, and 93.6% of all the visitors were hymenopterans and lepidopterans. The other pollinators were not observed in a large enough numbers (26 of 408) for meaningful interpretation, so they were removed from the analysis.
We divided the remaining pollinators into three groups: *Bombus* spp. (bumble bees), *Apis mellifera* (Western Honey Bees), and lepidopterans (butterflies and moths). *Bombus* spp. comprised 65%, *A. mellifera* comprised 18%, and lepidopterans comprised 10% of the 408 observed visitors. The most common *Bombus* species observed in both *A. syriaca* populations was *Bombus griseocollis*. Diurnal lepidopterans included *Danaus plexippus* (Monarch Butterfly), several swallowtail species, including *Pterourus glaucus* (Eastern Tiger Swallowtail), and hesperiids including *Epargyreus clarus* (Silver-spotted Skipper). During nocturnal observations, 100% of the visitors were moths and the majority were geometrids and noctuoids. We collected 51 control bagged inflorescences over the sampling period.

*Pollinator behavior and floral display*

There was a strong correlation between VT and NFVOFI ($r = 0.42$, $P = 2.2 \times 10^{-16}$), and many of the relationships between VT and NFVOFI, and floral-display sizes were similar. There was a significant main effect of NFPFI and pollinator taxon on VT ($\chi^2 = 17.8$, $P = 8.6 \times 10^{-8}$ and $\chi^2 = 13.7$, $P = 0.0002$, respectively, fig. 1A) and pollinator taxon on NFVOFI ($\chi^2 = 11.3$, $P = 0.003$, fig. 1B and C). *Bombus* spp. visited the most flowers, followed by *A. mellifera* and the lepidopterans. Lepidopterans spent the most time on the focal inflorescence, followed by *A. mellifera* and *Bombus* spp. There was a significant overall interaction between pollinator taxon and NFPFI for VT ($\chi^2 = 16.8$, $P = 0.0002$). Specifically, VT increased as NFPFI increased for *Bombus* spp. and *A. mellifera*, but decreased for lepidopterans (fig. 1A). There was also an interactive effect of NFPS and NFPFI on NFVOFI ($\chi^2 = 7.4$, $P = 0.006$). Generally, controlling for NFPS, NFVOFI increased as NFPFI increased. However, for low through intermediate NFPFI, as NFPS increased NFVOFI increased. But for high NFPS, NFVOFI decreased. On inflorescences with high NFPFI, NFVOFI generally decreased as NFPS increased.
Also, *Bombus* spp. visited stems with significantly greater NFPS and NFPFI than either lepidopterans or *A. mellifera* ($\chi^2 = 59.6, P = 1.1 \times 10^{-13}$ and $\chi^2 = 38.6, P = 2.1 \times 10^{-8}$, respectively, fig. 1C). However, all of the pollinator taxon ranges of NFPS, NFPFI, NFVOFI, and VT overlapped a great deal.

**Pollen deposition and removal**

There was no significant overall effect of pollinator taxon on insertion rate ($\chi^2 = 1.3, P = 0.52$), but there was a significant interaction between pollinator taxon and VT ($\chi^2 = 12.7, P = 0.0001$), and pollinator taxon and NFVOFI ($\chi^2 = 6.04, P = 0.048$). However, there was also a significant three-way interaction among pollinator taxa, NFPFI, and NFVOFI ($\chi^2 = 6.7, P = 0.033$) which means the insertion rate as NFVOFI increased, was greater for *A. mellifera* than *Bombus* spp., but the number of insertions per focal flower decreased for *A. mellifera* ($\chi^2 = 7.1, P = 0.007$). There was also a three-way interaction among pollinator taxa, NFPFI and VT ($\chi^2 = 15.0, P = 0.0006$). The driver of this interaction was that, while *A. mellifera* inserted more pollinia than lepidopterans as VT increased for all NFPFI values, the rate of pollinia insertion became greater as NFPFI increased for both *A. mellifera* and lepidopterans ($\chi^2 = 7.3, P = 0.007$) (fig. 2A). This three-way interaction is not significant when contrasting just *A. mellifera* and *Bombus* spp. ($\chi^2 = 2.0, P = 0.16$). There was no significant relationship between the control insertions and floral display.

There was no overall significant effect of pollinator taxon on the number of pollinia removed ($\chi^2 = 3.5, P = 0.17$). This number increased with NFPFI and VT ($\chi^2 = 6.4, P = 0.01, \chi^2 = 3.9, P = 0.048$, respectively), and increased from 2008 to 2009 ($\chi^2 = 5.1, P = 0.02$). There was a significant interaction between pollinator taxa and VT ($\chi^2 = 6.5, P = 0.03$) because the number of removals increased faster for *A. mellifera* than for the other two pollinator taxa. Also, there was a significant three-way interaction among pollinator taxa, NFPFI and NFPS ($\chi^2 = 8.4, P = 0.02$) because for *Bombus* spp., the
number of removals increased at a greater rate, as NFPFI increased, when NFPS was higher.

Self-pollination rate

There was no overall significant difference in $S_{dp}$ between *A. mellifera* and *Bombus* spp. ($\chi^2 = 1.88, P = 0.17$). There was a significant effect of NFPFI on $S_{dp}$ ($\chi^2 = 8.3, P = 0.003$), and a nearly significant interaction between pollinator taxon and NFPFI ($\chi^2 = 3.7, P = 0.053$, fig. 3). $S_{dp}$ increased with NFPFI at a greater rate for *A. mellifera* than *Bombus* spp. For the method-of-moments estimation of self-pollination ($S_{mp}$), *A. mellifera* had the highest self-pollination rate, followed by *Bombus* spp., and the lepidopterans (fig. 4). Greater than 97.5% of the pairwise comparisons of the bootstrap estimates of $S_{mp}$ for *A. mellifera* were greater than the bootstrap estimated mean of $S_{mp}$ for *Bombus* spp.; therefore, $S_{mp}$ for *A. mellifera* was significantly greater than $S_{mp}$ for *Bombus* spp. The rest of the comparisons (*A. mellifera* and lepidopterans; *Bombus* spp. and lepidopterans) were not statistically different.

Pollinator effectiveness and importance

There was a significant overall effect of pollinator taxon on pollinator effectiveness ($PE$) ($\chi^2 = 25.2, P < 3.3 \times 10^{-6}$, fig. 5). The main source of the difference was between *A. mellifera* and *Bombus* spp. $PE$ was significantly greater for *A. mellifera* than *Bombus* spp. ($\chi^2 = 22.1, P = 5.0 \times 10^{-6}$). The mean $PI$ value for *Bombus* spp. and *A. mellifera* were not within the 95% C.I. for lepidopterans; therefore, $PI$ for lepidopterans was significantly greater than $PI$ for *Bombus* spp. or *A. mellifera*. SICPI was not statistically different between any of the pollinator taxa. However, qualitatively, lepidopterans and *Bombus* spp. had greater importance than *A. mellifera* when considering abundance, self-
pollination and SI (fig. 5). For the diurnal pollinators, *Bombus* spp. were the most important pollinators.

*Selection on floral-display size*

In general, there was stronger directional selection on floral-display size through male fitness than female fitness, and there were no significant univariate or multivariate selection gradients for NFPS (Table 1). The selection gradient through male fitness tended to be negative as floral-display size increased. There were no significant multivariate selection gradients through female reproduction. However, there was a nearly significant difference in the univariate selection gradients for *A. mellifera* and *Bombus* spp. on NFPFI through female fitness ($\beta_F = 0.9141675, \chi^2 = 3.24, P = 0.071$). There was also a significant difference between the selection gradients of *A. mellifera* and *Bombus* spp. for NFPFI through male fitness (Table 1).

**Discussion**

We quantified pollinator behavior, pollen deposition and the self-pollination rates of three pollinator taxa of *A. syriaca*. In terms of pollinator diversity, our results are consistent with those found in other studies of *Asclepias* spp., that while variable in terms of species and temporality, the majority of diurnal pollinations were by hymenopterans (Wyatt 1976; Willson and Bertin 1979; Kephert 1983; Wyatt and Broyles 1994; Fishbein and Venables 1996; Ivey et al. 2003). The most important pollinator taxon overall, however, was Lepidoptera. While there was variability in the quantity, quality and efficiency of pollen movement across taxa, our results are consistent with the observation that *A. syriaca*, along with other studied *Asclepias* spp. is generalist pollinated (Morse and Fritz 1983;
Fishbein and Venables 1996; Ivey et al. 2003). However, we did find differences in the behavior, pollen movement, and the self-pollen deposition of pollinators, and many of our results correlated with floral-display size, suggesting that there may be differential selection pressures on the floral-display size of *A. syriaca* due to various pollinator taxa.

Lepidopterans had the greatest variability in pollen deposition (fig. 2 and 5), and due to this variability it was difficult to draw any strong conclusions about the interaction between their behavior and floral display. Lepidopterans did spend the most time per visit on the focal inflorescence which may be due to increased nectar volume and sucrose production in *A. syriaca* at night (Willson and Bertin 1979; Morse and Fritz 1983; Southwick 1983). There was no statistical difference between the lepidopteran $S_{mp}$ and that of *A. mellifera* or *Bombus* spp. (fig. 3), but qualitatively it was intermediate to *A. mellifera* and *Bombus* spp., which is consistent with the insertion and removal data (fig. 2). When considering only visitor frequency, lepidopterans were the most important pollinators (fig. 2), which is due to the fact that all of the nocturnal pollinators were lepidopterans. However, when considering diurnal pollinators alone, lepidopterans were the least important, which is consistent with past studies of diurnal *Asclepias* pollinators (Fishbein and Venables 1996; Ivey et al. 2003). Bertin and Willson (1980) showed that nocturnal pollinators deposited less pollen, but produced the same number of fruit as diurnal pollinators and suggested that nocturnal pollinators carried higher-quality pollen. However, Morse and Fritz (1983) found that nocturnal pollinators were less likely to deposit pollinia or produce surviving fruit. Our results are comparable with Bertin and Willson (1980) in that nocturnal pollinators are important, but inconsistent with their insertion results. All of these results suggest that there may be great variability between populations in pollinator-mediated pollen deposition.

*Apis mellifera* had the highest rate of pollen deposition in *A. syriaca* (fig. 2). This is consistent with the results of Ivey et al. (2003) that also quantified pollen deposition in *A. incarnata*. *Apis mellifera* also had the strongest relationship between insertions and NFVOFI (fig. 2). Along with the
fact that *A. mellifera* visited fewer flowers per visit that *Bombus* spp., these results support the result that *A. mellifera* is the most effective pollinator (fig. 5). However, *A. mellifera* also had the highest self-pollination rate that was consistently high across NFPFI (fig. 3), and was the only pollinator to show a significant decrease in insertion rate as NFPFI increased (fig. 2). The high rate of self-pollination across NFPFI suggests that, due to their high efficiency of pollen movement (in terms of insertions and removals), *A. mellifera* exhaust outcrossed pollen that they carryover from other plants very quickly by re-depositing removed pollinia on the same stem. Despite a required amount of time needed for removed pollinia to change orientation before insertion is possible (Wyatt 1976), this result is certainly possible, because the mean time spent on the focal flowers for *A. mellifera* was well over 150 sec. Alternatively, the pollen deposited by *A. mellifera* could just represent a high rate of self-pollen carryover from other stems of the same plant due to the strong clonality of *A. syriaca*. *Apis mellifera* preferentially visit patches with large neighborhood size (Kunin 1997) and move between nearest neighbors despite interplant distance (Morris 1993) suggesting that clonal spatial autocorrelation in *A. syriaca* could result in extremely high self-pollen carryover by *A. mellifera*. The most obvious way to distinguish between within- and among-stem geitonogamy, and determine the mechanism of self-pollen deposition by *A. mellifera*, would be to male sterilize flowers by removing the pollinia from all of the flowers before allowing pollinators to visit the inflorescences.

*Bombus* spp. was the least effective pollinator (fig. 2 and 5), but had significant positive relationships between insertions and NFPFI. Additionally, there was not a significant interaction between NFVOFI and NFPFI for insertion rate, demonstrating that the insertion rate does not decrease as NFPFI and NFVOFI increases. This is despite the fact that *Bombus* spp. visit more flowers per inflorescence and visit inflorescences with higher NFPFI than the two other pollinator taxa (fig. 1). These results are comparable with many studies that have demonstrated bumble bees visit inflorescences with higher NFPFI (*e.g.*, Broyles and Wyatt 1995) and visit patches with higher floral-
display density (Kunin 1997).

The behavior results suggest a similarity in behavior between *A. mellifera* and *Bombus* spp., and in conjunction with the insertion and removal results demonstrate that the difference between the taxa is most likely in the efficiency of pollen deposition. This conclusion is supported by the interesting relationship between NFPFI and the rate of self-pollination (fig. 3), and is consistent with other studies of floral-display size and self-pollination where the rate of self-pollination increased as the number of flowers visited increased (Karron et al. 2004; Brunet and Sweet 2006; Karron et al. 2009). Another possible explanation for the difference between the self-pollination rates of *A. mellifera* and *Bombus* spp. could be different movement patterns between stems, which we did not measure. Nonetheless, this possibility is not supported by our behavioral data or the literature (Broyles and Wyatt 1995; Thompson 2001; Mitchell et al. 2004). Our findings make it clear that future studies must consider pollinator movement among stems to determine the interactive effects of pollinator movement and patch floral-display size on self-pollination rates. This is especially true for clonal plants such as *A. syriaca*, where spatial autocorrelation within a clone may exacerbate the consequences of preferential visitation to dense floral patches.

*Smp* was significantly higher for *A. mellifera* than *Bombus* spp., and *Sp* increased at a greater rate for *A. mellifera*, across the shared range of NFPFI, than for *Bombus* spp. (fig. 3 and 4). If considering the actual quantity of self versus outcross pollen deposited per pollinator per flower, *A. mellifera* is still a 30% more effective pollinator. However, when including pollinator abundance, self-pollination rate and *SI*, *Bombus* spp. became the much more important pollinator (fig. 5). Additionally, the relative abundance controlled number of self-pollinium depositions per flower was three times greater in *A. mellifera* than *Bombus* spp. Therefore, even despite the greater abundance of *Bombus* spp. pollinators, *A. mellifera* were still responsible for a greater total number of the self-pollinia insertions.

The high rate of self pollination along with the derived floral morphology that prevents non-
vector autogamy in *A. syriaca* means that a very high percentage of pollination events in this study were the result of geitonogamous insertions. Geitonogamy is viewed as disadvantageous, because when plants are self-incompatible, it results in pollen discounting (de Jong et al. 1993) and reduced seed set (Broyles and Wyatt 1993). *Asclepias syriaca* is highly self-incompatible, suggesting that pollen discounting could be very high in our study populations. However, if our populations are pollen limited, then these geitonogamous pollinations may be an inevitable consequence of selection for increased floral-display size and pollinator visitation. But, even if a plant population is pollen limited, there may still be a point where the detrimental effects of pollen discounting outweigh the benefits of increased floral-display size (Klinkhamer and de Jong 1993; Barrett 2003). Pollen limitation in *A. syriaca* is still a question, but in our populations the insertion rate for open pollinated inflorescences was high (1.5 insertions per flower). The high insertion rate, along with our population estimate of the self-pollination rate of 0.54 and the mean NFPFI of 62.5, results in an estimate of over 42 compatible inserted pollinia per inflorescence, which is much larger than the mean number of observed fruit per inflorescence (.93) (personal observation), suggesting that there is no pollen limitation. Unfortunately, pollen limitation is methodologically difficult to estimate (Zimmerman and Pyke 1988; Ashman and Morgan 2004) and probably varies spatially and temporally within a species (Wyatt and Broyles 1994). Nonetheless, our data suggest that in our study populations, selection may be stronger for reduced floral-display size as a result of *A. mellifera* pollinations rather than *Bombus* spp. pollinations, especially through male fitness.

*-* *mellifera* was introduced into North America approximately 400 years ago (Sheppard 1989). However, it is impossible to determine the frequency and duration of the interaction between *A. mellifera* and *A. syriaca* over those 400 years making it difficult to speculate on the long term influence of *A. mellifera* on the reproduction and floral-display size evolution of *A. syriaca*. But it is clear, from our results, that *A. mellifera* has the potential to reduce reproductive success, especially at high floral-
display sizes, and influence the evolutionary arch of *A. syriaca*. It may be beneficial to examine herbarium specimen of *A. syriaca*, and correlate inflorescence size with the time since the introduction of *A. mellifera* and the probability of exposure to them as pollinators in order to determine if there is any long term relationship between inflorescence size and *A. mellifera* pollination.

The target of selection on floral-display size, whether it is the inflorescence, total plant, or somewhere in between, in *Asclepias* is still an open question (Broyles and Wyatt 1997). While it is more evolutionarily appropriate to examine total plant fitness, and hence total plant floral-display size, it is also necessary to determine the unit of floral-display size that affects pollinator behavior. In our study, pollinator behavior, pollinia insertions and removals, and the self-pollination rate did vary with floral-display size. While there were relationships between behavior, pollen deposition and total ramet floral display, it is difficult to determine how whole plant floral display influences pollen deposition in large clonal plants like *A. syriaca*. What is clear, however, is that our results suggest that floral-display, at the focal-inflorescence level, has the greatest effect on pollinator behavior and self-pollen deposition.

In summary, there was large variability among pollinator taxa in behavior, pollen deposition, and self-pollination. The difference in self-pollination rate for *A. mellifera* and *Bombus* spp. differed substantially across floral-display size, and along with significantly different selection gradients suggest potentially differential selection pressures on floral-display size. Additionally, our results, along with others, show that *A. mellifera* is a very efficient at depositing pollen (Goulson 2003), but our results also show that *A. mellifera* deposited the largest proportion of self-pollen. These data, along with the fact that *A. mellifera* is an introduced bee (Sheppard 1989), suggest there may be a disconnect between the quantity and quality of pollen deposition from *A. mellifera*, which could seriously influences the reproductive success and evolutionary trajectory of *A. syriaca*.

Appendix A
Index of commonly used abbreviations

1. number of flowers per focal inflorescence (NFPFI)
2. number of flowers per stem (NFPS)
3. time spent visiting a focal inflorescence (VT)
4. number of flowers visited on focal inflorescence (NFVOFI)
5. direct estimation of the rate of self-pollination for a specific pollinator ($S_{dp}$)
6. method of moments rate of self-pollination for a specific pollinator taxon ($S_{mp}$)
7. probability of undetected outcross insertions ($\alpha$)
8. pollinator effectiveness ($PE$)
9. pollinator importance ($PI$)
10. self-incompatibility controlled pollinator importance ($SICPI$)

Literature Cited


Bertin, R. I., and M. F. Willson. 1980. Effectiveness of diurnal and nocturnal pollination of two


Fishbein, M., and D. L. Venable. 1996. Diversity and temporal change in the effective pollinators of


male and female reproduction in experimental populations of Wild Radish, *Raphanus sativus* L.

Evolution 45:268–280.


Figure 1: Various measures of floral-display size and behavior by pollinator taxa (*Apis mellifera*, *Bombus* spp., and lepidopterans). For all three graph legends, A represents *A. mellifera*, B represents *Bombus* spp. and L represents lepidopterans. A, time (sec) spend on the focal inflorescence (VT) versus the number of flowers per focal inflorescence (NFPFI) for all three pollinator taxa. B, the number of flowers visited on the focal inflorescence (NFVOFI) versus NFPFI for all three pollinator taxa. C, the mean value of NFPS, NFPFI, VT and NFVOFI for all three pollinator taxa (±1 SE).
Figure 2: The number of pollinium insertions and the number of pollinium removals versus the number of flowers on the focal inflorescence (NFPFI) for *Apis mellifera*, *Bombus* spp., and lepidopterans. For both graph legends, A represents *A. mellifera*, B represents *Bombus* spp. and L represents lepidopterans. A, the number of pollinium insertions versus NFPFI, and B, the number of pollinium removals versus NFPFI.
Figure 3: Probability of the observed family self-pollination rate ($S_{dp}$) versus the mean number of flowers on the focal inflorescence (FF) for *Apis mellifera* and *Bombus* spp. A family is defined as all pollinia inserted into flowers of the same plant. Predicted $S_{dp}$ is the solid line for *A. mellifera*, and is the dashed line for *Bombus* spp. Circles are the observed $S_{dp}$ values for *A. mellifera*, and triangles are observed $S_{dp}$ values for *Bombus* spp.
Figure 4: Modified method of moments self-pollination rate ($S_{mp}$) for *Apis mellifera* (A), *Bombus* spp. (B), and lepidopterans (L). The error bars are ±1 SE.
Figure 5: Pollinator effectiveness (PE), pollinator importance (PI), and self-incompatibility controlled pollinator importance (SICPI) for *Apis mellifera* (A), *Bombus* spp. (B), and lepidopterans (L). The units of PE are insertions per flower on the focal inflorescence. The error bars for PE are ±1 SE, and PI and SPI have 95% C.I.
Table 1: Female ($\beta_F$) and male ($\beta_M$) multivariate selection gradients and the difference between selection gradients for *Apis mellifera*, *Bombus* spp., the number of flowers per focal inflorescence (NFPFI) and the number of flowers per stem (NFPS). Male relative fitness was calculated as the number of pollinium removals, and female relative fitness was calculated as the number of pollinium insertions. The selection gradients were standardized using the standard deviation of each trait ($\pm$1 SE).

<table>
<thead>
<tr>
<th>Character</th>
<th>$\beta_F$, quasi-Poisson (SE)</th>
<th>$\beta_F$, least-squared (SE)</th>
<th>$\beta_M$, quasi-Poisson (SE)</th>
<th>$\beta_M$, least-squared (SE)</th>
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<tbody>
<tr>
<td>NFPFI</td>
<td>-0.5384 (0.7173)</td>
<td>-0.1191 (0.0670)</td>
<td>-1.618 (0.5245)**</td>
<td>-0.3376 (0.0204)</td>
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<td>NFPS</td>
<td>0.3657 (0.7249)</td>
<td>0.0237 (0.0396)</td>
<td>0.3873 (0.5024)</td>
<td>-0.0217 (0.0172)</td>
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<td><em>Apis mellifera</em> x NFPFI</td>
<td>-1.544 (1.641)</td>
<td>-0.2799 (0.3984)</td>
<td>-2.775 (0.9452)**</td>
<td>-0.6608 (0.2606)</td>
</tr>
<tr>
<td><em>Bombus</em> spp. x NFPFI</td>
<td>0.4676 (0.4642)</td>
<td>0.0417 (0.0670)</td>
<td>-0.4619 (0.5457)</td>
<td>-0.0144 (0.0204)</td>
</tr>
<tr>
<td><em>Apis mellifera</em> x NFPS</td>
<td>0.4056 (1.550)</td>
<td>0.0233 (0.1975)</td>
<td>0.6368 (0.8726)</td>
<td>-0.0489 (0.1724)</td>
</tr>
<tr>
<td><em>Bombus</em> spp. x NFPS</td>
<td>0.3257 (0.5823)</td>
<td>0.0241 (0.0396)</td>
<td>0.1377 (0.5618)</td>
<td>0.0055 (0.0172)</td>
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<tr>
<td><em>Bombus</em> spp. x NFPI - <em>Apis mellifera</em> x NFPFI</td>
<td>1.006 (0.7173)</td>
<td>0.1608 (0.4055)</td>
<td>1.156 (0.5245)*</td>
<td>0.3232 (0.2622)</td>
</tr>
<tr>
<td><em>Bombus</em> spp. x NFPS - <em>Apis mellifera</em> x NFPFI</td>
<td>0.3657 (0.7249)</td>
<td>0.0004 (0.2024)</td>
<td>-0.2495 (0.5024)</td>
<td>0.0272 (0.1731)</td>
</tr>
</tbody>
</table>

*P < 0.1; **P < 0.01; *P < 0.05; **P < 0.01.
Chapter 2:

Selection on multiple levels of floral-display size through female fitness and pollen dispersal

Introduction

Floral-display size influences the attractiveness and behavior of pollinators when visiting animal-pollinated plants and can greatly influence their reproductive success (de Jong and Klinkhamer 1994; Harder and Barrett 1996). Floral-display size can vary greatly within and among populations, and this along with the observation that limited resources can constrain floral-display size results in the expectation of trade-offs between different aspects of floral-display size, such as flower size and flower number (Worley and Barrett 2000). Empirical and theoretical considerations suggest inverse relationships between inflorescence size and number (Shoen and Dubuc 1990; Fishbein and Venables 1996) and flower size and number (Morgan 1993; Harder and Barrett 1996), as a result of the contrary effects of increased pollinator attraction with an increase in floral-display size and the concomitant increase in resource costs and self-pollination between flowers on the same plant (geitonogamy) (Klinkhamer and de Jong 1993; Karron et al. 2004; Brunet and Sweet 2006).

However, the interaction between floral-display size and plant reproduction goes beyond the direct relationships between pollinator attractiveness, resources and self-pollination at the single plant level. Factors such as plant density, population and patch size, and distance between patches can also influence pollinator behavior and plant reproduction. The detrimental effects of geitonogamous pollinations may be mitigated in large or dense patches as a result of pollinators' visiting a lower number of flowers in succession within a plant (Kunin 1993; Wyatt and Ivey 1999). Distance between patches may increase the potential negative effects of increased floral-display size by causing longer pollinator residency (Sih and Baltus 1987; Cresswell and Osborne 2004), but, on the other hand, patch
size may reduce self-pollination due to increased pollinator movement between patches when patch size is larger (Morris 1993; Cresswell and Osborne 2004).

These studies show that variation in reproductive success is effected by an individuals floral-display size traits, patch's floral-display size traits, population's floral-display size traits, or a combination of these traits, and suggests the importance of traits from the population down through the individual flower on fitness. The quantification of the effect of various levels of traits (e.g., patch, stem, and branch) on phenotypic selection is called contextual analysis, where a significant partial regression for a contextual-display trait provides evidence of an effect of that level of the trait on the fitness of an individual. While multilevel selection may be controversial in terms of its implications for group-level selection (Stevens et al. 1995), it is nevertheless useful in that it may help identify ecologically appropriate levels of selection (Heisler and Damuth 1995) and result in more accurate measures of selection at the individual level, especially if the group and individual level traits are correlated.

In studies of phenotypic selection, quantifying fitness of the whole plant is held as the standard (Janzen 1977; Harper 1980; Pan and Price 2002). However, the questions of what is an individual organism and what is the unit of selection are still an open questions (Pan and Price 2002) especially when the organism is modular (Folse III and Roughgarden 2010). Tuomi and Vuorisalo (1989) define individuals in modular organisms as “structural individuals [that] consist of repeated multi-cellular sub-units or 'modules,’” such as genets (individual plants), in more-derived clonal plants, that consist of ramet (stem) modules (Harper 1977). The argument that genets are the unit of selection are based on the fact that the ramets within a genet are genetically identical, resulting in coupled fitness interests, with the fitness of the genet as the sum of reproductive output of the ramets. However, from the perspective that ramets are the demographic unit, the genet is a kin group, not an individual, and the ramet is the domain of fitness (Folse III and Roughgarden 2010). Tuomi and Vuorisalo (1989) state
that ramets may be physiologically semi-autonomous and both genets and ramets may have distinct phenotypic traits that variably influence fitness and lead to differential selection. Additionally, the unit of fitness measure, in phenotypic selection, may depend on how the trait of interest influences fitness. For example, Bolstad et al. (2010) state that the reproductive success of a blossom can accurately quantify pollinator-mediated selection at the genet level if pollinators are unable to differentiate between plants in a patch except as a result of differences in blossom phenotype. Most likely, this is the case, in populations of clonal plants, where ramets of individual plants were interdigitated within a patch. The interdigitation of ramets, within a clonal population, makes it difficult to delineate individual plants and, consequently, requires all ramets in the population to be genotyped and assigned to a genet in order to properly estimate whole plant fitness. This requirement is difficult to achieve and may be impractical. Therefore, considering all of these factors, whether interpreted as the unit of selection, as a proxy for genet fitness, or for practical purposes, measuring selection at the stem level can be appropriate for clonal plants.

There is very little known about the potential for selection on multiple levels of floral-display size, but the few studies that examine it directly suggest that group and individual floral-display size can significantly effect female reproductive success (Stevens et al. 1995; Aspi et al. 2003). Additionally, studies that have investigated pollinator behavior, in the context of multiple-floral-display levels, point to the influence of contextual floral-display size on pollen deposition (Kunin 1993; Morris 1993; Grindeland and Sletvold 2005; Makino et al. 2006; Karron et al. 2009). However, the studies that have directly measured fitness (Stevens et al. 1995; Aspi et al. 2003) have considered only a few aspects of floral-display size such as stem number, stem density and stem height, and only at the group and individual levels. And the studies of pollinator behavior have not directly measured fitness. Therefore, in this study, we quantified fruit production and floral-display for over 90% of the stems in a population of the clonal, insect pollinated Asclepias incarnata ssp. pulchra (Swamp Milkweed) and
quantified phenotypic selection on floral-display size from the patch through the inflorescence and individual flower levels for female reproductive success.

Female reproductive success accounts for half of the fitness in hermaphroditic plants such as *A. incarnata*, and as a result, to fully examine the influence of floral-display size on fitness, the male portion of fitness must be considered. The most accurate method for estimating male fitness is through paternity analysis. However, paternity analysis is methodologically difficult because it requires many, highly polymorphic, genetic markers to ensure correct paternal assignment. An alternative method, that estimates pollen dispersal, is the Stepwise Analysis of Molecular Variance (StAMOVA) (Dyer et al. 2004). This method is based on the TWOGENER method described by Smouse et al. (2001) and uses the multilocus pairwise genetic distance between pollen haplotypes to partition variance in the pollen pool between external variables. This method allows researchers to partition genetic differentiation of the pollen pools between external variables, such as floral-display size, and determine how they influence pollen dispersal. The statistic estimated in this model is $\Phi_{st}$ which is the pollen pool equivalent of $F_{st}$, in that it estimates the difference in genetic diversity expected within a subpopulation (patches and stems) and the total populations. While this analysis is not equivalent to the estimates of phenotypic selection for female reproduction, it does tell us how floral-display size influences pollen dispersal. And pollen dispersal gives great insight into possible effects on male reproductive success, especially in a plant like *A. incarnata* that is clonal, partially self-incompatible, and experiences inbreeding depression (Lipow and Wyatt 2000). Therefore, to gain some insight into the possible effects of floral-display size on the male aspect of reproduction in *A. incarnata* we estimated the effect of floral-display size on pollen dispersal using the StAMOVA procedure.

**Methods**
Floral display and seed collection

We marked all stems in a population of *A. incarnata ssp. pulchra* (hereafter referred to as *A. incarnata*) located at Sky Meadow State Park in Fauquier County, VA in 2010. We counted all open flowers, throughout the flowering season (June through August), on all stems and inflorescences in the population and then recorded the patch, stem and branch location. We also measured the width of haphazardly-selected flowers on each stem, as a measure of flower size, using digital calipers. In September through October, when the fruit were mature, we counted the number of fruits produced on 91.3% of the stems, recorded the branch and inflorescence location, and collected seeds. The remaining 8.7% stems were lost due to storms or mowing.

Floral-display size and phenotypic selection

For our multiple-level model of phenotypic selection, we used group (patch) and individual (stem), branch and inflorescence measures of floral-display size and measured fitness at the stem level. Patch-display size traits were measured using the sum of the traits across the patch level. For example, for the trait number of flowers per patch we summed the total number of flowers on all stems within the patch (Table 1). Several other studies of the interaction between group and individual selection have measured the group traits as means of the individual traits in the group (Stevens et al. 1995; Weinig et al. 2007), however, in the context of floral-display size, using the sum of the traits is more appropriate (Aspi et al. 2003). While summing traits may be more meaningful for measuring floral-display size, and explaining fitness, it is not a trait of an individual, and the slope of the patch regression line should not be interpreted as a selection gradient. Therefore, to interpret the effect of patch display on stem fitness we looked at the interactions between only the patch factor and other
levels of floral-display size.

The pattern of trait measurement used for the patch level was also used for the within-stem, floral-display traits as well, except at the inflorescence level, where we also included mean number of flowers. All measures of floral-display size are shown in Table 1. We attempted to include all floral-display size measures from all of the levels, but the model was saturated due to high dimensionality. Since the measures of floral-display size at each level were highly correlated, we reduced dimensionality by performing a factor analysis, for each level of floral-display size, using a varimax rotation to maximize variation in the data explained by the factors (Anderson 1984; Stevens et al. 1995; Weinig et al. 2007). We used the first factor for each level of floral-display size only. For example, we produced one factor for patch floral-display size that included the variables number of stems per patch, number of branches per patch, number of inflorescences per patch and number of flowers per patch. The first factor generated for each level of floral display explained greater than 82% of the variance in all the included variables (Table 1). We also included flower width (mm), and average distance to other patches (m) as independent variables. All of the independent variables were standardized to a mean of one and standard deviation of 0.

Fruit production per stem, relativized using the population mean, was the response variable for our model of selection, and simple linear ($\beta$), quadratic ($\gamma_i$), and interactive ($\gamma_{ij}$) selection gradients were calculated using the least-squares (LS) method (Lande and Arnold 1983). However, relative fitness was overdispersed, so we separated the analysis into two sections. First we estimated the gradient as described, using the LS method, and then we tested the significance of the gradients using a quasi-Poisson generalized linear model (GLM). We report the gradient estimates for both the quasi-Poisson GLM and LS methods, and provide estimates of the LS standard errors from 1,000 bootstrap iterations using stem as the unit of re-sampling (Mitchell-Olds and Shaw 1987; Kruuk et al. 2002).
Pollen dispersal

To quantify pollen dispersal we genotyped 103 randomly selected seeds from 38 maternal stems with up to 12 microsatellites and we performed a StAMOVA (Dyer et al. 2004). We included the patch factor, stem factor, branch factor, inflorescence factor, floral width, average distance to other patches and all two-way interaction terms as independent variables in the model. Like any standard ANOVA model, adding independent variables to the model reduces the error sums of squares and, therefore, significance of the independent variables was estimated by permuting the elements of the independent variables among the sampled stems and comparing the observed reduction in the sums of squares of the independent variable, for the StAMOVA model, versus the distribution of the permuted reductions in the sums of squares (Dyer at al. 2004).

Results

Floral-display size and architecture and fruit production

There was a total of 194 stems in the population, with a total of 110,491 flowers, and 403 fruit produced. The floral-display size variables at each level of floral-display were highly correlated and resulted in high positive loadings for the first factor at each level (Table 1). High loadings can be interpreted as strong correlations between the variables and the factors.

Directional selection was significant for the stem level of floral-display. At the patch level, fruit production increased as the number of stems, flowers and branches in the patch decreased (Table 2). Alternatively, there was positive direct selection for stem-floral-display size. There was also direct negative selection for reduced distance between patches. Along with average distance to other patches,
flower width and branch factor had significant quadratic gradient terms. However, average distance to other patches shows no local maximum or minimum suggesting that the quadratic term was not relevant. The flower-width plot shows an internal minimum, purporting disruptive selection, and the branch factor plot shows a maximum, suggesting stabilizing selection (fig. 1a and b). However, there was a significant three-way interaction between the stem, branch and inflorescence factors (Table 2). The two-way interactions between the stem factor and the branch and inflorescence factor indicate there was significant positively correlated selection, but the three-way selection gradient is negative, indicating negatively correlated selection. At low stem-display size, there was weak positive correlation between branch- and inflorescence-display sizes (fig. 2), but as stem-display size increases, the fitness surface forms a saddle shape suggesting negatively correlated selection (fig. 2). This same essential pattern exists between branch- and inflorescence-display sizes when considering the three-way interaction with patch level display as well (fig. 3). Across patch size, when the inflorescence factor is large there is selection for smaller branch-display size and vice versa.

Floral-display size and pollen dispersal

Only 2 of the genotyped loci had any appreciable level of polymorphism, resulting in a low probability of excluding a single father. Due to the relatively low probability of exclusion, and the inability to confidently assign paternity, we did not perform paternity analysis. For the StAMOVA, the overall AMOVA estimate of \( \Phi_{st} \) for the pollen pool was 0.5024. When we controlled for all levels of floral-display, \( \Phi_{st} \) was reduced by 78% to 0.1095. Patch, stem, branch and inflorescence floral display significantly influenced pollen dispersal (\( \Phi_{st|patch} = 0.434, P = 0.001 \), \( \Phi_{st|stem} = 0.4343, P = 0.013 \), \( \Phi_{st|branch} = 0.433, P = 0.043 \), \( \Phi_{st|inflorescence} = 0.4219, P = 0.044 \)). Univariate analyses, of all levels, suggest that each level of floral-display size individually explains about 15% of the genetic structure found in the
pollen pool. Average distance to other patches did not affect pollen dispersal and none of the floral-display size interactions were significant.

Discussion

Our results show that floral-display size traits, from the patch through the individual flower level, can significantly affect female reproductive success and pollen dispersal. For female reproductive success, there was significant stem selection on floral-display size traits, and when group size decreased, fruit production increased. Our results are consistent with several other studies that have found significant effects of group density and size traits on fitness (Stevens et al. 1995; Aspi et al. 2003; Donohue 2004; Weinig 2007) in addition to other studies that have considered multilevel selection in the context of kin cooperation, root competition, or both (Murphy and Dudley 2009; Biernaskie 2011). Aspi et al. (2003) considered multilevel selection on floral-display size by measuring patch size and stem height. They found selection for reduced number of stems at the patch level that was antagonistic to selection at the individual level and attributed that result to increased herbivory with an increase in patch size. They also found positive direct selection for stem height at the patch and individual level and suggest that was a result of pollinator attraction. Our results, that include several more aspects of floral-display size, are consistent with results found in studies that have investigated patch size and pollinator behavior (Kunin 1993; Wyatt and Ivey 1999), indicating that pollinators may reduce the number of flowers visited on a stem when patch size is large and causes lower fruit production. Another possibility, however, is that patch residency increased with patch-display size and higher rates of geitonogamy resulted in reduced fruit production (Barrett 2003). This possibility is also supported by our results that fitness decreased as the distance between patches increased.
While there was one significant three-way interaction involving patch floral-display size, the important aspect of that interaction was between the branch and inflorescence factors (fig. 3). Specifically, the relationship between the branch and inflorescence factors is consistent across patch size, but only becomes apparent when patch-display size is controlled. Additionally, the consequences of significant multilevel selection are usually posited in terms of its effect on the appearance of “soft” selection (i.e., phenotypic selection in the context of relative trait values) and the validity of the law of “constant yield” of patches, which is defined as equal reproductive success across patches (Wallace 1968; Stevens et al. 1995; Weinig et al. 2007). However, our results indicate that selection is stronger at the stem level than the patch level (table 2), and that patch selection does not balance stem level selection. Therefore, despite the size of the patch-floral-display, net selection is for increased stem floral-display size, and “soft” selection is not present in this population. Additionally, fruit production at the patch level is significantly correlated with group size ($r = 0.48$, $P = 0.0001$) and does not support the law of “constant yield.” These results, in conjunction with the selection results, further show that patch-level-floral display does not play a large role in selection on floral-display size of individual plants in our population. Nonetheless, it is clear, that in our study, patch-floral-display size is an ecologically important aspect of female reproduction and essential to the accurate quantification of selection on floral-display size.

Along with the group level selection results, there was significant simple linear and correlated selection on floral-display size at many other levels within the individual (Table 2). The significant positively correlated selection on inflorescence size and stem-display size suggests that when stem display size is large, the negative effects, in terms of increased geitonogamous pollinations, as a result of large inflorescence size may be mitigated by reduced pollinator residency due to more flowers on the rest of the stem (Klinkhamer and de Jong 1993; Kunin 1993). Additionally, there was an interaction between stem-display size and branch-display size that points to direct correlated selection, and
suggests there may not actually be stabilizing selection at the branch level (fig. 1b).

Complicating interpretation of the above interactions further, is the fact that there was a three-way interaction between the stem-, branch-, and inflorescence-display size (Table 2). Where, at small stem-level-display sizes, there was a positive correlation between branch- and inflorescence-display size, but as stem-display size increases the correlation between inflorescence- and branch-display sizes becomes negative (fig. 2). We can hypothesize from these results that there may be a trade-off between inflorescence size and inflorescence number in larger plants due to an optimization of resources that balances between attraction to pollinators and geitonogamous insertions (Haig and Westoby 1988; Klinkhamer and de Jong 1993). While in smaller plants, it may be advantageous to increase display size either through both inflorescence number and inflorescence size. The reason that this interaction occurs between the branch and inflorescence levels may reflect a physiological relationship at the branch level. Watson and Casper (1984) state that plants may be divided into integrated physiological units, where exchange of resources between units is limited. These integrated units may be at different levels of plant organization, including the ramet, branch and inflorescence (Watson 1986), and for A. incarnata resources movement may be limited to within a branch, resulting in resource trade-offs occurring at the branch level (Casper and Neisenbaum 1993).

The evolution of inflorescence size has been considered in great detail for Asclepias (see Wyatt and Broyles 1994), and Broyles and Wyatt (1990) found that in A. exaltata (Poke Milkweed), inflorescence size was not as important as total plant floral-display size in determining male or female reproductive success. That conclusion is inconsistent with our results on female reproductive success, and may reflect differences in the display architecture of A. incarnata and A. exaltata. However, it seems that inflorescence size would influence female reproductive success in A. exaltata more that A. incarnata because inflorescences are more spatially separated on stems of A. exaltata, and could result in pollinator behavior that correlates more closely with inflorescence size (Broyles and Wyatt 1995).
Nonetheless, our results for *A. incarnata* suggest that, at least for female reproductive success, inflorescence-display size plays a significant role in fitness, but that role is specific to the context of display size at the stem level and may be lost if not considered in conjunction with other levels of floral-display size.

While we found no evidence of trade-offs between flower size (width) and flower number, we did find a significant quadratic selection gradient for flower width. This result provides evidence for divergent selection on flower width. Galen et al. (1987) suggest that disruptive selection on flower size is a result of pollinator taxa of different sizes preferentially pollinating size appropriate flowers.

*Asclepias incarnata* is pollinated by a diverse group of pollinators that vary greatly in size, including bumble bees, Western Honey Bees, Carpenter Bees, flies and lepidopterans (Ivey et al. 2003). The pollination system of *Asclepias* is evolutionarily derived and requires animal vectors to move aggregates of pollen grains (called pollinia) among flowers (Wyatt and Broyles 1994). A pollinium is removed from a flower when a pollinator's limb, or other body part, catches on a grooved organ called the corpusculum (Wyatt 1978). This floral morphology implies that there may be an important relationship between pollinator size and pollen removal that possibly could result in disruptive selection if there is a dichotomy in pollinator size as well. Ollerton et al. (2003) found a weak positive correlation between pollinator size and the number of pollinia carried by the pollinator in nine *Asclepias* species, suggesting that there may be at least some relationship between pollinator size and pollination success.

For our results on male reproduction, in terms of pollen dispersal, we saw significant multilevel statistical effects. While several studies have investigated hierarchical effects on population structure and even pollen-pool structure (Dyer et al. 2004), none have expressly looked at the effects of floral-display size on $\Phi_{st}$. Our patch-level results are consistent with those found by Dyer and Sork 2001 where stand density, but not individual density, affected pollen pool structure. When controlling for
population architecture they found a proportionately similar reduction in $\Phi_{st}$ (10%) that we found for each level of floral-display size that we measured, but a much lower overall reduction than we found when considering all levels of floral-display size. While our results do not permit direct examination of selection through male fitness, they do suggest that display-size has a large effect on the genetic structure of the pollen pool. And further, in light of the knowledge that *A. incarnata* is at least partially self-incompatible and experiences inbreeding depression, there could be a potentially large negative effect on male reproductive output (Lipow and Wyatt 2000).

The limited results that we have on the male aspect of reproduction limit the conclusions we can make about that portion of reproduction. Beyond the obvious need for paternity analysis, to more accurately quantify male fitness, future studies should estimate the pollen-pool size by patch and stem in order to more directly associate a linear relationship between floral-display size and pollen dispersal. In addition, it is important to note that StAMOVA estimates only the difference between the average pollen donor for each mother, and so it will also be important to measure the variance in maternal pollen pools because that may actually be more important to understanding transmission of genes to the next generation (Dyer et al. 2004; Dyer 2009; Dyer unpublished).

In conclusion, our results suggest that there are significant effects of floral display on both male and female reproduction at several different levels of floral-display size. While the presence of antagonistic selection between patch- and stem-display sizes is not evident in our population, it is clear that patch floral-display size is very important to the fitness of *A. incarnata*. And when examining the evolution of floral-display, especially in terms of female function, it is important to consider the interactions and correlations between floral-display from the stem through the inflorescence level.

**Literature Cited**


de Jong, T. J., and P. G. L. Klinkhamer. 1994. Plant size and reproductive success through female and


Figure 1: Relative Fitness versus $A$, standardized flower width and $B$, the branch factor for *Asclepias incarnata*. Relative fitness was calculated from fruit production and is relativized to the mean fruit production of the population. Flower width and the branch factor are standardized to mean = 0, and standard deviation = 1.
Figure 2: Relative fitness surface of the branch factor versus inflorescence factor as the stem factor increases from left to right in *Asclepias incarnata*. A, the stem factor equals the minimum population value; B, the stem factor equals the median population value; C, the stem factor equals the maximum population value. Relative fitness is fruit production relativized to the mean fruit production of the population. The branch and inflorescence factors are standardized to mean $= 0$, and standard deviation $= 1$. 
Figure 3: Relative fitness surface of the branch factor versus the patch factor as the inflorescence factor increases from left to right in *Asclepias incarnata*. A, the inflorescence factor equals the minimum population value; B, the inflorescence factor equals the median population value; C, the inflorescence factor equals the maximum population value. Relative fitness is fruit production relativized to the mean fruit production of the population. The branch and patch factors are standardized to mean = 0, and standard deviation = 1.
Table 1: Correlation coefficients ($r$) between floral-display size variables within each floral-display size level (patch, stem, branch, inflorescence) and loadings for floral-display factors (patch factor, stem factor, branch factor, inflorescence factor) that were calculated from factor analyses for each level of floral-display size in *Asclepias incarnata*.

<table>
<thead>
<tr>
<th>Patch-level floral-display size</th>
<th>Number of inflorescences</th>
<th>Number of branches</th>
<th>Number of stems</th>
<th>Patch factor</th>
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<tbody>
<tr>
<td>Number of flowers</td>
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<td>0.839</td>
<td>0.648</td>
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<td>0.884</td>
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<th>Number of inflorescences</th>
<th>Number of branches</th>
<th>Stem factor</th>
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<tbody>
<tr>
<td>Number of flowers</td>
<td>0.971</td>
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<td>0.963</td>
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<td>Number of inflorescences</td>
<td>0.827</td>
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<td>0.972</td>
</tr>
<tr>
<td>Number of branches</td>
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<td></td>
<td>0.928</td>
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<td>Number of flowers</td>
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<tr>
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<table>
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<th>Inflorescence-level floral-display size</th>
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<th>Inflorescence factor</th>
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<td>Total number of flowers</td>
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<td>0.924</td>
</tr>
<tr>
<td>Mean number of flowers</td>
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<td>0.828</td>
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Table 2: Standardized linear (β) and significant non-linear (γ) selection gradients for the patch factor, stem factor, branch factor, inflorescence factor, flower width and average distance to other patches in *Asclepias incarnata*. While we report the slope of the regression parameter of patch factor, it is a summed group characteristic and cannot be interpreted as a selection gradient.

**Linear selection gradients (β)**

<table>
<thead>
<tr>
<th>Character</th>
<th>β, quasi-Poisson (SE)</th>
<th>β, least-squares (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patch factor</td>
<td>-0.326 (0.158)*</td>
<td>-0.316(0.174)</td>
</tr>
<tr>
<td>Stem factor</td>
<td>0.547 (0.106)**</td>
<td>0.764 (0.152)</td>
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<tr>
<td>Branch factor</td>
<td>0.055 (0.143)</td>
<td>0.015 (0.157)</td>
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<tr>
<td>Inflorescence factor</td>
<td>-0.126 (0.159)</td>
<td>-0.090 (0.148)</td>
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<tr>
<td>Flower width</td>
<td>0.144 (0.132)</td>
<td>0.167 (0.160)</td>
</tr>
<tr>
<td>Average distance to other patches</td>
<td>-0.266 (0.140)*</td>
<td>-0.271 (0.152)</td>
</tr>
</tbody>
</table>

**Significant non-linear selection gradients (γ)**

<table>
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<th>Character</th>
<th>γ, quasi-Poisson (SE)</th>
<th>γ, least-squares (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branch factor</td>
<td>-0.449(0.259)*</td>
<td>-0.209 (0.162)</td>
</tr>
<tr>
<td>Flower width</td>
<td>0.191(0.086)*</td>
<td>0.301 (0.120)</td>
</tr>
<tr>
<td>Average distance to other patches</td>
<td>-0.430(0.208)*</td>
<td>-0.220 (0.230)</td>
</tr>
<tr>
<td>Stem factor x Branch factor</td>
<td>0.752(0.317)*</td>
<td>1.00 (0.324)</td>
</tr>
<tr>
<td>Stem factor x Inflorescence factor</td>
<td>0.755(0.352)*</td>
<td>0.300 (0.312)</td>
</tr>
<tr>
<td>Flower width x Average distance to other patches</td>
<td>-0.500(0.285)*</td>
<td>-0.071(0.216)</td>
</tr>
<tr>
<td>Patch factor x Branch factor x Inflorescence factor</td>
<td>0.882(0.556)*</td>
<td>0.190 (0.416)</td>
</tr>
<tr>
<td>Stem factor x Branch factor x Inflorescence factor</td>
<td>-0.632(0.385)*</td>
<td>-0.910 (0.383)</td>
</tr>
</tbody>
</table>

*P < 0.1; *P < 0.05; **P < 0.01.
Chapter 3:

Geitonogamy and Mating Patterns in Monoecious, Pollen-bearing Clonal Plants

Introduction

Plant clonal growth, which may broadly be described as an individual's proliferation through the production of more than one shoot (i.e., ramets), is very common in vascular plants, which have up to 80% of species exhibiting vegetative propagation in some way (Klimes et al. 1997). The advantages of the combination of clonal (asexual) and sexual proliferation manifest themselves through a plant's ability to allocate its resources between these two types of reproduction when ecologically suitable (Ronsheim and Bever 2000). However, despite these advantages, studies using molecular markers have shown that clonal growth, in terms of clone spatial arrangement and size, may deleteriously affect patterns of pollen dispersal (e.g., Reusch 2001; Wilson et al. 2005; Mori et al. 2009).

Clonal plants show a wide variety of ramet spatial arrangements that depend on many factors including clone-age structure, competition, habitat disturbance, and habitat type (Brodie 1995; de Kroon and Hutchings 1995; Kudoh et al. 2001; Jacuemyn et al. 2005), but, in general, vary from a random distribution through positive spatial autocorrelation (Heywood 1991), meaning ramets are spatially aggregated within a clone. The spatial arrangement and size of clones (number of ramets), particularly positive autocorrelation and increased clone size, may interfere with pollen dispersal by increasing the rate of geitonogamy, which is defined as “pollen movement” (operationally defined in our study as both the transport and deposition of pollen) among the flowers on a single plant (Klinkhamer and de Jong 1993). Geitonogamy is disadvantageous for both self-compatible and self-incompatible plants because unlike intra-floral autogamy, geitonogamy occurs via a vector (as does xenogamy or outcross-pollination) and is expected to result in pollen discounting (reduction in the
amount of pollen available for outcrossing), less reproductive assurance, inbreeding depression, or any combination of the three (Lloyd and Schoen 1992; Klinkhamer and de Jong 1993).

Generally, geitonogamy increases with floral-display size (Harder and Barrett 1995). Plant clonal growth can result in large floral-display sizes that may increase the rate of geitonogamy through interramet geitonogamy (g, i.e., self-pollination among ramets of the same genet) (Handel 1985; Eckert 2000). To date, studies of the effects of clonality on pollen dispersal have only focused on clone size and found that geitonogamy increases as clone size increases (Reusch 2001; Routley et al. 2004; Wang et al. 2005; Wilson et al. 2005; Clark-Tapia et al. 2006; Trapnell and Hamrick 2006; Mori et al. 2009), and they have not considered a wide number of possible clone-size distributions (numbers of ramets per genet) and clonal diversity (e.g., clonal richness, defined as the number of genets in the population divided by the total number of ramets in the population) combinations that occur in natural populations, nor developed a framework for detailed theoretical considerations of the influence of these population parameters on pollen dispersal (Charpentier 2002). The exception is Reusch (2001) that included estimates of clonal richness and found that the selfing rate increased as clonal richness decreased. Therefore, general expectations from the literature are limited to the intuitive observations that increased geitonogamy occurs as a result of increase clone size and decreased clonal richness. In view of this, the goal of our study is to simulate pollen movement in clonal plants and examine the rate of geitonogamy for a range of clonal spatial structures, diversities, and clone-size distributions (allocation of ramets per genet) in order to generate additional expectations and a theoretical framework for the influence of clonality on pollen deposition.

We used an individual-based model of pollen dispersal that accounts for the spatial arrangement of clonal, monoecious plants. First, we simulated a range of spatial and clone-size distributions of the plants. Second, we used a pollen-dispersal distribution to examine the effect of spatial arrangement on pollen movement. Last, we investigated g with regard to factors including clone density, radius, and
Methods

Clonal Spatial Arrangement

We followed the methods of Robledo-Arnuncio and Austerlitz (2006) to simulate clonal spatial arrangement. We used a Poisson-cluster-point process to model the plant spatial autocorrelation because it produces the spatial aggregation patterns found in natural populations of many plant species (Plotkin et al. 2000; Shimatani 2002). Spatial statistical models, like the Poisson-cluster-point process have been used widely to describe and model spatial autocorrelation in clonal plants (Oborny and Cain 1997). As described by Plotkin et al. (2000) the Poisson-cluster-point process is a descriptive statistical model and does not specifically model any biological process that causes aggregated plants based on plant growth, movement (e.g., generative and architectural models), foraging, or a combination of these factors (Oborny and Cain 1997). While Plotkin et al. (2000) and Robledo-Arnuncio and Austerlitz (2006) focused on trees, there seems to be no significant difference between plant type and spatial aggregation in nature, therefore this model is applicable to herbaceous plants, shrubs and trees (Peterson and Jones 1997). We specified a global-population density ($d$, see Appendix A for an index of commonly used abbreviations), and the Poisson-cluster-process permitted us to simulate a population within a square sample region (plot area) consisting of clones that are each a cluster of ramets. Each clone is defined by its radius ($r$) and ramet density ($\mu$). To obtain randomly dispersed clones, we used a Poisson process to randomly place ramets within the plot area.

Clone-size Distribution
The number of ramets within each genet of a population (i.e. clone-size distribution) is typically left skewed (Arnaud-Haond et al. 2007) meaning that within a population, there are many more genets with a small number of ramets than those with larger numbers. As a result of this skewed distribution, we modeled the clone-size distribution using the power-law distribution that is called the Pareto distribution (Pareto 1897 in Vidondo et al. 1997 and Ueda et al. 2004), because it fits the pattern of clonal membership found in most studies (Arnaud-Haond et al. 2007). The Pareto distribution is described by the equation

\[ N_{\geq X} = aX^\beta, \]  

where \( N_{\geq X} \) is the number of ramets in the population belonging to clones containing \( X \), or more, ramets, \( a \) is the scale parameter, and \( \beta \) is the slope of the line (Arnaud-Haond et al. 2007). As \( \beta \) increases, the probability of larger clone sizes decreases, and clones become more similar in size. We used the Pareto distribution to generate a randomly distributed vector that specified the number of ramets within each clone. We assumed that all ramets were reproductively mature and that the ortet, the common ancestor of all ramets within a clone (Wright 1976), was still present and equivalent to a ramet with regard to its pollination.

**Pollen Dispersal**

We simulated pollen movement using a pollen-dispersal distribution assuming that pollen movement depends on the relative spatial arrangement of ramets. We used a two-parameter \((a, b)\), bivariate, exponential power distribution as the pollen-dispersal distribution.
\[ p(x,y) = \frac{b}{2\pi a^2 \Gamma(2/b)} \exp \left[ -\left( \frac{\sqrt{x^2+y^2}}{a} \right)^b \right] \]  \hspace{1cm} (2)

(Clark 1998), where \( p(x,y) \) is the probability that pollen is transported from one individual ramet to another that are \( \sqrt{x^2+y^2} \) distance apart, \( \Gamma \) is the gamma function, \( a \) is the distance parameter, and \( b \) is the shape parameter of the distribution. This distribution is used because it fits the kurtotic distribution that is highly consistent with pollen dispersal data (Clark 1998) and was utilized successfully with the Poisson Clustering Model (Robledo-Arnuncio and Austerlitz 2006).

**Geitonogamy**

The goal for this study is to determine how the spatial arrangement of ramets within and among clonal plants possibly influences their rate of geitonogamy. Therefore, we calculated the average rate of pollen movement between ramets within a clone (interramet geitonogamy, \( g \)) as

\[ g_j = \sum_{i=1}^{N} J_{ij} \lambda_{ij}, \]  \hspace{1cm} (3)

where \( N \) is the number of ramets in the population, and \( \lambda_{ij} \) is described as

\[ \lambda_{ij} = \frac{p_{ij}}{\sum_{k=1}^{N} p_{kj}}, \]  \hspace{1cm} (4)
where $p_{ij}$ is the probability that a pollen grain moves from ramet $i$ to ramet $j$, and $J_{ij}$ is an indicator function where $J_{ij} = 1$ if ramets $i$ and $j$ are in the same clone (but not the same ramet), and $J_{ij} = 0$ if the modeled ramets are not in the same clone or are the same ramet (Robledo-Arnuncio and Austerlitz 2006). Therefore, $g$ measures only the interramet rate of pollen movement among ramets.

**Clonal Diversity**

We calculated several statistics from the simulations that describe clonal diversity including clonal richness, an evenness index called Simpson's complement index (based on Simpson's complement) that controls for clonal richness, and Simpson's reciprocal (Arnaud-Haond et al. 2007) to help with the interpretation of the geitonogamy results in light of the intrapopulation heterogeneity of the clones.

Clonal richness was calculated as the number of genets in the population divided by the total number of ramets in the population (Ellstrand and Roose 1987). Simpson's complement index is a useful measure of evenness because it is independent of richness, and it is described by

$$V = \frac{D - D_{\text{min}}}{D_{\text{max}} - D_{\text{min}}},$$

(5)

where $D$ is Simpson's complement

$$D = 1 - L$$

(6)

and $L$ is Simpson's index
\[ L = \sum_{i=1}^{G} p_i^2, \]  

(7)  

where \( p_i^2 \) is the probability of randomly sampling two ramets of the same clone. \( D_{\text{min}} \) and \( D_{\text{max}} \) are the approximate minimum and maximum of Simpson's complement given \( G \) and \( N \).

\[
D_{\text{min}} = \left[ \frac{|2N - G(N-1)|}{N^2} \right] \frac{N}{N-1} \quad \text{(8)} \\
D_{\text{max}} = \left[ \frac{G-1}{G} \right] \frac{N}{N-1} \quad \text{(9)} 
\]

(Hurlbert 1971), where \( G \) is the number of clones in the population (\( i.e., n \)), and \( N \) is the number of ramets in the population. Simpson's reciprocal is the reciprocal of \( L \) (Hill 1973; Arnaud-Haond et al. 2007).

**Effective Pollen Pool Size and Pollen Dispersal Distance**

In order to understand the role of spatial arrangement and isolation by distance in pollen movement, we quantified the effective pollen pool size \( (N_{\text{ep}}) \), axial-dispersal standard deviation \( (\sigma_p) \), and mean pollen-dispersal distance (Robledo-Arnuncio and Austerlitz 2006) for the simulated populations. We numerically calculated all of these statistics from the simulated populations and compared them to the analytical expected values of randomly distributed populations (those with no
spatial aggregation) of the same size with the goal of determining how clonal spatial arrangements can influence isolation by distance.

$N_{ep}$ is the pollination equivalent of the effective neighborhood size statistic (Wright 1946). This statistic is the inverse of the probability that a ramet is pollinated twice by pollen of the same other ramet in a continuous population (Austerlitz and Smouse 2001). The equation for $N_{ep}$ assuming a population of randomly dispersed ramets is

$$N_{ep}^{rnd} = 4^{1/b} \pi a^2 \Gamma \left( 1 + \frac{2}{b} \right) d_e$$

(Austerlitz et al. 2004). We computed $N_{ep}$ from the simulations as

$$N_{ep}^j = \left( \sum_{i=1}^{N} \lambda_{ij}^2 \right)^{-1}$$

(Robledo-Arnuncio and Austerlitz 2006), where $\lambda_{ij}^2$ is from eq. (4) and is the probability that a ramet ($i$) is pollinated twice by the same ramet ($j$).

Then, to determine the interaction between spatial arrangement of intraclonal and interclonal ramets and pollen movement, we split $N_{ep}$ between intra- and interclonal elements ($N_{ep, wc}$ and $N_{ep, ac}$, respectively). $N_{ep, wc}$ is defined as the inverse of the probability of a focal ramet being pollinated by the same ramet twice among the intraclonal pollen pool, and $N_{ep, ac}$ as the inverse of the probability of a focal ramet being pollinated by the same other ramet twice for the interclonal portion of the pollen pool. $N_{ep, ac}$ is calculated as
\[ N_{\text{ep,ac}}^j = m_j^2 \left( \sum_{i=1}^{N} I_{ij} \lambda_{ij}^{-2} \right), \text{ for } m_j \neq 0 \] (12)

(Robledo-Arnuncio and Austerlitz 2006). \( m \) is the probability that pollen is deposited from an interclonal ramet and is described by

\[ m_j = \sum_{i=1}^{N} I_{ij} \lambda_{ij}, \] (13)

where \( I_{ij} \) is an indicator function with \( I_{ij} = 1 \) if ramets \( i \) and \( j \) are interclonal, and \( I_{ij} = 0 \) if the ramets are intraclonal. \( N_{\text{ep,wc}} \) is calculated as

\[ N_{\text{ep,wc}}^j = \sum_{i=1}^{N} \left( 1 - I_{ij} \right) \left( \frac{1 - m_j}{\lambda_{ij}} \right)^2, \text{ for } m_j \neq 1 \] (14)


We also delineated how spatial arrangement affects the axial-dispersal standard deviation because this is a more complete description of the effects of isolation by distance of pollen movement than \( N_{\text{ep}} \). The equation for the expected axial-dispersal standard deviation, assuming a random distribution of ramets for an exponential dispersal curve is

\[ \sigma_{p}^\text{rnd} = a \sqrt{\frac{\Gamma(4/b)}{2\Gamma(2/b)}} \] (15)
(Clark et al. 1999). For the simulated populations we calculated $\sigma_p$ as

$$\sigma_p^j = \sqrt{\frac{1}{2} \sum_{i=1}^{N} \lambda_{ij} \delta_{ij}^2}$$  \hspace{1cm} (16)$$

(Crawford 1984), where $\lambda_{ij}$ is described by eq. (4) and $\delta_{ij}^2$ is the Euclidean distance separating the $i$th and $j$th ramets. Additionally, we computed the mean pollen-dispersal distance because it is a more intuitive measure of dispersal distance than $\sigma_p^{rnd}$. The analytical equation for a randomly distributed population is

$$dd_{p}^{rnd} = d \left[ \frac{\Gamma(3/b)}{\Gamma(2/b)} \right]$$  \hspace{1cm} (17)$$

(Austerlitz et al. 2004). The numeric values for mean dispersal distance were calculated from the simulated populations as

$$dd_{p} = \sum_{i=1}^{N} \lambda_{ij} \delta_{ij},$$  \hspace{1cm} (18)$$

where $\lambda_{ij}$ is described by eq. (4), and $\delta_{ij}$ is the Euclidean distance separating the $i$th and $j$th ramets. All statistics from the simulations except for richness, evenness, and Simpson's reciprocal were calculated using the average value for all ramets from a central subplot of ramets of an area of 100 $d^{-1}$ (meaning on average the inner 100 ramets) to avoid edge effects.
Simulation Specifications and Parameterization

We report the ranges of the demographic and pollen-dispersal parameters of our simulation, and focus on the ratio of parameter values rather than their actual values for two reasons. First, because in these models, it is fundamentally more meaningful to examine the relationship between parameters than the actual values, and second, using ratios aids in reducing computation time without reducing the applicability of the model to other parameter values. In particular, we used only one global density value in our simulation and varied all the other parameters with regard to that density. A single global density greatly reduced the number of parameter combinations and computational effort, but did not reduce the scope of inference of the simulation because the same results can be simulated with various global population densities when the appropriate ratios of all other demographic and dispersal statistics are held constant. For example, consider two populations of clones, with equal levels of spatial autocorrelation, each with a total of 10,000 ramets ($N$). Population-1 has an area of 10,000,000 m$^2$, a global density of 0.001 ramets per m$^2$, and a mean pollen-dispersal distance of 200 m. Population-2 has an area of 2,000,000 m$^2$, a global density of 0.005 ramets per m$^2$, and a mean dispersal distance of 40 m. These two populations are functionally the same because they share the same ratio of dispersal distance to plot area (ratio = 1:50,000). Other population variables (i.e., $N_{ep}$) may change magnitude as well, but the relationships between them will stay the same.

We simulated populations using a random distribution of ramets up to a ratio of global to intrACLonal density ratios ($\mu/d$) of 20, which represents a wide range of positive spatial autocorrelation. When the ramets are randomly distributed, the mean probability that the nearest neighbor is part of the same clone as the focal ramet is 0, and when $\mu/d = 20$ the probability is > 0.95. We used a global density of 0.0025 which is within the observed range of plant densities (Plotkin et al. 2000; Robledo-Arnuncio and Austerlitz 2006). For the pollen-dispersal distribution, we investigated curves that
ranged from leptokurtotic \( (b = 0.8) \) through platykurtotic \( (b = 2.0) \), and we varied the scale parameter \( (a) \) to span a wide range of dispersal distances: from 10 times the mean nearest-neighbor distance \( (nnd) \) through 40 times \( nnd \) (giving a \( \sigma_p^{nnd} \) range from 87 through 346).

We used the above specified values of the dispersal distribution \( a \) and \( b \), along with the global density of 0.0025, in order to maximize the range of investigated parameter values while keeping computation time reasonable and maintaining biological meaning. Explicitly, the area of a population plot and the global density must be appropriate for the dispersal distance. If dispersal distance is too large for the plot area, then the dispersal distribution is truncated and not properly simulated. Therefore, dispersal area is limited by plot size, and plot size is limited by computation time. In order to increase plot size, while keeping density constant, the population size must increase. We limited the population size to 10,000 ramets, which in turn limited the plot size and dispersal distance, to keep the computer memory and time requirements reasonable. Also, it is not biologically relevant to have a mean dispersal distance that is smaller than the mean distance to the nearest-neighbor, so the minimum dispersal distance is limited by the distance to the nearest-neighbor, which is determined by the global density. We simulated populations with randomly aggregated ramets and found that the mean dispersal distance had to be approximately 10 times greater than the mean \( nnd \) for the isolation by distance statistics \( (i.e., N_{ep}) \) to match the expected analytical values.

We used a range of Pareto distribution slopes from \( \beta = 0.5 \) through 3.0 which is consistent with what is found in natural plant populations (fig. 1, which shows representative samples of simulated spatial aggregation patterns of clones and their ramets when Pareto \( \beta \) is 0.5 and 3.0) (Arnaud-Haond et al. 2007). The clonal richness of the populations ranged from 0.0002 through 0.90. We investigated clonal richness only up through 0.90 because it was difficult to consistently generate random populations that fit the clonal distributions for values that exceeded this number. The minimum
The richness of 0.0002 is the lowest possible richness that was meaningful for this study because it results from only two clones in a population of 10,000 ramets. The ranges of Simpson's reciprocal and evenness were determined by the clone-size distribution.

We ran 100 iterations of all combinations of the population statistics described above. Expressly, for each combination of the ranges of the two demographic parameters (density ratio and radius), dispersal parameters \((a, b)\), and Pareto \(\beta\), we calculated values for the focal statistics (evenness index, \(g\), \(N_{cp}\), \(N_{cp, wc}\), \(N_{cp, wc}\), \(\sigma_p\), and Simpson's reciprocal). To test the appropriateness of our theoretical predictions (the analytical statistics calculated above), we simulated populations assuming a random spatial arrangement of ramets in the population for all combinations of the simulation parameters described above. Under the above parameter specifications, the simulated populations’ effective pollen pools and axial-dispersal standard deviations closely resembled the theoretical values as seen in eqq. (10) and (15), respectively, indicating that the population size and the individual-based model are applicable for representing pollen movement under spatial aggregation and clonal structure (Robledo-Arnuncio and Austerlitz 2006).

Results

Clonal Spatial Aggregation

The value of \(g\) increases as clone radius increases (fig. 2A). This occurs because in the smaller radius range \((r < 5\%\) of the plot-side length, or for \(d = 0.0025\), as \(r < 100\)) as the radius increases and \(\mu\) is held constant, the number of ramets in each clone increases and the distance between clones increases resulting in a higher proportion of intrACLonal pollinations. For smaller radii, the total
effective intra- and interclonal pollen pool sizes increase as well (fig. 2B, C, and D). \( N_{\text{ep,wc}} \) increases because as radius increases, the clone size increases resulting in a decreased probability of pollination of a focal ramet by another ramet twice. \( N_{\text{ep,ac}} \) increases because as radius increases and clone size increases, the distance between clones increases causing the proportion of pollen from interclonal pollen donors to decrease. These results are more clear under conditions of larger Pareto \( \beta \) values where the clone size is more even than under those of smaller Pareto \( \beta \) values where clone size is less even. The increase in pollen pool size at small radii is even more evident when \( \mu/d \) is higher (results not shown, but see Robledo-Arnuncio and Austerlitz 2006).

When the radius further increases (\( r > 5\% \) of the plot-side length) \( N_{\text{ep}} \) decreases because the clones become farther apart and ramets also increase in distance from each other as well (fig. 2B) resulting in a increased probability of pollination from fewer ramets. \( N_{\text{ep,ac}} \) decreases because while the proportion of pollen from interclonal ramets decreases, assuming all pollinations are interclonal (1*

\[
\sum_{i=1}^{N} I_{ij} \lambda_{ij}^{-2}
\]

), resulting in a larger interclonal pollen pool, the proportion of interclonal pollinations \( (m) \) decreases at a greater rate. This occurs because, as radius and clone size increase, distance between clones increases, decreasing the probability of pollen deposition. However, the portion of ramets that are interclonal decreases, resulting in increased probability of pollen deposition (smaller interclonal pollen pool) and decreased \( m \). \( N_{\text{ep,wc}} \) increases again because clone size increases; however, it increases at a lower rate because the ramets within the clone are becoming farther apart and each new ramet added to the clone (while density remains constant) becomes a smaller portion of the probability of intrACLonal pollination (fig. 2D). As a result of the increase in \( N_{\text{ep,wc}} \), \( g \) also continues to increase (fig. 2A).

However, when clone size is held constant, as radius and density ratio increases \( g \) decreases
(fig. 3A). Figure 3A shows the difference in \( g \) with regard to two density ratios (\( \mu/d = 1 \) and 20) and when Pareto \( \beta = 0.5 \) and 3.0. The radius of a clone is calculated from the number of ramets in the clone \( (n) \) and their intracloinal density (\( \mu \)) as \( r = \sqrt{(n/\pi \mu)} \). Therefore, when \( \mu \) increases and \( n \) is constant, the radius decreases. In other words, this graph demonstrates the change in \( g \) when radius increases and clone size is constant. Across clone sizes, the magnitude of the decrease (difference in \( g \) between \( \mu/d = 20 \) and \( \mu/d = 1 \)) in \( g \) initially increases because the rate of increase in \( N_{ep, wc} \) is greater than the rate of increase in \( N_{ep, ac} \). But as clone size further increases, the decrease in \( g \) decreases because clone size and radius become much greater than the dispersal distance, and isolation by distance decreases.

Dispersal distance remains fairly constant as radius increases (fig. 4A) for \( \mu/d = 1 \), but initially decreases for \( \mu/d = 20 \) because of the high number of nearby ramets under high intracloinal-density conditions. The excess of proximate ramets decreases as the radius increases resulting in an increases in \( \sigma_{p} \cdot g \) decreases as the dispersal distance increases (fig. 4B) because a higher dispersal distance reduces the effects of isolation by distance and results in more pollinations from interclonal ramets.

**Clone-size Distribution**

There is a definite relationship between \( g \) and clone-size distribution. As the Pareto \( \beta \) increases, \( g \) decreases (fig. 2A) because as Pareto \( \beta \) decreases, the proportion of individual ramets in large clone-size classes increases (fig. 5) resulting in an increased \( N_{ep, wc} \) (fig. 2C) and decreased \( N_{ep, ac} \) (fig. 2D). Even though the clonal richness is constant among Pareto \( \beta \) values, when Pareto \( \beta \) values are small, the population is more similar to a population with fewer and larger clones. The magnitude of the clone-size-distribution effect is fairly constant across density ratios, but the proportionate change in \( g \) across Pareto \( \beta \) values increases as the density ratio decreases (with the change in \( g \) for \( \mu/d = 20 \) being 8%,
and the change in $g$ for random populations being 61%). This occurs because $N_{ep, wc}$ increases with density ratio, resulting in an increase in the overall rate of $g$ (fig. 6A).

When clone size is held constant and radius increases, the decrease in $g$ has a higher peak in the middle clone sizes for the higher Pareto $\beta$ populations (fig. 3A). $g$ shows this greater decrease because clones are more even in size for the higher Pareto $\beta$ values resulting in less distance between clones so as radius increases, $N_{ep, ac}$ increases (fig. 3B), while the changes in $N_{ep, wc}$ follow a similar pattern between the clonal structure types (fig. 3C).

Dispersal distance is influenced by clone-size distribution as well. Its effect on dispersal distance depends on the spatial aggregation of the clones. For $\mu/d = 1$, $\sigma_p$ is equal for all Pareto $\beta$ values (fig. 4A). However for $\mu/d = 20$, the decrease in $\sigma_p$ at smaller radii is less for populations with smaller Pareto $\beta$ values (fig. 4A) because the larger clones have larger radii, a greater average distance to intraclonal ramets, and less isolation by distance than populations with larger Pareto $\beta$ values. The dispersal distance and clone-size distribution also influence $g$. It is consistently greater in populations with smaller Pareto $\beta$ values than those with larger Pareto $\beta$ values across the range of pollen dispersal distances (fig. 4B).

*Clonal Diversity*

Clearly clone size is important with respect to geitonogamy especially when intraclonal ramets are spatially aggregated. This relationship is demonstrated in figure 6A where the expected $g$ for randomly dispersed clone ramets is much lower than the observed $g$ for aggregated clones and randomly dispersed ramets (from the simulations) for a given clonal richness (which is a proxy for clone size). The difference in $g$ when Pareto $\beta = 0.5$ and Pareto $\beta = 3.0$ is largest at lower and
intermediate clonal richness ($R$) values because that is where the largest clone sizes are possible, and the differences in clone-size distributions are maximized. When $R$ approaches 1, the differences in clone-size distribution are more restricted because the range of possible clone sizes is limited (e.g., when $R = 1$, all clones sizes are 1).

Evenness increases as Pareto $\beta$ values increase (evenness, $V$ for Pareto $\beta = 0.5$ is 0.947 and for Pareto $\beta = 3.0$ is 0.996); however, this index is not the best measure of heterogeneity in these populations because the large number of clones and large population size results in the index's approaching 1. The more appropriate measure of heterogeneity differences between the simulated populations is Simpson's reciprocal. It may be understood as the number of clones that are necessary to have the same heterogeneity as observed in the clonal population (Hurlbert 1971; Arnaud-Haond et al. 2007). Simpson's reciprocal increases as Pareto $\beta$ increases (fig. 6B). When Pareto $\beta = 3.0$, this index increases rapidly as clonal richness increases because the clones are more even, and each clone represents a relatively equal portion of the ramet population (fig. 6B). However, when Pareto $\beta = 0.5$, Simpson's reciprocal does not increase rapidly over most of the range of clonal richness because as $R$ increases, the population still has a few large clones that represent a large portion of the ramet population. When $R = 1$, Simpson's reciprocal for all Pareto $\beta$ values equals $N$, and as a result, Simpson's reciprocal for Pareto $\beta = 0.5$ rapidly increases as $R$ approaches 1.

Discussion

We simulated spatially-aggregated, clonal-plant populations using a Poisson cluster process modeled after the study of Robledo-Arnuncio and Austerlitz (2006) to examine the effects of clonality on pollen movement in monoecious plants. We modeled the pollen movement using a bivariate exponential power distribution and clone-size distribution using a Pareto distribution (Arnaud-Haond et
In our model, the overall results indicate that clonal diversity, spatial aggregation, and clone-size distribution can strongly influence pollen movement.

We demonstrate that clone-size distribution (described by the slope of the Pareto distribution) affects interramet geitonogamy (g). Specifically, as Pareto β values decrease, the variation in clone size increases, and the frequency of large clones increases despite no change in average clone size, richness, radius, or density. This results in an increased probability of intrACLonal pollination, and, therefore, a difference between the extreme Pareto distribution values (β = 0.5 and β = 3.0) in N_{ep,wc} of up to 15% for μ/d = 1 and 64% for randomly distributed populations, and a difference in g of up to 20% for μ/d = 1 and 61% for randomly distributed populations. Consequently, having a higher Pareto β value could negatively affect reproduction, especially if the plants are self-incompatible or partly self-compatible through fully self-compatible and have inbreeding depression (Barrett 2003). Despite the potential population-wide effect that lower Pareto β values have on geitonogamy, these clonal distributions could perhaps be advantageous for small clones. Larger clones may increase floral-display size near smaller ones and, as a result, increase pollinator-visititation rate to smaller clones which could then increase their pollen movement without the negative consequences of high g (Klinkhamer et al. 1989). It is also important to note that the number of clones with one ramet (where g is 0) is higher in clones with a Pareto β value of 0.5 than clones with a Pareto β of 3.0 (fig. 5).

The clone-size distribution of a population may reflect many aspects of its life history including age or maturity. For example, a population of the tree Prunus ssiori established after a volcanic eruption in 1739 has a low Pareto β of 0.88 after relatively few generations due to this tree's long generation time (Nagamitsu et al. 2004). On the other hand, a population of the orchid Cremastra appendiculata that has been undisturbed in a Korean national park for hundreds of years has a Pareto β of 1.94 (Chung et al. 2004). Recently established populations (in terms of generation time) may have lower Pareto β values due to establishment of only a few clones that were initially recruited as
propagules, while older populations may have more clones.

This simulation suggests that intraclonal aggregation can increase \( g \) to a greater degree than expected from the classic clonal-diversity measure clonal richness. This pattern is a result of an increased proportion of pollen movement from intraclonal ramets (fig. 2). Within our parameter ranges, at low clonal richness levels, \( g \) could be almost two times greater than the expected rate of \( g \) in populations where interclonal ramets are randomly interspersed. These results are consistent with the hypothesis that interspersed clonal growth forms may be an advantageous mechanism that increases floral display while minimizing the effect of \( g \) (Charpentier 2002) and in agreement with empirical studies that show some spatial arrangements (e.g., interdigitated clones) have reduced geitonogamy as compared to others (Mori et al. 2009).

Geitonogamy has played a central role in the hypotheses concerning the evolution of clonality and clonality's effect on mating in plants (Barrett 2003). Specifically, clonality has been postulated to increase reproductive assurance especially when a species is subject to strong pollen limitation (Burd 1994). Some species of clonal plants may be subject to higher levels of pollen limitation because they are found in disturbed and stressed habitats more frequently than non-clonal plants (Klimes et al. 1997), but there may be an upper limit of clone size in which geitonogamy results in a fitness cost that is too high (Barrett 2003).

However, clonal propagation may be beneficial in other ways that offset the mating costs of geitonogamy. For example, in zoophilous (animal-pollinated) plants, some studies have illustrated that large floral displays increase the number of visitors, but the percent of flowers visited per pollinator often decreases as floral-display size increases (Harder and Barrett 1996), suggesting that pollen import and export do not increase with floral-display size. Even though large floral displays may not increase pollen import and export on a per-flower basis, total male reproductive success may increase because large-floral displays produce more pollen than smaller ones (Eckert 2000).
Besides floral-display size and reproductive assurance, clonality has many possible benefits, including enhanced anchoring in substrate, resource accretion and storage, decreased risk of total clone death, and invasion of disturbed habitats (Ericksson and Jerling 1990; Grace 1993; Fahrig et al. 1994; van Groenendael et al. 1996). These numerous potential advantages of clonality suggest many paths for the evolution of clonal growth in plants and that geitonogamy is just a repercussion of the pollen movement between spatially associated ramets (Eckert 2000; Porcher and Lande 2005; Vallejo-Marin 2007). Our study illustrates that it is critical to consider the complex interplay of clonal spatial aggregation and clone-size distribution in addition to diversity when considering the interaction of clonality, mating, and geitonogamy (Honnay and Jacquemyn 2008).

An implicit assumption of this model is that the pollen-dispersal distribution is constant and does not change with regard to clonal spatial aggregation. Therefore this model may not always be the most appropriate one for zoophilous systems in which pollinator behavior may change with such aggregation (e.g., Morris 1993). However, other studies have shown that pollinator time spent on an aggregation of ramets increases with clone size and spatial isolation, resulting in lower rates of interclonal pollination, which is consistent with this model (Robledo-Arnuncio and Austerlitz 2006). Despite this dispersal-distribution simplification, our results explicitly show the effects that clonality can have on the overall rate of geitonogamy based on a combination of intra- and interramet self-pollination. For example, Klinkhamer and de Jong (1993) described a fairly intuitive model of geitonogamy in which self-pollination increases as the number of flowers on a plant (for our study, clone) increases. What they did not consider is how spatial arrangement of ramets may influence the pollen carried over from other clones or intraclonal ramets. If one considers this pollen carryover, the amount of self-pollen brought to a focal ramet as a result of g can significantly affect the rate and relative quantity of total self-pollen (g plus intraramet geitonogamy) that is deposited on the ramet (fig. 7).
In addition to pollinator behavior, many other factors that may affect pollen movement including self-incompatibility, male fitness (measured as pollen production), phenology (Robledo-Arnuncio and Austerlitz 2006), and plant sexuality (e.g., dioecious and monoecious) are not included in our model. Nonetheless, these factors seem to limit the movement of pollen, and if they are consistent throughout the populations, will not affect the fundamental relationships between clonal structure and pollen dispersal (Robledo-Arnuncio and Austerlitz 2006).

Our model is the first to provide a broad framework for understanding the role of clonal growth in pollen dispersal and to provide some general expectations for the role of geitonogamy in clonal mating systems. Moving forward, it is most important to put this study into a population genetics framework by including genetic data, increase our knowledge of pollinator behavior in spatially aggregated plant populations to assess the appropriateness of possible pollen-dispersal kernels better, and simulate the spatial arrangement of the clones using models based on the actual growth and movement of the plants.

Appendix A

Index of commonly used abbreviations

1. density of the population \((d)\)
2. mean density of ramets with genets \((\mu)\)
3. the slope of the exponential distribution that describes the clone-size distribution \((\text{Pareto } \beta)\)
4. the average rate of pollen movement between ramets within a clone \((\text{interramet geitonogamy, } g)\)
5. the inverse of the probability that a ramet is pollinated twice by pollen of the same other ramet \((\text{effective pollen pool size, } N_{ep})\)
6. inverse of the probability of a focal ramet being pollinated by the same other ramet twice for the interclonal portion of the pollen pool (interclonal pollen pool, $N_{ep, ac}$)

7. the inverse of the probability of a focal ramet being pollinated by the same ramet twice among the intraclonal pollen pool (intraclonal pollen pool, $N_{ep, wc}$)

8. the two dimensional variation in pollen dispersal distance (axial-dispersal standard deviation, $\sigma_p$)

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Figure 1: Representative samples of the simulated spatial aggregation patterns of clones and their ramets. In A, the Pareto β is 0.5 and in B, the Pareto β is 3.0. Both plots have the same number of clones. The ratio of the mean clone radius and plot-side length is 0.009, and the density ratio ($\mu/d$) is 20 in both cases to illustrate the clonal distributions well. As Pareto β increases the probability of larger clone sizes decreases, and A has more larger clones than B.
Figure 2: Interramet geitonogamy ($g$) and three different parameters of effective pollen pool size versus the ratio of mean clone radius to plot-side length. $\mu/d$ is 1, and the ratio of mean pollen dispersal distance and mean nearest-neighbor distance ($dd/nnd$) is 25 (which corresponds to $\sigma_p = 217$) in all graphs. We used the ratio of clone radius to plot-side length because this allowed us to show data that may be interpreted for all densities because different densities have different radii and plot areas, but
the same ratio of radius to plot-side length. All graphs are means across the range of the pollen-dispersal distribution shape parameter ($b$). In all graphs the solid lines represent simulated populations in which the Pareto $\beta$ is 0.5, and the dashed lines represent populations in which the Pareto $\beta$ is 3.0. $A$, $g$ versus the ratio of radius to plot-side length; $B$, the ratio of the calculated mean pollen pool size and the expected pollen pool size ($N_{ep}: N_{ep}^{m;}$) versus the ratio of radius to plot-side length; $C$, the intraclonal effective pollen pool size ($N_{ep, wc}$) versus the ratio of radius to plot-side length; and $D$, interclonal effective pollen pool size ($N_{ep, ac}$) versus the ratio of radius to plot-side length.
Figure 3: Difference between $\mu/d = 20$ and $\mu/d = 1$ for interramet geitonogamy ($g$), interclonal effective pollen pool size ($N_{ep,ac}$), and intraclonal effective pollen pool size ($N_{ep,wc}$) versus clone size. The radius
of a clone is determined by its number of ramets \( (n) \) and its density \( (\mu) \) \( r = \sqrt{\frac{n}{\pi \mu}} \). When \( \mu \) increases and \( n \) is constant, the radius decreases. All graphs are for \( \mu/d = 1 \), averaged across all ranges of the pollen-dispersal distribution shape parameter \( (b) \), and are for \( dd/nnd \) is 25 (corresponding to \( \sigma_{nd} = 217 \)). In all three graphs the solid line represents the relationship when Pareto \( \beta = 0.5 \), and the dashed line represents the relationship when Pareto \( \beta \) is 3.0. \( A \), difference in \( g \) versus clone size, \( B \), difference in \( N_{ep, ac} \) versus clone size, and \( C \), difference in \( N_{ep, wc} \) versus clone size.
Figure 4: The ratio of axial-dispersal standard deviation calculated from our simulations and expected axial-dispersal standard deviation ($\sigma_p : \sigma_p^{md}$) versus the ratio of clone radius to plot-side length (A) and interramet geitonogamy ($g$) versus mean dispersal distance for three nearest-neighbor-distance ratio values ($dd/nnd$) (B). We used the ratio of clone radius to plot-side length because this allowed us to show data that may be interpreted for all densities because different densities have different radii and plot areas, but the same ratio of radius to plot-side length. Both graphs are averaged over the entire range of the pollen-dispersal distribution shape parameter ($b$). In B, $g$ for $dd = 10nnd$, $dd = 25nnd$ and $dd = 40nnd$ correspond to $\sigma_p^{md} = 87, 217, \text{ and } 346$, respectively. In A, the solid line represents the relationship when Pareto $\beta = 0.5$, the dashed line represents the relationship when Pareto $\beta$ is 3.0, and the lines with open diamonds represent the relationship when $\mu/d$ is 20 while the lines with open triangles represent the relationship when $\mu/d$ is 1. In B, $\mu/d$ is 1, the black bar represents the relationship when Pareto $\beta$ is 0.5, and the white bar represents the relationship when Pareto $\beta$ is 3.0.
Figure 5: Clone-size distributions when Pareto $\beta$ is 0.5 ($A$) and Pareto $\beta$ is 3.0, ($B$). Data are for clone sizes 1 through 25. The histograms are averaged over the ranges of the pollen-dispersal distribution shape parameter ($b$), global density ($d$), clone density ($\mu$), and expected axial-dispersal standard deviation ($\sigma_p^{\text{rnd}}$).
Figure 6: Interramet geitonogamy ($g$) and expected $g$ versus clonal richness ($A$) and Simpson's reciprocal versus clonal richness ($B$). In both graphs, the solid line represents the relationship when Pareto $\beta = 0.5$, and the long dashed line represents the relationship when Pareto $\beta = 3.0$. Both graphs are for the entire range of Pareto $\beta$, pollen-dispersal distribution shape parameter ($b$), global density ($d$), clone density ($\mu$) and expected axial-dispersal standard deviation ($\sigma^{rd}_p$). In $A$, $g$ is for populations with both positively spatially autocorrelated and randomly spaced ramet populations, and expected $g$ is for randomly spaced ramet populations that are completely even. Expected $g$ was calculated as
\[ \left( \frac{N}{NR} \right)^{-1} \] where \( R \) is clonal richness and \( N \) is the number of ramets in the population. Both the numerator and denominator have a one subtracted from them to remove a focal ramet. Open diamonds represent randomly dispersed populations, xs represent the means over all positively spatially autocorrelated populations, and the short-dashed line with triangles represents expected \( g \).
Figure 7: Self-pollination ($S$) versus number of flowers visited on a focal ramet ($f$) with regard to the entire range of interramet geitonogamy ($g$) values. Increasing $g$ significantly changes the rate of self-pollen movement by pollinators within the ramet. We calculated graph values using a simple model of pollen movement based on $g$, a rate of pollen movement per flower ($K_1$), and a rate of pollen removal per flower ($K_2$) (Klinkhamer and de Jong 1993). Specifically the proportion of self-pollen deposited
As the number of flowers visited increases, was calculated as  
\[ S(f) = \frac{K(f) + s(f)c}{K(f) + s(f)c + t(f)c} \]
where \( c \) is the relative abundance of pollen on the body of the pollinator as compared to pollen available per flower, \( K_0 \) is the proportion of pollen on the pollinator from intraramet geitonogamy, \( s_0 \) is the proportion of self-pollen from \( g \), and \( t_0 \) is the proportion of outcross pollen still on the pollinator after visiting \( f \) flowers. \( K_{(f+1)} = K_0 + (K_2(1-K_1)) \), \( s_{(f+1)} = s_0 - (s_0K_1) \), and \( t_{(f+1)} = t_0 - (t_0K_1) \) where  
\[ s_0 = (g) - (s_0K_1), \quad t_0 = (1-g) - (t_0K_1), \]  
and \( K_{(f-1)} = K_2 \). The values of \( K_1 \) and \( K_2 \) for this graph are from personal observation of the animal-pollinated, clonal *Asclepias syriaca* (Common Milkweed). \( K_1 = 0.11, K_2 = 0.026, \) and \( c = 0.115 \).