Patterns and Consequences of Competition for Pollination between Introduced and Native Species with Different Floral Traits

by

Benjamin R. Montgomery

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Doctoral Committee:

Associate Professor Beverly J. Rathcke, Chair
Professor Deborah E. Goldberg
Professor Earl E. Werner
Emily D. Silverman, U.S. Fish and Wildlife Service

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Abstract

Competition for pollinators and avoidance of heterospecific pollen transfer have been assumed to be important factors promoting the evolution of specialized (restricted) floral morphologies. To test these assumptions, I performed field and greenhouse studies on prairie plants in Iowa. I also examined whether self-incompatibility reduces detrimental effects of heterospecific pollen. In a field study, neither *Sisyrinchium campestre* (Iridaceae) with an unrestrictive floral morphology nor *Viola pedatifida* (Violaceae) with a restrictive floral morphology showed a decrease in fruit or seed set when growing near patches of introduced *Euphorbia esula* (Euphorbiaceae) indicating that competition for pollination was weak or absent. In a survey of 29 species, plants with restrictive floral morphologies experienced less pollinator overlap with *Euphorbia* and received significantly less *Euphorbia* and other heterospecific pollen than those with unrestrictive (open) floral morphologies, as predicted. However, flowers with unrestrictive morphologies had significantly larger stigmas, and the density of heterospecific pollen (per stigmatic area) did not differ significantly between floral morphologies. Thus, correlated effects of stigma size may explain the patterns of heterospecific pollen receipt.

Large quantities of *Euphorbia* pollen added to stigmas prior to conspecific pollen had no effect on fecundity for two self-incompatible species and reduced fecundity for three self-compatible species, as predicted. However, two other self-compatible species were not significantly affected, indicating that this relationship needs further testing. Additional experiments with two species demonstrated that *Euphorbia* pollen receipt is unlikely to affect fecundity in nature because reducing the quantity of *Euphorbia* pollen applied or eliminating the time delay between *Euphorbia* and conspecific pollen application reduced effects of heterospecific pollen receipt.

To further explore the effects of relative abundance, pollinator visitation rates, and pollen carryover on competition by interspecific pollen loss, analytical and simulation models were developed which indicate that rare plants receiving few visits are most affected by pollen loss. Furthermore, increased pollen carryover is expected to result in the same average pollen receipt, but with a more uniform pollen distribution. This could increase or decrease competition by pollen loss, depending on the relationship between pollen receipt and pollination success.

Introduction

Differences among plant species in floral morphology, color, phenology and breeding systems, and the degree to which these differences have been shaped by interactions with pollinators have long intrigued biologists. One long-standing explanation for this diversity of floral traits is that it results from selection to avoid competition for pollination (Robertson 1895, Waser 1983). Competition for pollination has been invoked to explain divergence in flowering period (e.g. Robertson 1895, Stiles 1977, Brown and Kodric-Brown 1979, but see Poole and Rathcke 1979), flower color and floral morphology (reviewed in Levin 1978 and Waser 1983, also see Fishman and Wyatt 1999, Miyake and Inoue 2003). A logical first test of this hypothesis is whether interspecific competition for pollination affects plant reproduction, although the effect of competition for pollination on selection can also be measured directly (Caruso 2000). An indication that plants may experience competition for pollination is provided by studies of pollinator limitation, reviews of which have shown that the fruit or seed set of many populations are limited by inadequate pollinator service (Burd 1994, Ashman et al. 2004). If pollinator service is inadequate, then anything further decreasing the quantity or quality of pollinator service could further reduce fecundity.

Competition for pollination is of ecological interest because the interaction may differ considerably from competition for other abiotic or biotic resources. In particular, the resource – pollinators – are highly mobile, allowing interactions to occur across considerable distances. Additionally, for outcrossing species, pollination is only successful if the pollinator delivers pollen from another conspecific. This results in the potential for positive frequency dependence in mixed communities, where more common flowers may receive increased visitation rates (Levin 1972, Thomson 1978, Rathcke 1983) and may be disproportionately likely to be successfully pollinated (Levin and Anderson 1970, Feinsinger et al. 1986, Feinsinger et al. 1991, Kunin 1993, Kunin and

Iwasa 1996, but see Caruso 1999). However, for potentially self-pollinating species, prior visit history may be less important (Motten 1982, Kunin 1997).

Ecological effects of competition for pollination may have applied implications. This is true for conservation because rare plants may be especially prone to inadequate pollinator service (Kunin 1997). Furthermore, introduced plants have been integrated into plant-pollinator networks (Memmott and Waser 2002), and in a variety of cases this integration has resulted in decreased fruit or seed set of native species (Chittka and Schürkens 2001, Brown et al. 2002, Ghazoul 2004, Larson et al. 2006), although such effects are not always detected (Aigner 2004). In agriculture, it has also been observed that pollinator visits to weeds in preference to crop plants can lead to reduced pollination service (Free 1968).

The term competition for pollination actually subsumes a variety of mechanisms (Rathcke 1983, Waser 1983), all of which share the outcome that increased floral abundance of one species results in reduced pollination success of another species. Competition for pollination can occur when species compete for pollinator visits themselves, and this has been detected in several studies (Gross and Werner 1983, Jennersten and Kwak 1991, Chittka and Schürkens 2001, Brown et al. 2002, Ghazoul 2004), although other studies have instead detected facilitation (Laverty 1992, LaRosa et al. 2004, Moeller 2004). Additionally, if pollinators make interspecific flights, species can exchange pollen (Kephart 1983, Bell et al. 2005), and interspecific exchange can result in reduced fecundity (Waser 1978a, Harder et al. 1993, Caruso and Alfaro 2000, Brown and Mitchell 2001). This reduced fecundity may result from stigma clogging (Galen and Gregory 1989), pollen allelopathy (Murphy 2000), premature stigmatic closure (Waser and Fugate 1986), or production of unviable hybrids (Randall and Hilu 1990, Fishman and Wyatt 1999). However, receipt of heterospecific pollen does not necessarily reduce fecundity (Kwak and Jennersten 1991, Gross 1996, Kasagi and Kudo 2005, Moragues and Traveset 2005). Finally, interspecific pollinator flights can lead to loss of pollen to heterospecific stigmas, resulting in reduced conspecific pollen receipt (Campbell and Motten 1985).

Plants' susceptibility to experiencing competition for pollination may be influenced by a variety of traits, depending in part on the mechanisms by which the plants compete. For example, competition by any mechanism could be minimized by differences in flowering phenology or primary pollinators (Waser 1983). Competition could also be reduced by decreased reliance on pollinator service overall, for example by increased reliance on self-pollination or asexual propagation (Motten 1982, Bond 1994, Fishman and Wyatt 1999). When heterospecific pollen receipt occurs, a large stigmatic surface area could minimize competitive effects because a smaller portion of the stigma would be occluded by or in the immediate vicinity of a given amount of heterospecific pollen (Kohn and Waser 1985). It has been hypothesized that pollen carryover could help to maintain population success when competition occurs by loss of pollen to heterospecific flowers, (Feldman et al. 2004). Finally, traits promoting pollinator constancy could decrease competition resulting from heterospecific pollen receipt or conspecific pollen loss (Levin and Anderson 1970, Waser 1983, 1986).

Complex and restrictive floral morphologies could decrease competition, especially when competitors have unrestrictive morphologies. Avoidance of competition is likely to occur in this case because different pollinator guilds tend to visit flowers with easy access to floral rewards and flowers with restricted access. Additionally, when pollinator overlap between these groups does occur, competition may be minimized because pollinators to complex and restrictive flowers tend to exhibit greater constancy than to simple and unrestrictive morphologies (Waser 1983). This decreases the probability of both heterospecific pollen receipt and conspecific pollen loss.

Because many traits could buffer plants from experiencing competition for pollination it may be hypothesized that plants with any one such trait that minimizes competition may be less likely to also exhibit other such traits (Pleasants 1980). For example, Kunin and Shmida (1997) found that rare plants were either likely to have either simple flowers that were self-compatible, thereby allowing pollinators to be effective despite making interspecific flights, or they were likely to be self-incompatible but have deep flowers which restrict access to floral rewards. It may be similarly hypothesized that flowers with restrictive morphologies are less likely to receive heterospecific pollen than unrestrictive flowers, so restrictive flowers may be less reliant on large stigmas as a mechanism to minimize effects of heterospecific pollen receipt.

Although hypotheses have been put forth to predict how various traits influence a plant's response to competition for pollination, few studies have investigated whether particular traits influence competition in the manner hypothesized. Instead, many studies have focused on competitive effects for a single species or between species pairs, making it hard to identify which traits or ecological conditions influence the degree of competition (but see McLernon et al. 1996). Other studies have compared competitive responses among species with different traits, but only for small groups of species (Feinsinger et al. 1986, Murcia and Feinsinger 1996, Fishman and Wyatt 1999, Larson et al. 2006).

Modeling approaches have also been used productively to investigate the potential for competition for pollination and to generate hypotheses concerning which traits are likely to influence response to competition for pollination. For example, models have demonstrated that competition by loss of pollen is likely to be particularly severe for rare plants, plants receiving few visits, and plants with inconstant pollinators (Levin and Anderson 1970, Straw 1972, Waser 1978b, Campbell 1986). However, previous models have included simplifications that could exaggerate the potential for competition for pollination to influence fruit or seed set. Previous models have also suggested that pollen carryover may mitigate the effect of competition for pollination (Feldman et al. 2004), but unrealistic assumptions call this result into question.

In this dissertation, I seek to build on previous studies of competition for pollination by considering responses of several species to a putative competitor in a comparative context, and by developing novel models of competition for pollination. The empirical component of this research (Chapter 1 through Chapter 4) looks at indicators of the competitive effect of an introduced plant on the pollination of simultaneously flowering species with different traits on a Loess Hill prairie in western Iowa. The introduced plant, *Euphorbia esula* L. (Euphorbiaceae, leafy spurge), is native to Eurasia and has only been present in Iowa since the late 1800's (Huerd and Taylor 1998). Consequently, there has been relatively little opportunity for selection among other species for traits that minimize the competitive effect of *Euphorbia*. *Euphorbia*'s inflorescence is morphologically complex, composed of male and female flowers subtended by involucres with nectaries. Nonetheless, the structure is remarkably similar to a simple flower with an unrestrictive morphology, as the nectar source is located below and in close proximity to the anthers and stigmas. As a result, patterns of pollen transfer and competition from *Euphorbia* to simultaneously flowering species may reasonably represent patterns from less complex flowers with unrestrictive morphologies. Furthermore, *Euphorbia* is visited by a diverse array of pollinators and another study has detected pollen transfer from *Euphorbia* to simultaneously flowering prairie species (Larson et al. 2006).

The first two chapters investigate the effects of *Euphorbia* on the pollination of two native species with contrasting reproductive traits. *Sisyrinchium campestre* (Iridaceae) is self-incompatible with an unrestrictive morphology whereas *Viola pedatifida* (Violaceae) is self-compatible with a restrictive morphology. The difference in floral morphologies suggests that *Sisyrinchium* is more likely to share pollinators and receive interspecific flights from *Euphorbia*, and self-incompatibility is hypothesized to render *Sisyrinchium*'s pollination success more dependent on pollinator service than is *Viola*'s pollination success. *Viola* also produces cleistogamous flowers, which pollinate in bud without opening, which render these flowers immune to competition for pollination.

To investigate the potential for competition between *Euphorbia* and these species, I observed pollinators to all three species to investigate overlap. For *Sisyrinchium*, I also investigated whether close proximity to *Euphorbia* decreased visit rates compared to plants at least 10 m from *Euphorbia* (visit rates were too low to *Viola* for such comparisons to be made). I measured heterospecific pollen receipt from *Euphorbia* to each of these species and performed hand-pollination studies to investigate the effect of heterospecific pollen receipt. Finally, to assess overall competitive effects of *Euphorbia* on the pollination of these species, I measured fruit and seed set of both species in close proximity or more distant from *Euphorbia*. To gauge whether the differences in success near *Euphorbia* were attributable to differences in pollination, for *Sisyrinchium* I compared the success of hand-pollinated flowers to unmanipulated flowers at each distance. This also allowed determination of whether *Sisyrinchium* experienced pollinator limitation at the study site. For *Viola*, hand-pollinations on a large scale were impractical, so I instead compared the success of chasmogamous flowers, which are open to pollination, to the success of cleistogamous flowers, which necessarily self-pollinate.

In chapters 3 and 4, I investigated aspects of competition for pollination with *Euphorbia* across a broader array of species to test whether floral restrictiveness influenced patterns and effects of heterospecific pollen receipt. Specifically, in chapter 3, I tested the hypothesis that flowers with restrictive morphologies would receive less heterospecific pollen than flowers with unrestrictive morphologies. To test this hypothesis, I compared pollen loads of *Euphorbia* and all other heterospecific pollen across 29 species, which were categorized as having restrictive or unrestrictive morphologies.

Additionally, I hypothesized that if flowers with unrestrictive morphologies receive more heterospecific pollen, then selection to minimize negative effects of heterospecific pollen receipt would be stronger on these plants. Greater stigmatic area is hypothesized to be one adaptation to minimize effects of heterospecific pollen, so I also tested the hypothesis that flowers with unrestrictive morphologies have a greater stigmatic area. For a subset of seven species with variation in floral restrictiveness and breeding systems (including the *Sisyrinchium* and *Viola* experiments previously described), I tested whether receipt of *Euphorbia* pollen affects fecundity. Comparisons among these seven study results then suggest whether floral restrictiveness and breeding system are predictive of the effect of receiving heterospecific pollen.

The degree to which interspecific pollen loss causes competition for pollination is less easily measured empirically, especially for large cross-species comparisons. Instead, I used analytical and simulation models to investigate how different floral traits are expected to influence competition by pollen loss. In particular, I present two analytical models with different assumptions that include competition by pollen loss and also incorporate pollen carryover. The models suggest different hypotheses about the effect of pollen loss on competition, and I work to reconcile these differences with a simulation models that allows incorporation of different functions relating pollen receipt to fruit and seed set. This model suggests that the relationship between pollen receipt and fruit and seed set is critical in determining whether pollen carryover increases or decreases the effect of competition by pollen loss.

Together, these studies are intended to advance our understanding of competition for pollination from a list of case studies with mixed outcomes toward the development of testable hypotheses about how different traits affect a species' susceptibility to experiencing competition.

Chapter 1

Pollination of *Sisyrinchium campestre* (Iridaceae) in Prairies Invaded by the Introduced Plant *Euphorbia esula* (Euphorbiaceae)

ABSTRACT This study investigates the breeding system and pollination biology of the native prairie perennial *Sisyrinchium campestre* (Iridaceae, blue-eyed grass) and the potential for the introduced plant *Euphorbia esula* (Euphorbiaceae, leafy spurge) to interfere with its pollination. *Sisyrinchium* was determined to be self-incompatible. Visit rates by pollinators were lower for *Sisyrinchium* near *Euphorbia* than for flowers greater than ten meters away, indicating competition for pollinator visits. However, supplemental hand pollinations of *Sisyrinchium* detected no evidence of pollen limitation of fruit or seed set either near to or far from *Euphorbia*. Although nearly one-fourth of stigmas sampled contained *Euphorbia* pollen, hand-pollination experiments detected no effect of *Euphorbia* pollen receipt on fruit or seed set, whether *Euphorbia* pollen was applied immediately or two hours prior to application of *Sisyrinchium* pollen. Overall, this study suggests that *Euphorbia* does not reduce *Sisyrinchium*'s pollination success despite competing for pollinator visits and being a source of heterospecific pollen on *Sisyrinchium* stigmas.

INTRODUCTION

Interspecific competition for pollination occurs when the fecundity of one species is diminished in the presence of a simultaneously flowering species due to pollinator-mediated interactions. Competition may occur by three mechanisms: competition for pollinator visits (e.g. Chittka and Schürkens 2001, Brown et al. 2002), pollen loss to flowers of other species resulting in decreased pollination success of the donor species(e.g. Campbell and Motten 1985), and foreign pollen deposition causing decreased success of recipient flowers (Waser 1978b, Rathcke 1983) Competition by foreign pollen transfer may occur either by stigma clogging (e.g. Brown and Mitchell 2001) or pollen allelopathy (Kanchan and Chandra 1980, Murphy 2000).

Competition for pollination may be particularly strong between native and invasive plants because there has been less opportunity for selection for traits, such as divergence in flowering time, that reduce competition. Indeed, several studies have documented competitive effects of invasive plants on the pollination of simultaneously flowering native species (Brown and Mitchell 2001, Chittka and Schürkens 2001, Brown et al. 2002, Ghazoul 2004), although not all native species are necessarily affected and facilitation may occur instead (e.g. Moragues and Traveset 2005). As species invasions and habitat fragmentation continue to create novel flowering communities, the strength of competition for pollination by all of these mechanisms is likely to increase (Kephart 1983).

Leafy spurge (*Euphorbia esula* L.; *Euphorbia*ceae; hereafter *Euphorbia*) is an introduced species that may exert a competitive effect on the pollination of native species. *Euphorbia* is of Eurasian origin, and in North America, it is particularly abundant in the central and northern Great Plains (Watson 1985). Its presence is frequently associated with decreased diversity of and abundance of native forbs (Belcher and Wilson 1989, Butler and Cogan 2004). *Euphorbia* is abundant on Iowa's Loess Hills prairies, where steep slopes and well-drained soils create ideal semiarid habitat conditions, and the use of herbicides to control *Euphorbia* is limited by the potential for water contamination, and high densities of native plants that could be adversely affected (Huerd and Taylor 1998). The potential for competition for pollination with native forbs is suggested by the high abundance of *Euphorbia* in areas with a diversity of native species, and *Euphorbia*'s open floral morphology, which allows visits by a wide array of pollinators. The plant provides pollen and nectar rewards and is visited by a wide range of pollinators, but especially Diptera and Hymenoptera (Selleck et al. 1962, Messersmith et al. 1985, Larson et al. 2006). Its clonal growth form leads to high flowering densities, especially in well-established clones. A study in North Dakota has shown that some native species receive fewer visits and fewer conspecific pollen grains per stigma when growing in areas invaded by *Euphorbia* compared to non-invaded areas (Larson et al. 2006).

Blue-eyed grass (*Sisyrinchium campestre* E. Bickn; Iridaceae; hereafter *Sisyrinchium*) is a common perennial in Iowa's Loess Hills prairies, and its flowering period overlaps with that of *Euphorbia*. Like *Euphorbia*, *Sisyrinchium* flowers have open morphologies and therefore they may potentially share pollinators. However, neither *Sisyrinchium*'s pollination biology nor the potential for *Euphorbia* to interfere with its pollination has been investigated. Studies of congeners have shown that a range from complete self-incompatibility to complete self-compatibility occurs in other members of the genus (Ingram 1968, Henderson 1976, Cholewa and Henderson 1984).

The goal of this study was to investigate the pollination biology of *Sisyrinchium* and determine whether it experiences competition for pollination with *Euphorbia*. To determine whether *Euphorbia*'s presence affects visit rates, pollinator visits to *Sisyrinchium* were observed in areas either interspersed with *Euphorbia* or at least ten meters distant. To determine whether *Sisyrinchium* experiences pollen limitation and whether the degree of limitation is influenced by proximity to *Euphorbia*, the fruit and seed set of supplementally hand-pollinated flowers were compared to unmanipulated control flowers either nearby or distant from *Euphorbia*. In order to assess the possibility that improper pollen receipt from *Euphorbia* decreases *Sisyrinchium*'s pollination success, foreign pollen counts were conducted on naturally pollinated stigmas, and greenhouse experiments were conducted in which reproductive success was compared between outcrossed flowers and flowers pollinated with *Euphorbia* pollen prior to outcrossing. Finally, as part of this greenhouse study, the breeding system of

Sisyrinchium was investigated, as to our knowledge no published information about this exists.

METHODS

Sisyrinchium campestre was studied at The Nature Conservancy's Broken Kettle Grasslands Reserve in Plymouth County, northwestern Iowa (42.709° N, 96.579° W) during the spring of 2004. The study was conducted in an open area with a mix of prairie and European grasses, and prairie and introduced forbs. *Euphorbia esula* was the only abundant introduced forb in flower during the study. The area was subject to grazing, though not during the study, and had been burned during the previous summer.

Pollinator observations

To determine whether the presence of *Euphorbia* affects visitation rates to *Sisyrinchium*, visits were observed for *Sisyrinchium* growing either near (< 0.5 m) or distant from (> 10 m) a *Euphorbia* patch. Visitors were observed during afternoons between 3 May and 22 May, with observation pairs consisting of sequential 15-minute observations, one at each distance. Plots were haphazardly established with a 1 m diameter frame, ensuring that plots within an observation pair had similar *Sisyrinchium* densities. Up to three observation pairs were performed per day, and locations were shifted to avoid multiple observations of the same plots within a day. In total, there were 16 pairs of observation periods, amounting to 8 hours of observations over the course of the study. Visitors, excluding ants, were identified in the field to higher level groups (e.g. solitary bees, flies). Visit rates were determined by dividing the total number of flower visits during an observation period by the number of *Sisyrinchium* flowers in the plot, thus counting visits by a single pollinator to multiple flowers as multiple visits.

To verify that floral abundances were similar, the number of *Sisyrinchium* flowers was compared between plots with or without *Euphorbia* with a t-test paired by observation period. Visit rates per *Sisyrinchium* flower were calculated as visits per flower per observation period and were also compared between plots with or without *Euphorbia* with a t-test paired by observation period.

Pollen limitation of fruit and seed set

To determine whether *Euphorbia*'s presence affects the pollination success of *Sisyrinchium*, pollen limitation of fruit and seed set was examined for plants near and far from *Euphorbia*. Two sites, approximately 150 m apart, were selected, each of which had abundant *Sisyrinchium* and patches with and without *Euphorbia*. In each site, fourteen pairs of *Sisyrinchium* plants were chosen in a patch with high densities of *Euphorbia* and in a patch 10 m from the nearest flowering *Euphorbia* (112 plants total). In areas without *Euphorbia*, plants were selected by choosing the nearest two plants to each meter mark along a transect. Due to the patchy distribution of *Sisyrinchium* within *Euphorbia* stands, in areas with *Euphorbia*, plants with a conspecific within 0.5 m were selected haphazardly and paired by proximity. Within a pair, plants were randomly assigned to either supplemental hand-pollination (HP) or untreated control (Control) groups. The number of conspecifics with inflorescences within one-half meter of each plant pair was counted. Fruits and flowers open prior to the start of the study were labeled with acrylic paint on the ovary and excluded. Thereafter, plants were monitored daily or every other day throughout the remainder of the flowering season.

For hand-pollinated plants, all flowers open at the time of monitoring received supplemental hand-pollination and were monitored for fruit and seed set. For use as a pollen source, in both sites several *Sisyrinchium* located between the study patches were bagged with bridal veil netting, and each day anthers from several bagged plants were collected in a glass vial and agitated to release and mix the pollen. Hand pollinations were always performed with pollen from the same site using a paintbrush, taking care to minimize self pollen from landing on stigmas. Pollination success was confirmed with a hand-lens. Hand-pollinated flowers were labeled in one color, and flowers that senesced without having received hand-pollination (senesced) were labeled in another color. Flowers on control plants were monitored and labeled on the same schedule as HP plants but without distinguishing between open and senesced flowers. Because fruit and seed set could change during the flowering season, the season was divided into three flowering periods of nine, eight, and fourteen days, respectively, with different paint colors used for each period.

Fruit set was determined by counting the number of developing ovaries about one week after labeling, by which time aborted ovaries had abscised. Fruit set was calculated as the proportion of flowers that initiated fruit development. Fruits were collected three to four weeks after flowering, when they were mature, and were stored in 70% ETOH. All undamaged collected fruits with intact pericarps were checked to ensure seeds were present; if no seeds were present, the fruit was treated as an abortion when calculating fruit set, and the zero count was excluded when calculating seed set. Seed set per developed fruit was determined by counting seeds from two randomly chosen undamaged fruits, whenever available, for each combination of plant, flower status (open or senesced), and time period. Counts from only one fruit were used when additional fruits were unavailable. Overall, including both treatments, seed counts from 481 fruits were included in the analysis. Only 44 of 112 plants produced flowers during the third period, so analysis of fruit and seed set was restricted to the first two time periods. Analyses of fruit and seed set excluded senesced flowers (unpollinated) on HP plants.

To analyze the effects of pollination treatment, *Euphorbia*'s presence, and time period on fruit set, a mixed model was fit using the generalized linear mixed effects (GLME) procedure assuming a binomial distribution with the logit function and using the correlatedData library in S-Plus 6.0. Fruit set in this analysis is represented by the numbers of fruits and aborted flowers (i.e. fruit set) for each plant in each time period. In this analysis, site was treated as a random effect, while time period, *Euphorbia* presence, and pollination treatment were treated as fixed effects, and *Sisyrinchium* density was included as a covariate. A model including all interactions between the fixed effects was initially fit, but interactions between time period and the other fixed effects were not even marginally significant ($P > 0.10$), so were dropped from the model. The interaction between *Euphorbia* presence and pollination treatment was retained, because this value indicates whether the degree of pollen limitation of fruit set differs between the two distances from *Euphorbia*.

A similar analysis was conducted to determine whether proximity to *Euphorbia* influenced whether *Sisyrinchium* experienced pollen limitation of seed set. For analysis of seed set, counts were square-root transformed to meet model assumptions of normality and constant error variance, but for ease of interpretation figures depict untransformed values. This analysis was conducted using a linear mixed effects (LME) model in S-Plus, with transformed average seed set as the response variable, pollination treatment, time period, and *Euphorbia* presence included as fixed effects, and site treated as a random effect. Interactions with time period were again not significant, so were dropped from the model.

Flowering phenology

Euphorbia's flowering was monitored in ten 0.25 square-meter plots in each of four *Euphorbia* patches, two patches of which included *Sisyrinchium* plants used in the pollinator limitation study. In each patch, plots were situated every meter along a haphazardly placed transect. Monitoring was repeated every three to five days, noting the number of ramets in each plot with open male or female flowers. The flowering curve was calculated by averaging the number of flowering ramets across all forty plots. *Sisyrinchium*'s flowering phenology was constructed based on the proportion of monitored *Sisyrinchium* plants in the pollinator limitation study with new flowers since the last inspection (flowers generally remained open for one day, and inspections were performed daily or on alternate days).

Incidence of Euphorbia pollen on Sisyrinchium stigmas

To determine whether *Sisyrinchium* stigmas receive *Euphorbia* pollen under natural conditions, 118 stigmas were collected from 58 plants between May 7 and May 28, 2004 at distances ranging from 0 – 14 m from the nearest flowering *Euphorbia*. From one to three stigmas per plant were stored in microcentrifuge tubes in a 9:1 mixture of 70% ethyl alcohol to glycerin. In the laboratory, stigmas were either individually mounted on slides with basic fuchsin gel (Kearns and Inouye 1993), or individually acetolyzed in a microcentrifuge tube after which the pollen was similarly mounted in basic fuchsin gel (Kearns and Inouye 1993). In a paired comparisons, the two techniques did not significantly differ in the quantity of pollen recovered (unpublished data). Additional pollen that fell off stigmas during storage was recovered from the storage vials and added to the pollen counts from stigmas. Pollen was identified with a compound scope at 400X magnification, and *Euphorbia* pollen grains were counted. To avoid pseudoreplication, pollen counts from multiple stigmas on the same plant were averaged prior to analysis. The effect of distance to the nearest flowering *Euphorbia* on incidence of *Euphorbia* pollen grains was analyzed using linear regression after square-root transforming average *Euphorbia* pollen incidence per plant, with weights assigned according to the number of stigmas analyzed from each plant. For *Sisyrinchium* within 0.5 m of flowering *Euphorbia*, the effect of *Euphorbia* flowering ramet density on average *Euphorbia* pollen incidence (square-root transformed) was investigated with a linear model weighted by the number of stigmas analyzed from each plant.

Breeding system and effect of heterospecific pollen transfer

To determine the breeding system and effect of *Euphorbia* pollen incidence on *Sisyrinchium* reproductive success, a greenhouse experiment was conducted with plants transplanted from McCormack Prairie in O'Brien County, Iowa, about 100 kilometers west of Broken Kettle Grasslands Reserve. The experiment was conducted at the University of Michigan's Matthaei Botanical Gardens. After overwintering plants in a cold frame until late February 2004, *Sisyrinchium* plants were enclosed in groups of up to eight plants under bridal-veil nets, and the forty-four plants that flowered were used in the experiment. Several potted *Euphorbia* were similarly treated to use as a pollen source. The study included the following four hand-pollination treatments, each applied to one flower per plant: self-pollinate (Self), cross-pollinate (Cross), heterospecific pollen transfer of *Euphorbia* followed by cross-pollinate (HPT+Cross), and untreated control (Control).

Pollinations were performed on the first day a flower opened. All pollinations were performed by transferring pollen with cleaned fine-tipped forceps. Control flowers were left unmanipulated. Self-pollinations were performed by transferring self-pollen from the anthers to the stigma within flowers autogamously. Cross pollinations were performed by sequentially transferring pollen from flowers on two different plants to the selected stigma. HPT+Cross treatments were performed by transferring pollen from one or two dehisced *Euphorbia* anthers in the same manner, then cross pollinating the flower as in the cross pollination treatment. Flowers were labeled with a dot of acrylic paint on the ovary. Pollination treatments were performed between March 19 and April 1, and fruits were collected on April 18, by which time seeds were mature. Fruits were stored in 70% ETOH until seeds were counted.

Because fruit set was zero for some treatments, effects of pollination treatment were analyzed with Fisher's exact test, which does not allow estimating random differences among plants. To focus exclusively on the effect of *Euphorbia* pollen transfer on fruit set relative to cross-pollinated flowers, fruit set was reanalyzed including only the cross pollination and HPT+Cross treatments, using a GLME model assuming binomial variance as detailed in earlier analyses, and including plant as a random effect to account for variation among plants. Seed set, which was square-root transformed prior to analysis, was compared between the Cross and HPT+Cross treatments with a LME model assuming a normal distribution, and including plant as a random effect.

A follow-up study was performed on April 7 and 8 to determine whether the effect of *Euphorbia* pollen depended on its timing of application. The study included twenty plants, on each of which one flower was pollinated with *Euphorbia* pollen immediately preceding conspecific pollination, as in the earlier study while another flower on each plant pollinated with *Euphorbia* pollen approximately two hours prior to conspecific pollination. *Euphorbia* pollen application was scheduled such that conspecific pollen was applied at the same time for both treatments. All methods were otherwise identical to the prior study. Fruit set and seed set were analyzed with GLME and LME models, respectively, as in the prior study.

RESULTS

Flowering phenology

Both species had begun flowering by 27 April, when monitoring began, and *Sisyrinchium* finished flowering while *Euphorbia* remained in flower (Figure 1.1). Reduced flowering of *Sisyrinchium* during its flowering season corresponded to periods of cold or rainy weather.

Pollinator Visitations

Observations suggested limited pollinator sharing between *Euphorbia* and *Sisyrinchium*. Halictid bees and other solitary bees comprised the majority of visits to *Sisyrinchium*, while ants, syrphids, and other flies accounted for most visits to *Euphorbia* (Table 1.1). However, all groups of visitors made some visits to both species. *Sisyrinchium* floral abundance was similar between plots with and without *Euphorbia* (paired t-test, $t = -0.909$, *d.f.* = 15, $P = 0.37$). Visit rates per flower per 15-minute time period to plots without *Euphorbia* were (± s.e.) 1.02 ± 0.29, and were significantly higher than the visit rates to plots with *Euphorbia* of 0.29 ± 0.12 (paired t-test, $t = 2.2463$, *d.f.* = 15, $P = 0.04$).

Fruit and Seed Set

Sisyrinchium fruit set was significantly higher near than distant from *Euphorbia* (0.71 ± 0.03 v. 0.68 + 0.03, respectively) but there was no significant main effect of time period or of supplemental hand-pollination (Table 1.2a). There was also no significant interaction between *Euphorbia*'s presence and pollination treatment, indicating that pollen limitation of fruit set was not detected at either distance from *Euphorbia* (Figure 1.2, Table 1.2a). *Sisyrinchium* seed set was significantly higher in the first time period than second (13.5 ± 0.7 v. 10.5 ±0.6, respectively). However, there were no significant effects of distance from *Euphorbia*, pollination treatment, or the interactions between these variables, indicating that pollen limitation of seed set was not detected overall or at either distance from *Euphorbia* (Figure 1.3, Table 1.2a).

Incidence of *Euphorbia* pollen on *Sisyrinchium* stigmas

Euphorbia pollen was found adhered to 22% of *Sisyrinchium* stigmas, and with the conservative assumption that all unattached *Euphorbia* pollen recovered within each tube originated from only one stigma stored within that tube, *Euphorbia* pollen occurred minimally on 38% of stigmas. Including dislodged pollen, an average (± s.d.) of 1.6 (± 2.4) *Euphorbia* pollen grains were found per *Sisyrinchium* stigma, with a maximum of seven *Euphorbia* grains associated with a single stigma. Excluding one observation with disproportionate leverage, *Euphorbia* pollen incidence declined with distance to the nearest flowering *Euphorbia* (*Euphorbia* pollen incidence square-root transformed r^2 =

0.13, $F_{1,55}$ = 8.08, P = 0.006). However, *Euphorbia* pollen was detected at all distances measured (Figure 1.4), and one plant received nearly six pollen grains despite being located 10 m from *Euphorbia* (inclusion of this point in regression did not substantially change curve fit, but reduced fit to r^2 = 0.06, $F_{1,56}$ = 3.40, P = 0.07). The density of flowering *Euphorbia* ramets was positively correlated with *Euphorbia* pollen grain receipt for *Sisyrinchium* plants with flowering *Euphorbia* ramets within 0.5 m (r^2 = 0.24, $F_{1,25}$ = 7.99, P = 0.009) (Fig. 1.5).

Breeding system and effect of heterospecific pollen transfer (HPT)

Fruit set was significantly affected by pollination treatment, with no fruit set in either the self pollination or unmanipulated treatments, and high fruit set in the cross pollination and HPT+Cross treatment (P < 0.001, Fisher's exact test, Table 1.3). When the analysis was restricted to the HPT+Cross and Cross treatments, fruit and seed set were similar (Table 1.3), and did not significantly differ for either treatment variable (Table 1.4a,5b). In the study investigating the timing of *Euphorbia* pollen application, fruit set was 60% when *Euphorbia* pollen was applied two hours prior to conspecific pollination, and 55% when applied immediately before conspecific pollination, and this difference was not significant (Table 1.4c). Seeds were counted from eleven fruit per treatment, and seed set averaged (± s.e.) 22.3 ± 4.0, and 23.5 ± 4.6 seeds when applied two hours before or immediately prior to conspecific pollination, respectively, and this difference was not significant (Table 1.4d).

DISCUSSION

Euphorbia was found to compete with *Sisyrinchium* for pollinator visits, but this competition did not translate to decreased fruit or seed set near *Euphorbia*. This outcome may be explained by the finding that neither *Sisyrinchium*'s fruit nor seed set was pollen limited during the study. Consequently, even if *Euphorbia*'s presence decreased conspecific pollen receipt by competition for pollinator visits or by pollen loss to *Euphorbia*, such a decrease would not necessarily decrease fruit or seed set. It is not clear why *Sisyrinchium*'s fruit set increased in the vicinity of *Euphorbia*, but the similarity of fruit set patterns between plants receiving supplemental pollination and control flowers indicates that this pattern does not relate to different rates of pollinator visits or pollen receipt. Instead the pattern could be attributable to differences in soil moisture or nutrients or a variety of other abiotic variables with the capacity to affect fruit set.

This study does not support the hypothesis that *Euphorbia* decreases the pollination success of *Sisyrinchium*. The finding of *Euphorbia* pollen associated with more than one-third of *Sisyrinchium* stigmas suggests the potential for competition by heterospecific pollen transfer. However, even when present, *Euphorbia* pollen grains occluded only a small fraction of the stigmatic surface, and artificial transfer of large quantities of *Euphorbia* pollen failed to reduce fruit or seed set relative to uncontaminated flowers. This suggests that neither stigmatic clogging nor pollen allelopathy are a likely result of improper pollen transfer from *Euphorbia*.

Although several studies have detected competition for pollination (Waser 1978a, Campbell 1985b, Murphy and Aarssen 1995b, Caruso 2000, Chittka and Schürkens 2001, Brown et al. 2002, Bell et al. 2005), other studies have failed to detect such competition (Caruso 1999). Other studies have detected competition for pollination, but only for a subset of species investigated (Feinsinger et al. 1986), a subset of years, or a subset of indicators of competition (Feinsinger et al. 1991, Caruso 2001, Ghazoul 2004). Other studies have instead detected facilitation for pollination (Laverty 1992, LaRosa et al. 2004, Moeller 2004). This variation in study outcomes may result in part from differences in floral attributes, such as floral morphology, and differences in the degree to which focal species rely on outcrossing, as species more reliant on self-pollination may be more buffered from competitive effects than species more reliant on outcrossing (Feinsinger et al. 1991, Fishman and Wyatt 1999). However, in this study competition for pollination was not detected despite *Sisyrinchium*'s reliance on outcrossing. Additionally, variation in outcomes may result from variation in target or neighbor density (Thomson 1978, Kunin 1993), or the spatial scale examined. *Sisyrinchium* flowers were abundant during peak flowering which may have allowed pollinators to specialize on this floral resource, thus reducing competition with *Euphorbia*.

Similarly, several previous studies have detected decreases in fruit or seed set upon artificially transferring foreign pollen to stigmas (Waser 1978a, Galen and Gregory 1989, Caruso and Alfaro 2000, Brown and Mitchell 2001), but other studies have not detected this effect (e.g. Campbell and Motten 1985, Kwak and Jennersten 1991, McGuire and Armbuster 1991). Differences in the pollen chemistry of donor flowers may partly account for why only a subset of studies have detected an effect (Murphy 2002). The breeding system may also affect outcomes, as outcrossing plants are likely to have larger stigmatic areas due to decreased certainty of pollination success (Cruden and Millerward 1981). This could result in a smaller proportion of a stigmatic surface being occluded by heterospecific on outcrossing plants than primarily self-pollinating species (Kohn and Waser 1985). Because *Sisyrinchium* is self-incompatible and presents pollen immediately beneath its receptive stigma, its stigmas are likely adapted to function despite receipt of conspecific incompatible pollen, for example by having a large stigmatic surface relative to area required for adherence of enough pollen grains to bring about complete pollination.

Considered together, the results of this study and that of Larson et al. (2006) indicate that *Euphorbia* affects the pollination of simultaneously flowering species in some but not all cases. Larson et al. (2006) detected a significant decrease in visit rates near *Euphorbia* for *Linum lewisii* across two study seasons and for *Campanula rotundifolia* in one of two study seasons. These species and *Sisyrinchium* have open, unrestrictive floral morphologies. In contrast, overall visit rates to *Oxytropis lambertii*, which has a restricted morphology, were not affected by *Euphorbia* in their study. This suggests that flowers with an open floral morphology, are more likely to experience competition for pollination with *Euphorbia*, which also has an open morphology. However, in the Larson et al. (2006) study, visit rates by halictid bees were lower for all three species near *Euphorbia,* and conspecific pollen receipt rates were lower for both unrestrictive *Linum* flowers and restrictive *Oxytropis* flowers, indicating that restrictive floral morphologies do not necessarily prevent competition for pollinators that visit both open and restrictive flowers.

Larson et al. (2006) did not measure effects on fruit or seed set, so it is unknown whether changes in visit rates or conspecific pollen receipt rates resulted in decreased fecundity. For *Sisyrinchium*, the close proximity of anthers and stigma prevented attaining conspecific pollen counts free of contamination from anthers, so conspecific pollen receipt rates could not be compared between studies. Future studies measuring both pollen receipt rates and fecundity would provide stronger evidence of whether *Euphorbia* is likely to interfere with the pollination of simultaneously flowering species.

Another important difference between this study and that of Larson et al. (2006) is the spatial separation of invaded and uninvaded areas. In the Larson et al. (2006) study, uninvaded areas were at least 100 m distant from the nearest flowering *Euphorbia*, but in this study uninvaded areas were separated by as little as 10 m. This small distance is justifiable given that pollinators typically fly short distances to nearby flowers (Levin and Kerster 1969a), and in this study, expected *Euphorbia* pollen receipt was reduced by more than 70% ten meters distant from *Euphorbia* compared to immediately adjacent to it (see Figure 1.4). This reduction is greater than the 50% reduction in heterospecific pollen receipt for every 10 m separating adjacent populations of lepidopteran pollinated *Phlox* (Levin 1971), perhaps because small flies and bees tend to fly shorter distances between flowers than lepidopterans (Herrera 1987). Nonetheless, *Euphorbia* pollen was detected more than 10 m from *Euphorbia*, suggesting that future studies could benefit from further separating plots with and without *Euphorbia*.

This study, and that of Larson et al. (2006) indicates that the presence of flowering *Euphorbia* can decrease visit rates and act as a source of pollen contamination for simultaneously flowering native plants. Although *Sisyrinchium* did not experience decreased fecundity when nearby *Euphorbia* or when artificially exposed to *Euphorbia* pollen, other species that are pollen limited or that are more affected by foreign pollen receipt may experience greater competitive effects. This suggests that timing *Euphorbia* management prior to full flowering may help to restore pre-invasion plant pollinator interactions and minimize the potential for other species to experience competition for pollination.

Table 1.1 Total number of visitors observed from each group to *Euphorbia* and to *Sisyrinchium* in *Euphorbia*'s presence and absence. Parenthetical numbers indicate percent of visits each taxa represents out of total for column.

	Plant Species		
	Euphorbia present		*Euphorbia* absent
Insect group	*Euphorbia*	*Sisyrinchium*	*Sisyrinchium*
Green halictid bees	1 (5%)	8 (33%)	12 (31%)
Other solitary bees	2 (10%)	11 (46%)	17 (44%)
Apis mellifera	1 (5%)	--	--
Syrphids	6 (30%)	2 (8%)	6 (15%)
Other flies	10 (50%)	1 (4%)	0
Moths	--	1 (4%)	0
Unidentified	--	1 (4%)	4 (10%)
Total	**20**	**24**	**39**

Table 1.2 A. Results of generalized linear mixed effects (GLME) analysis of effects of specified factors and interactions on fruit set of *Sisyrinchium*, with site included as a random effect. B. Results of linear mixed effects model of effects of specified factors and interactions on seed set across the first two time periods, with site included as a random effect.

Factor	Numerator d.f.	Denominator d.f.	*F*	*P*
A. Fruit set				
Intercept	1	200	73.25569	< 0.0001
Euphorbia	1	200	6.74290	0.0101
Treatment	1	200	0.39759	0.5291
Time period	1	200	2.20673	0.1390
Euphorbia by treatment	1	200	0.01151	0.9147
B. Seed set				
Intercept	1	179	944.790	< 0.0001
Euphorbia	1	179	1.791	0.183
Treatment	1	179	0.203	0.653
Time period	1	179	9.424	0.003
Euphorbia by treatment	1	179	0.044	0.835

Table 1.3 Fruit and seed set of *Sisyrinchium* flowers exposed to one of four pollination treatments: cross pollination (Cross), pollination with *Euphorbia* immediately followed by cross pollination (HPT+cross), self pollination (Self), and untreated control (Control).

	Fruit Set			Seed Set		
Treatment	Mean	s.e.	N	Mean	s.e.	N
Cross	0.79	0.06	43	18.5	2.5	34
HPT+cross	0.86	0.05	43	20.9	2.2	33
Self	0.00	n.a.	43	n.a.	n.a.	n.a.
Control	0.00	n.a.	43	n.a.	n.a.	n.a.

Table 1.4 Significance tests of effect of pollination treatment on *Sisyrinchium* fruit set. A. First study, fruit set including only Cross and HPT+cross treatments B. First study, seed set including only Cross and HPT+cross treatments C. Second study, fruit set including *Euphorbia* pollen applied two hours before or immediately before conspecific pollen.

Factor	Numerator d.f.	Denominator d.f.	*F*	*P*
A. Fruit set including only Cross and HPT+cross treatments				
Intercept	1	42	26.68	<0.0001
Cross v. HPT+cross	1	42	3.34	0.07
B. Seed set including only Cross and HPT+cross treatments				
Intercept	1	25	309.56	<0.0001
Cross v. HPT+cross	1	25	1.22	0.28
C. Fruit set, pollen application timing study				
Intercept	1	19	0.78	0.39
Immediate v. delay	1	19	0.11	0.75
D. Seed set, pollen application timing study				
Intercept	1	5	61.35	< 0.001
Immediate v. delay	1	5	0.04	0.85

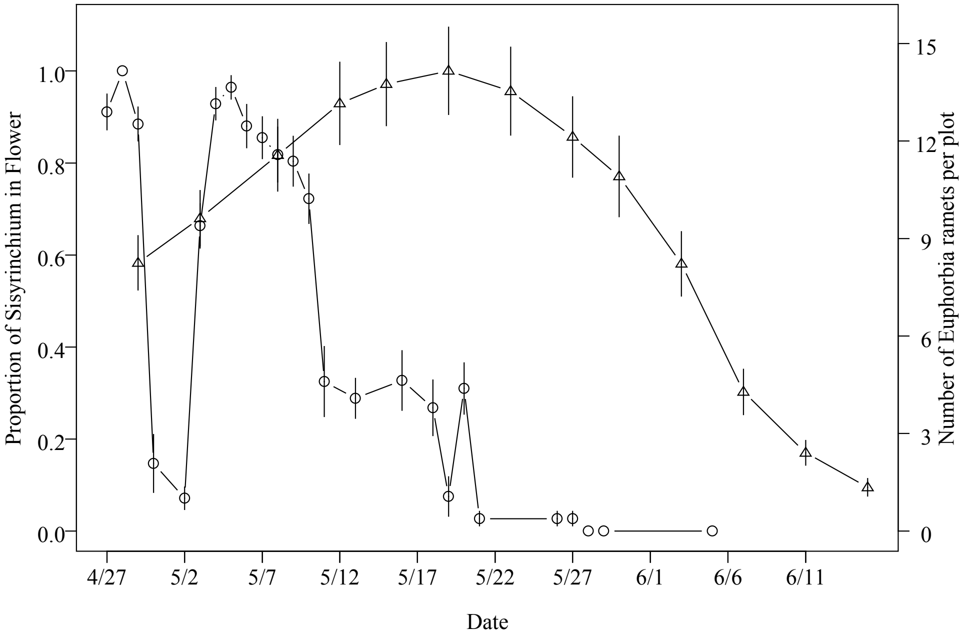

Figure 1.1 Phenology curves showing proportion (± s.e.)of *Sisyrinchium* plants in pollinator limitation study with new flowers (circles) and average number (± s.e.) of *Euphorbia* ramets in flower per 0.5 square meter plot (triangles).

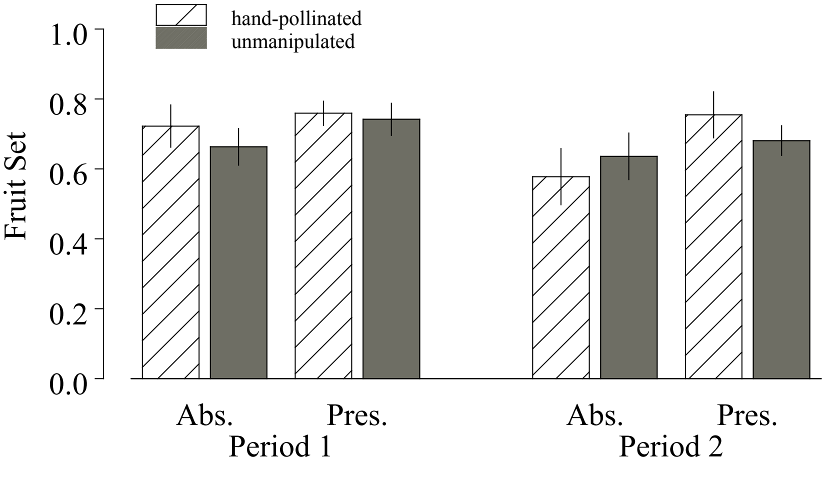

Figure 1.2 Effect of treatment on fruit set per flower (± s.e.) in the absence (Abs.) and presence (Pres.) of *Euphorbia*, for each the first two time periods. See Table 1.2 for significance testing.

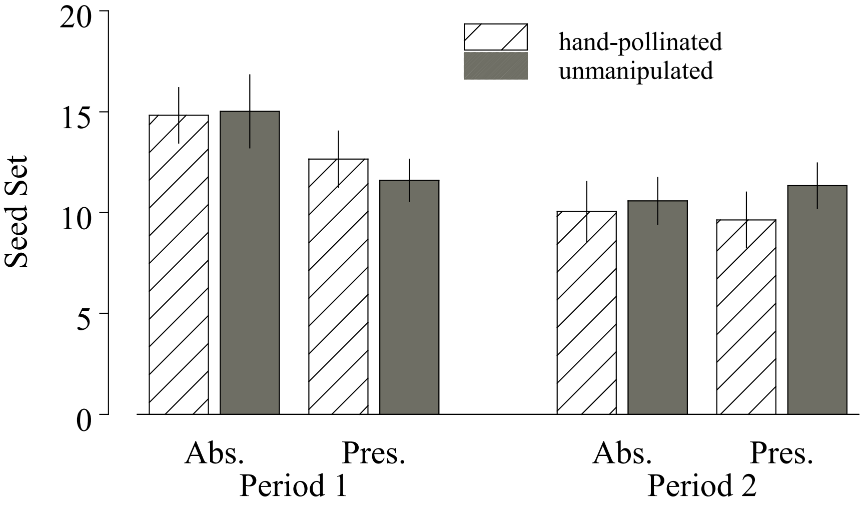

Figure 1.3 Effect of treatment on seed set per fruit (+ s.e) in the absence (Abs.) and presence (Pres.) of *Euphorbia*, for each of the first two time periods.

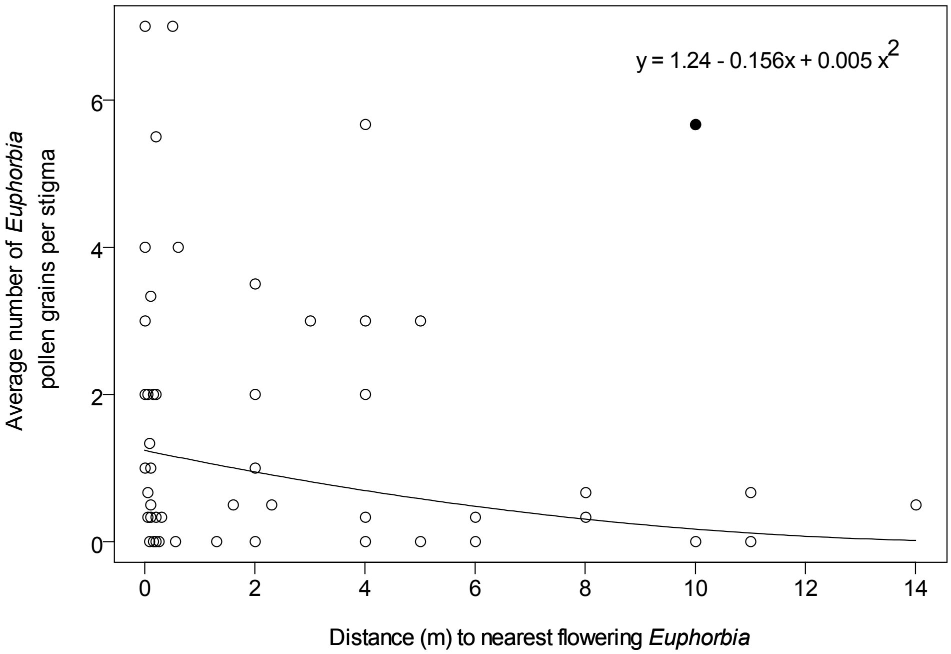

Figure 1.4 Average number of *Euphorbia* pollen grains received per stigma for plants at a range of distances to the nearest *Euphorbia* flowering ramet. Curve fit based on regression of square root of pollen receipt as a function of distance. Filled point excluded from regression.

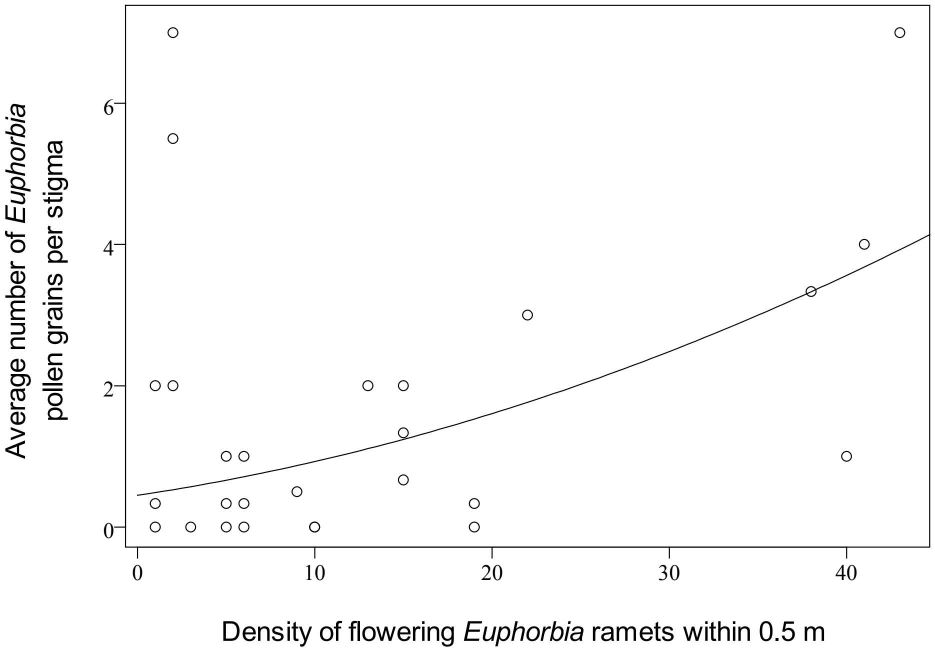

Figure 1.5 Average number of *Euphorbia* pollen grains received per stigma for plants with a range of densities of *Euphorbia* flowering ramets within a 0.5 m radius.

Chapter 2

Effect of Introduced *Euphorbia esula* on the Pollination of *Viola pedatifida*, a Species with Cleistogamous and Chasmogamous Flowers

ABSTRACT Interspecific competition for pollination has the potential to affect the pollination success of chasmogamous flowers but not cleistogamous flowers, potentially leading to increased reliance on cleistogamous flowers in the presence of competing species. This study assesses the potential for a competitive effect of the invasive plant *Euphorbia esula* (leafy spurge, Euphorbiaceae) on the pollination of the native plant *Viola pedatifida* (prairie violet, Violaceae), which has both chasmogamous and cleistogamous flowers. Both plants were mostly visited by solitary bees, and *Euphorbia* pollen was found on most *Viola* stigmas, suggesting the potential for competition. Additionally, in heterospecific pollen transfer experiments, application of *Euphorbia* pollen prior to conspecific pollen led to substantially reduced seed set. Unexpectedly, fruit set was higher for *Viola* near *Euphorbia* despite increased *Euphorbia* pollen receipt, and seed set did not vary with distance from *Euphorbia*. However, there was no significant interaction between distance from *Euphorbia* and flower type suggesting that the cause of increased fruit set near *Euphorbia* is unrelated to pollination. The results of study suggest that *Viola* and *Euphorbia* interact through shared pollinators, but there is no evidence that *Euphorbia* reduces the fecundity of *Viola* or that this interaction leads to selection for increased reliance on cleistogamous flowers.

INTRODUCTION

Competition for pollination may involve three mechanisms: competition for pollinator visits, pollen loss to flowers of other species causing decreased seed set of the donor species, and heterospecific pollen transfer causing decreased seed set of the recipient species (Rathcke 1983). Competition for pollinator visits has been detected in several studies (e.g. Chittka and Schürkens 2001, Brown et al. 2002, Larson et al. 2006), as has competition mediated by pollen loss to other species (e.g. Campbell and Motten 1985). Finally, competition mediated by heterospecific pollen transfer occurs when foreign pollen is improperly deposited on a stigma, resulting in reduced pollination success by conspecific pollen due to stigma clogging (e.g. Waser 1978a, Brown and Mitchell 2001) or pollen allelopathy (Kanchan and Chandra 1980, Murphy 2000). As species invasions and habitat fragmentation continue to bring together novel flowering communities, the strength of competition for pollination by all of these mechanisms is likely to increase (Kephart 1983). Models suggest that competition for pollination may have substantial ecological effects, leading to competitive exclusion for plants with overlapping flowering phenologies or to coexistence for species with different times of peak flowering (Levin and Anderson 1970, Ishii and Higashi 2001).

Flower types may differ in their susceptibility to experiencing competition for pollination. In particular, chasmogamous flowers, which open and may receive pollinator visits, are more susceptible to experiencing competition than are cleistogamous flowers, which self-pollinate in bud without exposure to pollinators (Fishman and Wyatt 1999). One reason for this is that cleistogamous flowers are less likely to receive heterospecific pollen (Randall and Hilu 1990, Murphy and Aarssen 1996).

For many species, chasmogamous and cleistogamous flowers are produced simultaneously or sequentially on the same plant (Lord 1981). For chasmogamous flowers, fruit and seed set may be limited by either a lack of pollination or a lack of other resources (Burd 1994). In contrast, pollination success is assured for cleistogamous flowers, so their fruit and seed set may only be limited by a lack of other resources. Consequently, competition for pollination can be tested for by comparing patterns of fruit and seed set between chasmogamous and cleistogamous flowers in areas with or without a putative competitor. Fruit set, and the number of seeds per fruit may differ between chasmogamous and cleistogamous flowers within a given population, as has been demonstrated for multiple *Viola* species (Redbo-Torstensson and Berg 1995, Culley 2002). However, differences in seed set per fruit between flower types tend to mirror differences in the number of ovules per flower (Berg 2003). Thus, detection of competitive effects should be based on whether the ratios of fruit set and seed set between chasmogamous and cleistogamous flowers change between environments with or without the putative competitor, rather than on whether fruit and seed set differ between flower types overall.

It has been hypothesized that species with similar floral traits may be more likely to experience competition from heterospecific pollen transfer than species with very different flowers. This is because pollinators transition among flower types less when flowers have different morphologies (Heinrich 1979, Lewis 1986), and the placement of pollen on visitors is hypothesized to be more similar among flowers with similar morphologies, leading to an increased likelihood of foreign pollen transfer (Murcia and Feinsinger 1996). However, patterns and effects of heterospecific pollen transfer among dissimilar species have rarely been investigated, so the extent to which different morphologies prevent receipt of heterospecific pollen is not well known.

Chasmogamous *Viola* flowers may be likely to receive heterospecific pollen from dissimilar flowers because, despite their restrictive floral morphology, many species are primarily pollinated by generalist solitary bees, though some rely heavily on bumblebees, syrphid flies and bombyliid flies (Beattie 1974, Freitas and Sazima 2003). Evidence that *Viola* is prone to heterospecific pollen receipt comes from a study of three *British Viola* species, for which all observed pollinators carried heterospecific pollen (Beattie 1971). Additionally, heterospecific pollen, mostly from nearby flowering species, was commonly found on all three *Viola* species, and represented up to 7% of pollen found in *Viola* stigmatic cavities (Beattie 1969). Of the four common sources of heterospecific pollen in Beattie's (1969) study, two species had restrictive tubular morphologies similar to *Viola*, but the other two had unrestrictive morphologies dissimilar from *Viola*. Based on this evidence, Beattie (1971) hypothesized that heterospecific pollen receipt could affect fecundity, but evidently, this was not tested.

Leafy spurge (*Euphorbia esula* L.; *Euphorbia*ceae; hereafter *Euphorbia*) is an introduced species that may exert a competitive effect on the pollination of native species. *Euphorbia* is of Eurasian origin, and in North America, it is particularly abundant in the central and northern Great Plains (Watson 1985). Its presence is frequently associated with decreased diversity and abundance of native forbs (Butler and Cogan 2004), and some evidence suggests that it exerts an allelopathic effect on competitors (Steenhagen and Zimdahl 1979, Olson and Wallander 2002). *Euphorbia* is abundant on Iowa's Loess Hills prairies, where steep slopes and well-drained soils create ideal semiarid habitat conditions and herbicide use is discouraged because there is potential for water contamination and native plant populations could be adversely affected(Huerd and Taylor 1998). The plant provides both pollen and nectar rewards and is visited by a wide range of pollinators, but especially Diptera and Hymenoptera (Selleck et al. 1962, Messersmith et al. 1985, Larson et al. 2006). Its clonal growth form leads to high flowering densities, especially in well-established clones. A previous study in North Dakota has shown that some other native species receive fewer visits and fewer conspecific pollen grains per stigma when growing in areas invaded by *Euphorbia* compared to uninvaded areas (Larson et al. 2006).

Prairie violet (*Viola pedatifida* G. Don; Violacaeae, hereafter *Viola*) is one species that flowers simultaneously with *Euphorbia*. *Viola* occurs primarily on mesic to dry prairies (Christiansen and Muller 1999). *Viola* produces both chasmogamous and cleistogamous flowers, with chasmogamous flowers occurring from late April through late May at the study site, and cleistogamous flowering beginning in mid-May and lasting into June (B. Montgomery, unpublished data). *Viola* occurs within and nearby clones of *Euphorbia*, and this proximity increases the potential for competition. The floral morphology of *Viola* is zygomorphic and restrictive, whereas the morphology of *Euphorbia* is actinomorphic and unrestrictive. However, despite this difference, *Euphorbia* relies on generalist pollinators (Selleck et al. 1962), as do many *Viola* species (Beattie 1974), so there is potential for *Viola* to experience competition for pollination from *Euphorbia*.

If the fecundity of chasmogamous *Viola* flowers is reduced from competition with *Euphorbia*, this could lead to increased reliance on cleistogamous flowers due to phenotypic plasticity or selection. For another *Viola* species, it has been experimentally demonstrated that preventing pollinator visits to chasmogamous flowers leads to decreased chasmoganous fruit set, and increased production of cleistogamous flowers, demonstrating phenotypic plasticity (Redbo-Torstensson and Berg 1995). Selection for increased reliance on cleistogamous flowers could occur if relative reliance on chasmogamous and cleistogamous flowers varies and plants relying more heavily on cleistogamous flowers are more fecund near *Euphorbia*. This mechanism has been invoked to explain the evolution self-pollinating populations of *Arenaria uniflora* sympatric with a competing congener (Fishman and Wyatt 1999).

To assess the potential for competition for *Euphorbia* to compete for pollination with *Viola*, a series of experiments was conducted. The effect of *Euphorbia* pollen receipt on *Viola* was investigated by comparing the fruit and seed set of *Viola* flowers hand-pollinated with *Euphorbia* and *Viola* pollen to those receiving only *Viola* pollen. Pollinator overlap between *Viola* and *Euphorbia* was investigated by observing pollinators to both species over two field seasons. To determine whether *Viola* receives *Euphorbia* pollen, the pollen content of *Viola* stigmas was determined for individuals growing near to or several meters from *Euphorbia*. To determine whether proximity to *Euphorbia* results in reduced fecundity, patterns of fruit and seed set of chasmogamous and cleistogamous *Viola* flowers were determined for plants growing near to or several meters from *Euphorbia*. Finally, the breeding system of *Viola pedatifida* is not known, so to investigate this, breeding system studies were performed in the field and greenhouse.

METHODS

Breeding System Studies

Viola pedatfida's breeding system was investigated with field and greenhouse experiments. The field study was conducted in the spring of 2003 at McCormack Prairie (O'Brien County, Iowa). On 21 May, 54 plants with at least one chasmogamous bud were found and assigned to one of four treatment groups: cross-pollinate (Cross), self-pollinate (Self), open and unmanipulated (Open), or enclosed and unmanipulated

(Enclosed). For all treatments except open, plants were enclosed under bridal veil netting. Open flowers were labeled but not manipulated. Pollinations were performed on 24 and 25 May on one flower per plant by transferring pollen three times per flower on the tips of fine-pointed forceps, which were cleaned with alcohol between flowers. For self-pollinations, pollen from another flower on the same plant (geitonogamous pollen) was used when available; otherwise, pollen from the same flower was used. Cross-pollinations were performed by independently transferring pollen from three donor flowers that were either bagged or recently opened. Flowers were labeled with acrylic paint on the upper penduncle. Flower status was surveyed and bags were removed on 4 June, by which time petals had abscised and fruit development was apparent. Developing fruits were left to allow for seed maturation, but subsequent animal removal of fruits prevented collection of fruits for seed counts.

In 2005, another breeding system study was performed in the greenhouse with 37 plants transplanted from McCormack Prairie in order to confirm results from the field study and to determine seed set. One flower from each plant was assigned to one of three treatments: cross-pollinate (Cross), self-pollinate (Self), or enclosed and unmanipulated (Enclosed). For cross-pollinations pollen was independently transferred with an insect pin from donor flowers on two other plants to the recipient stigma. For self-pollinations, pollen was transferred with a clean insect pin from another flower on the same plant (geitonogamously) when available, or from the same flower (autogamously). Pollinations were performed between 21 and 30 March; fruit set was determined on 9-10 April, and fruits were collected on 18 April. Fruits were stored dry in coin envelopes, and seeds were counted. Smaller, soft seeds were included in the counts, but very small undeveloped ovules were not counted.

Heterospecific Pollen Transfer (HPT) Studies

In order to determine whether receipt of *Euphorbia* pollen on *Viola* stigmas decreases fruit and seed set, greenhouse experiments were performed from February through April of 2004 and 2005. *Viola* plants came from two populations. Plants from Iowa were transplanted from wild populations in O'Brien County Iowa in April of 2003, and were brought to Matthaei Botanical Gardens (Ann Arbor, Michigan) in July of 2003.

Plants from Wisconsin were purchased from AgroEcol in 2002, and were maintained in a greenhouse during the summer of 2003. Plants were potted into 7.5 cm square pots, and were fertilized using Osmocote fertilizer during the growing season. *Euphorbia* was transplanted from Iowa into larger pots during the summers of 2002 – 2004 and treated similarly. All plants were overwintered in cold-frames until mid-February of 2005, when they were moved inside. Flowering of both species began by mid March.

The 2004 study was performed at Matthaei Botanical Gardens and included 20 plants with chasmogamous flowers from each population (ie. field-collected and purchased). To prevent insect visitations, groups of up to eight plants were enclosed under bridal veil netting during flowering. The first two flowers from each plant were assigned one of two treatments: conspecific pollen only (Conspecific), or transfer of heterospecific (*Euphorbia*) pollen transfer from *Euphorbia* followed immediately by conspecific pollen (HPT Prior). Each treatment order was assigned randomly to half of the plants from each population, and plants with only one chasmogamous flower received only the first treatment. Flowers that opened prior to netting and extra flowers on a given plant were not used. Flower treatments were labeled with acrylic paint on the upper sepal.

Pollinations were performed between 13 and 28 March on the second or rarely the third day that the flower was open enough for the stigma to be visible. To transfer *Euphorbia* pollen, a dehissed *Euphorbia* anther was excised and touched to the *Viola* stigma. *Viola* pollen was gathered by probing the anthers and the cup formed by petals beneath the ovary with the tip of fine-pointed forceps. Pollen was then transferred to the recipient stigma on forceps tips. This procedure was repeated for two donor flowers on different plants. Yellow *Euphorbia* pollen was easily visible on stigmas, and receipt of *Viola* pollen was inferred by the reduction in the pollen load on the forceps as the white grains could not be seen on the stigma.

To determine fruit set, flowers were monitored until the ovary showed clear signs of swelling or until the flower aborted. Fruits were collected on 12 April, at which time a few fruits had dispersed their seeds. The remaining fruits were stored individually in 70% ETOH. Seed set was determined by counting developed or developing seeds in each fruit, excluding very small, undeveloped ovules.

The 2005 heterospecific pollen transfer (HPT) study was performed in the Kraus Natural Sciences greenhouse (Ann Arbor, Michigan). The HPT study was performed on the first 32 plants from Iowa and the first eight plants from Wisconsin to produce chasmogamous flowers. Three treatments were included: outcrossing with two other flowers from the same population (Conspecific); outcrossing with two flowers followed by *Euphorbia* pollen (HPT after); and *Euphorbia* pollen followed by outcrossing (HPT prior). Treatment order was varied and randomly assigned among plants such that each treatment was represented in approximately equal frequency among first, second, and third flowers. Plants producing fewer than three chasmogamous flowers received a subset of treatments, and additional flowers were left unmanipulated. Pollinations were performed on the second day a flower was open, between 17 – 25 March.

Unlike the 2004 study, *Viola* pollen was applied using the tip of a insect pin, and *Euphorbia* pollen was applied using the tip of a dissecting needle. As a sham control, flowers receiving only conspecific pollen were prodded with a clean dissecting needle prior to application of pollen. Application of *Euphorbia* pollen occurred a few minutes before or after application of conspecific pollen. Flowers were labeled as in the 2004 study.

Fruit set was determined on 9-10 April, and fruits were collected between 10-15 April 2005 and stored dry in coin envelopes. Seed set was determined by counting the number of seeds per set fruit, including smaller, soft seeds, but excluding very small undeveloped ovules.

Pollinator Observations

Visitors to *Viola* and *Euphorbia* were observed at The Nature Conservancy's Broken Kettle Grasslands Reserve (Plymouth County, Iowa, 42.709° N, 96.579° W) during the spring of 2005 and 2006 as part of a larger study investigating pollinator overlap between *Euphorbia* and simultaneously flowering species. During 2005, observations of *Viola* occurred between 27 April and 20 May, and observations of *Euphorbia* occurred between 27 April and 16 June. During 2006, observations of both species occurred between 27 April and 18 May. In both years, pollinators were observed by placing a 1 m diameter sampling frame around haphazardly selected plants and observing all visits to flowers of any species within the plot for fifteen minutes. New visits were counted if a pollinator transitioned to a new plant or ramet. Occasional additional visits to *Viola* were recorded when observed.

Field Study of Proximity to Euphorbia and Reproductive Success

Viola's pollination success was investigated during the spring of 2004. The study was conducted in an open area with a mix of native and introduced grasses and forbs. *Euphorbia esula* was the only abundant introduced forb in flower during the study. The study was conducted in an area subject to late summer grazing that had been burned during the previous summer. To study the effect of *Euphorbia* on the pollination of *Viola*, four *Viola* patches were selected with patches including between 35 and 110 *Viola* plants within ten m, and few or no conspecifics within at least a 15 m around the patch. In two patches *Viola* was interspersed with *Euphorbia* (*Euphorbia* status = present), whereas in the remaining two patches, *Viola* was on average at least 15 m from the nearest flowering *Euhorbia* and never closer than 7 m (*Euphorbia* status = absent).

Twenty-five *Viola* plants were selected per patch, with haphazard selection of plants in the three smaller patches. In the largest patch, plants were selected by establishing two parallel transects and randomly selecting one plant within a 0.5 m radius at 1.5 m intervals. Plants were labeled with a small white garden stake nearby and a flag 0.5 m away. For each plant, the distance to the nearest reproductive *Euphorbia* ramet and the number of reproductive *Euphorbia* ramets within 0.5 m was determined. Flowers already senesced at the beginning of the study (April 27) were labeled and excluded from analysis. Plants were surveyed for newly mature flowers at the initiation of the study and subsequently on six or seven day intervals until June 14. Flowers were identified as being chasmogamous (with showy petals and an extended style) or cleistogamous (without showy petals and with stigma appressed to ovary) and were labeled with dots of acrylic paint along the peduncle to identify flowers within a plant. Stigmas were collected from all chasmogamous flowers during subsequent surveys once petals had abscised, and were stored individually in microcentrifuge tubes in a 9:1 solution of 70%

ethanol:glycerin. Fruit status (aborted, fruit, or damaged) was noted during subsequent surveys, and mature fruits were collected and stored in 70% ethanol for seed counts.

In the laboratory, 94 stigmas (43 from near *Euphorbia*, and 51 distant from *Euphorbia*) were acetolyzed to isolate pollen, and the resulting pollen was mounted in basic-fuschin gel (Kearns and Inouye 1993). Additionally storage tubes were centrifuged, and dislodged pollen was similarly mounted in basic-fuschin gel. Pollen was identified to species when possible and later aggregated into the categories of conspecific, *Euphorbia*, or other foreign pollen. Counts from stigmas and storage tubes were combined prior to analysis.

Statistical Analyses

The field breeding system study was analyzed using a general linear model (GLM) with a binomial distribution and the logit-link function in S-Plus 7. Treatment was significant overall, so all six pairwise treatment combinations were compared individually with Fisher's exact test, with significance compared to Bonferroni-adjusted critical values. For the greenhouse breeding system study, fruit set was analyzed using a generalized linear mixed effects model (GLME) in S-Plus 7.0, using the correlatedData library, and including plant as a random effect. Seed set in the greenhouse breeding system study was analyzed using ANOVA omitting plant as a variable because for about two-thirds of plants, a seed count could be made for only one of the two treatments (due to the plant producing only one chasmogamous flower or one flower aborting).

Fruit set in the 2004 and 2005 HPT studies were analyzed with GLME models, including plant as a random effect and fixed effects of treatment and population (Iowa or Wisconsin). Interaction terms between fixed effects were initially included but were dropped if they failed to reach marginal significance. For the 2004 and 2005 HPT studies, seed set was square-root transformed to improve normality prior to analysis, but graphs depict the untransformed data. For both years seed set results were analyzed with a mixed effects ANOVA, with population (Iowa or Wisconsin) included as a random effect and treatment as a fixed effect. For analyses of seed set, plant was not included as a random effect because few plants produced a fruit for both treatments. To allow a more similar comparison with the 2004 HPT results, the 2005 HPT fruit set and seed set results were reanalyzed without the HPT-after treatment, which was not performed in 2004.

Conspecific pollen receipt in the field study was analyzed using a general linear model (GLM) with *Euphorbia* status (presence or absence of reproductive ramets) as a main effect and site nested within *Euphorbia* status. The number of *Euphorbia* pollen grains per stigma was analyzed in S-Plus using the MASS library, with a generalized linear model (GLM) with a negative binomial variance and log link function. In the analysis, *Euphorbia* status in the patch was treated as a fixed effect, site differences were nested within *Euphorbia* status, and the number of conspecific pollen grains per stigma was treated as a covariate. Interactions between the number of conspecific pollen grains and both factors were also initially included in the model, and non-significant interactions were dropped.

Fruit set was analyzed including both flower types (chasmogamous or cleistogamous) using a GLM with a binomial variance logit link function, with flower type and *Euphorbia* status included as main effects with an interaction term, and site nested within *Euphorbia* status. Fruit set was similarly analyzed for chasmogamous flowers only. Seed set (seeds per developed fruit) was analyzed in the same manner as fruit set, but with a GLM with a Gaussian variance function.

Pollen counts and fruit set were known for a subset of 90 flowers. For these flowers, the effect of conspecific pollen receipt, *Euphorbia* pollen receipt, and other heterospecific pollen receipt was investigated. This was analyzed as a binomial GLM (as above) with each category of pollen count included as an independent variable and fruit set per stigma included as the dependent variable. Pollen counts and seed set were determined for 40 flowers. The effects of conspecific, *Euphorbia*, and other heterospecific pollen receipt on seed set were investigated for these flowers with a linear multiple regression model including these three pollen counts as independent variables and seed set as a dependent variable.

RESULTS

Breeding System Studies

In the field study, several flowers were eaten before results could be determined, and the actual sample sizes used in analyses are included under the x-axis of Figure 2.1. There was an overall effect of treatment on fruit set (Fisher's exact test, $N_{total} = 54$, $P = 0.0001$), and post-hoc comparisons indicated that the fruit set for open flowers and hand-pollinated cross flowers was significantly higher than for enclosed unmanipulated flowers, which had no fruit set, but other differences were not significant (Figure 2.1).

In the greenhouse breeding system study, fruit set differed significantly among all three treatments, with the highest fruit set for cross flowers, intermediate fruit set for self flowers, and very low fruit set for enclosed flowers (Figure 2.2, Table 2.1). Seed set per developed fruit did not differ significantly between cross and self treatments (ANOVA, $F_{1,27}=0.078$, $P = 0.78$), and the seed set of the only enclosed flower to set fruit was three seeds, less than 10% of the average seed set of the other treatments.

Heterospecific Pollen Transfer (HPT) Studies

In the 2004 HPT study, stigmas in the HPT prior treatment contained an average of 103 *Euphorbia* pollen grains, while flowers in the conspecific treatment received almost no *Euphorbia* pollen (Table 2.2). Conspecific pollen receipt did not differ significantly between HPT prior and conspecific flowers ($F_{1,44} = 0.86$, $P = 0.36$, Table 2.2). In the 2004 HPT study, fruit set was not significantly affected by population (Wisconsin or Iowa), treatment, or their interaction (Figure 2.3, Table 2.3a). However, seed set per developed fruit was more than 50% lower for flowers treated with HPT prior relative to conspecific (Figure 2.4), a significant difference (fixed effect of treatment in GLME model, $F_{1,24} =12.31$, $P = 0.002$).

In the 2005 HPT study, fruit set was significantly lower overall for plants from Wisconsin than Iowa, but there was no significant difference among the three treatments, (Table 2.3b, Figure 2.5). Results were similar when the HPT after treatment was omitted from analysis (analysis not shown). Seed set was not affected by population, but there was a marginally significant trend ($P = 0.06$) for treatment to affect seed set, with the seed set of HPT prior fruits lower than that of conspecific or HPT after fruits (Table 2.4, Figure 2.6). When the HPT after treatment was excluded to compare more directly with the 2004 results and results of studies of other species, the effect of treatment became significant ($P = 0.04$), and population became marginally significant ($P = 0.07$).

Pollinator Observations

In 2005, twelve visits to *Viola* were observed over eight hours and ten minutes of observations, and 71 visits to *Euphorbia* were observed over 29 hours and 45 minutes of observation during the same time period (Table 2.5). In 2006, eleven visits to *Viola* were observed over 7 hours and 15 minutes, and 87 visits were observed to *Euphorbia* over 11 hours and 45 minutes. In both years most visits to both *Viola* and *Euphorbia* were from solitary bees, suggesting the potential for overlap in pollinators.

Field Study of Proximity to Euphorbia and Reproductive Success

Conspecific pollen receipt did not differ significantly between areas with or without *Euphorbia*, or between patches with the same *Euphorbia* status (Table 2.6). However, *Euphorbia* status (ie. presence or absence of *Euphorbia*) significantly affected *Euphorbia* pollen receipt for *Viola*, as receipt rates were higher in patches with reproductive *Euphorbia* (Table 2.7). *Euphorbia* pollen was found on 69% of stigmas in patches without *Euphorbia*, with an average receipt of (+ s.e.) 2.2 + 0.4 grains, and *Euphorbia* pollen was found on 86% of stigmas in patches with *Euphorbia*, with an average receipt of 8.2 + 1.1 grains. Flowers receiving more conspecific pollen also received significantly more *Euphorbia* pollen (Table 2.7). Interactions between conspecific pollen receipt and each factor did not approach significance, so were dropped.

Fruit set was significantly higher for cleistogamous than chasmogamous flowers, and was significantly higher for patches near *Euphorbia*, but there was no significant interaction between the flower type and *Euphorbia* status (Figure 2.7, Table 2.8a). Considering only chasmogamous flowers, fruit set was higher in areas with *Euphorbia* and differed between patches with the same *Euphorbia* status (Table 2.8b). Seed set was significantly higher for cleistogamous than chasmogamous flowers, but did not significantly differ according to *Euphorbia* status, nor was there a significant interaction between flower type and *Euphorbia* status (Figure 2.8, Table 2.9). There were, however, significant differences in seed set between patches with the same *Euphorbia* status. Patterns and significances were similar when chasmogamous flowers were analyzed alone (results not shown).

In the analysis of fruit set as a function of pollen receipt, conspecific pollen receipt was significantly positively correlated with fruit set, but the effects of *Euphorbia* pollen receipt and other foreign pollen receipt were not significant (Table 2.10). For the analysis of seed set as a function of pollen receipt, there was no significant relationship between conspecific pollen, *Euphorbia* pollen, or other foreign pollen and seed set (results not shown).

DISCUSSION

The results demonstrate that *Euphorbia* and *Viola* share pollinators, resulting in *Euphorbia* pollen receipt on *Viola* stigmas. Additionally, experimental transfer of *Euphorbia* pollen to *Viola* stigmas results in reduced seed set per fruit. However, despite these findings, surveys of fruit and seed production from cleistogamous and chasmogamous flowers growing near to or several meters from *Euphorbia* do not support the hypothesis that *Viola* chasmogamous flowers experience competition for pollination from proximity to *Euphorbia*. It is possible that no effect was found because fifteen meters was inadequate to functionally separate *Viola* from *Euphorbia*. However, *Viola* several meters distant from *Euphorbia* received substantially less *Euphorbia* pollen than intermixed *Viola*, suggesting that this degree of separation could substantially reduce competition if heterospecific pollen receipt was the operative mechanism. Other studies have also found that most pollinators fly short distances between flowers (Levin and Kerster 1969b, Waser 1982), and that separation by several meters is adequate to substantially reduce heterospecific pollen receipt (Levin 1971).

Pollinator sharing between *Euphorbia* and *Viola* occurs despite very different morphologies. Inflorescences of *Euphorbia* are unrestrictive, actinomorphic and yellow, whereas *Viola* flowers are restrictive, zygomorphic and deep blue or purple. Nonetheless, observations show that both species receive a majority of their visits from solitary bees. Solitary bees include a diversity of species, so potentially there could be little overlap in the bee species visiting each plant. However, observations suggest that both plants receive visits from an overlapping range of bee species, and this observation is corroborated by the presence of *Euphorbia* pollen on the majority of *Viola* stigmas. The receipt of *Euphorbia* pollen on *Viola* stigmas is similar to Beattie's (1969) finding that other species of *Viola* receive heterospecific pollen from nearby flowers with a range of floral morphologies. Together, these results suggest that despite *Viola*'s restrictive floral morphology, it relies on generalist pollinators.

Additional evidence from previous studies suggests that pollen movement can occur between flowers with different morphologies. A study of pollen transfer among three insect-pollinated species found that one species received more foreign pollen from a neighboring species with similarly shaped flowers than from a neighbor with dissimilar flowers, but the reverse was true for another species (Arroyo and Dafni 1992). These results indicating that heterospecific pollen transfer occurs among dissimilar flowers reinforce an emerging view of pollination systems as being more generalized than was previously thought (Waser et al. 1996).

Several studies in other systems have also found reduced fecundity resulting from heterospecific pollen receipt, as was found in this study when *Euphorbia* pollen was transferred by hand to *Viola* (Waser 1978a, Galen and Gregory 1989, Murphy and Aarssen 1995b, Caruso and Alfaro 2000, Brown and Mitchell 2001). This result has been found for closely related species apparently due to the production of aborted unviable hybrid seeds (Fishman and Wyatt 1999). However, other studies have found no significant reduction in reproductive success with heterospecific pollen receipt (Campbell and Motten 1985, Armbruster and McGuire 1991, Kwak and Jennersten 1991).

Despite *Viola* receiving *Euphorbia* pollen in the field, fruit set for chasmogamous flowers was higher near *Euphorbia* (although seed set was unaffected by proximity), a pattern suggestive of facilitation of *Euphorbia* on *Viola*'s pollination. However, cleistogamous flowers showed a similar increase in fruit set near *Euphorbia*, which is necessarily unrelated to interactions for pollination. Also, analyses including both chasmogamous and cleistogamous flowers and proximity to *Euphorbia* showed no significant interaction between flower type and *Euphorbia*'s presence, which indicates that the mechanism leading to increased fruit set near *Euphorbia* is unrelated to interactions for pollination and may reflect patterns of resource availability, such as good microsite conditions. Comparisons of fruit and seed set between chasmogamous and cleistogamous flowers could give misleading results if the fruit or seed set of cleistogamous flowers increased in response to decreased success of chasmogamous flowers. However, in this study, chasmogamous and cleistogamous flowers both showed increased success near *Euphorbia*. In a study of *Viola hirta*, Redbo-Torstensson and Berg (1995) found that decreased pollination success of chasmogamous flowers led to increased production of cleistogamous flowers, but the study did not provide evidence of changed fruit set or seed set per fruit. Proximity to *Euphorbia* did not affect conspecific pollen receipt, which further indicates that proximity to *Euphorbia* does not lead to increased pollination success. Instead, different resource environments or other differences among sites are likely to have created this pattern of higher fruit set near *Euphorbia*.

There is an apparent discrepancy between the results of the greenhouse study, which detected a competitive effect of *Euphorbia* pollen receipt on seed set, and the field study, in which increased heterospecific pollen receipt did not result in a no decrease in seed set for *Viola* near to *Euphorbia*. This discrepancy is likely a result of the different quantities of *Euphorbia* pollen received between the two studies. In the 2004 greenhouse study, flowers in the heterospecific pollen receipt study received on average 103 *Euphorbia* pollen grains. In contrast, *Viola* stigmas near *Euphorbia* in the field received an average of only eight and a maximum of 26 *Euphorbia* pollen grains. Additionally, the order of receipt of *Euphorbia* pollen may be important, as shown by the trend in the 2005 greenhouse study toward decreased seed set only when *Euphorbia* pollen is received prior to *Viola* pollen. For natural pollen receipt, conspecific pollen may be received first or simultaneously with heterospecific pollen, and this could result in the heterospecific pollen having less effect.

The breeding system studies show that *Viola* depends on pollinators for fruit set. In the field study no enclosed flowers set fruit and only a few set fruit in the greenhouse. *Viola* is self-compatible, as both self and cross pollination treatments resulted in similar fruit and seed set, indicating a lack of inbreeding depression. Other *Viola* species have also been found to be self-compatible, with a range of capabilities for self-pollinating in the absence of visits (Knuth 1908, Valentine 1962, Culley 2000, 2002, Berg 2003).

Competitive effects of *Euphorbia* on the pollination of *Viola* could lead to selection for increased reliance on cleistogamy; conversely, facilitative effects could lead to selection for increased reliance on chasmogamous flowers. However, this study did not find strong evidence that proximity to *Euphorbia* affects the success of chasmogamous *Viola* flowers under natural conditions, so this study does not indicate that *Euphorbia* is likely to influence selection on the degree of cleistogamy in *Viola*.

Table 2.1 Regression coefficients of treatment contrasts from GLME for *Viola* fruit set for breeding system study, greenhouse, 2005.

Factor	Value	s.e.	d.f.	t-value	*P*
Intercept	-1.044	0.529	44	-1.97	0.055
Self v. cross	1.306	0.342	44	3.82	0.0004
Self v. enclosed	-1.685	0.267	44	-6.32	<0.0001

Table 2.2 Pollen receipt (± s.e.) for flowers in 2004 heterospecific pollen transfer study.

	Euphorbia		*Viola*	
Treatment	mean	s.e.	mean	s.e.
HPT Prior	103.1	16.8	159.0	17.3
Conspecific	0.3	0.1	189.0	25.7

Table 2.3 For heterospecific pollen transfer (HPT) studies, regression coefficients from binomial GLME testing fixed effects of pollination treatment and plant population (Iowa or Wisconsin) on fruit set. Individual plants were treated as blocks and included as a random effect. A. 2004 results. B. 2005 results.

Factor	Value	s.e.	d.f.	t-value	*P*
A. 2004					
Intercept	0.776	0.538	27	1.44	0.16
Conspecific v. HPT Prior	0.069	0.495	27	0.14	0.89
Population	-1.689	0.678	38	-2.49	0.017
B. 2005					
Intercept	-0.436	0.421	70	-1.04	0.3
Population	-2.176	0.759	41	-2.87	0.007
Conspecific v. HPT After	0.901	0.476	70	1.90	0.06
Conspecific v. HPT Prior	0.772	0.472	70	1.63	0.10

Table 2.4 For 2005 HPT study, ANOVA table of effects of plant population (Iowa or Wisconsin) and pollination treatment on *Viola* seed set.

Factor	numerator d.f.	denominator d.f.	F-value	*P*
Intercept	1	21	605.87	< 0.0001
Population	1	26	1.74	0.198
Treatment	2	21	3.21	0.061

Table 2.5 Numbers of visits of different visitor groups to *Viola* and *Euphorbia* over two years.

Species	Solitary bee	Social bee	Wasp	Lepidoptera	Diptera	Coleoptera	Hymenoptera	Total
2005 Results								
Viola	10	1	0	0	0	1	0	12
Euphorbia	36	2	3	0	22	7	1	71
2006 Results								
Viola	9	0	0	2	0	0	0	11
Euphorbia	65	0	0	0	22	0	0	87

Table 2.6 Results of GLM of effects of *Euphorbia* status (whether flowering *Euphorbia* was present in the vicinity) and site within *Euphorbia* status on the quantity of conspecific pollen received.

Factor	d.f.	Deviance explained	Resid. d.f.	Resid. deviance	*F*	*P*
Intercept			93	1232774		
Euphorbia status	1	637.65	92	1232136	0.047	0.83
Site in *Euphorbia* status	2	3444.60	90	1228691	0.126	0.88

Table 2.7 Results of negative binomial GLM of effects of *Euphorbia* status (whether flowering *Euphorbia* was present in the vicinity), site within *Euphorbia* status, and quantity of conspecific pollen on the quantity of *Euphorbia* pollen received (negative binomial $\theta = 1.4$).

Factor	d.f	Deviance explained	Resid. d.f	Resid. deviance	*P*(Chi)
Intercept			93	164.13	
Euphorbia status	1	40.48	92	123.65	< 0.001
Conspecific	1	8.55	91	115.11	0.003
Site in *Euphorbia* status	2	6.11	89	108.99	0.047

Table 2.8 A. Results of binomial GLM of effect of flower type, *Euphorbia* status (flowering *Euphorbia* present or absent), and site within *Euphorbia* status on fruit set. B. Results including only chasmogamous flowers.

Factor	d.f.	Deviance explained	Resid. d.f.	Resid. deviance	*P*(Chi)
A. All flowers					
Intercept			178	270.71	
Flower Type	1	48.48	177	222.23	<0.001
Euphorbia status	1	8.43	176	213.80	0.004
Flower Type:*Euphorbia* status	1	0.002	175	213.80	0.97
Site in *Euphorbia* status	2	0.45	173	213.35	0.80
B. Including only chasmogamous flowers					
Intercept			91	133.76	
Euphorbia status	1	6.40	90	127.36	0.01
Site in *Euphorbia* status	2	8.18	88	119.18	0.02

Table 2.9 Results of GLM of effects of flower type, *Euphorbia* status, the interaction of these factors, and site within *Euphorbia* status on seed set.

Factor	d.f.	Deviance explained	Resid. d.f.	Resid. deviance	F	*P*(F)
Intercept			124	28320.5		
Flower Type	1	1131.9	123	27188.7	6.34	0.013
Euphorbia status	1	410.8	122	26777.8	2.30	0.13
Flower type:*Euphorbia* status	1	122.8	121	26655.0	0.68	0.41
Site in *Euphorbia* status	2	5289.3	119	21365.7	14.73	< 0.0001

Table 2.10 Results of binomial GLM of effects of conspecific, *Euphorbia*, and other heterospecific pollen counts on fruit set.

Factor	d.f.	Deviance explained	Resid. d.f.	Resid. deviance	*P*(Chi)
Intercept			87	120.35	
Conspecific pollen	1	4.60	86	115.75	0.032
Euphorbia pollen	1	0.21	85	115.55	0.65
Other heterospecific pollen	1	0.01	84	115.54	0.94

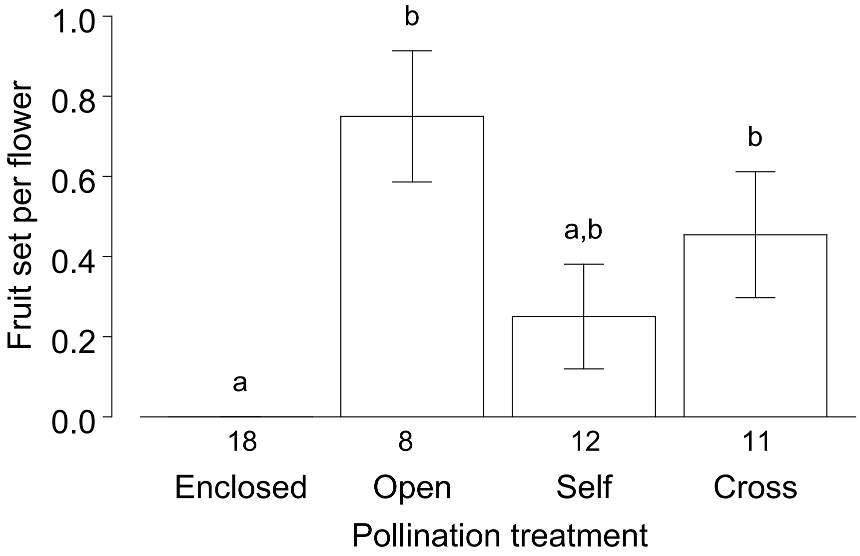

Figure 2.1 Fruit set (fruits / flowers) (± s.e.) of *Viola* exposed to four pollination treatments at McCormack Prairie, 2003. No fruit set occurred in the Enclosed treatment. Numbers below bars indicate sample size of flowers with known results. Different letters indicate *Bonferroni adjusted* significant differences at $P < 0.05$.

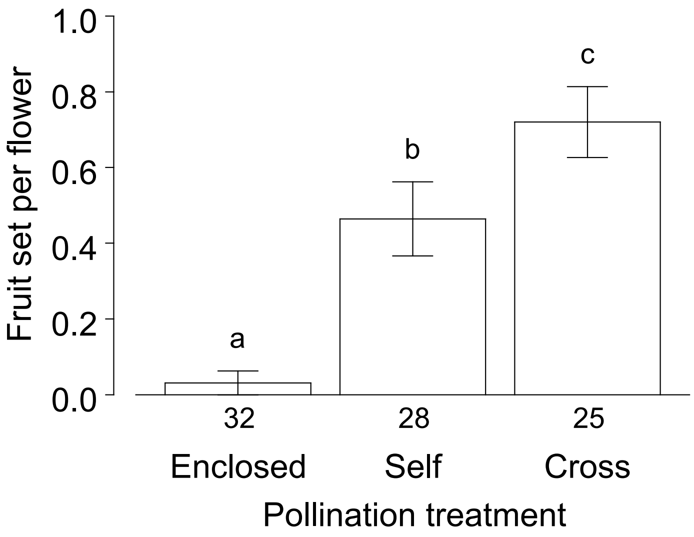

Figure 2.2 Fruit set (± s.e.) of *Viola* with three pollination treatments in the greenhouse, 2005. Numbers below bars indicate sample size, and different letters indicate significant contrasts at $P < 0.05$.

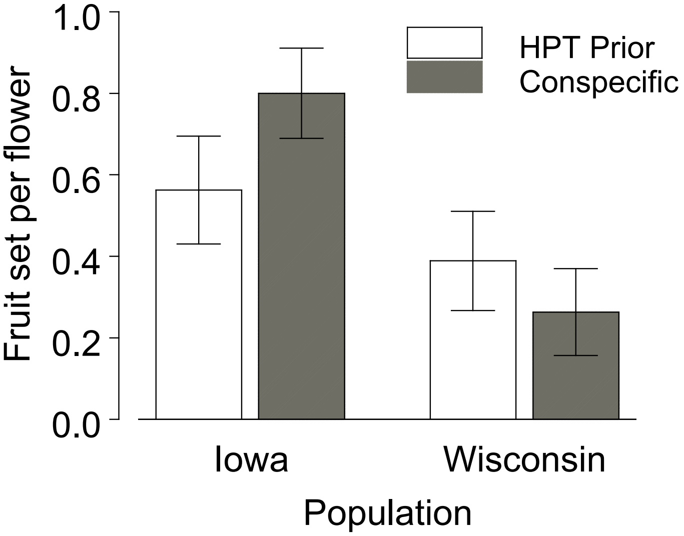

Figure 2.3 Fruit set (± s.e.) of *Viola* flowers, for HPT Prior and Conspecific treatments from Iowa or Wisconsin populations, for greenhouse 2004 study.

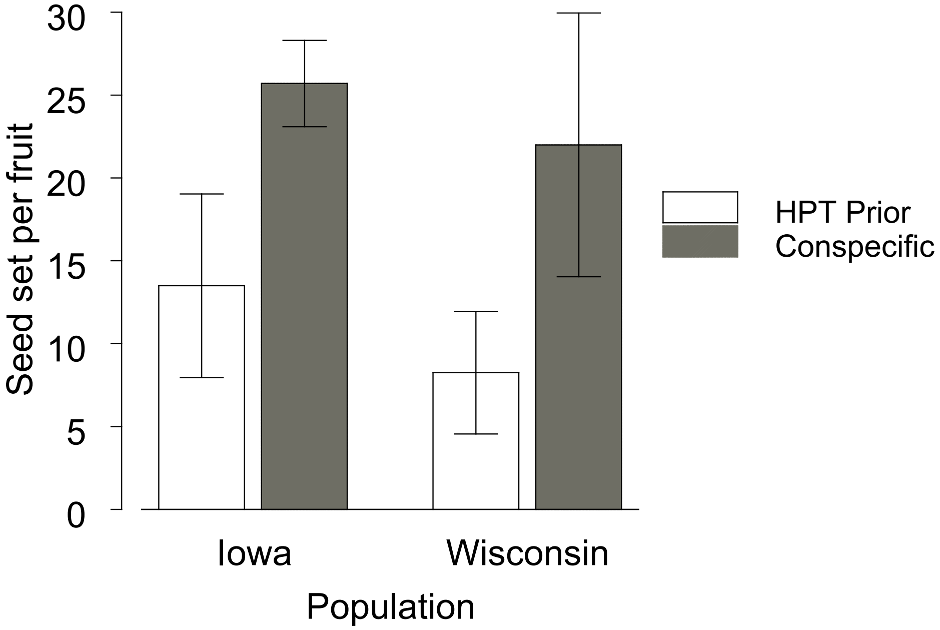

Figure 2.4 Seed set per fruit (± s.e.) of *Viola* flowers from Iowa or Wisconsin populations for HPT-Prior and Conspecific treatments, greenhouse 2004.

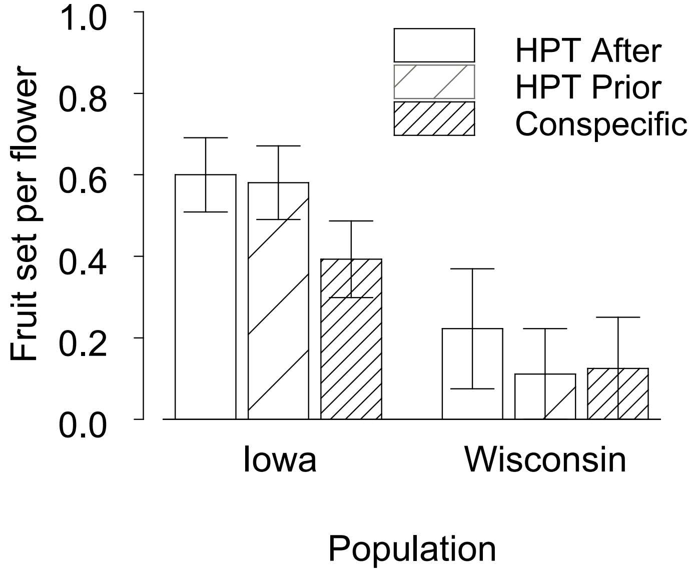

Figure 2.5 Fruit set (± s.e.) of *Viola* flowers from Iowa or Wisconsin population for three pollination treatments for greenhouse 2005 study.

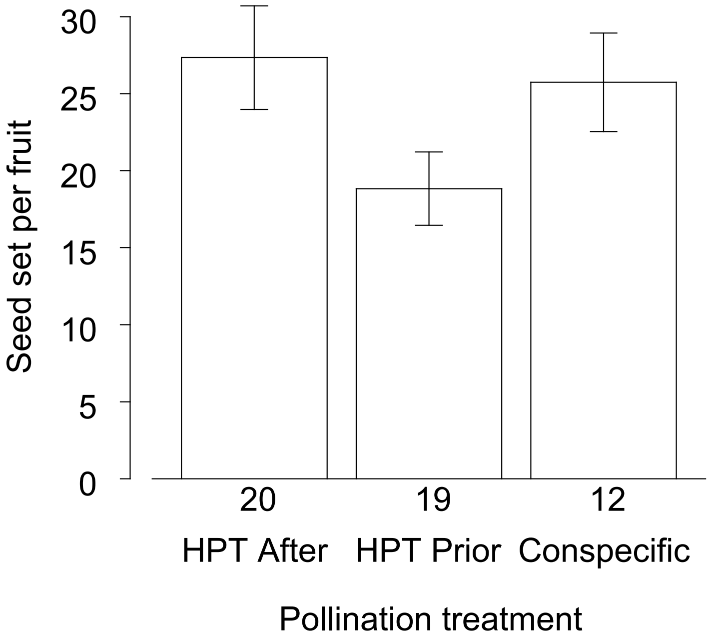

Figure 2.6 Seed set per fruit (± s.e.) of *Viola* flowers for three pollination treatments (cross-pollinated = Conspecific, *Euphorbia* pollen applied after cross pollination = HPT After, *Euphorbia* pollen applied before cross pollination = HPT Prior), combining results from both populations, for greenhouse 2005 study.

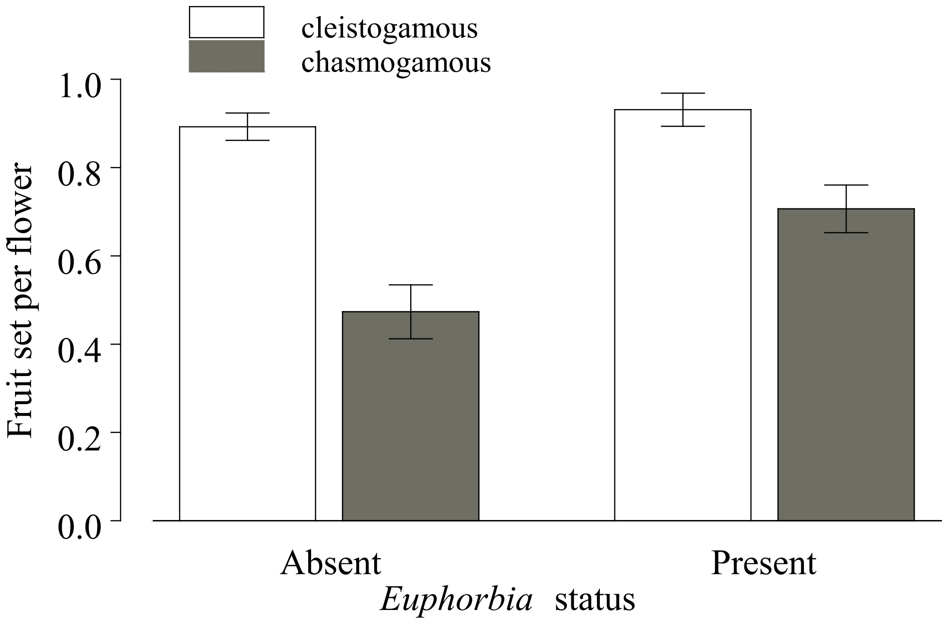

Figure 2.7 Effect of *Viola* flower type, and presence or absence of *Euphorbia* on *Viola* fruit set (± s.e.).

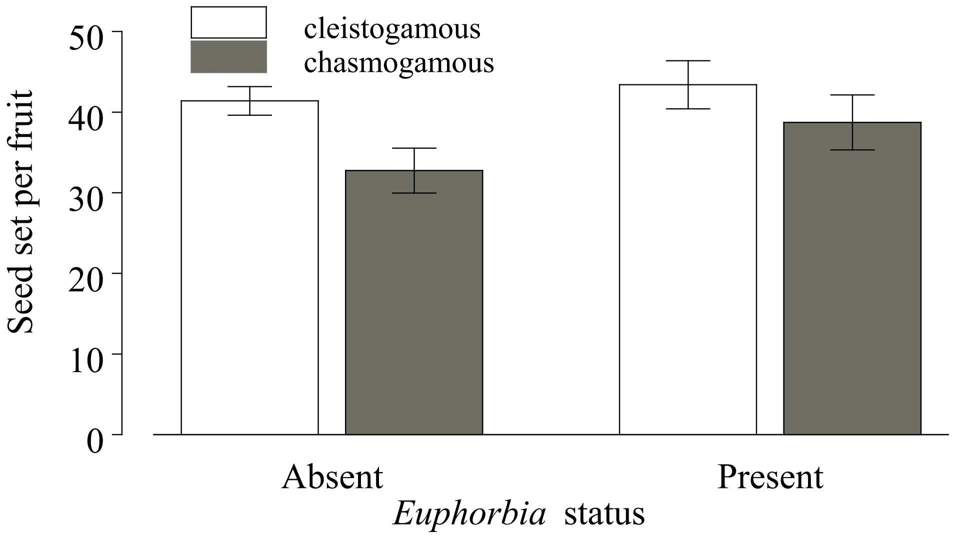

Figure 2.8 Effect of flower type, and presence or absence of *Euphorbia* on seed set per fruit. (± s.e.).

Chapter 3

Patterns of Heterospecific Pollen Receipt for Prairie Plants with Different Floral Traits

ABSTRACT Flowers may receive heterospecific pollen from simultaneously flowering species with shared pollinators, but the effect of floral morphology on heterospecific pollen receipt has received little attention. To investigate whether floral restrictiveness influences patterns of heterospecific pollen receipt, I determined patterns of pollinator visitation and rates of heterospecific pollen receipt for 29 species with a range of floral morphologies flowering simultaneously with the introduced plant *Euphorbia esula* (leafy spurge, Euphorbiaceae) which has an unrestrictive morphology. Pollinator overlap was significantly greater between *Euphorbia* and other species with unrestrictive than restrictive morphologies. As hypothesized, flowers with unrestrictive morphologies received significantly more *Euphorbia* pollen, more heterospecific pollen excluding *Euphorbia*, and a greater diversity of pollen per stigma than flowers with restrictive morphologies. However, stigmatic surface area was significantly larger for flowers with unrestrictive morphologies, and the density of *Euphorbia* and other heterospecific pollen per stigmatic surface area did not significantly differ between flowers with restrictive and unrestrictive morphologies. These results show that that stigmatic surface area is larger for flowers with unrestrictive morphologies and support the hypothesis that larger stigma size is as an adaptation to prevent stigma clogging and hence reduce theis potentially harmful effect of heterospecific pollen receipt.

INTRODUCTION

Because most pollinators are generalists (Waser et al. 1996), pollinators may deposit heterospecific pollen as well as conspecific pollen (Rathcke 1983, Waser 1983). Heterospecific pollen may reduce pollination success by a variety of mechanisms (Kanchan and Chandra 1980, Rathcke 1983, Waser 1983, Fishman and Wyatt 1999, Murphy 2002), so patterns of heterospecific pollen receipt have potential ecological and evolutionary implications. From an evolutionary perspective, in cases for which heterospecific pollen receipt leads to reduced pollination success, selection may favor traits that minimize heterospecific pollen receipt (Feinsinger 1983) or its effects (Kohn and Waser 1985).

Heterospecific pollen receipt is a common phenomenon, with reports of heterospecific pollen occurring on from 4% to 81% of stigmas, but little work has investigated what influences this variation (McLernon et al. 1996, Waites and Aagren 2004). Ecological factors, including relative floral abundance, heterospecific proximity and density, as well as pollinator visit rates and floral constancy, can influence rates of heterospecific pollen receipt (Levin 1971, Kephart 1983, Campbell 1986, Feinsinger et al. 1986, Kunin 1993, Larson et al. 2006).

Floral morphology could also influence rates of heterospecific pollen receipt through multiple mechanisms. Flowers with restrictive morphologies tend to exclude generalist pollinators (Heinrich 1975b, Ostler and Harper 1978) and are more likely to attract specialist pollinators or pollinators that exhibit constancy, defined as transitions between conspecific flowers within flowering bouts beyond expectations due strictly to preference for that species (Feinsinger 1983, Waser 1983, 1986). Additionally, restrictive morphologies could decrease receipt of wind-dispersed pollen if they physically shield the stigma. Consequently, restrictive flowers may be expected to have more specialized pollinators and to receive less heterospecific pollen (Faegri and van Der Pijl 1979, McLernon et al. 1996, Fishman and Wyatt 1999). Because heterospecific pollen receipt can reduce reproductive success, selection for avoidance of heterospecific pollen could explain why some plant taxa have evolved restrictive morphologies. However, the prediction that flowers with restrictive morphologies receive less heterospecific pollen has remained virtually untested (but see Feinsinger et al. 1986, McLernon et al. 1996)

Similarity of floral morphology could also increase heterospecific pollen receipt because increased similarity can lead to increased overlap in pollinator species (Brown and Kodric-Brown 1979, Arroyo and Dafni 1992), and deposition of pollen on the same locations on the pollinator (Sprague 1962, Levin and Berube 1972, Brown and Kodric-Brown 1979, Waser 1983). Additionally, pollinators may be more likely to transition between similar than dissimilar flowers (Kunin 1993, Chittka et al. 1997) because extra learning time is required for transitions between dissimilar flowers (Heinrich 1975b, Lewis 1986). Thus, the prediction that flowers with restrictive morphologies receive less heterospecific pollen may depend in part on the floral morphology of coflowering species (Feinsinger 1983) as well as on pollinator overlap and behavior.

An alternative evolutionary strategy to avoiding heterospecific pollen receipt is possessing traits that minimize effects of heterospecific pollen receipt. It has been hypothesized that possessing a large stigmatic surface is one trait that helps to minimize effects of heterospecific pollen receipt because a given amount of heterospecific pollen occludes a smaller fraction of a large stigma than a small one (Cruden and Millerward 1981, Kohn and Waser 1985). If, as hypothesized, flowers with unrestrictive morphologies receive more heterospecific pollen than flowers with restrictive morphologies, then it may be hypothesized that flowers with unrestrictive morphologies should have larger stigmas to cope with higher rates of heterospecific pollen receipt.

In this study, I measure heterospecific pollen receipt in the field for 29 species and relate natural levels of heterospecific pollen receipt to floral morphology, and pollinator overlap. I focus on patterns of pollinator overlap and heterospecific pollen receipt from *Euphorbia esula*, an abundant introduced plant with an unrestrictive floral morphology. I test the hypotheses that restrictive morphologies reduce pollinator sharing and pollen receipt, especially from flowers with unrestrictive morphologies. Specifically, I test the predictions that pollinator overlap and heterospecific pollen receipt rates from *Euphorbia* and other sources are lower for species with restrictive than unrestrictive morphologies. Additionally, I test whether patterns of pollinator overlap with *Euphorbia* and an index of *Euphorbia*'s relative floral abundance are predictive of patterns of heterospecific pollen transfer. Finally, I also test whether, as predicted, stigmas are smaller for species with restrictive morphologies. In the next chapter, I follow up this study by investigating effects of heterospecific pollen receipt from *Euphorbia* to a range of species with different floral traits.

METHODS

Study System

This study was conducted at Broken Kettle Grasslands Preserve in Iowa's Loess Hills (Plymouth County, 42.709° N, 96.579° W). The site includes native mixed grass prairie interspersed with introduced grasses and forbs. A major introduced species in the study area is *Euphorbia esula* L. (Euphorbiaceae; leafy spurge). *Euphorbia*'s morphology consists of a cyathium of unisexual pistillate and staminate flowers and subtending bracts with nectaries. Despite this unusual floral morphology, the floral morphology is non-restrictive, with open access to the nectar, anthers and stigma.

Because *Euphorbia* is introduced from Eurasia, establishing in midwestern North America in the late 1800s (Dunn 1985), it is unlikely that selection resulting from competition for pollination with *Euphorbia* has substantially influenced the floral morphology of the studied species. Instead, *Euphorbia*, by virtue of its abundance and unrestrictive morphology, allows assessment of other species' susceptibility to heterospecific pollen receipt before floral trait evolution is likely to have occurred.

Twenty-nine species flowering simultaneously and in close proximity to *Euphorbia* were included in the study, of which 21 were native (Table1). Nearly all of these species were herbaceous dicots, although one monocot (*Sisyrinchium campestre* E. Bickn; Iridceae) and one shrub (*Ceanothus americana* L. Rhamnaceae) were included.

Floral Morphologies

Flowers were categorized as having a restrictive or unrestrictive morphology by first assigning all flowers whose stigma was level with or exerted beyond the level of the petals as having an unrestrictive morphology. For flowers with stigmas not exerted beyond the petals, the perpendicular distance between the edge of the stigma and the petal (hereafter tube radius) was measured with calipers for three flowers per species. Flowers with a tube radius of at least 2 mm were classified as unrestrictive, whereas flowers with a radius of 2 mm or less were classified as restrictive.

Flowering Phenology

The flowering phenologies of the study species were investigated in 2006 by establishing 49 rectangular plots measuring 5 m by 10 m in an approximately 400 m by 240 m area. Nine plots were spaced at equal intervals, with 35 m between plots, along five east-west transects approximately parallel to the dominant contour of the slope. Transects were separated by 35 m, and plot positions were staggered along the east-west axis to form a checker-board of plots. Three adjoining plots were arranged with the same spatial pattern on a spur of higher quality prairie in an area otherwise heavily dominated by dense *Bromus* grass. A final plot was in a similar area ca. 400 meters away, included in order to measure the phenology of *Comandra*, which occurred only rarely in the contiguous area. Within each plot, a square one-quarter m subplot was established with flags in the northwest corner of each plot.

From April 26 through June 19, 2006, plots were surveyed every seven or eight days, and the number of plants of each species with flowers in each plot was counted or estimated. Additionally, for each species except *Euphorbia* in flower, the numbers of flowers were counted on the three plants closest to the northwest corner, with aster heads counted as individual flowers. For *Euphorbia*, to increase sampling of different genets within each plot, flowering status was instead monitored on the flowering ramets closest to the northwest, north central, and northeast points in each plot were counted. For each of these three ramets, the number of cyathia with male flowers was counted, as well as the number with male or female flowers. Observations of flowering phenology were supplemented by noting when species were observed in flower outside of the phenology plots but within the confines of the study area.

Pollinator Overlap and Visits

To assess the overlap in pollinators between *Euphorbia* and co-flowering species, pollinator observations were performed during the 2005 and 2006 field seasons. Observations were performed by haphazardly placing a circular 1 m diameter sampling frame and observing visits to flowers of all species within the frame, typically for 15 minutes. In 2005, the order of observations was haphazard, but staggered among species in flower. In 2006, the order of observations was established by choosing species randomly, without replacement, from a list of all species in flower at the time of observation, and repeating the process once all species in flower had been observed. For each observation, the sampling frame was placed around the focal species, but other species in the plot were simultaneously observed. Observations during both years were made between 7:00 am and 6:00 pm, avoiding periods of rain. In 2005, 74.25 hours of pollinator observations were performed split among three observers, and in 2006, 86.50 hours of observations were performed, split among two observers. Pollinator visits were counted as new visits each time a pollinator arrived at a new plant or ramet, but consecutive visits to flowers on a ramet or plant were not counted. Additionally, observations of pollinators to species for which few visits had been observed were noted as they were observed during the course of other field work.

In 2005, a small subset of pollinators was captured to aid in identification, but most identifications were made to higher level taxonomic or morphological groups based on field observations. In 2006, pollinators were captured whenever possible to do so without damaging the plants being observed, with captured insects used to verify field identifications to higher level groups. Both captures and field identifications were included in the analysis.

Heterospecific Pollen Receipt

Amounts of heterospecific pollen receipt were measured by collecting stigmas of 29 species with overlapping phenology with *Euphorbia* (see Table 1 for list of species, morphologies, and nativity to the United States). Most stigmas were collected during the spring of 2005, and additional stigmas of some species were collected in 2006. Most collections were made in a ca. 400 m by 400 m section of prairie demarcated by fencing and two ridgelines, but to maximize the number of species sampled, collections of five species present but not abundant within this area were sampled in adjoining areas. Flowering was clustered spatially and temporally, and an emphasis was placed on collecting as many species flowering simultaneously with *Euphorbia* as possible.

Stigmas were collected haphazardly, but to minimize confounding spatial variation in the data, an effort was made to collect from individuals less than three meters from flowering *Euphorbia*, and to collect from several different patches of each species. Similarly, to minimize confounding temporal variation, an effort was made to collect from all species in flower on each collection day, and to collect stigmas from each species over several collection days.

Stigmas were dissected from flowers using cleaned forceps in the field and were stored in 0.5 ml microcentrifuge tube in a solution of 9:1 70% ethyl alcohol: glycerin. Stigmas from up to three flowers per plant were collected, depending on availability, and were stored in the same vial. For ten samples of each species, stigmas from one flower were transferred in the laboratory to a 2.0 ml microcentrifuge tube, and the stigmas were acetolyzed according to the standard procedure (Kearns and Inouye 1993), modified for small sample volumes. Pollen was then transferred to a microscope slide by melting basic fuschin jelly (Kearns and Inouye 1993) into the bottom of the microcentrifuge tube and allowing the jelly to solidify. Additionally, pollen that became dislodged during storage was collected by removing remaining stigmas, centrifuging the microcentrifuge tube, drawing off the solution, and transferring the remaining pollen to a microscope slide using the process previously described.

All heterospecific pollen on each slide was counted and identified to species or morphospecies, primarily based on size and shape, using a compound binocular scope at 600 magnification. Additionally, darkness and surficial characters were used as secondary identification characteristics. Although not all species were distinguishable, *Euphorbia* pollen was distinct from all other species, both in acetolyzed and non-acetolyzed samples. Three individuals performed pollen counts, with periodic cross-checking of identifications. Each individual performed counts of similar numbers of samples of each species. Conspecific pollen receipt was not analyzed because for most species it was not possible to differentiate between outcrossed pollen and self pollen that was deposited naturally or transferred during dissections.

To estimate the surface areas of stigmas for each species, between five and eleven stigmas per species (mean ± s.e.= 9.0 ± 1.4, minimum = 5 stigmas),were mounted in fuchsin jelly on a microscope slide and photographed using a dissecting scope at 20 to 60 magnification. Stigmatic surface area was then estimated as the two dimensional size of the stigma in the photograph, adjusted for magnification power and for the number of stigmatic lobes if only a subset were measured. Analyses were performed with ImageJ (Rasband 2006).

The number of morphospecies of pollen on each slide was determined by counting the number of different heterospecific pollen types apparent, regardless of whether or not they could be identified to species. The appearance of pollen is affected by acetolysis. Because pollen adhering to stigmas was acetolyzed, whereas dislodged pollen was not, it was not always possible to determine whether the morphospecies from the stigma sample were the same as the morphospecies from the dislodged pollen sample. Consequently, the number of morphospecies of pollen present was calculated as the maximum value between the stigma and dislodged pollen sample. The estimate of pollen diversity is likely an underestimate both because dislodged and stigmatic pollen could have different species composition, and because different species may have not been distinguished.

Data Analysis

To quantify flowering phenologies, the flowering density of each species in each plot was calculated as the product of the number of ramets in flower and the average number of flowers per ramet. Average flowering density for each species was calculated by averaging flowering density across all plots. When identical maximum flowering densities occurred in consecutive weeks, maximum peak flowering was estimated to occur halfway between these dates. For species whose maximum recorded flowering was during the first or last survey, peak flowering was assumed to occur on the survey date. For *Euphorbia*'s phenology, the same process was used, but including only counts of cyathia with male flowers in order to best gauge the relative ability over the flowering season for *Euphorbia* to act as source of heterospecific pollen to other species.

An index of *Euphorbia* flowering intensity was created in order to determine whether patterns of *Euphorbia* pollen receipt to other species were attributable to differences in the availability of *Euphorbia* pollen over the study period. The index, ranging from 0 to 1.0 was created by dividing each survey date's average *Euphorbia* flowering density by the maximum average flowering density attained by *Euphorbia*.

For pollinator observations, visits were tallied within each species across 2005 and 2006 because for several species, few visits were recorded within each year. Visits by ants, true bugs and most beetles were excluded from analysis because these groups are unlikely to transport pollen among plants; however, visits by soldier beetles (family Cantharidae) were retained because they were observed to carry pollen and move between plants. Three plant species (*Phlox pilosa*, *Galium aparine*, and *Medicago lupulina*) were excluded from analysis because fewer than five visits were observed to each. Pollinator overlap between *Euphorbia* and all other remaining species was calculated with the Morisita-Horn overlap index (Magurran 1988), using EstimateS (Colwell 2005). The hypothesis that pollinator overlap is greater between *Euphorbia* and species with unrestrictive morphologies than restrictive morphologies was tested using a one-sided t-test, after confirming homogeneity of variance with Levene's test, and visually confirming normality of residuals.

Analyses of heterospecific pollen receipt were based on counts of *Euphorbia* pollen grains, other heterospecific pollen, and number of morphospecies of pollen. To combine counts of dislodged and stigmatic pollen for each sample, the count of dislodged pollen for each pollen category was divided by the number of stigmas collected for that plant, then added to the count of stigmatic pollen. Species averages for each measure were calculated by averaging pollen receipt for each pollen category across the ten samples analyzed.

Counts of average *Euphorbia* pollen receipt per stigma for each species were transformed by the natural log of one plus average receipt to improve the normality of residuals. *Euphorbia* pollen receipt was then compared among species with unrestrictive and restrictive morphologies using a one-tailed t-test to test the *a priori* prediction that flowers with unrestrictive morphologies will receive more heterospecific pollen. The number of heterospecific pollen grains, excluding *Euphorbia,* was compared between morphologies using a one-tailed t-test after log-transformation. Finally, the number of morphospecies of heterospecific pollen was also compared with a one-tailed t-test. In all cases, homogeneity of variance was confirmed with Levene's test, and residuals were inspected for normality.

Phenological differences among species meant that the abundance of *Euphorbia* as a pollen donor was potentially greater for some species than others. To test this possibility, the index value for *Euphorbia* flowering intensity on the date of peak flowering was calculated for each species, interpolating between values for survey dates when necessary, and this value was used as an estimate of *Euphorbia* flowering intensity for each species. Multiple linear regression, including floral restrictiveness, pollinator overlap, and *Euphorbia* flowering intensity as independent variables, was used to determine whether greater pollinator overlap and greater *Euphorbia* flowering intensity led to greater *Euphorbia* pollen receipt per stigma. *Euphorbia* pollen receipt per stigma was again log-plus-one transformed prior to analysis. Three species for which pollinator overlap was not measured due to inadequate sample size were excluded from this analysis.

Stigma size was estimated as the median measured size for each species. Median stigma size was chosen in order to minimize the impact of occasional stigma mounts at unfavorable angles which could result in under-estimates of stigmatic surface area. Stigma size was compared between flowers with unrestrictive and restrictive morphologies using a two-sided t-test on log-transformed measures, confirming homogeneity of variance with Levene's test and normality of residuals. The effect of stigmatic area and floral restrictiveness on *Euphorbia* and total heterospecific pollen receipt were investigated with separate GLMs for each response variable, dropping non-significant interaction terms. Pollen densities per stigmatic area were determined by dividing average pollen counts by median stigma size for each species. The density of *Euphorbia* pollen per area of stigma was not normally distributed even after a variety of transformations, so comparisons were performed with a two-sided Wilcoxon rank-sum test. The density of all heterospecific pollen, and heterospecific pollen excluding *Euphorbia* per stigmatic area were compared between restrictive and unrestrictive flowers using t-tests on log-transformed counts, after confirming normality of residuals and homogeneity of variance. The effects of stigmatic area and floral restrictiveness on patterns of *Euphorbia* pollen receipt were further analyzed using a GLM after log-plus-one transforming *Euphorbia* pollen counts. The same analysis was also performed with log-transformed counts of other heterospecific pollen as the dependent variable.

RESULTS

Floral Morphologies

Sixteen species were categorized as having an unrestrictive morphology, whereas 13 species were categorized as having a restrictive morphology.

Flowering Phenologies

The flowering phenologies of the 29 study species and other unsampled species to flower are shown in Figure 3.1. *Euphorbia* flowered throughout the duration of the floral surveys, from 26 May through 19 June, and maximum flowering for male flowers occurred in late May (Figure 3.2).

Pollinator Overlap

Across both field seasons, a total of 1,031 floral visits were observed, including 202 visits to *Euphorbia* (Table 3.1). Excluding *Euphorbia* and the three species for which too few visits were observed to allow analysis, an average (± s.d.) of 32 ± 24 visits were observed for the remaining 26 species. Hymenopterans were the most frequent visitors (687 visits), and of these species, the most frequent visitors were Agapostemon / *Augochorella* (family Halictidae, subfamily Halictinae) (178 visits), *Dialictus* (family Halictidae, subfamily Halictinae) (192 visits), other solitary bees (197 visits), and *Bombus* (bumblebees, 72 visits). The category of 'other solitary bees' included Megachilids; non-metallic halictini bees (family Halictidae, subfamily Halictinae); *Hylaeus* (family Colletidae, subfamily Hylaeinae), as well as other taxa. Dipterans were the next most frequent visitors, with most visits by syrphids (86 visits) and other flies (93 visits). Lepidopterans (moths and butterflies) accounted for 138 visits, with 67 visits by clear-winged moths. Finally, 'soldier beetles' accounted for 27 visits.

Pollinator overlap with *Euphorbia*, measured by the Morista-Horn index, ranged from a minimum of 0.03 for *Astragulus crassicarpus* to 0.90 for *Sisymbrium loeselii*, with an overall average (± s.d.) of 0.53 (± 0.22) (Table 3.2). Pollinator overlap was significantly greater for flowers with unrestrictive than restrictive morphologies (t = 2.642, d.f. = 26, *P* = 0.007).

Patterns of heterospecific pollen receipt

Rates of *Euphorbia*, other heterospecific, and total heterospecific pollen per stigma, as well as stigma size and *Euphorbia* and other heterospecific pollen densities per mm of stigmatic surface are shown for individual species in Table 3.3. Species with unrestrictive morphologies received significantly more *Euphorbia* pollen per stigma than species with restrictive morphologies (Table 3.4, *t* = 2.028, d.f. = 27, *P* = 0.026). Similarly, species with unrestrictive morphologies received significantly more other heterospecific pollen, whether excluding *Euphorbia* pollen (Table 3.4, *t* = 2.055, d.f. = 27, *P* = 0.025), or including it (*t* = 2.290, d.f. = 27, *P* = 0.015). The richness of morphospecies of heterospecific pollen was also significantly greater than for flowers with unrestrictive than restrictive morphologies (Table 3.4, t = 3.501, d.f. = 27, *P* = 0.0008).

In a model of *Euphorbia* pollen receipt per stigma as the response variable, neither pollinator overlap nor the abundance of *Euphorbia* pollen sources significantly affected the response variable, and floral restrictiveness had a marginally significant effect (Table 3.5). Dropping terms did not substantially change results for remaining variables (results not shown). Nevertheless, when effects of floral restrictiveness and pollinator overlap on *Euphorbia* pollen receipt are jointly considered, it is apparent that flowers with open morphologies have greater pollinator overlap with *Euphorbia* and receive more *Euphorbia* pollen per stigma, although pollinator overlap explains less than 10% of the variation in *Euphorbia* pollen receipt (Figure 3.3).

The stigmatic areas of flowers with restrictive morphologies were significantly smaller than flowers with unrestrictive morphologies, (*t* = 2.7426, d.f. = 27, *P* = 0.01, Table 3.6). *Euphorbia* pollen receipt per stigma increased with increasing stigmatic area (Figure 3.4). The effect of stigmatic area was significant, explaining 50% of the variation in *Euphorbia* pollen receipt across all species, and the effect of floral restrictiveness was not statistically significant once differences in stigma size were accounted for (Table 3.7). Upon exclusion of *Convolvulus*, which had an exceptionally large stigmatic area, only 12% of variation in *Euphorbia* pollen receipt was explained by stigmatic area.

After adjusting for stigmatic area, *Euphorbia* pollen receipt did not differ significantly between restrictive and unrestrictive morphologies (Table 3.6; $Z = 0.790$, $P = 0.43$). Similar results were observed for total heterospecific pollen receipt (Table 3.5), whether excluding *Euphorbia* pollen ($t = -0.454$, d.f. = 27, $P = 0.65$), or including it ($t = -0.279$, d.f. = 27, $P = 0.78$).

DISCUSSION

This study supports the prediction that flowers with unrestrictive morphologies receive more heterospecific pollen than flowers with restrictive morphologies. This was found specifically for pollen from *Euphorbia*, which itself has an unrestrictive morphology, but also for all other heterospecific pollen, which includes pollen from flowers with a range of morphologies. Furthermore, a greater richness of pollen morpho-species was found on flowers with unrestrictive morphologies. These results are consistent with the hypothesis that restrictive morphologies reduce heterospecific pollen receipt. At a different site, Larson et al. (2006) also found lower incidence of *Euphorbia* pollen, total heterospecific pollen, and diversity of heterospecific pollen for two legumes with restrictive morphologies than four other species with unrestrictive morphologies.

Heterospecific pollen receipt has been found to reduce fruit or seed set in a variety of studies (Waser 1978a, Harder et al. 1993, Caruso and Alfaro 2000, Brown and Mitchell 2001), although some studies have not detected reductions (Kwak and Jennersten 1991, Gross 1996, Kasagi and Kudo 2005, Moragues and Traveset 2005). This reduced fecundity may result from stigma clogging (Galen and Gregory 1989), pollen allelopathy (Murphy 2000), premature stigmatic closure (Waser and Fugate 1986), or production of unviable hybrids (Randall and Hilu 1990, Fishman and Wyatt 1999). Because heterospecific pollen receipt can reduce fruit and seed production, avoidance of heterospecific pollen receipt may have selected for restrictive floral morphologies.

Reduction in diversity of heterospecific pollen received could be important because not all pollen is allelopathic (Murphy and Aarssen 1989), but receipt of even a few grains of allelopathic pollen can substantially affect fruit and seed production (Murphy 2000). Thus, receipt of pollen from fewer species could decrease the chance of receipt from an allelopathic species.

Although *Euphorbia* pollen was found on the stigmas of most species, it was typically present only in small quantities (Table 3.3). Similar results were found by Larson et al (2006), who found average receipt rates of no more than 0.5 grains per stigma (back calculated from Larson et al. Figure 3), and two species in their study received no *Euphorbia* pollen. This indicates that *Euphorbia* pollen is unlikely to reduce the fruit of seed of other species as a result of stigma clogging, and competitive effects resulting from competition for ovules or formation of unviable hybrids is unlikely because there are no co-flowering congeners at the site. However, receipt of *Euphorbia* pollen could reduce success if the pollen is allelopathic, or if other species react to it with premature stigma closure or similar mechanisms.

Receipt rates of total heterospecific pollen were substantially higher for some species in this study, reaching 172 pollen grains for *Convolvulus arvensis*, and smaller absolute receipt rates nevertheless translated to high densities per stigmatic area for a variety of species. Larson et al. (2006) found a similar range of rates, reaching a maximum of approximately 260 pollen grains for a population of *Campanula rotundifolia* L. (Campanulaceae), a species with an unrestrictive morphology. Waites and Agren (2004) found even higher rates, with a range of average heterospecific pollen receipt from about 100 grains in some populations of *Lythrum salicaria* L. (Lythraceae)to more than 2000 pollen grains in others, accounting for 9%-81% of total pollen receipt. Arroyo and Dafni (1993) reported an average heterospecific pollen receipt rate of about six grains per stigma for the pin morph of *Narcissus assoanus* Dufour (Amaryllidaceae), and lower rates for the other morph and another species sampled. Galen and Newport (1988) found receipt of approximately 8 heterospecific pollen grains for *Polemonium viscosum* Nutt. (Polemoniaceae), accounting for 18% of pollen received. In a cross-species comparison, McLernon et al. (1996) reported that on average 10% of pollen was heterospecific, with up to approximately 35% for one species, but absolute numbers of pollen grains were not reported.

Receipt of *Euphorbia* pollen by the different species was not correlated with *Euphorbia* flowering intensity. *Euphorbia*'s peak flowering occurred approximately midway through the study period, which could result in some species being exposed to greater potential than other species for interspecific pollen transfer from *Euphorbia*. However, the index of *Euphorbia* flowering intensity did not significantly correlate with *Euphorbia* pollen receipt. This lack of correlation may be accounted for the collection of stigmas from each species throughout its flowering period rather than just near peak flowering. McLernon et al. (1996) similarly found that for most species heterospecific pollen receipt did not correlate well with the flowering phenology of heterospecifics, perhaps because phenological patterns across the entire site do not represent well local abundance of heterospecific flowers around sampled individuals.

The observed reduction in heterospecific pollen receipt to flowers with restrictive morphologies could be attributable to multiple mechanisms. First, pollinator overlap was lower between *Euphorbia* and flowers with restrictive than unrestrictive morphologies, which could lead to fewer opportunities for pollinators to deposit *Euphorbia* pollen onto flowers with restrictive morphologies. Pollinator visits to *Euphorbia* overlapped less with flowers with restrictive than unrestrictive morphologies because *Euphorbia* received a low proportion of visits from *Bombus* and lepidopterans, both of which groups tended to visit flowers with restrictive morphologies. Additionally, dipterans comprised approximately one-third of visits to *Euphorbia*, and dipterans were more frequent visitors to flowers with unrestrictive than restrictive morphologies.

Although pollinator overlap with *Euphorbia* and *Euphorbia* pollen receipt were both greater for flowers with unrestrictive morphologies, pollinator overlap failed to significantly predict *Euphorbia* pollen receipt to individual species. Overlap may not be a good indicator because sharing a small number of taxa that make frequent interspecific flights could result in a high rate of heterospecific pollen receipt even if most visitors are not shared between species. Conversely, having many shared pollinator taxa would not lead to heterospecific pollen receipt if pollinators exhibit labile preferences, choosing a species to visit depending on floral abundance and reward rates, or if pollinators exhibit constancy over consecutive floral visits due to costs of transitioning between species (Heinrich 1979, Waser 1986). Pollinator constancy is expected to be greater to flowers with restrictive morphologies (Heinrich 1975a, Gegear and Laverty 1998, Stout et al. 1998), and increased constancy would reduce the opportunity for heterospecific pollen transfer even under conditions of substantial pollinator overlap (Levin and Anderson 1970, Straw 1972, Heinrich 1975a). Additionally, pollinator overlap could be a poor predictor of heterospecific pollen receipt if differential pollen placement leads to minimal pollinator sharing even when visitors make interspecific flights (Sprague 1962, Levin and Berube 1972, Brown and Kodric-Brown 1979, Waser 1983).

Restrictive floral morphologies could block wind-pollinated pollen from landing on stigmas, reducing heterospecific pollen receipt from some donor species. Not all heterospecific pollen was identified to species, but pine pollen, which is wind-dispersed, was found in several samples, confirming that wind pollination contributed to heterospecific pollen receipt. However, this would not account for patterns of *Euphorbia* pollen receipt, because *Euphorbia* pollen is sticky and unlikely to disperse by wind (Selleck et al. 1962).

A final explanation for increased heterospecific pollen receipt for flowers with unrestrictive morphologies is that stigma size was significantly larger for these species than flowers with unrestrictive morphologies. Because larger stigmas are likely to contact a larger portion of a pollinator's body, they are likely to acquire more pollen on each visit (Cruden and Millerward 1981), which could include heterospecific pollen. This explanation is supported by the finding that per unit of stigmatic area, flowers with restrictive morphologies tended to receive less heterospecific pollen both from *Euphorbia* and from all other sources, although the difference was not statistically significant. Large stigma size could itself be an adaptation to mitigate effects of heterospecific pollen receipt, because a smaller portion of the stigma would be occluded by receipt of a similar amount of pollen (Cruden and Millerward 1981, Kohn and Waser 1985).

The results of this study contrast with those of McLernon et al. (1996), who found increased heterospecific pollen receipt to zygomorphic flowers, which have restrictive morphologies, relative to actinomorphic flowers, which have unrestrictive morphologies. The difference between their study and my study might be explained by different relative abundances of zygomorphic and actinomorphic flowers at the two sites, as six of the nine most common species at their site were restrictive legumes. For example, increased densities of restrictive zygomorphic flowers could have created more opportunities for heterospecific pollen transfer among zygomorphic flowers at their site. Arroyo and Dafni (1992), found that flowers morphs with more restrictive morphologies (thrum flowers) tend to contaminate other similar morphs, and flowers with less restrictive morphologies (pin morphs) tend to receive pollen from other flowers with unrestrictive morphologies. This finding further supports the hypothesis that even if flowers with unrestrictive morphologies generally receive more heterospecific pollen, restrictive flowers may in fact receive more heterospecific pollen in communities dominated by similar restrictive flowers.

Another challenge in interpreting the results of McLernon et al. (1996) is that in their study, all six species with restrictive morphologies were legumes, and two of the three unrestrictive flowers were congeners (*Potentilla*), making it difficult to separate effects of morphology from other differences among related species. The sample of 29 species across 15 families in this study allows greater confidence that the patterns detected are representative of different floral morphologies, not particular taxonomic groups.

Nonetheless, in the analyses presented here, species have been treated as independent observations, but phylogenetic constraints on species traits could introduce non-independence among species responses. For example, the seven legumes (Fabaceae) included in the study all were classified as having restrictive morphologies, whereas the six composites (Asteraceae) were classified as having unrestrictive morphologies. This limitation could be addressed in future studies either by creating a design with phylogenetically constrained comparisons or by manipulating floral restrictiveness with natural or artificial flowers in order to achieve variation without associated phylogenetic correlations.

A variety of factors not examined here could also influence patterns of heterospecific pollen receipt. In particular, floral density is expected to influence the rate of pollinator transitions between species, and consequently heterospecific pollen receipt rates (Levin and Anderson 1970, Kephart 1983, Rathcke 1983, Campbell 1986).

Additionally, interplant spacing of conspecific and heterospecific flowers may influence heterospecific pollen receipt (Waser 1978b, Kephart 1983, Kunin 1993, Stout et al. 1998, Caruso 2002).

This study has demonstrated that flowers with unrestrictive morphologies receive more heterospecific pollen, yet many species have unrestrictive floral morphologies suggesting that there are benefits. Unrestrictive morphologies increase the diversity of potential pollinators, thus allowing higher visitation rates and increasing the likelihood of adequate pollinator service when one pollinator group becomes rare (Waser et al. 1996). These advantages of generalization may outweigh benefits of specialization, particularly for common or self-compatible species, but rare outcrossing plants are disproportionately likely to have restrictive flowers, presumably because benefits of increased pollinator constancy outweigh costs of a reduced pool of potential visitors (Kunin and Shmida 1997). Thus, deleterious effects of heterospecific pollen receipt may be one factor that has selected for increasingly restrictive morphologies, but the net direction of selection will ultimately depend on a variety of factors favoring unrestrictive or restrictive morphologies.

Table 3.1 List of species for which pollen counts were made, with type of symmetry, restrictedness of floral morphology and whether native or introduced into the contiguous United States, organized alphabetically within family.

Family	Species	Floral Symmetry	Floral Restrictiveness	U.S. Nativity
Asteraceae	*Erigeron strigosus*	Radial	Unrestrictive	Native
Asteraceae	*Achilea millefolium*	Radial	Unrestrictive	Introduced
Asteraceae	*Nothocalais cuspidata*	Radial	Unrestrictive	Native
Asteraceae	*Senecio plattensis*	Radial	Unrestrictive	Native
Asteraceae	*Tragopogon dubius*	Radial	Unrestrictive	Introduced
Asteraceae	*Taraxacum officinale*	Radial	Unrestrictive	Introduced
Boraginaceae	*Lithospermum canescens*	Radial	Restrictive	Native
Boraginaceae	*Lithospermum incisum*	Radial	Restrictive	Native
Brassicaceae	*Sisymbrium loeselii*	Radial	Restrictive	Introduced
Convolvulaceae	*Convolvulus arvensis*	Radial	Unrestrictive	Introduced
Fabaceae	*Astragulus crassicarpus*	Bilateral	Restrictive	Native
Fabaceae	*Astragulus lotiflorus*	Bilateral	Restrictive	Native
Fabaceae	*Medicago lupulina*	Bilateral	Restrictive	Introduced
Fabaceae	*Melilotus officinalus*	Bilateral	Restrictive	Introduced
Fabaceae	*Oxytropis lambertii*	Bilateral	Restrictive	Native
Fabaceae	*Psoralea esculentum*	Bilateral	Restrictive	Native
Fabaceae	*Vicia americana*	Bilateral	Restrictive	Native
Iridaceae	*Sisyrinchium campestre*	Radial	Unrestrictive	Native
Lamiaceae	*Scuttelaria parvula*	Bilateral	Restrictive	Native
Linaceae	*Linum rigidum*	Radial	Unrestrictive	Native
Onagraceae	*Gaura coccinea*	Bilateral	Unrestrictive	Native
Oxalidaceae	*Oxalis stricta*	Radial	Unrestrictive	Introduced
Polemoniaceae	*Phlox pilosa*	Radial	Restrictive	Native
Rhamnaceae	*Ceanothus americanus*	Radial	Unrestrictive	Native
Rubiaceae	*Galium aparine*	Radial	Unrestrictive	Native
Santalaceae	*Comandra umbellata*	Radial	Unrestrictive	Native
Scrophulariaceae	*Castilleja sessiliflora*	Bilateral	Unrestrictive	Native
Scrophulariaceae	*Penstemon albidus*	Bilateral	Unrestrictive	Native
Violaceae	*Viola pedatifida*	Bilateral	Restrictive	Native

Table 3.2 Total number of pollinators visits observed per flower species in 2005 and 2006 shown for pollinator categories.

Species	*Agapostemon* & *Augochorella*	*Ceratina*	*Dialictus*	Andrenid	Other Solitary Bee	*Apis*	*Bombus*	Wasp	Syrphidae	Bomylidae	Other flies	Clear wing Moth	Other moths or butterflies	Soldier beetle	Total
Euphorbia esula	33	3	42	-	36	2	-	5	17	1	49	-	7	7	202
Achilea millefolium	-	1	-	-	9	-	10	-	4	-	2	6	4	15	51
Astragulus crassicarpus	-	1	-	-	-	-	7	-	1	-	-	6	1	-	16
Astragulus lotiflorus	5	-	-	-	2	-	8	-	-	-	-	9	2	-	26
Castilleja sessiliflora	7	-	24	-	7	-	-	-	-	-	-	1	-	-	39
Ceanothus americanus	20	1	7	-	18	-	1	1	1	-	2	-	1	-	52
Comandra umbellata	3	-	-	-	1	-	10	-	4	-	5	6	2	-	31
Convolvulus arvensis	4	-	-	-	11	-	-	-	5	-	5	-	2	4	31
Erigeron strigosus	1	2	-	-	3	-	-	-	2	-	3	-	-	1	12
Galium aparine	-	-	-	-	1	-	-	-	-	-	1	-	-	-	2
Gaura coccinea	2	-	1	-	2	-	-	-	-	-	1	1	2	-	9
Linum rigidum	4	-	3	-	7	1	1	1	2	-	2	-	7	-	28
Lithospermum canescens	2	-	1	-	2	-	7	-	-	1	3	10	9	-	35
Lithospermum incisum	5	1	2	-	2	-	-	-	-	-	2	-	-	-	12
Medicago lupulina	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Melilotus officinalus	2	1	1	1	6	-	1	-	1	-	6	-	2	-	20
Nothocalais cuspidata	22	6	1	-	6	-	-	-	-	-	-	-	-	-	35
Oxalis stricta	5	-	5	-	10	-	-	-	27	-	-	-	-	-	42
Oxytropis lambertii	10	1	2	1	5	-	15	-	-	-	3	14	17	-	68
Penstemon albidus	11	1	8	-	11	-	4	-	-	-	-	7	1	-	43
Phlox pilosa	1	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Psoralea esculentum	1	-	1	-	1	-	4	-	-	-	-	1	-	-	8

…Table 3.2 continued…

	Agapostemon & Augochorella	*Ceratina*	*Dialictus*	Andrenid	Other Solitary Bee	*Apis*	*Bombus*	Wasp	Syrphidae	Bomylidae	Other flies	Clear wing Moth	Other moths or butterflies	Soldier beetle	Total
Scuttelaria parvula	1	1	-	-	5	-	-	-	-	-	-	-	-	-	7
Senecio plattensis	9	9	6	-	32	-	-	-	1	-	1	-	3	-	61
Sisymbrium loeselii	3	-	5	1	4	-	-	2	3	-	2	-	1	-	20
Sisyrinchium campestre	15	-	77	-	14	1	1	-	3	-	1	1	1	-	114
Taraxacum officinale	2	1	2	-	-	-	-	-	9	-	3	-	7	-	24
Tragopogon dubius	4	-	-	-	1	-	-	-	-	-	1	-	-	-	6
Vicia americana	2	-	-	-	1	-	2	-	6	-	1	3	2	-	17
Viola pedatifida	4	5	4	-	5	-	1	-	-	1	-	2	-	-	22
Total	178	34	192	3	195	4	72	9	86	3	93	67	71	27	1,034

Table 3.3 Pollinator overlap as calculated by Morisita-Horn index, and values of heterospecific pollen receipt for *Euphorbia* and other study species. Other heterospecific pollen includes all heterospecific pollen except *Euphorbia*; total heterospecific pollen includes *Euphorbia* and other sources of heterospecific pollen; morphospecies represents the richness of pollen morphospecies per sample; stigma size is calculated as described in methods; *Euphorbia* and other heterospecific pollen are calculated as counts per stigma divided by stigma area.

		Pollen per stigma					**Pollen density per mm area**	
Species	Morisita-Horn Index	*Euph-orbia*	Other hetero-specific	Total hetero-specific	Morpho-species	Stigma area (mm)	*Euph-orbia*	Other hetero-specific
Euphorbia esula	*n.a.*	12.63	4.7	4.7	8	0.384	0.0	12.3
Achilea millefolium	0.339	0.5	2.3	2.8	2.2	0.039	12.9	60.2
Astragulus crassicarpus	0.033	0.167	0.7	0.8	1	0.029	5.8	23.1
Astragulus lotiflorus	0.219	0	0.4	0.4	0.6	0.042	0.0	10.3
Castilleja sessiliflora	0.615	0	22.2	22.2	2.9	0.291	0.0	76.3
Ceanothus americanus	0.721	0.633	4.4	5.0	2.7	0.052	12.2	85.0
Comandra umbellata	0.400	0.233	1.2	1.4	1	0.030	7.7	38.6
Convolvulus arvensis	0.755	21.5	150.5	172.0	6	1.947	11.0	77.3
Erigeron strigosus	0.753	0.267	1.7	2.0	1.4	0.023	11.4	73.9
Galium aparine	n.a.	0.033	1.4	1.5	1.9	0.013	2.5	109.2
Gaura coccinea	0.748	0.667	29.1	29.8	4	0.642	1.0	45.4
Linum rigidum	0.718	4.8	91.1	95.9	5.3	0.246	19.5	370.7
Lithospermum canescens	0.295	0.067	1.4	1.4	1.5	0.105	0.6	13.0
Lithospermum incisum	0.800	0	1.8	1.8	0.8	0.057	0.0	30.8
Medicago lupulina	n.a.	0.233	1.0	1.3	1.2	0.008	28.9	128.0
Melilotus officinalus	0.843	0.2	2.0	2.2	1.6	0.010	19.7	193.3
Nothocalais cuspidata	0.452	3.467	4.7	8.2	3	0.287	12.1	16.5
Oxalis stricta	0.316	1.967	3.5	5.4	2.5	0.187	10.5	18.6
Oxytropis lambertii	0.354	0	2.3	2.3	1.5	0.048	0.0	46.4
Penstemon albidus	0.679	0.833	12.5	13.4	4.5	0.069	12.0	180.2

…Table 3.3 continued...

		Pollen per stigma				Pollen density per mm area		
Species	Morisita-Horn Index	*Euph-orbia*	Other hetero-specific	Total hetero-specific	Morpho-species	Stigma area (mm)	*Euph-orbia*	Other hetero-specific
Phlox pilosa	n.a.	0.067	1.9	2.0	1.3	0.270	0.2	7.2
Psoralea esculentum	0.283	0.2	14.9	15.1	2.2	0.027	7.3	547.0
Scuttelaria parvula	0.414	2.4	5.7	8.1	4.1	0.006	375.3	883.4
Senecio plattensis	0.578	0.667	2.2	2.9	2.8	0.037	17.8	59.7
Sisymbrium loeselii	0.897	1.317	19.0	20.3	2.8	0.109	12.1	174.4
Sisyrinchium campestre	0.568	0.533	2.3	2.8	2	0.045	11.8	50.5
Taraxacum officinale	0.486	0.267	4.3	4.5	2.1	0.152	1.8	28.2
Tragopogon dubius	0.533	3.467	3.9	7.4	2.4	0.818	4.2	4.8
Vicia americana	0.415	0.1	8.0	8.1	1.9	0.052	1.9	153.5
Viola pedatifida	0.629	3.95	45.1	49.1	4.3	0.070	56.4	644.8

Table 3.4 Number of species sampled with unrestrictive or restrictive floral morphologies, and for each category average number (± s.e.) of *Euphorbia* pollen grains, other heterospecific pollen grains, and morphospecies of heterospecific pollen per species. Differences were statistically significant for each of three measures – see text for statistics.

Morphology	Number of species	*Euphorbia* pollen	Other hetero-specific pollen	Number of morphospecies
Unrestrictive	16	2.6 (1.3)	22.2 (10.2)	3.0 (0.3)
Restrictive	13	0.6 (0.3)	6.6 (3.4)	1.8 (0.3)

Table 3.5 Effect of floral restrictiveness, pollinator overlap, and *Euphorbia* flowering intensity on *Euphorbia* pollen receipt rates per stigma.

	d.f.	Sum of Squares	Mean Square	*F*	*P*
Floral restrictiveness	1	1.876	1.876	3.596	0.07
Pollinator overlap	1	0.277	0.277	0.531	0.47
Euphorbia flowering intensity	1	0.293	0.293	0.561	0.46
Residuals	22	11.489	0.522		

Table 3.6 Average stigmatic area in mm^2 ($\pm$ s.e.), and density ($\pm$ s.e.) of *Euphorbia* pollen and other heterospecific pollen grains (grains per stigmatic area) for flowers with unrestrictive or restrictive morphologies. Difference in stigmatic area was statistically significant between floral categories, but differences in pollen densities were not - see text for statistics.

Morphology	Stigmatic area	*Euphorbia* pollen density	Other heterospecific pollen density
Unrestrictive	0.31 (0.13)	9.59 (1.52)	89.61 (6.01)
Restrictive	0.06 (0.02)	40.72 (31.52)	214.52 (25.17)

Table 3.7 Effect of stigmatic area and floral restrictiveness on receipt of a. *Euphorbia* pollen and b. all other heterospecific pollen. *Euphorbia* pollen receipt was transformed by the natural log plus one, and other heterospecific pollen receipt was natural log transformed prior to analysis.

	d.f.	Deviance explained	Residual d.f.	Residual. deviation	*F*	*P*
A. *Euphorbia* pollen receipt						
Null			28	14.750		
Stigmatic area	1	7.832	27	6.918	30.534	< 0.001
Floral restrictiveness	1	0.249	26	6.669	0.972	0.333
B. Other heterospecific pollen receipt						
Null			28	58.634		
Stigmatic area	1	17.921	27	40.712	12.115	0.002
Floral restrictiveness	1	2.252	26	38.461	1.522	0.23

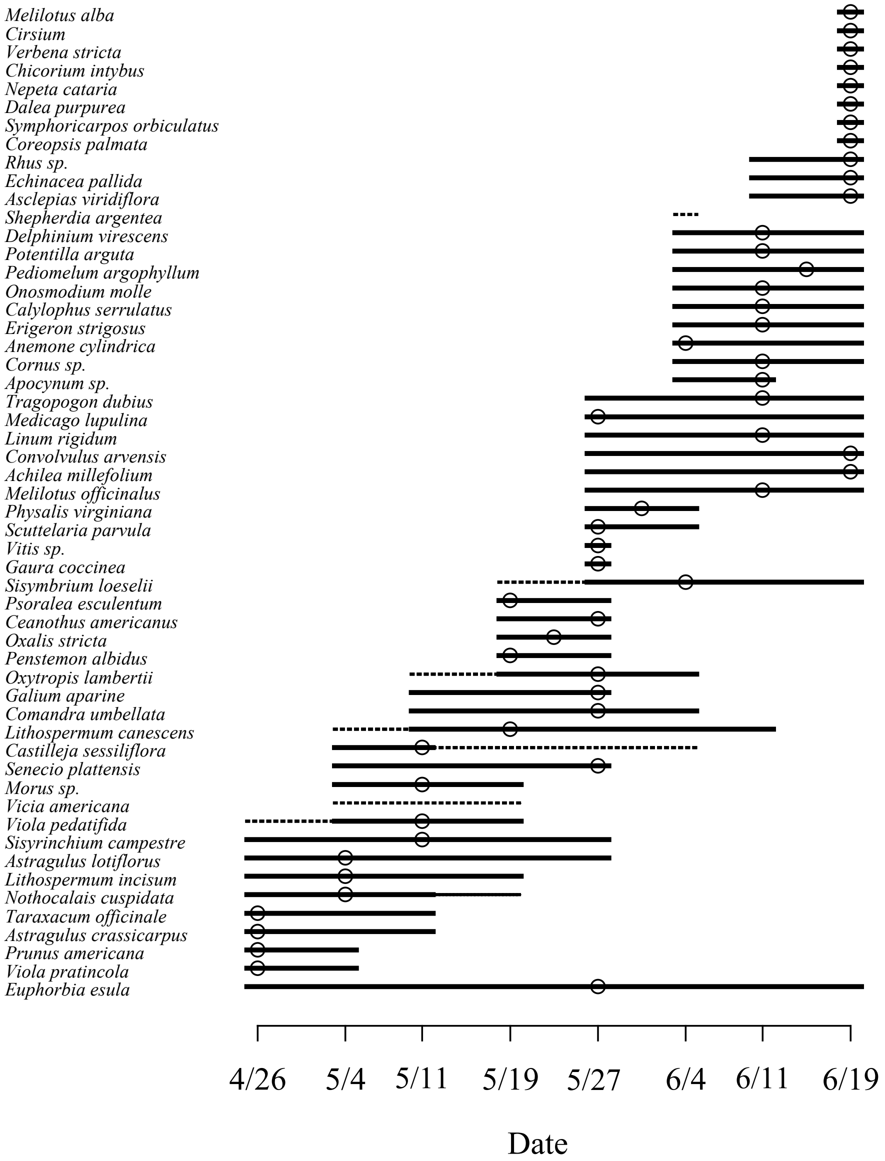

Figure 3.1 Flowering phenologies *of Euphorbia,* 29 study species, and other plants blooming at study site, for spring 2006. Solid line indicates period of flowering within plots, and dotted line indicates flowering in the study area but not within plots. Circles indicate survey date of peak flowering, or median date if same maximum flowering occurred in consecutive weeks.

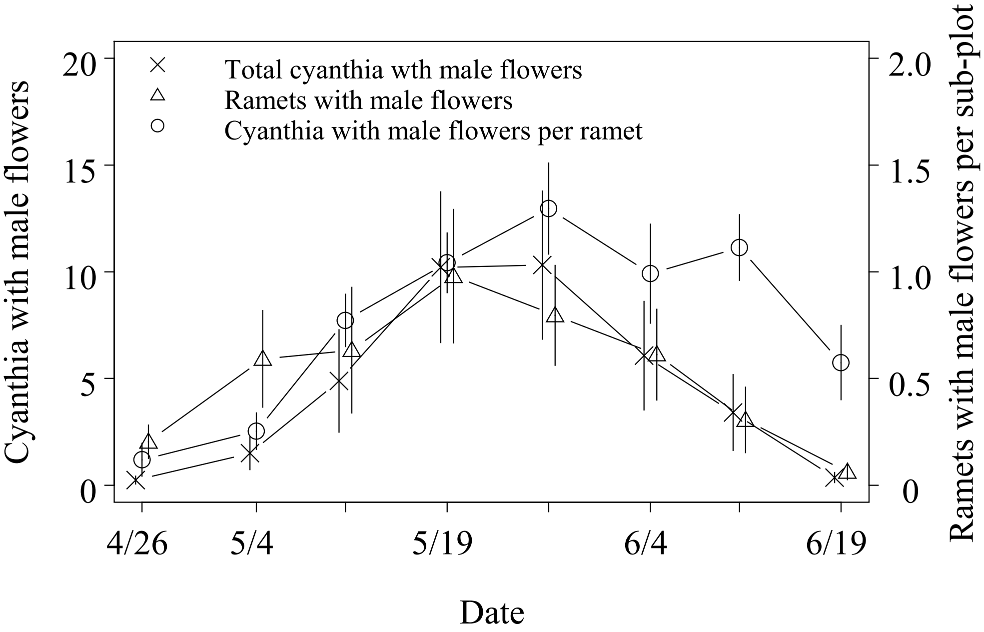

Figure 3.2 *Euphorbia* flowering phenology, including the number of cyathia with male flowers per flowering ramet (circles), the number of ramets with male flowers per 0.25 m^2 subplot (triangles), and the total number of cyathia with male flowers per subplot (x).

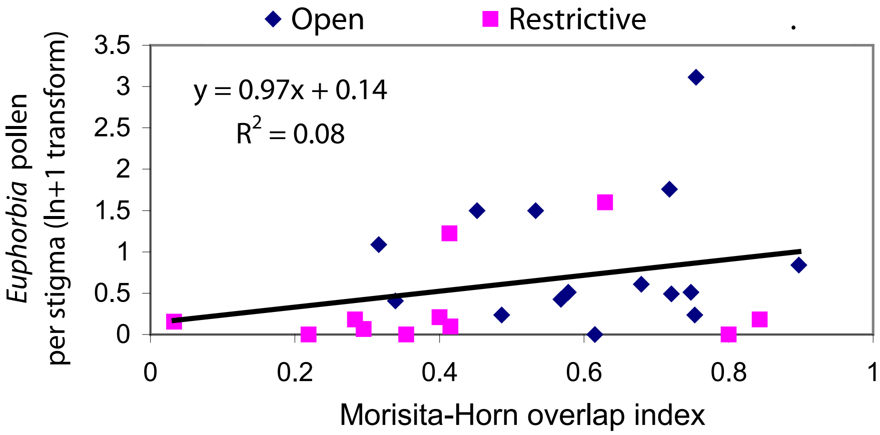

Figure 3.3 Relationship between floral restrictiveness and pollinator overlap on average *Euphorbia* pollen receipt per stigma (ln + 1 transformed). Diamonds indicate averages for species with open morphologies; squares represent averages for species with restrictive morphologies. The regression line includes both morphologies.

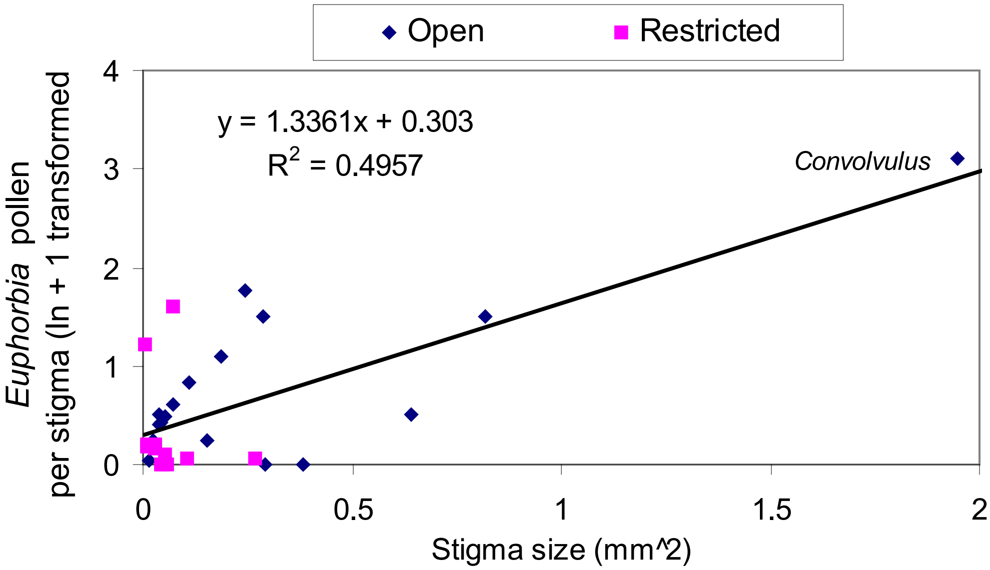

Figure 3.4 Relationship between floral restrictiveness, and pollinator overlap on average *Euphorbia* pollen receipt per stigma (ln + 1 transformed). Diamonds indicate averages for species with open morphologies; squares represent averages for species with restrictive morphologies. The regression line includes both morphologies.

Chapter 4

Effects of Heterospecific Pollen Receipt from *Euphorbia esula* on Prairie Plants with Different Floral Traits

ABSTRACT Flowers may receive heterospecific pollen from simultaneously flowering species, potentially resulting in reduced fruit or seed set. However, there has been a paucity of research concerning whether effects of heterospecific pollen receipt are similar across multiple species with different breeding systems and floral traits. I investigated this question by looking at effects of heterospecific pollen receipt from *Euphorbia esula* (leafy spurge, Euphorbiaceae) on fruit and seed set of seven species with a range of floral traits. In hand-pollination experiments, receipt of *Euphorbia* pollen significantly decreased fruit or seed set for three species, *Linum rigidum* (stiff flax, Linaceae), *Viola pedatifida* (prairie violet, Violaceae), and *Zizia aurea* (golden alexander, Apiaceae). The effect of *Euphorbia* pollen receipt varied according to the order, timing and magnitude of pollen application, with stronger effects associated with earlier application of *Euphorbia* pollen relative to conspecific pollen and with application of larger quantities of *Euphorbia* pollen. For four other species, receipt of *Euphorbia* pollen did not result in a statistically significant decrease in fruit or seed set. These results suggest that heterospecific pollen receipt may be harmful in some instances, but in other cases plants pollination success is unaffected, particularly for low levels of heterospecific pollen receipt. Study results suggest that traits associated with self-incompatibility may mitigate effects of heterospecific pollen receipt, but no evidence was found that plants naturally receiving more heterospecific pollen are less affected by its receipt.

INTRODUCTION

Because most pollinators do not specialize exclusively on a single plant species (Waser et al. 1996), pollinators may deposit heterospecific pollen as well as conspecific pollen (Rathcke 1983, Waser 1983). Heterospecific pollen receipt can result in reduced pollination success, leading to reduced fruit or seed set (Waser 1978a). This effect of heterospecific pollen receipt can occur through a variety of mechanisms, including stigma clogging (Galen and Gregory 1989, Randall and Hilu 1990), pollen allelopathy (Kanchan and Chandra 1980, Murphy and Aarssen 1989, Murphy 2000), and premature stigma closure (Waser and Fugate 1986). Similarly, receipt of pollen from closely related species may reduce seed production due to abortion of inviable hybrids (Stucky 1985, Wang and Curzan 1998, Fishman and Wyatt 1999). In cases for which heterospecific pollen receipt leads to reduced pollination success, selection may favor traits that result in reduced heterospecific pollen receipt (Brown and Kodric-Brown 1979, Feinsinger 1983) or that minimize the negative effects of heterospecific pollen receipt (Kohn and Waser 1985).

As documented in the previous chapter, heterospecific pollen receipt is common (McLernon et al. 1996, Waites and Aagren 2004). A variety of floral traits, including the flowering phenology and restrictiveness of floral morphology, could influence the foreign pollen receipt rates (Rathcke 1983, Feinsinger et al. 1986). Additionally, distance and density of neighboring heterospecifics can influence pollen exchange (Beattie 1969, Levin 1971, Kephart 1983).

Studies have found a range of responses to heterospecific pollen receipt, from substantial reductions in fruit or seed set to no significant effect (references in McLernon et al. 1996). This range of outcomes suggests that the degree to which heterospecific pollen reduces pollination success may depend on the timing or quantity of heterospecific pollen receipt (Murphy and Aarssen 1989, Caruso and Alfaro 2000) or on traits of the recipient flower (Kohn and Waser 1985) or the donor pollen (Murphy 2000). Despite this range in outcomes, only a few studies have investigated effects of heterospecific pollen receipt for different application quantities or timing (Waser and Fugate 1986, Kwak and Jennersten 1991, Caruso and Alfaro 2000), for different taxa of heterospecific pollen (Galen and Gregory 1989, Murphy and Aarssen 1995a), or for different recipient species with different floral traits (Harder et al. 1993). Such studies could help us make predictions when heterospecific pollen transfer is likely to have substantial effects on pollination success.

Self-incompatibility is one trait that may correlate with tolerance for (experiencing less harm from) heterospecific pollen receipt. For example, recipient flowers exhibiting self-incompatibility may have adaptations to prevent harm from self-pollen, and these same mechanisms might prevent harm from receipt of heterospecific pollen. This can be a consequence of unilateral interspecific incompatibility which refers to when pollen tubes of self-incompatible species successfully penetrate the stigmas of closely related self-compatible species, whereas pollen tubes are unsuccessful in the reverse crosses (Harder et al. 1993). Flowers with less restrictive morphologies tend to receive more heterospecific pollen (chapter 3). If heterospecific pollen receipt selects for traits that mitigate effects of this receipt, then it can be hypothesized that plants with unrestrictive morphologies and high rates of heterospecific pollen receipt should be less affected by application of heterospecific pollen than flowers with restrictive morphologies. In addition, a fixed quantity of heterospecific pollen would occlude a smaller portion of a large stigma than a small stigma, so larger stigmas could also be a trait that mitigates effects of heterospecific pollen receipt (Cruden and Millerward 1981).

In this study, I perform experimental hand pollinations to investigate the effects of heterospecific pollen from the introduced species *Euphorbia esula* L. (leafy spurge, Euphorbiaceae) on seven native species that vary in breeding system, floral restrictiveness, and stigma size. Because *Euphorbia* has only been present in Iowa since the late 1800s (Huerd and Taylor 1998), there has presumably been little opportunity for simultaneously flowering species to evolve traits that minimize effects of *Euphorbia* pollen receipt. By using one pollen from one donor species for all heterospecific pollen transfer experiments, I remove an important source of variation that complicates comparisons of results among other studies. For some recipient species in this study, I also vary the quantity and timing of *Euphorbia* pollen application to determine the sensitivity of results to these differences. Additionally, I investigate the breeding system of species for which information is lacking.

METHODS

Study System

The study was conducted on plant species at Broken Kettle Grasslands Preserve in the Iowa's Loess Hills (Plymouth County, 42.709° N, 96.579° W). For the hand-pollination studies, seven species with different floral traits were selected, of which six species occurred in the area used for stigma collections (chapter 3). Species are referred to by their genus throughout the chapter. The same site used for stigma collections, as descriped in chapter 3, was used for hand-pollination studies of four recipient species, and other species were studied under greenhouse conditions. The site includes native mixed grass prairie interspersed with introduced grasses and forbs, and is heavily invaded by *Euphorbia*.

Breeding Systems of Study Species

I conducted breeding system studies on most species because published information was inadequate or not available. *Euphorbia* itself requires pollinator visits for success, and is self-compatible, although self-pollination reduces seed set success compared to cross-pollination (Bakke 1936, Selleck et al. 1962, Selbo and Carmichael 1999). My experiments demonstrate that *Sisyrinchium campestre* Bickn. (Iridaceae, blue-eyed grass) is self-incompatible (chapter 1) and *Viola pedatifida* L. (Violaceae, prairie violet) is self-compatible but benefits from pollinator visits (chapter 2). *Lithospermum canescens* (Michx) Lehm (Boraginaceae, hoary puccoon) is distylous, with pin and thrum morphology, and is likely self-incompatible (Johnston 1952, Molano-Flores 2004).

Information on the breeding system of the remaining species was lacking or inadequate. The breeding system of *Zizia aurea* L. (Apiaceae, golden alexanders) was unknown, but the congener *Zizia trifoliata* is andromonecious (with male and hermaphroditic flowers on the same plant), strongly protogynous (stigma receptivity occurs before anther dehiscence), and self-compatible, resulting in a 5-10% self-pollination rate (Lindsey 1982). Piehl (1965) found evidence for self-compatibility of

Comandra umbellata L. (Santalaceae, bastard toadflax), based on fruit production of a single isolated greenhouse clone, but suggested that more work was needed to verify this conclusion. No previous research on the breeding system of *Linum rigidum* Pursh. (Linaceae, stiff flax) has been found, and both self-compatibility and self-incompatibility have been found among congeners (Kearns and Inouye 1994, Richards 1997). To my knowledge, previous research has not investigated the breeding system of *Oxytropis lambertii* Pursh (Fabaceae, locoweed) or congeners.

Heterospecific pollen transfer studies were performed in the field on four of these species (*Comandra*, *Linum*, *Lithospermum*, and *Oxytropis*). Another two species (*Sisyrinchium* and *Viola*) were common at the study site, but for logistical reasons, experiments were conducted on transplanted plants from other regions in the greenhouse. Finally, the seventh species (*Zizia*) is reported to grow at Broken Kettle Grasslands Preserve (Scott Moats pers. comm.), but was not found on the drier hillsides where the study was conducted. However, elsewhere *Zizia* has been observed growing in close proximity to *Euphorbia* (B. Montgomery, unpublished data). It was also studied in the greenhouse using plants transplanted from another location in northwestern Iowa.

Breeding Systems and Effects of Heterospecific Pollen Transfer (HPT) Studies

Because details of the pollination experiments differed, each species is discussed separately. Studies of breeding systems and effects of *Euphorbia* pollen receipt on *Sisyrinchium* and *Viola* are described in chapters one and two, respectively. Studies of the remaining five species are described here.

i. *Comandra umbellata*

The breeding system of *Comandra* and effects of *Euphorbia* pollen receipt on *Comandra* were studied in the field during the 2005 field season. The breeding system study of *Comandra* consisted of 30 plants split between two areas 50 m apart. Fifteen plants were enclosed with wire frames and bridal veil netting, and fifteen plants were left exposed to pollinators (open-pollination). Enclosures prevented access for most pollinators, but ants and thrips could access flowers. For open-pollinated plants, the first

8 flowers on each were labeled and monitored. Up to twelve flowers on enclosed plants were randomly assigned one of three treatments: cross-pollinate; self-pollinate; and unmanipulated, with twice as many flowers assigned to the unmanipulated treatment as to cross- and self-pollinate. Pollinations were performed from 10 May through 18 May, on the first day without substantial precipitation that a flower was open, as defined by the stage at which most petals were either vertical or angled outwards.

Hand-pollinations were performed, usually in the morning, by transferring pollen on forceps tips, which were cleaned with alcohol between pollinations. *Comandra* is clonal, so cross-pollinations were always performed between patches to minimize geitonogamous pollination. In total 292 flowers were included, with 119 exposed flowers, 85 enclosed unmanipulated flowers, and 44 flowers each in the cross-pollinate and self pollinate treatments. Bags were removed and fruit set was determined on 29 May by swelling at the base of the ovary. Remaining fruits were collected on 9 June. Because only one seed is produced per fruit, seed set was not measured separately.

The HPT study of *Comandra* was performed on 19 ramets, divided between the two areas used in the breeding system study. Ramets were enclosed with bridal veil netting supported by wire. After initiating the experiment on 4 May, plants were monitored daily for new flowers. On each plant, flowers were randomly assigned one of three treatments: unmanipulated; cross-pollinate; or a heterospecific pollen transfer (HPT) treatment consisting of pollination with *Euphorbia* followed by conspecific pollination. Pollinations were performed on the first dry day a flower was open. Up to six flowers were randomly assigned the umanipulated treatment, and up to three flowers were assigned to both the cross-pollinate and HPT treatments, with reduced replication on plants with fewer flowers. In total, cross-pollinate and HPT treatments each included 56 flowers, and unmanipulated included 115 flowers.

Unmanipulated flowers were labeled with paint on the ovary and corolla but not otherwise treated. HPT flowers were pollinated with *Euphorbia* pollen, and after a delay of between two and three hours, they were pollinated with conspecific pollen collected from the distant area. All pollinations were performed by transferring pollen on forceps tips, which were cleaned with ethyl alcohol between treatments. For both species, enough pollen was applied to be visible with a hand lens over most of the stigma. Cross-pollinate flowers were touched with clean forceps tips in the manner used to apply *Euphorbia* pollen, and after a similar time delay conspecific pollen from the distant area was applied to stigmas. Pollinations were performed from 7 May through 17 May. Exclosure bags were removed and fruit set was determined on 27 May, with remaining fruits harvested on 9 June.

ii. *Oxytropis lambertii*

The breeding system and effect of *Euphorbia* pollen receipt on *Oxytropis* was investigated with field studies in the spring of 2005 and 2006. In 2005, on 19 May 16 plants were enclosed with bridal veil netting supported by wire frames. Enclosures prevented visits by most pollinators, but thrips and less commonly ants were present in enclosures. Previously opened inflorescences were removed, leaving at least three unopened inflorescences per plant, and pollinations were performed on up to the next four inflorescences to open. Hand pollinations were performed between 20 and 28 May.

Four flowers were assigned without replacement to one of four pollination treatments per inflorescence: enclosed unmanipulated, self-pollinate, cross-pollinate, or heterospecific pollen transfer (HPT) from *Euphorbia* followed later by cross-pollination. Flowers were pollinated in the order in which they opened (typically from the base of the stem upward), and treatment orders for each inflorescence were pre-determined in a manner that varied the order among plants and inflorescences within plants. Flowers were pollinated in the morning or early afternoon on the first dry day that the lateral wing petals were observed to have opened sufficiently to allow access to the stigma.

Techniques for hand-pollinations were similar among treatments, and all flowers were labeled with acrylic paint on their calyx and stalk below the flower. To perform self-pollinations, keel petals were depressed to expose the stigma, and clean forceps were used to remove excess pollen from around the stigma, in the process pollinating the stigma. Additionally, the stigma was rubbed gently with forceps tips to mimic other treatments. For cross-pollinate flowers, keel petals were depressed to expose the stigma, after which the anthers were removed and the stigma was gently rubbed with clean forceps. Two to four hours later, conspecific pollen was applied with a natural hair paintbrush. A conspecific pollen supply was created by collecting pollen in a microcentrifuge tube from at least ten unopened flowers from at least four plants. For HPT flowers, the same steps were followed, except that following anther removal, *Euphorbia* pollen from about two anthers was applied using forceps tips, followed two to four hours later by application of conspecific pollen.

Enclosure bags were removed on 7 June, by which time all experimental flowers had either initiated fruit development or aborted, allowing determination of fruit set. Developing fruits were harvested at two times before seed dispersal began: earlier pollinated flowers were harvested on 25 June and later pollinated flowers were harvested on 29 June. Fruits were stored in coin envelopes. In the laboratory, seeds were counted, including only large well formed seeds. Seed set was similar between the cross-pollinate and HPT treatments, so seeds from these treatments were weighed in order to maintain an objective criteria for what constituted a developed seed. Since there was a gradual gradation between small, clearly undeveloped and large developed seeds, a series of threshold weights were established above which the seed would count as being developed. Statistical results did not meaningfully change significance levels across this range of thresholds, so a threshold size of 0.4 mg was arbitrarily chosen as the threshold weight to be considered a seed.

In 2006, an additional *Oxytropis* pollination study was conducted in the field to investigate effects of smaller quantities of *Euphorbia* pollen receipt. Four treatments were included: cross-pollinate, enclosed unmanipulated, application of large amounts of *Euphorbia* pollen (HPT large), and application of smaller amounts of *Euphorbia* pollen (HPT small). The study was initiated on 21 May, and again included 16 plants enclosed with netting supported by wire frames in the same area as the previous year's study. Four inflorescences were selected per plant, and treatments were applied in varying order to each inflorescence in the order in which flowers opened. Treatments were performed similarly to 2005, except for the HPT small treatment, in which a small amount of *Euphorbia* pollen was applied with a natural hair paintbrush cut down to a few bristles. Pollinations were performed between 22 May and 1 June. Nets were removed on 17 June, and fruits were collected from 25 June to 29 June. All seeds were weighed, and those at least 0.4 mg were counted as developed seeds.

iii. *Lithospermum canescens*

The effect of heterospecific pollen transfer on *Lithospermum* was investigated with a field study in 2005. On 26 May, eighteen ramets were enclosed in anchored mesh bags. Thereafter, on days without rain, plants were checked for open healthy flowers on a rotating basis of three to five plants per day. Flowers were included in the study only if the stigmas and ovules were light in color and not shriveled. Flowers were randomly assigned to one of three treatments: enclosed unmanipulated, cross-pollinated, or pollinated with *Euphorbia* pollen then later cross-pollinated (HPT). Each treatment was applied to three to five flowers per plant, expect for three plants for which a lack of healthy flowers or subsequent exclusion due to florivory led to inclusion of fewer flowers. In total, the unmanipulated, cross-pollinate, and HPT treatments included 56, 52, and 53 flowers, respectively.

Corollas were removed from all treatments, including the unmanipulated control, to facilitate pollination treatments for both treatment groups. For the HPT treatment, *Euphorbia* pollen, collected from the anthers of nearby plants, was lightly brushed onto *Lithospermum* stigmas with forceps tips. For cross-pollinate treatments, stigmas were lightly brushed with clean forceps tips in the same manner. Then for both HPT and cross-pollinate flowers, two to four hours later, outcrossed pollen was applied to the stigma in the same manner. This *Lithospermum* is distylous (pin and thrum), so pollinations were performed with pollen from an individual of the other morph. Forceps were cleaned with ethyl alcohol between pollinations, and flowers were labeled with acrylic paint on their sepals.

Exclusion bags were removed around 29 May, and fruit and seed set were determined on 11 June. *Lithospermum* flowers typically have four ovaries, which each may form a separate nutlet, and scars of dispersed fruits are apparent, allowing determination of fruit set after dispersal. For equivalence with other study species, all of which make at most one fruit per flower, fruit set for *Lithospermum* was defined as a flower developing at least one ovary. Seed set per flower was determined as the number of developed nutlets per flower, irrespective of fruit set. Finally, seed set per successful flower was determined as the number of nutlets per flower that produced at least one nutlet.

iv. *Zizia aurea*

The breeding system and effect of heterospecific pollen transfer on *Zizia* was studied with greenhouse experiments in 2005 and 2006. In 2003, *Zizia* were transplanted from a restoration site in O'Brien County, northwestern Iowa into 1-quart pots. Plants were maintained outside except during experiments, when they were transferred to a cool greenhouse (maximum temperature of 16° C). Plants were fertilized regularly and watered below the flowers throughout the experiments.

For the 2005 study, plants were moved to the greenhouse on 10 February. Thirteen inflorescences on nine plants were selected haphazardly and labeled with acrylic paint on the pedicel. For each selected umbel, four outer sub-umbels were selected and labeled with paint on the pedicel. Within each sub-umbel, six outer flowers representing five treatments were selected and labeled with paint on the ovary. The following treatments were each represented with one flower per sub-umbel: self pollinate, cross-pollinate, *Euphorbia* pollen followed after a two to four hour delay with conspecific pollen (HPT 2 hour delay), and *Euphorbia* pollen followed immediately by conspecific pollen (HPT-immediately-prior). Additionally, two flowers on each sub-umbel were left unmanipulated. Treatments were assigned to flowers quasi-randomly by assigning treatments in a varied pre-determined order from a randomly chosen starting flower.

Euphorbia pollen application was usually performed around 13:00, and other pollinations were performed around 16:00. *Euphorbia* pollen was applied to both stigmas on each flower using the point of a dissecting needle. Conspecific pollinations were performed by lightly rubbing two anthers from different plants against the stigmas. Pollination success for both treatments was visibly apparent. Fruit set was determined initially by observing ovary enlargement in late May. Fruits were collected on 12 July, by which time a few fruits had dispersed. Fruits were weighed after drying in an oven at 60° C for a week. A histogram of fruit weights divided into one mode of fruits less than 2 mg, which appeared undeveloped, and another mode of heavier developed fruits, so 2 mg was considered the minimum threshold to count as a fruit. Additionally, dispersed ovaries that had been determined to be swollen in earlier surveys were counted as fruits.

For the 2006 study, plants were transferred to the greenhouse in early February, and pollinations were performed starting on 21 March. The self-pollinate and HPT-immediately-prior treatments were omitted. Instead, in addition to the unmanipulated treatment, a two-factorial design was used to vary *Euphorbia* and conspecific pollen application. *Euphorbia* pollen was either omitted or applied in small or large quantities, and conspecific pollen was applied in small or large quantities, leading to six treatment combinations. Each treatment was applied to one outer flower per sub-umbel on six sub-umbels per umbel, using the assignment process described for 2005. On sub-umbels with extra outer flowers, a second unmanipulated flower was also labeled. Ten umbels were treated across nine plants, for a total of 470 flowers.

To perform pollinations, large amounts of *Euphorbia* pollen were transferred from a slide to both stigmas using cleaned forceps tips. To apply small amounts of pollen, whether *Euphorbia* or conspecific, pollen was initially spread on a slide, then collected and applied using a single hair acrylic paint brush. A hand lens was used to confirm the transfer of pollen. Stigmas of flowers receiving only conspecific pollen were touched with cleaned forceps tips to mimic application of large amounts *Euphorbia* pollen. To apply large amounts of conspecific pollen, a dehisced anther was rubbed across the stigmas. All conspecific pollen was outcross pollen, and came from the same donor plant for all treatments to a given inflorescence. *Euphorbia* pollen application and sham controls were applied two to four hours prior to conspecific pollen application, and pollinations were timed such that all conspecific pollen application occurred at the same time for a given inflorescence.

To determine fruit set, sub-umbels were harvested in early June and stored separately in coin-envelopes. After drying at room temperature, fruits were weighed individually. As in 2005, the distribution of weights was apparently bimodal, with one mode corresponding to undeveloped ovaries less than 2 mg, and a second mode of larger developed fruits. Consequently, aborted flowers and remaining ovaries less than 2 mg were treated as failures, and larger remaining ovaries were counted as fruits.

v. *Linum rigidum*

The breeding system and effect of *Euphorbia* pollen receipt on the pollination of *Linum* was investigated with field studies during the summers of 2005 and 2006.

To investigate *Linum*'s breeding system, on 29 May 2005, ten *Linum* plants were enclosed with mesh bags supported by wire frames. Thereafter, plants were checked daily for newly opened flowers, which were assigned randomly without replacement to one of three pollination treatments: leave unmanipulated, cross-pollinate, or self-pollinate. Once all treatments were represented equally on a plant, the randomization process was repeated. Flowers were excluded if they were in contact with the bag, if they developed abnormally, or if flowers had already self-pollinated. Each treatment was performed on at least one flower per plant, except for two plants which produced few flowers and lacked any flowers in the unmanipulated treatment. Overall 20 or 21 flowers were included in each treatment.

Pollinations were performed in the morning or early afternoon, during dry periods from 30 May through 15 June. Unmanipulated flowers, were not treated except to collect one anther for use as a pollen source. For self pollinations, pollen was transferred autogamously from anthers to each stigma using forceps tips until multiple pollen grains were apparent on each stigma. Afterwards, a subset of anthers were removed to be used as a pollen source. Cross pollinations were performed by mixing pollen from at least three plants in a microcentrifuge tube and applying the mixture to each stigma. Flowers were labeled with acrylic paint on their sepals and subtending stem. Enclosure bags were removed on 22 June. At this time, fruit set was determined and mature fruits were collected and stored in microcentrifuge tubes. Remaining fruits were collected as they matured through 30 June, at which time remaining fruits were collected. Seed set per fruit was later determined in the laboratory.

For the 2005 study of effects of *Euphorbia* pollen receipt on *Linum*, on 26 May, twenty-four *Linum rigidum* plants were enclosed in mesh bags supported by wire frames. Thereafter, plants were checked daily for newly opened, unpollinated flowers, which were assigned to one of three pollination treatments: unmanipulated , cross-pollinate, or heterospecific pollen transfer (HPT) of *Euphorbia* pollen followed later by pollination with outcrossed *Linum* pollen. Treatment were assigned randomly without replacement, repeating the process once each treatment was represented with equal numbers on a given plant. Each treatment was performed on at least one flower per plant.

Pollinations were performed in the morning or early afternoon during dry periods from late May through early June. To prevent self-pollination prior to treatments, flowers in the cross pollinate and HPT treatments were emasculated. At this time, *Euphorbia* pollen was applied to flowers by lightly touching anthers to all five stigmas and spreading the pollen with cleaned forceps tips. As a control, cross pollinate flowers were similarly rubbed with forceps tips. Then, two to four hours later, cross pollinate and HPT flowers were pollinated with a mixture of conspecific pollen from previously collected anthers, applied with a paintbrush. Flowers were labeled as in the breeding system study. In late June, enclosure bags were removed, fruit set was determined, and mature fruits were collected into microcentrifuge tubes. Less mature fruits were checked periodically through 30 June, at which time remaining fruits were collected for seed counts.

In 2006, two related studies of *Linum* pollination were conducted. As in 2005, both included a cross-pollinate treatment and a heterospecific pollen transfer treatment in which a large amount of *Euphorbia* pollen was applied two to four hours prior to conspecific pollen. The first study, investigating effects of quantity of heterospecific pollen, also included a third treatment of a smaller amount of *Euphorbia* pollen applied two to four hours prior to conspecific pollen. The second study , investigating effects of timing of heterospecific pollen, included a third treatment of a large amount of *Euphorbia* pollen applied immediately prior to conspecific pollen. For the heterospecific pollen quantity study, flowers were treated on twelve plants. The timing study included flowers on 16 different plants, and additionally flowers from four plants used in the quantity study for which five pollinations per treatment had already been performed, for a total of 20 plants. For plants used in both studies, treatments flowers were labeled differently between studies, and data were separated between the studies so subsequent analyses of the studies would be independent.

In both the quantity and timing studies, up to five flowers were used per treatment per plant, depending on availability. Treatments were assigned randomly without replacement, repeating the process once a plant had equal numbers of each treatment.

For the heterospecific quantity study, the cross-pollinate and heterospecific pollen transfer with a large amount of *Euphorbia* pollen (HPT-large) treatments were performed as in the 2005 study. To transfer a small amount of *Euphorbia* pollen (HPT-small), *Euphorbia* pollen was transferred on the point of an insect pin, and its presence was visually confirmed on the stigma, then conspecific pollen was applied two to four hours later as for the other treatments. For the timing study, the cross-pollinate treatment and treatment of heterospecific pollen transfer with a time delay (HPT-delay) were performed as in the quantity study. The treatment of heterospecific pollen transfer without a time delay (HPT-immediate) was performed as for the HPT-delay treatment, except that *Euphorbia* pollen was applied immediately before cross-pollinations. Pollinations were timed such that all cross-pollinations were performed at the same time, regardless of when *Euphorbia* pollen was applied.

Pollinations were performed during mornings and early afternoons between 1 and 13 June for the quantity study, and between 3 and 14 June for the timing study. For both studies, a subset of stigmas were collected the next day, by which time they had senesced, and stored individually in 9:1 70% ethanol:glycerin for pollen counts. In total, 134 flowers were included in the quantity study, and 116 flowers were included in the timing study. For both studies, nets were removed and fruit set was determined on 18 June, after which plants were checked daily through 1 July for mature fruits, which were collected for seed counts as they matured. To determine the amount of *Euphorbia* and *Linum* pollen received in each treatment, a subset of 15 stigmas from each treatment were acetolyzed in microcentrifuge tubes with acetic anhydride and sulfuric acid, and the pollen was mounted in basic fuchsin jelly on slides for identification under compound microscopy (Kearns and Inouye 1993).

Statistical Analyses

Because details of the experiments varied, the statistical analysis is discussed separately for each species. All analyses were conducted in S-Plus 7.0.

Comandra

The study of *Comandra*'s breeding system was analyzed with a general linear model (GLM) specifying a binomial distribution, with the dependent variable representing the number of fruits and aborted flowers for each plant and treatment combination, and treatment included as a fixed effect. Plant was omitted from the analysis because the open-pollination treatment was performed on different plants than the three enclosed treatments. Seed set was not analyzed separately because fruits have only one seed. The *Comandra* HPT study was analyzed with a general linear mixed effects (GLME) model using the 'correlatedData' library in S-Plus, with a binomial distribution, specifying the logit-link function, and including ramet as a random effect and treatment as a fixed effect. Additionally, for comparison with other studies, the data were reanalyzed similarly, but omitting the unmanipulated treatment.

Oxytropis

Fruit set results from the 2005 *Oxytropis* study were analyzed with a GLME model as described above for *Comandra*. Seed set results across all four treatments were analyzed with a linear mixed effects model, including treatment as a fixed effect and plant as a random effect. Additionally, for consistency with other studies, fruit set and seed results were reanalyzed including only cross-pollinate and HPT treatments. Fruit set and seed set data from 2006 were analyzed as in 2005, including a separate analysis of only the cross pollinate and HPT large treatments. Additionally, for both fruit set and seed set results from the cross pollinate and HPT treatments from 2005 and 2006 were combined and analyzed together, initially including a treatment by year interaction term, which was dropped if non-significant or if the model failed to converge with the interaction included.

Lithospermum

For *Lithospermum*, fruit set was analyzed with a GLME model as described previously. Additionally, for consistency with other studies, successful flowering was reanalyzed including only cross-pollinate and HPT treatments. Seed set per successful flower was analyzed with a linear mixed effects model, excluding the unmanipulated treatment because only two flowers set fruit and there was no variability in results. Seed set per flower, irrespective of success, was analyzed with a GLME model including plant as a random effect, and with a Poisson distribution because the average number of seeds was low and seed number is bounded by zero. Seed set per flower was also reanalyzed excluding the unaminpulated treatment using a linear mixed effects model.

Zizia

For *Zizia*, developed fruits typically contain two seeds, so seed set was not analyzed separately from fruit set. For both years, fruit set was analyzed with a GLME model as described previously, including inflorescence as a random effect. For 2005, analyses were performed including all treatments and for consistency with other analyses, including only the cross-pollinate and HPT-large treatments. For 2006, the fruit set of unmanipulated flowers was compared to all flowers receiving conspecific pollen, regardless of conspecific or heterospecific pollen quantity. To investigate effects of pollination treatment among pollinated flowers, unmanipulated flowers were excluded and the remaining treatments were analyzed with a GLME model including main effects of heterospecific pollen transfer (none v. small v. large), conspecific pollen receipt (small v. large), and their interaction. The interaction term was determined to be non-significant, so was dropped from the model. For consistency with previous analyses, an additional analysis was performed comparing only the HPT-large and cross-pollinate (ie. no-HPT) treatments, including results from both large and small conspecific pollination treatments because there was no significant difference between them (see results). Finally, to investigate the effects of *Euphorbia* pollen receipt across both study years, the two data sets were analyzed together, including only the HPT-large and cross-pollinate treatments, with a GLME model including main effects of year and treatment, after dropping the non-significant interaction term.

Linum

For the 2005 *Linum* breeding system study, fruit set per flower was analyzed with a GLME model, as described previously, including plant as a random effect. The effect of treatment on *Linum* seed set was analyzed using the Kruskal-Wallis rank-sum test because residuals of linear mixed effects (LME) models deviated substantially from normality, even after a variety of transformations. Fruit set per flower for the *Linum* 2005 HPT study analyzed as in the breeding system study. Seed set per fruit for the *Linum* 2005 HPT study was analyzed with a LME model including plant as a random effect, after confirming approximate normality of residuals.

The 2006 *Linum* HPT studies were analyzed independently, except as described below. Conspecific pollen receipt was compared among treatments with ANOVA. The effect of treatment on fruit set in both the quantity and timing studies was analyzed with a GLME model including plant as a random effect, as previously described. For the quantity study, the effect of treatment on seed set was fit with a linear mixed effects model including plant as a random effect, and normality of residuals was visually confirmed. Seed set in the timing study was analyzed as in the quantity study after transforming seed set by 1.4 power to improve normality of residuals.

For comparison with other studies, the shared treatments (cross-pollinated and heterospecific transfer of large quantities of *Euphorbia* pollen with a time delay) among the 2005 and both 2006 *Linum* HPT studies were analyzed jointly to arrive at a single estimate of the effect of adding *Euphorbia* pollen in large quantities with a time delay. To accomplish this, data from the two treatments shared across both 2006 studies were combined. For the four plants used in both 2006 HPT studies, fruit and seed set results were combined prior to the analysis. Fruit set was analyzed with a GLME model, as previously described, including treatment and year as fixed effects and plant as a random effect. Seed set was analyzed similarly, but with a LME model, and confirming normality of residuals. For both analyses, the interaction term between fixed effects was dropped after confirming that it did not approach significance.

Cross-Species Analyses

To determine whether the response of the seven species to *Euphorbia* pollen receipt differed, fruit set results across species were analyzed in a single GLM analysis, including treatment and species as fixed effects. The data was simplified to include only two treatments: cross-pollinate and for most species application of large amounts of *Euphorbia* pollen with a time delay. For *Viola* and *Sisyrinchium*, a treatment with time delay and appropriate control treatment were not available, so the *Euphorbia* pollen application immediately prior to conspecific pollen was substituted. For *Sisyrinchium* this is justifiable because the effect of adding *Euphorbia* pollen with or without a time delay did not significantly differ. For *Viola*, this substitution is conservative because for other species effects tend to be even stronger when a time delay is included between the application of *Euphorbia* and conspecific pollen (see results and discussion). For three species (*Viola*, *Oxytropis*, and *Linum*), studies were performed in two years, and because there was no significant effect of year on fruit set in these studies, the two years' data were combined prior to analysis. Seed set per fruit was calculated for the same treatments for the five species for which seed set was determined independently of fruit set, again averaging across multiple years of data as necessary.

To determine whether there was an overall effect of *Euphorbia* pollen receipt on fruit set for all the study species generally, combined species data were reanalyzed with a GLME analysis including treatment as a fixed effect and species as a random effect. For the purpose of comparing effects of *Euphorbia* pollen receipt among studies, absolute effect size was calculated as the difference in fruit or seed set between treatment and control flowers. Relative effect size was calculated as natural log of treatment fruit set divided by control fruit set. For both, negative effect sizes indicate decreased fruit set for flowers receiving *Euphorbia* pollen relative to control flowers.

To investigate whether the effect of receipt of *Euphorbia* pollen on seed set differs among species, seed set per fruit was calculated for the five species for which seed set was determined independently of fruit set. The same two treatments were included as in the fruit set analysis, and results were averaged across multiple years of data as necessary. The effect of species, treatment, and their interaction on seed set was analyzed with a generalized least squares model, fit by restricted maximum likelihood with species and treatment included as fixed factors, and a heterogeneous residual variance structure for species, allowing different species to have different residual variance parameters. There was a significant interaction between species and treatment (see results), and determination of which of the ten species pairs differed significantly was made by comparing P-values to Bonferroni-adjusted values.

RESULTS

Comandra

For the breeding system study of *Comandra*, fruit set was low for all treatments, ranging from a low of 5% for enclosed unmanipulated flowers (no hand-pollination) to a high of 13% for cross-pollinated flowers (Figure 4.1A). In the breeding system study, there was not a significant effect of treatment on fruit set (5.4% of variance explained; 3,56 d.f., $P_{Chi} = 0.21$), although the effect became marginally significant ($F_{2,28} = 2.859$, $P = 0.07$) upon dropping the open-pollinated treatment (which was performed on different plants) and including plant as a random effect.

In the HPT study for *Comandra*, there was a significant effect of treatment ($F_{2,36} = 4.130$, $P = 0.024$), with fruit set lower in the unmanipulated treatment than the cross-pollinate and HPT treatments (Figure 4.1B). However, there was no significant difference between the cross-pollinate and HPT treatments, and removing the unmanipulated treatment did not affect this conclusion (analysis not shown).

Oxytropis

For *Oxytropis* in 2005, there was a significant effect of treatment on fruit set, with significantly higher fruit set for cross-pollinated flowers than self-pollinated and unmanipulated flowers, and lower fruit set for unmanipulated flowers than all other treatments ($F_{3,228} = 15.838$, $P < 0.0001$, Figure 4.2). There was no significant difference between the cross-pollinate and HPT treatments whether these treatments were analyzed with the other treatments or in isolation ($t_{15} = 0.515$, $P > 0.5$). For 2005, there was a significant effect of treatment on seed set, with significantly higher seed set for cross-pollinate than HPT, and significantly higher seed set for both of these treatments than either self-pollinated or enclosed unmanipulated flowers ($F_{3,101}$ = 19.623, $P < 0.0001$, Figure 4.3). Exclusion of the self-pollinate and unmanipulated treatment did not change the finding of higher seed set per fruit in the cross pollinate than HPT treatment ($F_{1,68}$ = 5.719, $P = 0.02$).

For *Oxytropis* in 2006, there was an overall significant effect of treatment on fruit set ($F_{3,237}$=10.533, $P < 0.0001$), due to substantially lower fruit set for enclosed unmanipulated flowers (Figure 4.2). However, there was no significant difference in fruit set between cross-pollinate and either HPT treatment, and the treatment effect remained insignificant when comparing just the cross-pollinate and HPT large treatment (results not shown). In 2006, seed set per fruit for unmanipulated flowers was substantially lower than for the other treatments, which all had similar seed set (Figure 4.3), but presumably due to the small sample size for this treatment (n=2), there was no significant overall effect of treatment ($F_{3,88}$ = 1.934, $P = 0.13$). When only the cross-pollinated and HPT-large treatments were analyzed across both years, there was no significant effect of year or treatment on fruit or seed set (Table 4.1).

Lithospermum

For *Lithospermum*, there was a significant effect of treatment on fruit set ($F_{1,35}$ = 9.921, $P = 0.003$), due to substantially lower success in the unmanipulated treatment (Figure 4.4A). However, there was no significant difference in successful flowering between cross-pollinate and HPT treatments, and this did not change upon excluding the unmanipulated treatment from the analysis ($F_{1,17}$ = 1.318, $P = 0.27$). Seed set per successful flower was lower, but not significantly so, for HPT compared to cross-pollinate ($F_{1,22}$ = 0.2623, $P = 0.61$, Figure 4.4B). Seed set per flower, irrespective of success, varied significantly with treatment ($F_{2,142}$ = 12.2465, P < 0.0001), due to substantially lower fruit set for the unmanipulated treatment (Figure 4.4C). However, there was no significant difference in seed set per flower when only the cross-pollinate and HPT treatments were analyzed ($F_{1,87}$ = 1.376, $P = 0.244$).

Zizia

For the *Zizia* 2005 study including all treatments, there was a significant effect of treatment ($F_{4,315} = 9.264$, $P < 0.0001$), due largely to substantially lower fruit set in the unmanipulated treatment compared to all others (Figure 4.5a). Fruit set was lower for the HPT-2-hours-prior treatment than the cross-pollinate treatment, but this difference was not significant, even upon excluding other treatments from analysis ($F_{1,97} = 2.964$, $P = 0.09$). In 2006, as in 2005, fruit set was significantly higher for pollinated than unmanipulated flowers ($F_{1,459} = 50.281$, $P < 0.0001$). Considering only hand-pollinated flowers, there was a significant effect of the quantity of *Euphorbia* pollen, with lower fruit set for flowers receiving large quantities of *Euphorbia* pollen relative to flowers receiving small amounts or no *Euphorbia* pollen (Figure 4.5b, Table 4.2). There was not a significant effect of the quantity of conspecific pollen applied, and the interaction term was also non-significant so was dropped from the model. For *Zizia* in 2006, when only the HPT-large and cross-pollinate treatments were included, application of *Euphorbia* pollen significantly decreased fruit set ($F_{1,230} = 6.052$, $P = 0.01$). When these treatments were analyzed across 2005 and 2006, fruit set was significantly lower for the HPT-large treatment than cross-pollinate, and neither year nor the interaction term was significant (Table 4.3).

Linum

For the breeding system study of *Linum*, there was a significant effect of treatment ($F_{2,16} = 4.322$, $P = 0.03$), with similar fruit set between cross- and self-pollinate treatments but significantly lower fruit set for unmanipulated flowers (Figure 4.6a). Seed set per fruit was not significantly affected by treatment, although there was a trend toward lower seed set for unmanipulated flowers ($X_2 = 5.632$, $P = 0.06$, Figure 4.6b). For the 2005 HPT study, there was a significant effect of treatment ($F_{2,46} = 4.459$, $P = 0.017$), with significantly lower fruit set in the HPT and unmanipulated treatments compared to the cross-pollinate treatment (Figure 4.7a). Seed set per fruit was also significantly affected by treatment ($F_{2,79} = 3.788$, $P = 0.027$), with significantly lower seed for flowers receiving *Euphorbia* pollen than cross-pollinated flowers, and intermediate seed set for unmanipulated flowers not significantly different from either other treatment (Figure 4.7b).

For the 2006 study of effects of *Euphorbia* pollen quantity on *Linum*'s pollination, pollen counts indicated that flowers assigned large quantities of *Euphorbia* pollen received substantially more than those assigned small quantities, and flowers in the cross-pollinate treatment received only trace amounts (Table 4.4a). Conspecific pollen retained on stigmas decreased with larger treatments of *Euphorbia* pollen (Table 4.4a), but differences between treatments were not significant ($F_{2,42} = 2.429$, $P = 0.10$). For the study of timing of *Euphorbia* pollen application, *Euphorbia* pollen receipt was similar whether it was added immediately prior or a few hours prior to conspecific pollen (Table 4.4b). However, conspecific pollen receipt varied significantly with treatment ($F_{2,34} = 8.219$, $P = 0.001$), with reduced conspecific pollen retention when *Euphorbia* pollen was applied with a time delay prior to conspecific pollen compared to the other two treatments, which did not differ significantly from each other (Table 4.4b).

For *Linum* in the *Euphorbia* quantity study, fruit set decreased from cross-pollinate to HPT-small and was lowest for HPT-large, but the effect of treatment did not approach significance ($F_{2,22} = 0.208$, $P = 0.81$, Figure 4.8a). There was, however, a significant effect of treatment on seed set per fruit ($F_{2,40} = 5.546$, $P = 0.008$), with similar seed set for cross-pollinate and HPT-small, but lower seed set for HPT-large (Figure 4.8b). For the timing study, although fruit set was lowest for the HPT-delay treatment and intermediate for the HPT-immediate treatment, there was no significant effect of treatment on fruit set ($F_{2,31} = 1.308$, $P = 0.29$, Figure 8c). For seed set in the timing study, there was a significant effect of treatment ($F_{2,44} = 4.595$, $P = 0.015$), with significantly lower seed set for HPT-delay relative to cross-pollinate, and intermediate success for HPT-immediate (Figure 4.8d).

In the combined analysis of 2005 and 2006 *Linum* results, both fruit and seed set were significantly lower for the HPT treatment than the cross-pollinate treatment, there was not a significant effect of year for either response variable, and both interaction terms were dropped for lack of significance (Table 4.5a,b).

Cross-species analysis

Fruit set per flower for control and treated flowers, as explained for the cross-species analysis, are summarized in Table 4.6a. *Linum* and *Zizea* experienced the largest absolute decrease in fruit set, and *Linum* and *Lithospermum* experienced the largest relative decrease upon receipt of large quantities of *Euphorbia* pollen. In the GLM including all seven recipient species, there were significant differences in fruit set among species, and significantly lower fruit set for flowers receiving *Euphorbia* pollen (Table 4.7). However, there was not a significant interaction between species and treatment. When the data was reanalyzed with a GLME with species included as a random rather than fixed effect, the effect of treatment was not significant ($F_{1,6} = 3.435$, $P = 0.11$).

In the cross-species comparison, there was a significant interaction between treatment and species, indicating that *Euphorbia* pollen receipt affected some species more than others (Table 4.8). *Euphorbia* pollen receipt decreased seed set for *Viola* significantly more than it did for *Oxytropis*, *Lithospermum* and *Linum*, and there was a marginally significant difference in the effect on *Viola* compared to *Sisyrinchium*, but the effect of treatment did not significantly differ between other treatment pairs. *Viola* and *Linum* experienced the largest relative decrease in seed set upon receipt of large quantities of *Euphorbia* pollen (Table 4.6b).

A summary of the floral restrictiveness, breeding system, natural *Euphorbia* pollen receipt rates, and effects of *Euphorbia* pollen receipt on fruit and seed set is provided in Table 4.9.

DISCUSSION

Of the native species studied here, three species had restrictive morphologies and the other four had unrestrictive morphologies. Two species were self-incompatible and the remainder could self-pollinate, albeit with reduced seed set for *Oxytropis*.

Some aspects of the breeding system studies merit attention. The low fruit set of *Comandra*, less than 20% across all treatments, is typical for the species (Piehl 1965), so does not reflect abnormal pollen or resource limitation at this study site. Thrips and ants were able to access flowers despite pollinator exclusion bags, and their presence may have led to autogamous or geitenogamous self-pollination of unmanipulated flowers, as seen in other study systems (Baker and Cruden 1991). Such visits or unaided self-pollination could explain low rates of fruit set seen for several of the species (*Comandra*, *Oxytropis*, *Zizea*, in this chapter, and *Viola* in chapter 2). For *Lithospermum*, pollinators were occasionally observed probing through bags, and occasional successes could account for the low fruit set of unmanipulated flowers. Finally, for *Linum*, flowers were frequently observed to self-pollinate without visitors, particularly on windy days, and this probably accounts for the relatively high fruit set of unmanipulated flowers. Bagged unmanipulated treatments were also included in most of the heterospecific pollen transfer studies, and low fruit and seed set across these treatments for all species not expected to be able to self-pollinate without pollinators confirm that exclusion of pollinators was generally successful.

Euphorbia pollen receipt significantly reduced fruit or seed set for three of the seven species tested (*Viola*, *Zizia* and *Linum*) (Table 4.9). For the other four species (*Sisyrinchium*, *Comandra*, *Lithospermum*, and *Oxytropis*), neither fruit nor seed set was significantly affected by *Euphorbia* pollen receipt overall. These results indicate that *Euphorbia* pollen, if received in large quantities, can reduce reproductive success, but that it does not necessarily do so. Results of previous heterospecific pollen transfer studies have varied, with many studies detecting a significant effect, and other studies not detecting an effect on fruit or seed set (e.g. Campbell and Motten 1985, Galen and Gregory 1989, Armbruster and McGuire 1991, McGuire and Armbruster 1991, Moragues and Traveset 2005). However, these studies have varied in study design and donor and recipient species, making it difficult to determine what factors lead to variation in study outcomes (but see Randall and Hilu 1990, Harder et al. 1993). In this study, the donor species (*Euphorbia*) was the same for seven recipient species, eliminating one potential source of variation in study outcomes.

The similarity in study designs for the seven species tested here helps to eliminate another potential source of variation in study outcomes. Although details of the study designs varied among plants, all study designs, except for *Viola* and *Sisyrinchium*, included a treatment with a large amount of *Euphorbia* pollen delivered hours in advance of conspecific pollen, and all designs included a cross-pollinate treatment. Results from

Viola and *Sisyrinchium* may nonetheless be included and compared with the other studies. For *Viola*, there was a significant negative effect on seed set of adding *Euphorbia* pollen immediately prior to conspecific pollen (see chapter 2). Because adding a time delay made the negative effect stronger for the other plant species, it is reasonable to assume that adding *Euphorbia* pollen to *Viola* with a time delay prior to application of conspecific pollen would have a similar or stronger effect. For *Sisyrinchium* neither fruit nor seed set was significantly different when *Euphorbia* pollen was added immediately prior to conspecific pollen compared to two hours prior to conspecific pollen. Thus, it can be inferred for *Sisyrinchium* that *Euphorbia* pollen receipt two to four hours prior to conspecific pollen would be unlikely to reduce fruit or seed set relative to conspecific cross-pollen treatments.

For fruit set, the statistical significance of results largely parallel effect magnitudes, as the two species whose fruit set was significantly reduced by *Euphorbia* pollen receipt, *Zizia* and *Linum*, experienced the greatest absolute difference in percent fruit set upon receipt of *Euphorbia* pollen. For *Lithospermum*, fruit set was not significantly affected by *Euphorbia* pollen receipt, and *Lithospermum* experienced a smaller absolute reduction in fruit set than *Linum* and *Zizia*. However, because *Lithospermum's* average fruit set was low, it experienced a greater relative reduction in successful flowering than *Zizia*. The GLM analysis of fruit set found no significant interaction between species and treatment, which fails to reject the possibility that for fruit set the studied species are similarly affected by receipt of large amounts of *Euphorbia* pollen. Furthermore, the GLME analysis of fruit set across species found no significant effect of treatment, which indicates that receipt of *Euphorbia* pollen receipt does not generally decrease fruit set.

For seed set per fruit, statistical significance parallels effect magnitudes even more closely than for fruit set. *Viola* and *Linum*, the two species whose seed sets were significantly affected by *Euphorbia* pollen receipt, experienced the largest relative reductions in seed set. The cross-species analysis of seed set indicated that effects of *Euphorbia* receipt differed among species, but the only statistically significant difference was that seed set was more affected for *Viola* than the other four species for which seed set was measured. Because there is a larger effect of *Euphorbia* pollen receipt on the seed set of *Viola* than the other species, it is worth considering traits that differ between *Viola* and the other species. Unlike the other species, *Viola* lacks an external stigmatic surface; instead, pollen germinates within the lumen of the style (Beattie 1969). Thus, the area available for pollen adherence may be more limiting than for other species. Additionally, pollen is drawn into the lumen during pollination (Beattie 1969), and clogging of the lumen by heterospecific pollen could prevent subsequent entry of conspecific pollen. *Euphorbia* may be particularly likely to clog the lumen, as it has sticky pollen that loosely adheres in masses connected by viscin threads (Selleck et al. 1962, Messersmith et al. 1985). Thus, *Viola* may be more vulnerable to heterospecific pollen clogging than the other species tested, for which pollen does not need to pass through a constricted opening.

Results showed some support for the hypothesis that self-incompatible species are less affected than self-compatible species by heterospecific pollen receipt (Table 4.9). Among the seven species studied here, the three species negatively affected by large quantities of *Euphorbia* pollen receipt (*Viola*, *Linum* and *Zizia*) are all self-compatible, whereas neither self-incompatible species (*Lithospermum* and *Sisyrinchium*) was negatively affected. This result supports the hypothesis that that traits associated with self-incompatibility may reduce the effects of heterospecific pollen receipt. However, two self-compatible species (*Comandra* and *Oxytropis*) were unaffected by heterospecific pollen receipt, so self-compatibility status alone does not completely predict the effect. Testing more species will be necessary to determine the generality of this relationship between breeding system and effects.

The hypothesis that a large stigmatic area buffers plants from negative effects of heterospecific pollen receipt was not supported. *Linum*, which has the largest stigmatic area of the species studied, experienced significant decreases in fruit and seed set upon receipt of *Euphorbia* pollen, whereas the three species with the smallest stigmatic areas (*Sisyrinchium*, *Comandra* and *Oxytropis*) were not significantly affected (Table 4.9). A possible explanation for why species with larger stigmas were not less affected by *Euphorbia* pollen receipt in this study is that more *Euphorbia* pollen was applied to large than small stigmas. For a fixed amount of heterospecific pollen, a smaller portion of a large stigma would be occluded than for a small stigma, but because proportionately more *Euphorbia* pollen was applied to larger stigmas in this study, stigmatic occlusion would not have been lessened for larger stigmas. Effects of stigma size may also have been obscured by the large quantities of heterospecific pollen applied. For example, *Linum* received approximately 3-fold more total heterospecific pollen in the HPT-small treatment and many fold more *Euphorbia* pollen in the HPT-large treatments than it received under natural conditions (compare *Euphorbia* pollen receipt in Table 4.4 versus Table 4.9). Thus, *Linum*'s large stigmatic surface area relative to the other species would not have mitigated effects of heterospecific pollen receipt in this study, even if it would have mitigated effects of receipt of lesser quantities of heterospecific pollen under natural conditions.

The hypothesis that species that receive more heterospecific pollen in nature will be less affected by it was not supported by this study. My studies show that *Linum* and *Viola* experienced the second and third highest rates of natural *Euphorbia* pollen receipt per stigma, respectively, among the 29 species examined, and even after adjusting for stigmatic area, both species were among the top five recipients of *Euphorbia* pollen (chapter 3). However, both of these species were negatively affected by *Euphorbia* pollen receipt. In contrast, *Sisyrinchium, Oxytropis*, *Lithospermum* and *Comandra*, which received substantially less heterospecific pollen, were not significantly affected by it. Subsequent tests of this hypothesis with lower rates of heterospecific pollen receipt, applied in equal quantities to all species, would provide a more powerful test of this hypothesis.

There was also no support for the hypothesis that species with restrictive morphologies are more affected by heterospecific pollen receipt. *Lithospermum* and *Oxytropis*, the two species with the most restrictive morphologies, did not show significantly reduced fruit or seed set upon application of *Euphorbia* pollen, whereas *Linum* and *Zizia*, both of which have unrestrictive morphologies, experienced significantly reduced fruit or seed set upon receipt of *Euphorbia* pollen. *Viola* has a restrictive morphology and was significantly affected by *Euphorbia* pollen receipt, but it does not support the mechanism because is exceptional in that it, unlike most other species with restrictive morphologies, naturally receives large amounts of heterospecific pollen. Thus, although *Viola* follows the predicted pattern, it does not conform to the logic of the hypothesis, namely that restrictive flowers are more affected by heterospecific pollen receipt when it occurs because they receive less heterospecific pollen under natural conditions.

These studies did not determine the mechanism of interference of *Euphorbia* pollen with the reproduction of *Viola*, *Zizia* and *Linum*. The lack of a significant effect of small amounts of *Euphorbia* pollen on the fruit or seed set of *Zizia* and *Linum*, the two species for which this was studied, suggests that if allelopathy is responsible, then the allelopathic constituents must be present only in low concentrations. Alternatively, *Euphorbia* may have clogged stigmatic surfaces, preventing adherence or germination of conspecific pollen. This explanation would also account for why *Euphorbia* pollen only significantly affected fruit or seed set of *Zizia* and *Linum* when applied in large quantities. The hypothesis of stigmatic clogging is supported by the finding that for *Linum* less conspecific pollen adhered on stigmas to which large quantities of *Euphorbia* pollen had been applied.

The greater negative effects for fruit and seed set of *Linum* and *Zizia* when an extended delay occurred prior to conspecific pollen receipt suggests that the germination of *Euphorbia* pollen is involved in the mechanism of interference. The importance of the time delay for *Linum* is also apparent in the finding that less conspecific pollen adhered if it was applied with a time delay after application of *Euphorbia* pollen. This effect of a time delay could be due to release of allelopathic chemicals during germination, by competition with conspecific pollen tubes for space in the style, or by causing the stigma to senesce prematurely. *Euphorbia* pollen was found to germinate on and penetrate the stigmatic surface of *Linum* (unpublished data). It is possible that these events elicited reactions in *Linum* associated with being successfully pollinated, leading the stigma to lose receptivity. Other studies have shown similar effects of time delays. The seed set of *Ipomopsis aggregata* (Polemoniaceae) was not reduced if heterospecific pollen from *Delphinium nelsonii* (Ranunculaceae) was applied simultaneously with conspecific pollen (Kohn and Waser 1985), but seed set was reduced by inducing early stigma closure if heterospecific pollen was applied several hours earlier than conspecific pollen (Waser and Fugate 1986). A related study found that seed set of *Ipomopsis aggregata* was also reduced by early application of heterospecific pollen from *Castilleja lilnariaefolia*

(Scrophulariaceae), but not when it was applied simultaneously (Caruso and Alfaro 2000).

Although time delay can increase negative effects of heterospecific pollen receipt on fruit or seed set, such a delay is not is not always necessary for heterospecific pollen to reduce pollination success. In this study, *Euphorbia* pollen applied immediately prior to conspecifc pollen negatively affected reproduction in *Viola*, and other studies have shown that heterospecific pollen reduces seed set even if applied simultaneously with conspecific pollen (Randall and Hilu 1990, Brown and Mitchell 2001). A time delay does not always lead to a reduction in reproductive success, as evidenced by a lack of a significant effect on *Lithospermum*, *Sisyrinchium*, <u>Comandra</u>, and *Oxytropis* in this study. Similarly, Kwak and Jennersten (1991) found that heterospecific pollen did not significantly affect seed set of *Viscaria vulgaris* (Caryophyllaceae), whether applied simultaneously with or several hours prior to conspecific pollen. As a whole, these various results suggest that heterospecific pollen receipt reduces seed set more if received a well in advance of conspecific pollen, but that timing of receipt is not the only factor influencing the effect of heterospecific pollen receipt.

The studies presented here show how different plant species respond to receiving large quantities of heterospecific pollen receipt. However, field studies show that in nature receipt rates of *Euphorbia* and other heterospecific pollen, are substantially lower than the quantities applied in these studies and therefore unlikely to affect the reproductive success. For *Linum*, for example, natural pollen receipt averaged only five *Euphorbia* pollen grains per stigma, approximately 2% as much as the pollen applied in the HPT-large treatments. Similarly, *Viola* naturally received an average of four *Euphorbia* pollen grains per stigma, compared to an average of 103 in the 2004 heterospecific pollen transfer study. Pollen counts were not performed in the other hand-pollination studies, but it is likely that similar discrepancies occur between natural and artificial pollen receipt rates for the other species. Thus, the study supports the contention of Brown and Kodric Brown (1979) that stigmatic occlusion is unlikely to naturally occur at high enough rates to substantially influence fruit or seed set.

Heterospecific pollen receipt may nonetheless be important if pollen is allelopathic, allowing it to have an effect at densities too low to substantially occlude the stigma. Even for pollen demonstrated to be allelopathic, natural pollen receipt rates may be too low to lead to allelopathic effects in some cases (Murphy and Aarssen 1989) but may be adequate to substantially reduce success in other cases (Murphy 2000). Subsequent studies may further improve our understanding of the effect of heterospecific pollen receipt by continuing to compare effects across species with different breeding systems, stigma sizes and natural rates of heterospecific pollen receipt. As more studies are performed, meta-analyses may also begin to shed light on what plant traits influence susceptibility to harm from heterospecific pollen receipt.

Table 4.1 Significance testing of fixed effects of year and pollination treatment on *Oxytropis* fruit and seed set, including only cross-pollinate and HPT-large treatments and results from 2005 and 2006.

	Numerator d.f.	Denominator d.f.	*F*	*P*
a. Fruit set				
Intercept	1	218	13.238	0.0003
Year	1	30	3.054	0.09
Treatment	1	218	0.002	0.968
b. Seed set				
Intercept	1	124	231.308	<0.0001
Year	1	29	1.204	0.28
Treatment	1	124	2.766	0.10

Table 4.2 Significance testing of fixed effects of conspecific pollen receipt and *Euphorbia* pollen receipt on *Zizia* fruit set for 2006 study. The unmanipulated treatment was excluded from this analysis.

	Numerator d.f.	Denominator d.f.	*F*	*P*
Intercept	1	348	3.132	0.078
Euphorbia Quantity	2	348	6.628	0.002
Conspecfic Quantity	1	348	0.029	0.87

Table 4.3 Significance testing of fixed effects of year and *Euphorbia* pollen receipt (large or none) on *Zizia* fruit set for the 2005 and 2006 studies combined.

	Numerator d.f.	Denominator d.f.	*F*	*P*
Year	1	22	0.264	0.61
Treatment	1	328	9.066	0.003

Table 4.4 Quantities (± s.d.) of *Euphorbia* and conspecific pollen found on stigmas for the 2006 *Linum* **a.** HPT quantity study and b. HPT timing study.

	Sample size	*Euphorbia* receipt mean	*Euphorbia* receipt s.d.	*Linum* receipt mean	*Linum* receipt s.d.
a. Quantity study					
Cross-pollinate	15	2.2	2.3	64.3	25.0
HPT-small	15	47.1	47.1	59.9	26.0
HPT-large	15	338.1	106.2	44.9	24.9
b. Timing study					
Cross-pollinate	11	3.1	2.6	61.2	24.1
HPT-immediate	13	259.8	80.3	71.0	28.1
HPT-delay	13	272.4	90.4	29.5	28.5

Table 4.5 Significance testing of fixed effects of year and *Euphorbia* pollen receipt (large or none) on *Linum* **a.** fruit set and **b.** seed set for the 2005 and 2006 studies combined.

	Numerator d.f.	Denominator d.f.	*F*	*P*
a. Fruit set				
Intercept	1	48	2.694	0.11
Year	1	50	2.094	0.15
Treatment	1	48	7.630	0.008
b. Seed set				
Intercept	1	100	817.2966	< 0.0001
Year	1	48	0.0007	0.9783
Treatment	1	100	24.5098	< 0.0001

Table 4.6 Fruit set per flower (**a**) and seed set per fruit (**b**) for control and treatment flowers for seven species with calculations of absolute and relative effect sizes. Absolute effect size is calculated as the difference in fruit or seed set between treatment and control flowers. Relative effect size is calculated as natural log of treatment fruit or seed set divided by control fruit or seed set. For both absolute and relative effect, negative values indicate reduced success upon receipt of *Euphorbia* pollen.

Species	Control	HPT	Absolute effect	Relative effect
a. Fruit set				
Comandra	0.107	0.125	0.018	0.154
Linum	0.631	0.469	- 0.162	- 0.297
Lithospermum	0.365	0.283	- 0.082	- 0.255
Oxytropis	0.643	0.640	- 0.003	- 0.004
Sisyrinchium	0.791	0.860	0.070	0.085
Viola	0.333	0.389	0.056	0.154
Zizia	0.646	0.511	- 0.134	- 0.234
b. Seed set				
Linum	8.39	6.43	-1.96	- 0.266
Lithospermum	2.21	2.00	-0.21	- 0.010
Oxytropis	*6.94*	*5.87*	*-1.06*	*- 0.166*
Sisyrinchium	*19.65*	*21.09*	*1.44*	*0.071*
Viola	25.04	16.10	- 8.94	- 0.442

Table 4.7 Significance testing of fixed effects of species, *Euphorbia* pollen receipt (large or none), and their interaction and on *Linum* fruit set.

	d.f.	Deviance explained.	d.f.	Residual Deviance	*P* (Chi)
Null			13	189.009	
Species	6	172.395	7	16.613	< 0.0001
Treatment	1	6.167	6	10.446	0.013
Species by treatment	6	10.446	0	0.000	0.11

Table 4.8 Significance testing of fixed effects of species, heterospecific pollen transfer of large amounts of *Euphorbia* pollen, and their interaction on seed set.

	Numerator d.f.	Denominator d.f.	*F*	*P*
Intercept	1	425	4180.95	< 0.0001
Species	4	425	82.23	< 0.0001
Treatment	1	425	10.32	0.0014
Species by treatment	4	425	5.74	0.0002

Table 4.9 Summary of floral morphology, stigmatic area, breeding system, natural rates of *Euphorbia* pollen receipt, and relative effects of *Euphorbia* pollen on fruit and seed set. Categorization of morphology, stigmatic area, and rates of *Euphorbia* pollen receipt are from study of natural incidence of *Euphorbia* pollen (chapter 3). For breeding system results, plants were considered self-compatible (s.c.) if the fruit and seed set of self- and cross-pollinated flowers were not significantly different; self-incompatible (s.i.) if fruit and seed set of self-pollinated flowers was near zero, and partially self-compatible (p.s.c.) if fruit and seed set were intermediate between s.c. and s.i..

Species	Floral Morphology	Stigmatic area (mm)	Breeding System	*Euphorbia* receipt rate	Relative effect Fruit set	Relative effect Seed set
Comandra	Unrestrictive	0.03	s.c.	0.233	0.154	na
Linum	Unrestrictive	0.25	s.c.	4.8	- 0.297	- 0.266
Lithospermum	Restrictive	0.11	s.i.	0.067	- 0.255	- 0.010
Oxytropis	Restrictive	0.03	p.s.c.	0.0	- 0.004	- 0.166
Sisyrinchium	Unrestrictive	*0.05*	s.i.	0.533	0.085	0.071
Viola	Restrictive	0.07	s.c.	3.95	0.154	- 0.442
Zizia	Unrestrictive	(t.b.d.)	s.c.	n.a.	- 0.234	n.a.

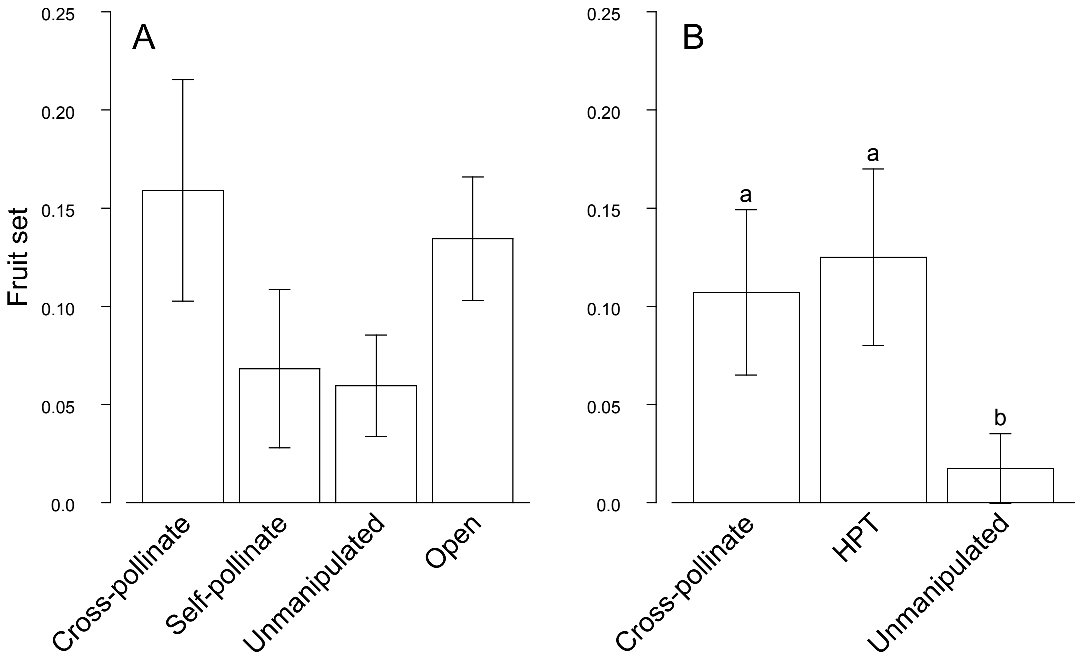

Figure 4.1 Fruit set per flower (± s.e.) for *Comandra* exposed to different pollination treatments. A. Breeding system study B. Heterospecific pollen transfer (HPT) study. Different letters indicate significant differences within each experiment at $P < 0.05$; for the breeding system study differences were not statistically significant.

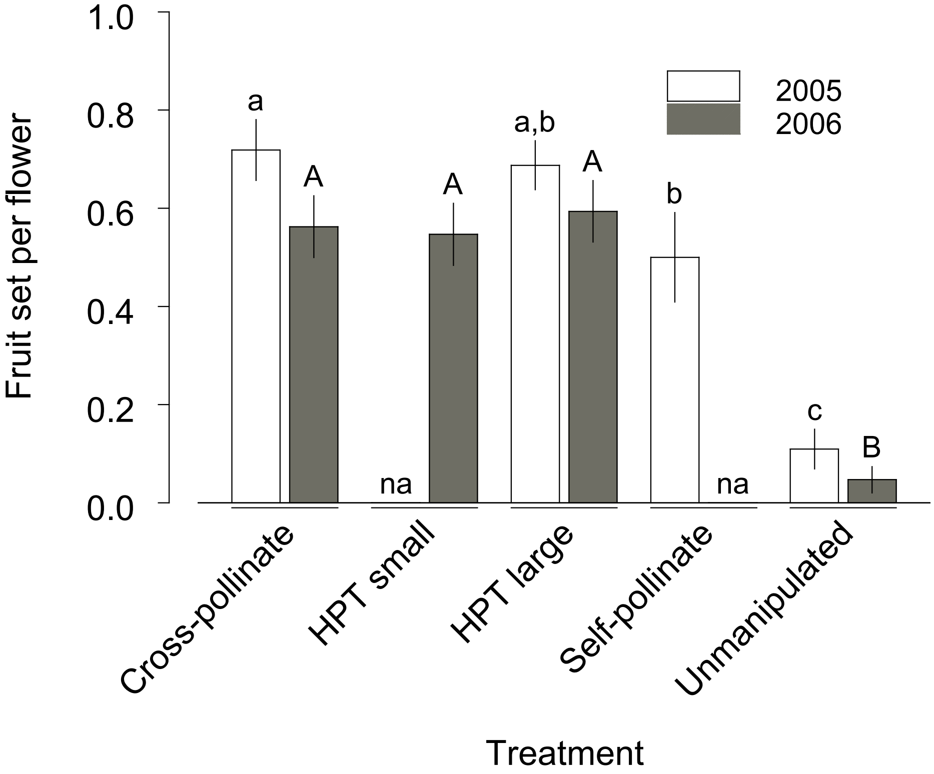

Figure 4.2 Fruit set per flower (± s.e.) in for *Oxytropis* exposed to five different pollination treatments, for 2005 and 2006. Treatments indicated 'na' were not performed in one year. Lowercase letters indicate significant differences between treatments in 2005 and uppercase letters indicate differences among treatments in 2006 at $P < 0.05$.

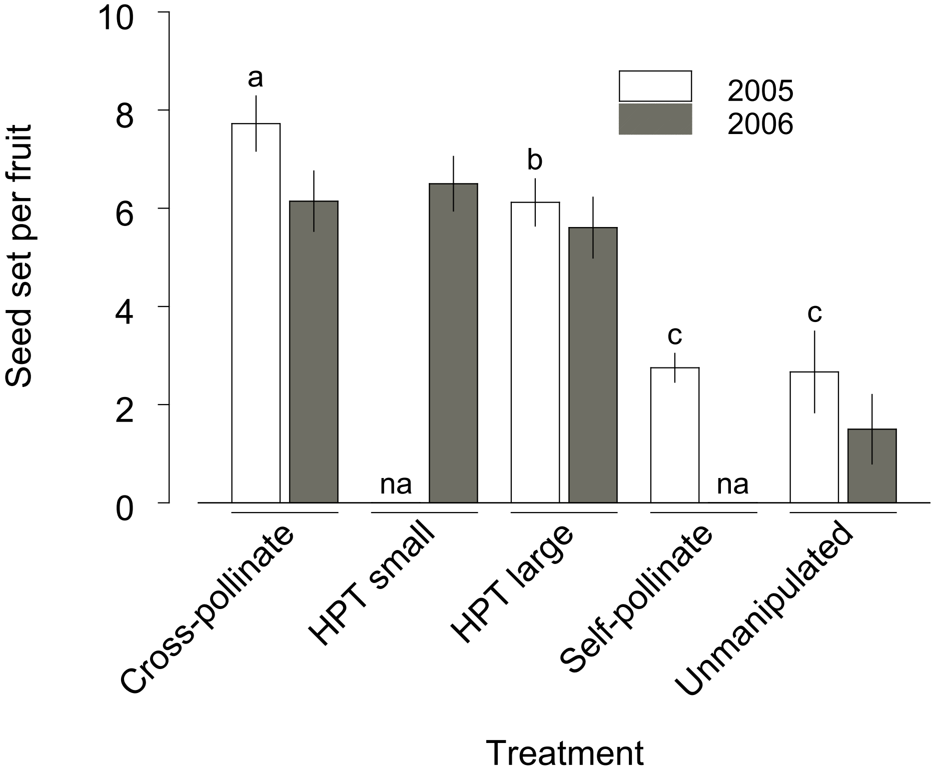

Figure 4.3 Seed set per fruit (± s.e.) in for *Oxytropis* exposed to five different pollination treatments, for 2005 and 2006. Treatments indicated 'na' were not performed in one year. Letters indicate significant differences between treatments in 2005 at $P < 0.05$. No significant effects occurred in 2006.

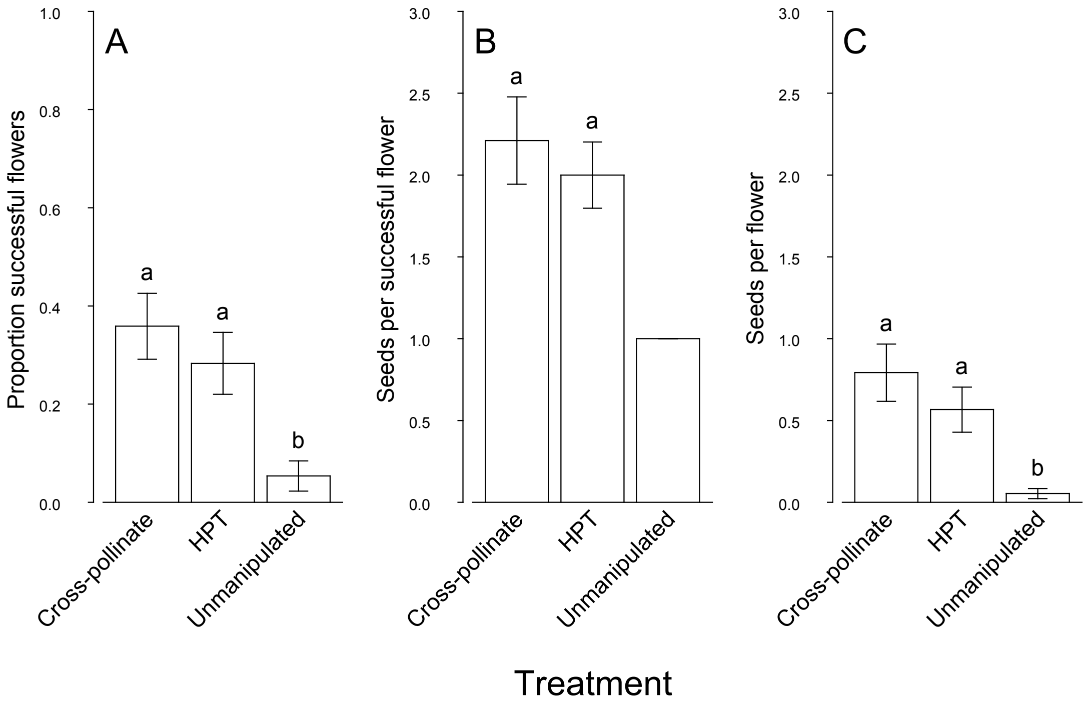

Figure 4.4 For *Lithospermum*, **A**. fruit set (proportion of flowers developing at least one fruit) (± s.e.), **B**. number of seeds per successful flower (± s.e.), and **C**. seeds per flower (± s.e.) for three pollination treatments. No error bar or letter indicating significant difference is shown for the unmanipulated treatment in pane B because the only two flowers to set fruit in this treatment each developed one seed.

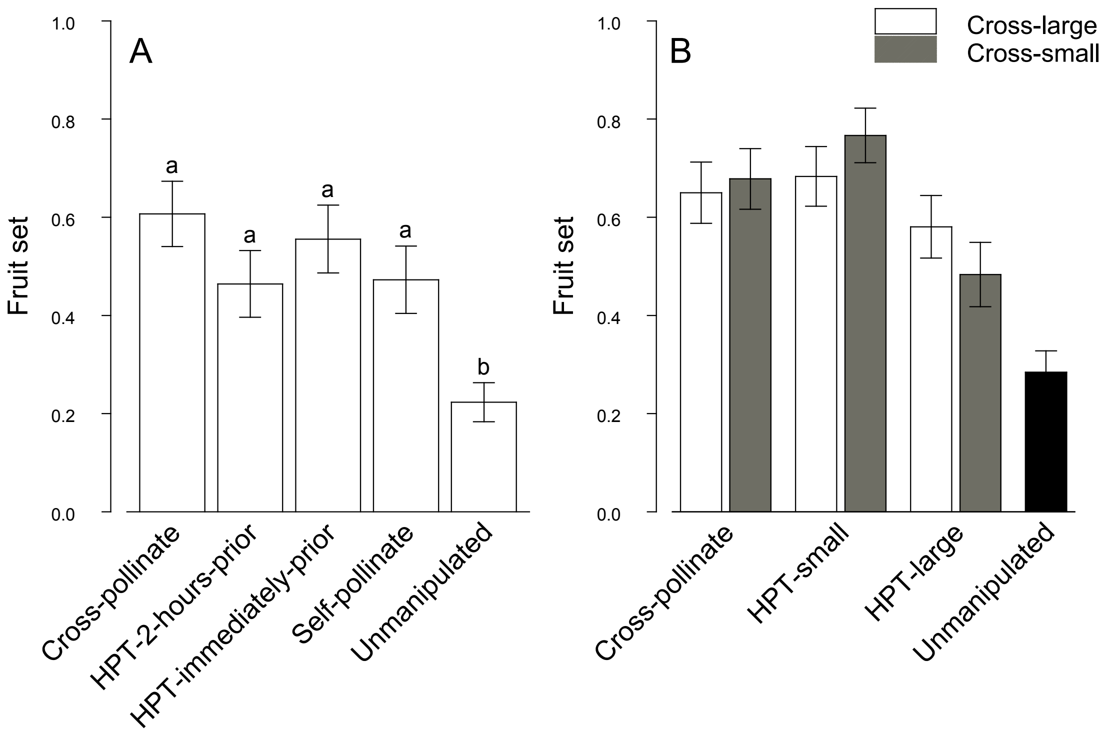

Figure 4.5 Fruit set (± s.e.) for *Zizia* **A**. for 2005 study, for five pollination treatments. Different letters indicate statistical differences at $P < 0.05$. **B.** for 2006 study, fruit set per flower (± s.e.) for unmanipulated flowers, and flowers receiving three different levels of *Euphorbia* pollen (large, small or none) and two different levels of conspecific pollen (large or small quantities). See results for significance testing.

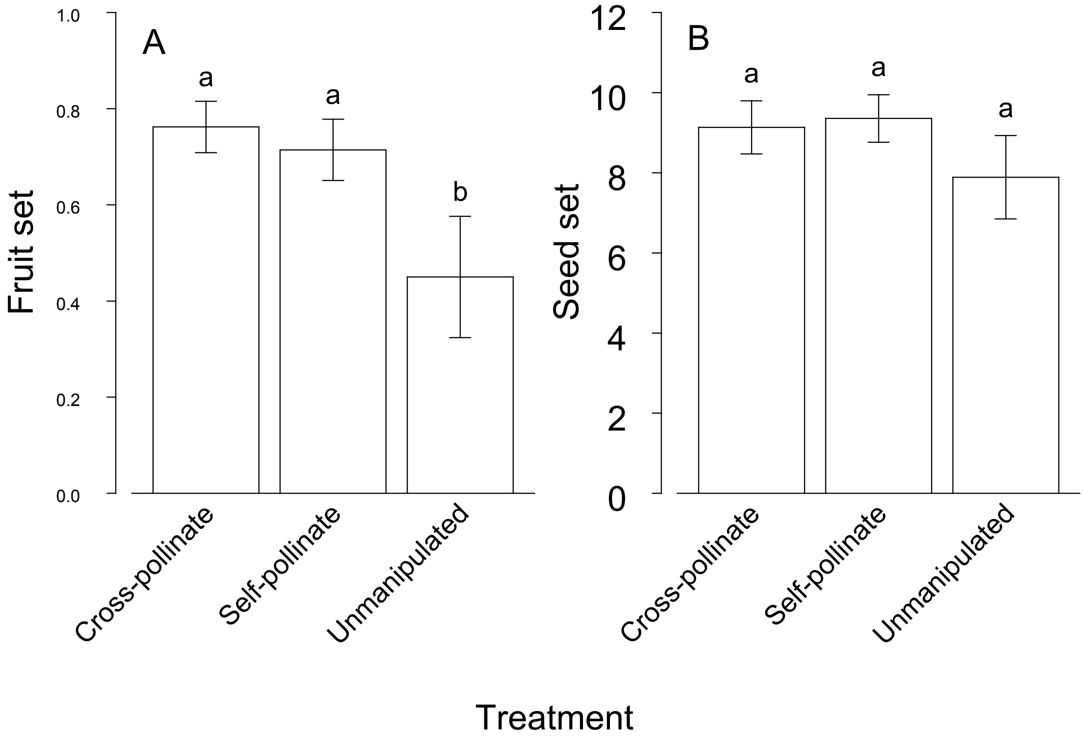

Figure 4.6 For the breeding system study of *Linum*, **A**. fruit set per flower (± s.e.), and B. seed set per fruit (± s.e.) for three pollination treatments. For both measures, different letters indicate statistical differences at $P < 0.05$.

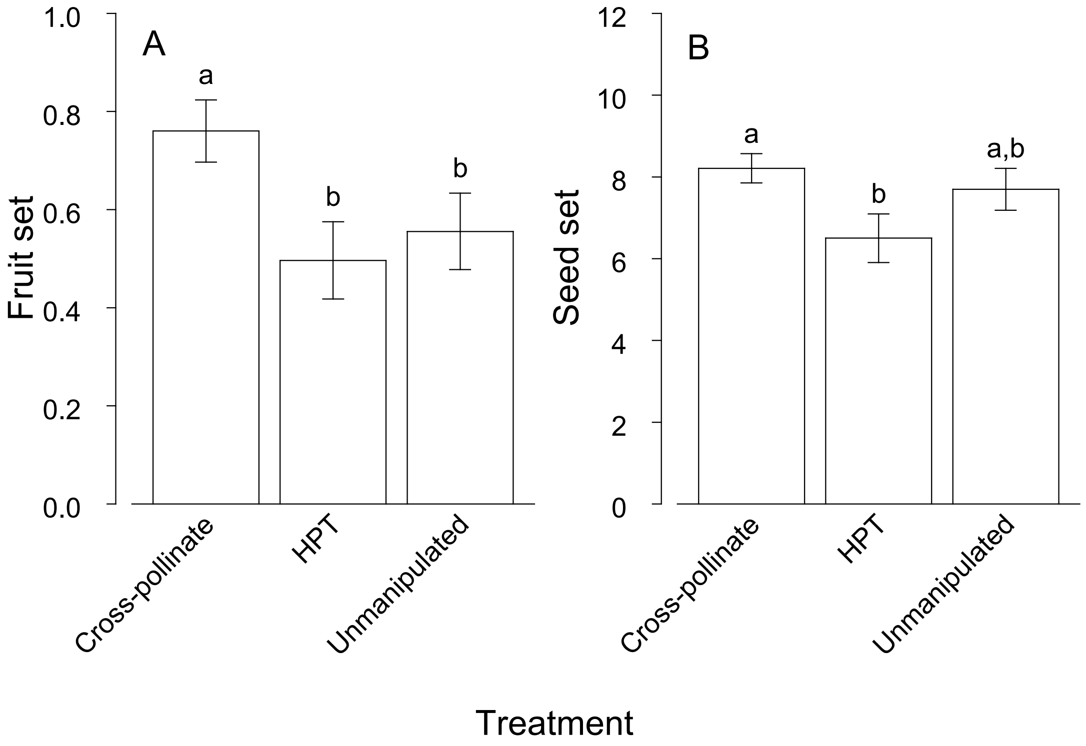

Figure 4.7 For *Linum* HPT study in 2005, **A**. fruit set per flower (± s.e.), and **B**. seed set per fruit (± s.e.) for three pollination treatments. For both measures, different letters indicate statistical differences at $P < 0.05$.

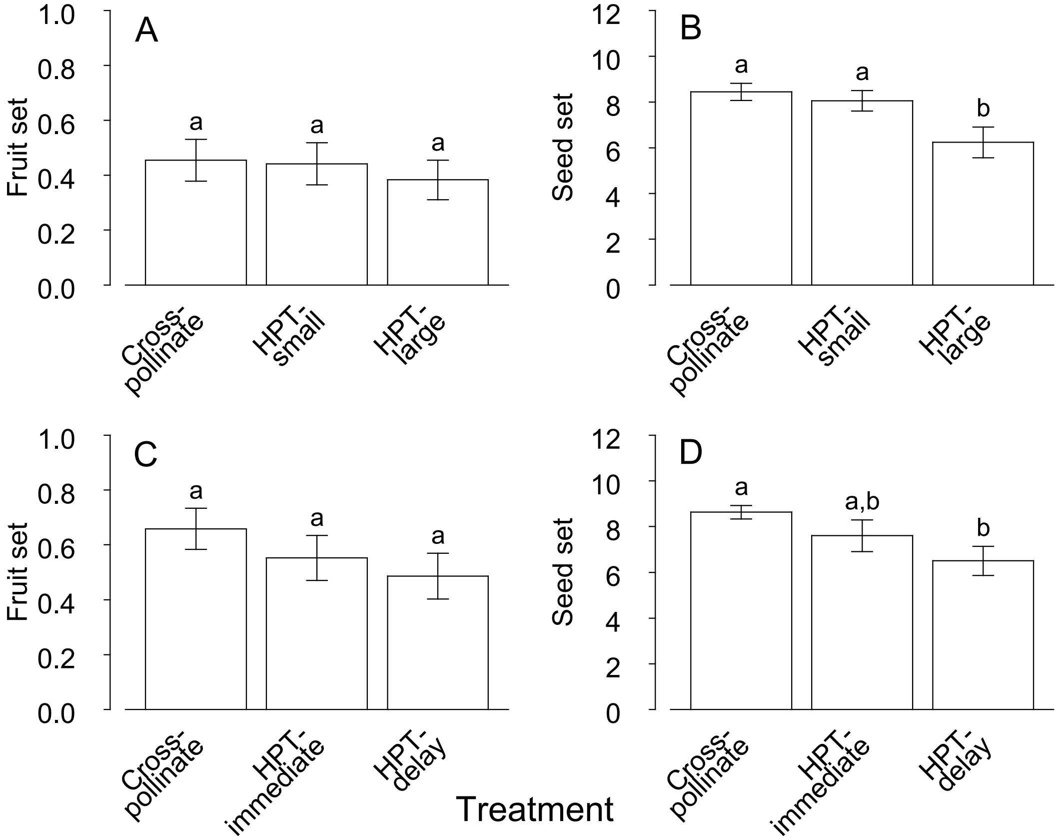

Figure 4.8 For *Linum* HPT studies in 2006, **A**. fruit set per flower (+ s.e.) for quantity study, **B**. seed set per fruit (+ s.e.) for quantity study, **C.** fruit set per flower (+ s.e.) for timing study, and **D.** seed set per fruit (+ s.e.) for timing study. In each case, different letters indicate statistical differences at $P < 0.05$.

Chapter 5

A Modeling Framework to Predict Effects of Pollinator Abundance, Constancy and Degree of Pollen Carryover on Competition by Interspecific Pollen Loss

ABSTRACT

Plant populations compete by pollen loss when interspecific pollen transfer leads to decreased conspecific pollen receipt, and consequently decreased fecundity. It has been suggested that pollen carryover mitigates this effect because pollen deposition over multiple flowers allows for successful pollination even if pollinators make intervening heterospecific visits. I present analytical and computational models to investigate how several factors influence competition by pollen loss. Like earlier models, my models demonstrate that increased relative floral abundance, visitation rates, and pollinator constancy decrease competition by pollen loss, but my results demonstrate that some earlier models overestimate the effect of pollen loss because they fail to account for diminishing returns of multiple visits. My analytical models demonstrate that carryover has no effect on average conspecific pollen receipt, but that carryover decreases competition if it does not come at a cost to earlier visited flowers. Additionally, I present a simulation model which indicates that carryover affects the frequency distribution of pollen receipt, and the effect of pollen carryover on competition by pollen loss depends on the relationship between pollen receipt and reproductive success. Rare flowers benefit from low carryover rates, especially if their pollen receipt thresholds are high, but common flowers with low thresholds benefit from increased carryover. Finally, parameterization of the simulation model with empirical values for an herb that has been demonstrated to experience competition by pollen loss shows that carryover may increase fecundity. Together, these models suggests that carryover reduces competition by pollen loss, but only in some instances, and further that pollen receipt thresholds, degree of carryover, and rarity are likely to be correlated traits in plants.

INTRODUCTION

Interspecific interactions among plants for pollination are of interest because they are predicted to lead to ecological effects, including competitive exclusion for simultaneously flowering plants (Levin and Anderson 1970) or coexistence for competing species with staggered flowering times (Ishii and Higashi 2001). Additionally, such interactions may lead to evolutionary effects, including selection on flowering phenology (Waser 1978a, Agren and Fagerstrom 1980, but see Kochmer and Handel 1986), floral morphology (Armbruster et al. 1994, Hapeman and Inouye 1997, Hansen et al. 2000), and floral specialization under some conditions (Feinsinger 1983, Kunin and Shmida 1997).

Competition by interspecific pollen loss occurs when pollinators deposit pollen of one species on flowers of a different species, resulting in reduced conspecific deposition to the initial species, and it is one of several mechanisms by which plants of different species may compete for pollination (Rathcke 1983, Waser 1983). Competition by interspecific pollen loss has been predicted by analytical models (Levin and Anderson 1970, Straw 1972, Campbell 1986) and simulation models (Waser 1978b). Furthermore, this mechanism has been detected in laboratory experiments (Feinsinger and Busby 1987, Feinsinger and Tiebout 1991, Murcia and Feinsinger 1996), and found to contribute to reduced fruit or seed set in the field (Campbell and Motten 1985). According to a previous model, pollen carryover, which refers to the retention of some pollen on a pollinator over multiple subsequent flowers, mitigates competition by pollen loss (Feldman et al. 2004), but this claim has not been systematically investigated. In this paper, I expand on previous models of competition by interspecific pollen loss by developing an analytical model and a closely related simulation model that allow investigation of the effects of several variables on the severity of competition by pollen loss. The variables investigated include plant relative abundance, pollinator visitation rates and constancy, degree of pollen carryover, and thresholds of pollen receipt necessary for pollination success. Additionally, the models presented here incorporate more realistic assumptions than some earlier models, as explained below.

The models developed here build upon the model of Levin and Anderson (1970), which represents two annual plant species that rely on shared generalist pollinators. Their model assumes that no carryover occurs, so only pollinator flights between conspecific flowers (i.e. intraspecific flights) result in pollination, and they assume that the relative fecundity of each species is proportional to the number of intraspecific flights each receives. With these assumptions and no pollinator preference, the model predicts positive frequency dependence and competitive exclusion of the minority species. Straw's (1972) model is similar, but considers a pollinator's choice of flowers as a first-order Markov process in which the flower species next visited is influenced by the species most recently visited. According to both models, pollinator constancy, defined as a tendency to visit another flower of the same species on the next visit more frequently than expected by chance alone, results in decreased competition and slower competitive exclusion.

These models of Levin and Anderson (1970), and Straw (1972) include the unrealistic assumption that fecundity increases linearly with the number of intraspecific flights. As acknowledged by Straw (1972), this assumption may be realistic for low visit rates, but for high visit rates pollination success is expected to asymptote because additional visits bring pollen to flowers that have already received adequate conspecific pollen. Pollen grains in excess of the number required to fertilize all ovules would not be expected to increase fruit or seed set, and consistent with this idea, many supplemental pollination studies fail to detect a significant increase in fruit or seed set (Burd 1994, Ashman et al. 2004). Furthermore, a variety of empirical studies have detected an asymptotic or decelerating relationship of fruit or seed set as a function of pollen grains received (e.g. Campbell 1986, Niesenbaum 1999, Cane and Schiffhauer 2003). If pollinators are saturating and pollen loss is the only mechanism of competition, then flowers of competing species should be equally successful regardless of relative abundance; however, the aforementioned models would predict success to be disproportionately high in the more common species. Subsequent analytical models and the models presented here incorporate an asymptotic relationship between visits and success by instead calculating the probability that a flower remains unpollinated after some number of independent pollinator visits (Campbell 1986, Ishii and Higashi 2001, Feldman et al. 2004).

Another possible relationship between pollen receipt and pollination success is that of a threshold below which fruit or seed set is disproportionately low, but above which it is disproportionately high (e.g. Cane and Schiffhauer 2003). This pattern may occur if low pollen receipt results in fewer ovules fertilized and developing fruits with few seeds are likely to abort (Stephenson 1981), or if a critical quantity of pollen exudates is required for pollen germination or penetration of the stigma (Brewbaker and Majumder 1961, Jennings and Topham 1971). Even when pollination success is an asymptotic function of conspecific pollen receipt, this may be approximated by a threshold below which fruit set or seed set is unlikely but above which it is likely. Because pollination success is likely to be a non-linear function of the quantity of pollen received, models of competition by pollen loss should consider the frequency distribution of pollen receipt as well as average receipt.

An unrealistic assumption of most previous models of competition by interspecific pollen loss is that no pollen carryover occurs, so non-consecutive visits to one species are assumed to not result in pollination. In contrast to early assumptions that all pollen acquired from one flower is deposited on the next, a variety of empirical studies demonstrate that pollinators typically distribute their pollen load over multiple flowers (e.g. Thomson and Plowright 1980, Price and Waser 1982, Morris et al. 1994). Feldman et al. (2004) show that pollen carryover may reduce competition by pollen loss because pollination can be successful even if a pollinator visits intervening heterospecific flowers between conspecific visits. However, their model may be unrealistic because it assumes that pollen carryover to later flowers comes at no cost to earlier visited flowers. Varying pollen carryover rates have also been incorporated into a simulation model of pollen loss (Campbell 1986), but this model represents two pollinator taxa that also vary in other ways, so the effects of different carryover rates are not isolated.

In this paper, I develop an analytical model of competition by pollen loss that includes pollinator constancy as a variable and accounts for diminishing returns of multiple pollinator visits, and I demonstrate that the results of Levin and Anderson's

(1970) model are a special case of the model presented here for the limit as the pollinator visitation rate approaches zero. In addition, I examine two related models that incorporate pollen carryover either 1. by assuming that pollen is carried over across some specified number of flowers or 2. that a constant proportion of pollen is deposited on each flower visited. These two approaches to modeling carryover lead to different conclusions about the effect of carryover on competition by pollen loss. To address these differences, I present a simulation model that incorporates pollen carryover and pollen loss and allows determination of the frequency distribution of pollen receipt. These frequency distributions help resolve the different conclusions from the two analytical models including carryover by showing that pollen carryover has no effect on average pollen receipt, but that carryover does affect the distribution of pollen. For some non-linear relationships between pollen receipt and pollination success, carryover is expected to reduce competition by pollen loss.

THE MODEL

Analytical model without pollen carryover

The initial model developed here is similar to that of Campbell (1986) for the condition of plants competing by pollen loss but not by stigmatic interference. Consider a community of two simultaneously flowering self-incompatible plants whose spatial distributions are well-mixed at the scale of typical pollinator flight distances. Like Levin and Anderson (1970), I consider generalist pollinators that visit both flowering species, and initially, I assume initially that the pollinators show no constancy. Consequently, the probability of a pollinator visiting a given species on any particular visit is simply the proportion of that flower in the entire community.

Once a flower has been pollinated, no additional benefit accrues from subsequent pollinator visits, an assumption not represented by the models of Levin and Anderson (1970) or Straw (1972). This assumption necessitates specifying a distribution of the number of pollinator visits each flower receives, which I represent with a Poisson distribution around some expected number of visits. For the time being, the model will also be limited to the scenario in which no pollen carryover occurs, so only conspecific flights are capable of leading to seed production. Then, for each visit of a pollinator to a flower of species *A*, the probability that the pollinator had most recently visited another flower of the same species is simply the proportion of species *A* in the entire community, represented by *a*. Thus, the probability that the pollinator most recently visited some other species is $[1 - a]$.

It follows that the probability, $P(s)$, that a flower is successfully pollinated after a given number of visits, *i*, is given by:

$$P(s \mid i) = 1 - (1 - a)^i \qquad (5.1)$$

Given a Poisson distribution of the number of visits received around some expected number of visits, λ, then the probability that a flower of species A receives exactly *i* visits can be calculated by:

$$P(i) = \frac{e^{-\lambda} \lambda^i}{i!} \qquad (5.2)$$

The probability that a flower is successfully pollinated then can be calculated as one minus the probability of failure, with the probability of failure calculated as the joint probabilities of receiving some number of visits *i* and being successfully pollinated having received *i* visits, summed across all possible numbers of visits:

$$P(s) = 1 - \sum_{i=0}^{\infty} [\frac{e^{-\lambda} \lambda^i}{i!} (1 - a)^i] \qquad (5.3)$$

where P_{sp} indicates the probability of successful pollination. By performing algebraic manipulation, and recognizing the infinite series to be a Taylor series expansion of e^x, where *x* equals $\lambda(1\text{-}a)$, equation (5.3) simplifies to:

$$P(s) = 1 - e^{-\lambda a} \qquad (5.4)$$

Thus, the probability of pollination success increases with an increase in the expected number of visits or the proportion of the community comprised by that species (Fig. 5.1).

To allow a full comparison with the model of Levin and Anderson, pollinator constancy can be incorporated into this model by assuming that upon leaving a flower, there is some probability (*c*) that a pollinator chooses to visit another flower of the same species. Otherwise, there is a probability (1-*c*) that the pollinator randomly chooses the next species to visit. Given these assumptions, the following equation may be used to calculate the probability of successful pollination for plants potentially receiving redundant pollinator visits (see Appendix A for derivation):

$$P(s) = 1 - e^{-\lambda(c+(1-c)a)} \tag{5.5}$$

As with the Levin and Anderson model, increased rates of pollinator constancy predict an increased probability of pollination success, indicating that competition by pollen loss is less severe (Fig. 5.2). As indicated by Fig 5.2., when pollinators are completely constant (c = 1), relative abundance has no effect on expected pollination success, and only flowers that receive no visits are unsuccessful.

Population Dynamic Model

Levin and Anderson (1970) initially presented their model of competition by pollen loss as a discrete-time population dynamic model of two competing annual plant species. The species were assumed to be identical in all respects, and the community was assumed to be saturated, such that the proportion of plants of each species in the next generation was equal to the proportion of seeds contributed by each species in the current generation. For comparison to their original model, the same approach is applied here, using the derived probabilities of seed set with and without pollinator constancy and pollen carryover.

The proportional abundance of a species in the next generation is assumed to equal the proportion of seeds in the current generation produced by that species out of all seeds produced by all species. To calculate this for a two-species community, it is first necessary to calculate an index of seed production for each species, A and B, by multiplying the proportion of the community each comprises by its per flower probability of seed set given its relative abundance, as determined by Equation 5.4. Then, to keep the overall community size constant, the index of seed production for each species is divided by the sum of the indices of seed production for both species, leading to the following equations:

$$a_{t+1} = \frac{a_t(1-e^{-a_t\lambda})}{\left[a_t(1-e^{-a_t\lambda}) + b_t(1-e^{-b_t\lambda})\right]}$$

(5.6 a,b)

$$b_{t+1} = \frac{b_t(1-e^{-b_t\lambda})}{\left[a_t(1-e^{-a_t\lambda}) + b_t(1-e^{-b_t\lambda})\right]}$$

Because $b_t = 1 - a_t$, these equations can be simplified to:

$$a_{t+1} = \frac{a_t(1-e^{-a_t\lambda})}{\left[a_t(1-e^{-a_t\lambda}) + (1-a_t)(1-e^{-(1-a_t)\lambda})\right]} \qquad (5.7)$$

To consider the dynamics of this system, it is useful to consider a graph of the a_t v. a_{t+1} (Fig 5.3). Wherever the function is over the line y = x, the population size of *a* is increasing, and wherever the function is below this line, the population size of *a* is decreasing. Furthermore, the greater the distance from the function to the line y = x, the faster the population size is changing.

Several important findings are apparent in Fig 5.3. First, as in the Levin and Anderson (1970) model, whichever species is initially more abundant increases monotonically, so competitive exclusion is inevitably approached. Second, when the expected number of visits is quite small, the shape of the curve resembles that in the Levin and Anderson model. The similarity between the two models can be demonstrated by considering that as λ approaches zero, then, using L'Hopital's Rule, Equation 7 approaches

$$a_{t+1} = \frac{a_t^2}{\left[a_t^2 + (1-a_t)^2\right]} \qquad (5.8)$$

This is equivalent to the Levin and Anderson models when pollinators exhibit no constancy. However, as the expected number of visits, λ, increases, the curve moves closer to the line y = x, indicating that the proportions of the two species change more slowly. In contrast, the Levin and Anderson model assumes that the rate at which competitive displacement is approached is independent of the expected number of visits. Thus, under the condition that pollinators display no constancy, the Levin and Anderson model may be considered a special case of the model presented here for the condition that the rate of pollinator visits approaches zero.

A population dynamic model that includes pollinator constancy may also be derived (see Appendix A). This model, like the Levin and Anderson model, demonstrates that increased pollinator constancy leads to slower rates of competitive exclusion (Fig 5.3). For any given rate of pollinator constancy, the model developed here predicts a slower rate of competitive exclusion than the Levin and Anderson model, and the Levin and Anderson model represents the limit of this model as the number of pollinator visits approaches zero (Appendix A).

Incorporating Pollen Carryover

Pollen carryover can be incorporated into the model in two different ways. First, it may be assumed that due to pollen carryover, a visit may be successful so long as the pollinator visited no more than some number, *h*, of heterospecific flowers since last visiting a conspecific flower. In this case, then as derived in Appendix B, the probability of successful pollination may be calculated as:

$$P(s) = 1 - e^{\lambda\left((1-a)^{h+1}-1\right)} \tag{5.9}$$

With pollen carryover, as without, the probability of pollination success increases with an increase in the expected number of visits or the proportion of the community comprised by that species (Fig 5.4). The probability of pollination success is, however, higher with pollen carryover than without, especially when the flower is rare (Fig 5.4).

It is useful heuristically to consider pollen carryover as occurring only over some predetermined number of intervening flowers, but this assumption does not well represent the quantities of pollen deposited by a pollinator over sequential visits. Instead, a second approach to incorporating carryover is based on the empirical finding that some fraction of the remaining pollen is deposited on each flower a pollinator visits, so pollen loads decay over successive visits (Thomson and Plowright 1980, Morris et al. 1994). It is expected that a pollinator carrying less conspecific pollen is correspondingly less likely to successfully pollinate a flower it is visiting, so it may be assumed that the probability of pollination success declines with increased numbers of intervening visits in a similar manner. Thus, for each pollinator visit it is necessary to determine both the probability that each earlier visited flower was conspecific and the amount of pollen remaining if the flower was conspecific.

The probability that each earlier visited flower is a conspecific remains *a*, the proportional abundance of the flower. Now, let *r* represent the fraction of the pollen picked up from a flower that is deposited on each subsequent visit. The amount of pollen remaining on a pollinator from a flower visited some number of visits, *j*, earlier depends on the fraction of the pollen load (1 - *r*) that is retained over each earlier visit and the number of visits between picking up pollen and reaching the current flower. The pollinator then deposits some fraction, *r*, of the pollen load remaining from that particular pollen source. Thus, the fraction, *f*, of the initial pollen load from a conspecific flower *j* visits earlier that is deposited on the flower currently being visited is:

$$f_j = r(1-r)^{j-1} \tag{5.10}$$

which is similar to Bateman's (1947) model of pollen carryover.

According to these assumptions, an arriving pollinator carries some fraction of the initial pollen deposited from all previously visited conspecific flowers, from the most recently visited flower (j=1) through many earlier visited flowers, approximated by (j= ∞). Because each earlier visited flower has a probability, *a*, of being conspecific, the expected total amount of conspecific pollen receipt ($PR_{|1}$) by the conspecific flower from one visit can be calculated by:

$$PR_{|1} = \sum_{j=1}^{\infty}[ar(1-r)^{j-1}] \tag{5.11}$$

which is a geometric series that simplifies to

$$PR_{|1} = a \tag{5.12}$$

This indicates that the expected pollen load deposited per visit with carryover is equal to the proportional abundance of conspecific flowers in the community.

In the model assuming no pollen carryover, it was assumed that a within-species pollinator visit always results in deposition of the entire pollen load and guarantees pollination success (*P(s)* = 1), whereas a pollinator visit with any intervening heterospecific flowers results in no conspecific pollen receipt and hence no chance of pollination success (*P(s)* = 0). To make this model with fractional pollen deposition comparable to the earlier model without carryover, it is assumed that the expected probability of pollination success per visit is proportional to the expected total fraction of a conspecific pollen load that is received on that visit, as represented by equation 12. According to this model, the sum of fractional pollen loads deposited on a stigma is always between zero and one, inclusively, even if all previously visited flowers are conspecific, allowing the sum to be reasonably treated as a probability.

Using equation 12 to represent the probability of pollination success on any particular visit, the probability of successful pollination given *i* visits if each visit contributes independently to the probability of pollination success may be calculated by:

$$P(s \mid i) = 1 - (1 - a)^i \qquad (5.13)$$

This probability is identical to the probability of pollination success in the earlier model assuming no carryover (Equation 1). It then follows that by incorporating a Poisson distribution of visits (Equations 2 and 3) and simplifying, the same expectation of pollination success is derived for this model including fractional pollen carryover as in the earlier model assuming no carryover, namely:

$$P(s) = 1 - e^{-\lambda a} \qquad (5.14)$$

This result indicates that, according to the assumptions of this model, pollen carryover does not affect the severity of competition mediated by pollen loss, and the expected probability of pollination success is identical regardless of what proportion of pollen is deposited on each flower visited. This result differs from the earlier model of carryover across a specified number of flowers (equation 9), which predicts that carryover increases the probability of pollination success.

Finally, the expected pollen receipt given a Poisson distribution of visits may be calculated as the expected amount of pollen received given *i* visits, weighted by the probability of receiving *i* visits. Because, each visit is expected to bring an amount of pollen represented by equation 11, total expected pollen receipt may be calculated by:

$$PR = \sum_{i=0}^{\infty}\left[\frac{e^{-\lambda}\lambda^{i}}{i!}\sum_{j=1}^{\infty}[ar(1-r)^{j-1}]\right] \tag{5.15}$$

which may be simplified to:

$$PR = \lambda a \tag{5.16}$$

Equation 16 indicates that expected pollen receipt does not vary with the degree of pollen carryover.

Simulation Model Including Pollen Carryover

Although the model of pollen carryover with proportional deposition of pollen loads suggests that the average pollen load is not influenced by the proportion of pollen deposited, the frequency distribution of pollen loads received by flowers may nevertheless vary with the proportion of pollen deposited. The distribution of net pollen loads received merits investigation to allow predictions of pollination success if success is not a linear function of the amount of pollen received. In particular, a plausible scenario for which pollination success would not be a linear function of pollen receipt is if success is unlikely below some threshold quantity of pollen received and equally likely above the receipt threshold.

To investigate aspects of the frequency distribution of pollen received, I created a simulation model in S programming language based closely on equation 15. The model includes an array of 600 plants whose pollen loads are determined. The algorithm first determines the number of visits each flower receives by either assigning a constant number of visits to all plants or by sampling from a Poisson distribution around a specified expected value.

Next, the amount of pollen each plant receives from each pollinator visit is determined. To accomplish this, a history of the last 70 flowers visited is independently simulated for each pollinator visit with each flower randomly assigned a conspecific or heterospecific identity according to the species' proportional abundance. For each conspecific in the visit history, it is calculated what proportion of the initial pollen load acquired on the pollinator is deposited on the current recipient flower. This is accomplished by multiplying by the term $r * (1-r)^{j-1}$ (Equation 10), where r represents the fraction of pollen deposited and j represents the rank of the flower in the pollinator's visit history (with $j = 1$ being the most recent flower visited). The sum of pollen deposited from all 70 flowers in the visit history is then determined, and if the flower is assigned multiple pollinator visits, the sum across all visit histories is calculated.

After repeating these steps for each plant, the mean and variance of pollen loads received for all 600 recipient plants is calculated. To compare patterns of pollen receipt across a range of pollen carryover values, the entire algorithm after determination of the number of pollinator visits to each flower is repeated ten times for values of r (pollen deposition rate) from 0.1 to 1.0 for each proportional abundance tested.

Simulation Model Results

Across ten proportional abundances and ten values of r, both linearly spaced from 0.1 to 1.0, and two expected numbers of visits (λ=1 or 4), the average pollen load predicted by the simulation model correlates with the expected value given by equation 16 ($PL_{sim} = 1.01\ PL_{pred} - 0.02$, $r^2 = 0.999$, n=200), confirming that the algorithm represents the analytical model well. On average, the pollen load predicted by the model deviated from the analytically calculated pollen load by less than 3%. As expected from Equation 16, the average received pollen load in these simulations was independent of the degree of pollen carryover but increased with plant relative abundance and the expected number of pollinator visits.

Although the degree of pollen carryover has no effect on average pollen receipt, it does affect the frequency distribution of pollen. For each combination of floral abundance and expected number of pollinator visits tested, the variance in pollen receipt increased with decreasing rates of pollen carryover. The effect of carryover on the frequency distribution of pollen is especially strong for low and consistent numbers of pollinator visits, as in simulations when all flowers receive exactly one visit (Fig. 5a-c). For flowers receiving one visit when there is a high degree of pollen carryover (small r value), pollen receipt is relatively even among all flowers. In contrast, when there is a low degree of pollen carryover (large r value), there is greater range of pollen loads received and a sharp transition between flowers receiving small and large pollen loads. These patterns hold true for a range of flower relative abundances, although relative abundance affects what proportion of flowers receive a small or large pollen load.

The distributions of pollen receipt loads allow determination of what proportion of flowers receive pollen loads equal to or greater than any specified amount. This translates to pollination success if below some threshold of pollen receipt flowers are likely to abort, but above that threshold, flowers are likely to successfully mature. When all flowers receive exactly one pollinator visit and pollen receipt thresholds are small, then a larger proportion of flowers are successful if there is a high degree of pollen carryover (ie. a low rate of pollen deposition) (Fig 5 a-c). Conversely, if pollen receipt thresholds are large, then a larger proportion of flowers are successful if there is a low degree of pollen carryover. For any relative abundance, there is some threshold where pollination success is equal regardless of the degree of carryover, indicated graphically by the intersection the respective cumulative frequency distributions.

For flowers that receive one pollinator visit, the threshold for which increased pollen carryover switches from increasing to decreasing success depends on plant relative abundance. Rare flowers benefit from pollen carryover only if their threshold is low; otherwise, pollination success is higher if less carryover occurs (Fig 5.5a). However, for very low thresholds, a higher number of rare flowers are expected to be successfully pollinated if a high degree of carryover occurs, whereas fewer flowers are expected to be successfully pollinated if little carryover occurs (Fig 5.5a). For plants of intermediate abundance, similar patterns hold, though the advantage of pollen carryover extends to higher pollen receipt thresholds (Fig 5.5b). Additionally, for very low thresholds, the benefit of pollen carryover is less for plants of intermediate abundance than for rare plants. Finally, very abundant flowers benefit from pollen carryover only if their pollen receipt threshold is very high (Fig 5.5c). For abundant flowers, for all but the highest thresholds, most flowers are successfully pollinated regardless of the degree of carryover, so a change in the degree of pollen carryover has little effect on the proportion of flowers successfully pollinated. If, however, the threshold of pollen receipt is quite high (> 0.8 pollen loads), then a substantially greater proportion of abundant flowers are successfully pollinated if there is little pollen carryover (Fig 5.5c).

Incorporation of a random number of pollinator visits into the simulation model increases the range of pollen loads received, especially for a greater number of expected visits (compare Fig 5.5, D-F with G-I). Among flowers receiving any particular number of visits, pollen carryover continues to effect the distribution of pollen loads received. However if the number of visits is allowed to vary, then for any given pollen receipt threshold, the degree of pollen carryover has a smaller effect on the proportion of flowers successfully pollinated than is the case if all flowers receive exactly one visit. This effect of pollen carryover is particularly small for common plants that are expected to receive a large number of visits (e.g. Fig 5.5 I). Nonetheless, even for flowers potentially receiving multiple visits, the degree of carryover may substantially effect the proportion of flowers successfully pollinated, particularly for rare or moderately abundant plants and low pollen receipt thresholds (e.g. Fig 5.5 D,E,G,H). Additionally, carryover may still be an important determinate of pollination success for rare plants with high receipt thresholds. In this case, the degree of carryover could be the difference between small proportion of plants or no plants being successfully pollinated.

Pollen carryover may affect expected reproductive success even without thresholds for pollination success, so long as the relationship between pollination receipt and reproductive success (fruit set or seed set) is non-linear. To illustrate this, I reanalyze data of Campbell (1986), who measured pollen deposition rates for the two most common visitors to *Stellaria pubera*, a plant that experiences competition by interspecific pollen loss. The original analysis investigated the effectiveness of these pollinators for plants in different spatial configurations, but also included differences in flight direction and flight distances, so effects of carryover alone were not isolated. To focus on the effect of pollen carryover, differences in other aspects of these species' pollinating behavior are not included here. The bee fly *Bombylius major* and inquiline bee *Nomada* were reported to both collect approximately 140 pollen grains upon visiting staminate *S. pubera*, and to deposit pollen at a rate of 23% and 47%, respectively. *Stellaria pubera* flowers are approximately twice as likely to be staminate as pistillate (Campbell 1985a), meaning that their abundance as a pollen source is two-thirds of their overall floral abundance. Pollen receipt in this simulation model was determined in the same way as for the earlier described model, using the observed pollen deposition rates specified above as well as more extreme rates of 10% or 90% in order to determine whether more extreme rates would lead to stronger effects. For this model, reproductive success is based on the empirically derived non-linear regression of seed counts as a function of pollen receipt, according to the equation $Y = 5.05(1-e^{-0.15X})$ which increases steeply for pollen receipt between zero and one-hundred grains, but asymptotes to approximately five seeds per flower for increasing quantities of pollen (Fig 6 in Campbell 1986). Results are analyzed for a community in which *S. pubera*'s relative abundance is either 15% (10% staminate) or 50% (33.3% staminate).

Simulations indicate that carryover increases fecundity of *S. pubera* and that the difference is greater for extreme values of deposition than moderate values (Fig 5.6). Additionally, differences in carryover lead to a greater difference in seed set for rare than common plants for both pairs of carryover values. This result that increased pollen carryover increases expected pollination success can be generalized to any situation in which there are decreasing marginal returns for pollination success of increased pollen receipt (e.g. asymptotic curves). This conclusion holds because as shown earlier increased pollen carryover leads to decreased variance in pollen receipt but no change in the average pollen receipt. For any curve with decreasing marginal returns, the decrease in success of those flowers that receive less than the average amount of pollen outweighs the increase in success of those flowers that receive more than the average amount of pollen. Thus, success is maximized when variance in pollen receipt is minimized, which occurs with a high degree of carryover.

DISCUSSION

Both the analytical and the simulation models presented here demonstrate that the effect of interspecific competition by pollen loss depends on several factors, including pollinator visitation rates and floral constancy, the plant's relative floral abundance, the degree of pollen carryover, and the relationship between a flower's pollen receipt and probability of pollination success. Some of these factors may be considered attributes purely of the plant (e.g. the relationship between pollen receipt and pollination success), or community (relative floral abundance), while others are jointly influenced by the plant and pollinators (e.g. floral constancy and degree of pollen carryover).

Despite the multiple factors involved, several generalizations may be reached about how these factors influence the severity of interspecific competition by pollen loss. First, in agreement with earlier models, the models presented here suggest that the severity of competition by pollen loss is strongest when visitation rates are low and diminishes with increased visitation rates. The models of Levin and Anderson (1970) and Straw (1972), which do not correct for redundant visits, overestimate the severity of competition by pollen loss for more realistic pollinator visitation rates. However, the analytical model presented here confirms their qualitative finding that increased pollinator constancy decreases the severity of competition for pollination. Additionally, the models presented here agree with earlier models that increased floral relative abundance decreases the severity of competition for pollination.

The effect of pollen carryover on competition by pollen loss is more complex. Whether pollen carryover affects competition by pollen loss depends on the relationship between the number of pollen grains received and pollination success. The analytical model demonstrates that the degree of pollen carryover has no effect on the expected number of pollen grains received per flower. This outcome makes intuitive sense because once a pollinator has acquired a pollen load from a pollinator, then so long as it visits other flowers in proportion to their relative abundances, it will deposit the same proportion of that pollen load on conspecifics whether it deposits all pollen at one time or smaller quantities of pollen over many consecutive flowers. However, the degree of carryover does affect whether a few flowers receive a large quantity of pollen or whether many flowers each receive less pollen. If pollination success is linearly dependent on the number of pollen grains received, then the population's pollination success would be unaffected by the degree of carryover. In other cases, such as when there are thresholds of pollination receipt necessary for pollination success, or when seed set otherwise varies non-linearly with pollen receipt, then changes in the distribution of pollen as a result of carryover may substantially affect the population's pollination success.

The results of the simulation model help to explain the apparently conflicting results between the two analytical models of pollen carryover. The simulation model predicts that pollination success is increased by pollen carryover when pollen receipt thresholds are low, especially for flowers that are rare or receive few visits. This benefit of carryover in this scenario results from carryover allowing more flowers to cross the low receipt threshold necessary for success while not reducing the pollen receipt of earlier visited flowers by enough that they drop below the threshold. These conditions are similar to assumptions implicit in the analytical model of carryover across a specified number of flowers, namely that receipt of more pollen by later visited flowers increases their success without substantially reducing the success of earlier visited flowers. Thus, the model of carryover across a specified number of flowers may be a useful approximation for flowers that have low pollen receipt thresholds. The simulation model also agrees with the analytical model with fractional pollen deposition inasmuch as both predict that average pollen receipt is not affected by the degree of carryover. Predictions only differ between this analytical model and the simulation model because the former assumes that pollination success is linearly dependent on pollen receipt, while the latter assumes a non-linear relationship. A linear relationship between pollen receipt and pollination success could conceivably occur for flowers that have many ovules relative to typical pollen loads and that ripen fruit regardless of the proportion of ovules fertilized. When applying these models of pollen loss to a specific plant, determination of the relationship between pollen receipt and pollination success is essential for choosing the appropriate analytical model or for parameterizing a simulation model, as done here and by Campbell for the data of Campbell (1986).

The simulation model is constructed around randomly determining pollinator visit numbers, and each pollinator's previous visit history from specified distributions rather than representing pollinators and plants as agents in a spatially explicit model. This approach was chosen to mimic as closely as possible the assumptions of the analytical model. However, an agent based model was also constructed that represented a single pollinator visiting each plant in an array in random order, with pollen acquired and deposited according to similar rules, and the outcome of the model was qualitatively similar to the simulation results presented here for the case of one pollinator visit per flower (B. Montgomery, unpublished data).

The new models presented here suggest important considerations for both empirical studies of competition by pollen loss and for modeling approaches generally. For studies of competition by pollen loss, our different model results emphasize the importance of investigating the relationship between pollen receipt and the probability of pollination success before predicting the importance of pollen loss in any given system. Additionally, the simulation model suggests that empirical investigations of the effect of carryover on competition for pollen loss are most likely to detect effects for rare plants that receive few pollinator visits. More generally, a comparison of the analytical and simulation models emphasizes the importance of considering the variation around the expected outcome predicted by a model. The analytical model, based solely on means, suggests that carryover is unimportant, while the simulation model, which captures the distribution in pollen loads received, suggests that carryover may be important even if it does not affect mean pollen receipt. This lesson parallels conclusions from optimal foraging models, in which the importance of investigating the distribution of foraging success was recognized only after a period of focusing solely on the expected foraging success (Pyke 1984), and echoes the advice of Lertzman and Gass (1983) that when investigating patterns of pollen transfer, it is important to consider variation as well as central tendencies.

The models presented here make several simplifying assumptions. First, they ignore the possibility of geitonogamous pollen transfer, the occurrence of which is decreased with increased pollen carryover (Geber 1985, Robertson 1992, Morris et al. 1994, Johnson and Edwards 2000). The results of these models are thus most directly applicable to plants with one or few flowers open and in female phase simultaneously, or to plants whose floral arrangement and phenology minimizes the possibility of geitonogamous self-pollination (Bertin and Newman 1993). In this paper, the degree of carryover only relates to the number of flowers receiving pollen or the proportion of pollen distributed to each flower from a pollen donor, and only for simplicity is it assumed that pollen is deposited on immediately following consecutive flowers. The model would equally apply to a scenario, such as is typical in orchids, in which pollen deposition is delayed for some period before deposition occurs, after which time pollen is deposited either at once or over a sequence of flowers (Johnson and Edwards 2000). As this scenario avoids geitonamy, the model could be applied to it even for plants with multiple inflorescences simultaneously open. A related phenomenon is that instead of a smooth decrease in the amount of pollen deposited over sequential flowers, pollen deposition may be hit-and-miss, with some flowers receiving little or no pollen interspersed with other flowers receiving decreasing amounts of pollen. This has been found in simulation models (Lertzman and Gass 1983), as well as empirical studies (e.g. Thomson and Plowright 1980, but infrequently in Waser and Price 1984, Rademaker et al. 1997). The models presented here can be interpreted to include flowers in the visit history that receive no pollen despite being visited. Effectively, these misses would just mean that the visitation rates functionally experienced by the plants would be lower than the rate determined by counting pollinator visits.

The analytical model of carryover with fractional pollen deposition, and the simulation model both assume that a constant fraction of pollen is deposited on each visit. However, it has been suggested from empirical studies that upon acquiring a pollen load pollinators deposit more pollen on both flowers visited soon afterwards and flowers at the end of the pollen deposition curve than is predicted by this "single-geometric" model (Morris et al. 1994, Harder and Wilson 1998). This may occur if pollen deposition rates vary according to the pollen's horizontal location on a pollinator, the pollen's depth in pollen layers on the pollinator, or variation in pollen deposition between pollinators (Lertzman and Gass 1983, Harder and Wilson 1998). Following previous models (e.g. Bateman 1947, Plowright and Hartling 1981, Campbell 1986, Robertson 1992, de Jong et al. 1993), I elected to maintain the single-geometric model for its simplicity and mathematical tractability, and suggest that the model captures patterns of pollen deposition with different carryover rates that are similar to more realistic curves that require more parameters.

The models presented here make another simplification in omitting terms to account for pollen loss that results from pollen falling or being groomed off pollinators in flight or from pollen being deposited on flower parts other than the stigma (see discussion in Harder and Barrett 1996). However, empirical studies have shown that only a small fraction of pollen acquired by pollinators is deposited on stigmas of conspecifics (Murcia and Feinsinger 1996, Rademaker et al. 1997). Implicitly, the models here assume that the same fraction of pollen acquired by pollinators is lost to these non-stigmatic pools, regardless of the degree of carryover, and that the model applies to the remaining fraction of pollen that the pollinator will deposit on stigmas. However, examining the effect of explicitly incorporating pollen loss to non-stigmatic pools into the models presented here may be a worthwhile endeavor.

The models developed here are constructed primarily as ecological models, predicting pollination success as a function of plant and pollinator abundances and plant traits, and they do not explicitly model selection. For the situation modeled here without the complication of geitonogamy, selection for maternal success should select for stigmas that acquire more conspecific pollen from each pollinator, at least up to a point at which no further benefit accrues. The models come closer to representing male fitness, as selection would favor pollen donors whose pollen is distributed in a manner that optimizes the number of stigmas that attain at least a threshold pollen load. However, to fully represent male fitness, it would be necessary to also take into account the number of ovules fertilized by each male for cases in which pollen from multiple flowers reaches the same stigma. Furthermore, for plants with multiple flowers open simultaneously, selection imposed by geitonogamy may lead to different patterns of selection (de Jong et al. 1993). Finally, it should be noted that the actual carryover rate may vary from the optimum for the plant because pollinator traits as well as plant traits may influence the degree of carryover (Waser and Price 1983, Geber 1985).

Although the model does not represent selection explicitly, it does suggest patterns that could be tested for in cross-species comparisons. Across species, pollen receipt thresholds are likely to vary with the number of ovules per flower, which vary across at least seven orders of magnitude from flowers with one ovule to orchid flowers, which may contain several million ovules (Johnson and Edwards 2000). The simulation model suggests that higher thresholds of pollen receipt should be associated with decreased rates of pollen carryover, whereas lower thresholds should be associated with increased carryover. Furthermore, it has been argued that flowers show adaptations for rarity (Kunin 1997) and the model suggests that the association between thresholds and the degree of pollen carryover should be strongest for rare flowers receiving few visits, and weaker for more common flowers or flowers receiving more visits. In this light, it is interesting to consider floral traits of orchids, which tend to be rare and require receipt of large numbers of pollen grains to fertilize the many ovules. Consistent with this model, orchid pollen is delivered in pollinaria, which leads to pollen being delivered in packages with large quantities of pollen, although even in orchids, some species exhibit fractional pollen deposition (Johnson and Edwards 2000).

This model considers only one mechanism by which plants may compete interspecifically for pollination – they may also compete by loss of visits (Chittka and Schürkens 2001, Brown et al. 2002) or by decreased pollination success due to receipt of interspecific pollen (Waser 1978a, Murphy and Aarssen 1995a). Other models have considered the joint effects of changes in visit rates and loss of pollen (Feldman et al. 2004), and pollen loss and interspecific pollen receipt (Waser 1978b, Campbell 1986) but further investigations may profitably investigate how these mechanisms interact to affect competition for pollination for varying degrees of pollen carryover and pollen receipt thresholds.

APPENDIX A

Incorporation of pollinator constancy into analytical models

1. No pollen carryover

If no pollen carryover occurs, then only conspecific pollinator flights result in pollination. A pollinator exhibits constancy and intentionally seeks out a conspecific flower with a probability *c*, but randomly chooses the next species of flower to visit with a probability (1-*c*), in which case it will randomly visit a flower of species A according to its proportional abundance (*a*). Then, as demonstrated by Levin and Anderson (1970), the overall probability of a pollinator making a conspecific flight upon leaving a flower of species A may be represented by:

$$P(s)_{|1} = c + (1 - c)a \qquad (5.A1)$$

Given i visits, the probability of successful pollination may be calculated as one minus the probability that each independent visit is unsuccessful, as represented by:

$$P_{sp|i} = 1 - [1 - (c + (1 - c)a)]^{i} \qquad (5.A2)$$

Assuming a Poisson distribution of visits about a mean of λ, the overall probability of pollination success may then be calculated as:

$$P_{sp} = 1 - \sum_{i=0}^{\infty} [\frac{e^{-\lambda} \lambda^{i}}{i!} (1 - (c + (1 - c)a))^{i}] \qquad (5.A3)$$

By performing algebraic manipulation, and recognizing the infinite series to be a Taylor series expansion of e^x, where x equals $\lambda[1-(c+(1-c)a)]$, it may be shown that equation (A3) simplifies to:

$$P_{sp} = 1 - e^{-\lambda(c+(1-c)a)} \qquad (5.A4)$$

2. Discrete-time population dynamic version of model including pollinator constancy

To develop a discrete-time population dynamic model including pollinator constancy, I start with the discrete time model lacking a term for pollinator constancy (Equation 16), but modify it by substituting the probabilities of a pollinator making a conspecific flight upon leaving a flower of species A or B with constancy taken into account. For species A, this is represented by equation A1, and for species B by the equation $P_{sp|1} = c + (1-c)(1-a)$, giving

$$A_{t+1} = \frac{A_t(1-e^{-\lambda(c+(1-c)a)})}{\left[A_t(1-e^{-\lambda(c+(1-c)a)}) + (1-A_t)(1-e^{-\lambda(c+(1-c)(1-a))})\right]} \tag{5.A5}$$

At the limit, as the expected rate of pollinator visits, λ, approaches zero, equation A5 approaches the Levin and Anderson model including pollinator constancy, namely:

$$A_{t+1} = \frac{A_t(c+(1-c)A_t)}{\left[c+(1-c)[A_t^2 + B_t^2\right]} \tag{5.A6}$$

APPENDIX B

Incorporation of pollinator carryover into analytical models:

Assume that a visit is successful so long as the pollinator has visited no more than some number, *h*, of interspecific flowers since last visiting a conspecific flower. Then for each pollinator visit, the probability that the pollinator did not visit a flower of the same species in either of the two most recent visits is given by $[1\text{-}a]^{h+1}$. For example, if it is assumed that pollen carryover can occur over one intervening flower (h=1), then the probability of pollination success from one visit is equal to the probability that at least one of the previously two visited flowers was conspecific, or $[1\text{-}a]^2$. The probability of a flower being successfully pollinated after *i* visits is then:

$$P_i = 1 - (1 - a)^{(h+1)i} \qquad (5.B1)$$

Consequently, the probability of a flower being successfully pollinated with pollen carryover over *c* intervening flowers can be calculated by modifying equation (3) to the following:

$$P_{sp} = 1 - \sum_{i=0}^{\infty} [\frac{e^{-\lambda}\lambda^i}{i!}(1 - a)^{(h+1)i}] \qquad (5.B2)$$

By recognizing that Equation (6) contains a Taylor series expansion, it may be demonstrated that

$$P_{sp} = 1 - e^{\lambda\left((1-a)^{h+1} - 1\right)} \qquad (5.B3)$$

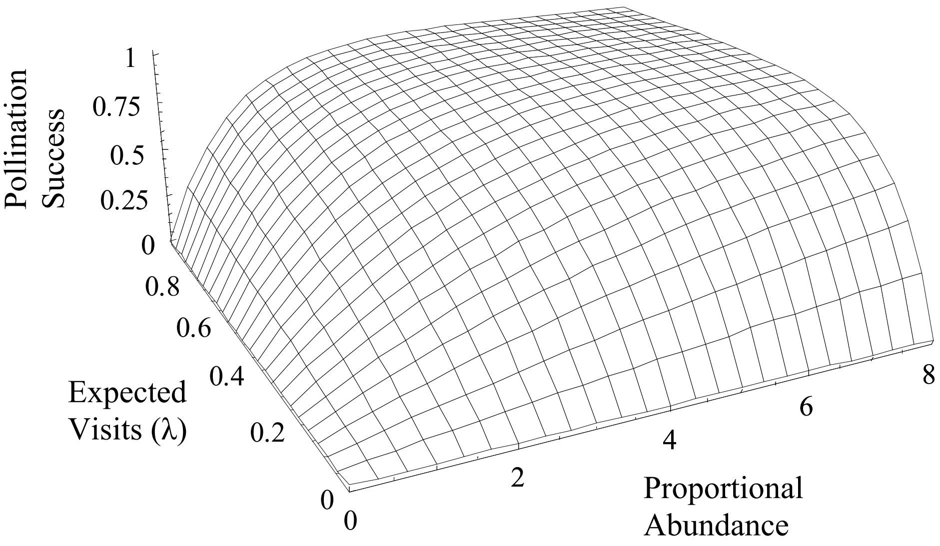

Figure 5.1 Probability of pollination success as a function of plant relative abundance and number of expected pollinator visits (λ) for case of no pollen carryover and no pollinator constancy.

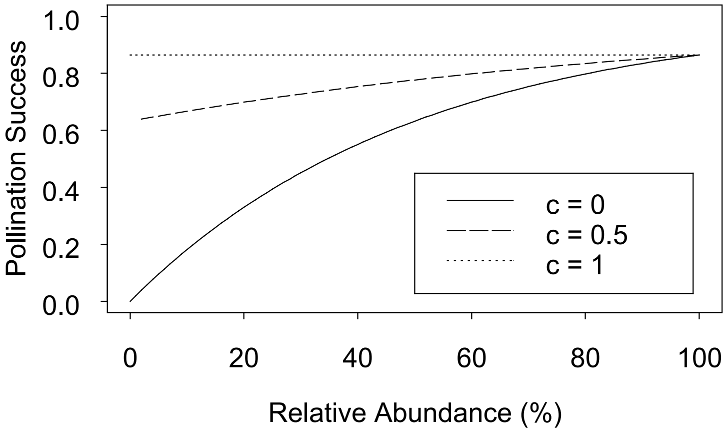

Figure 5.2 Probability of pollination success for three values of pollinator constancy, *c*, for a plant with 25% relative abundance (*a* = 0.25) and two expected visits per flower (λ = 2).

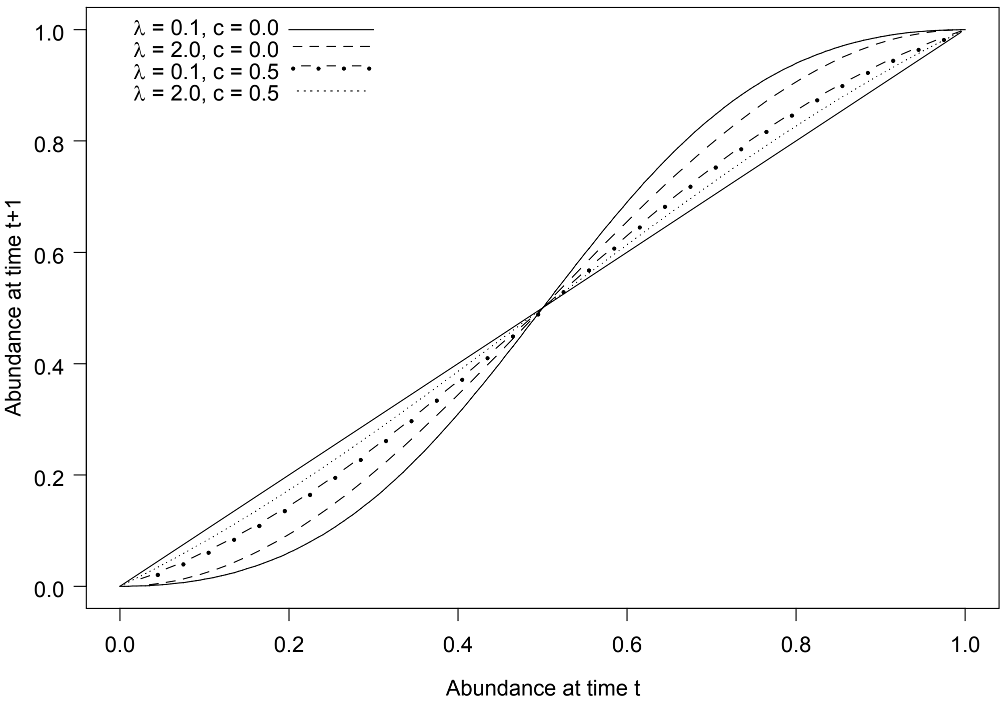

Figure 5.3 Discrete-time map of abundance of species *A* at time *t+1* as a function of abundance at time *t* for two expected numbers of pollinator visits (λ) and two rates of pollinator constancy (*c*).

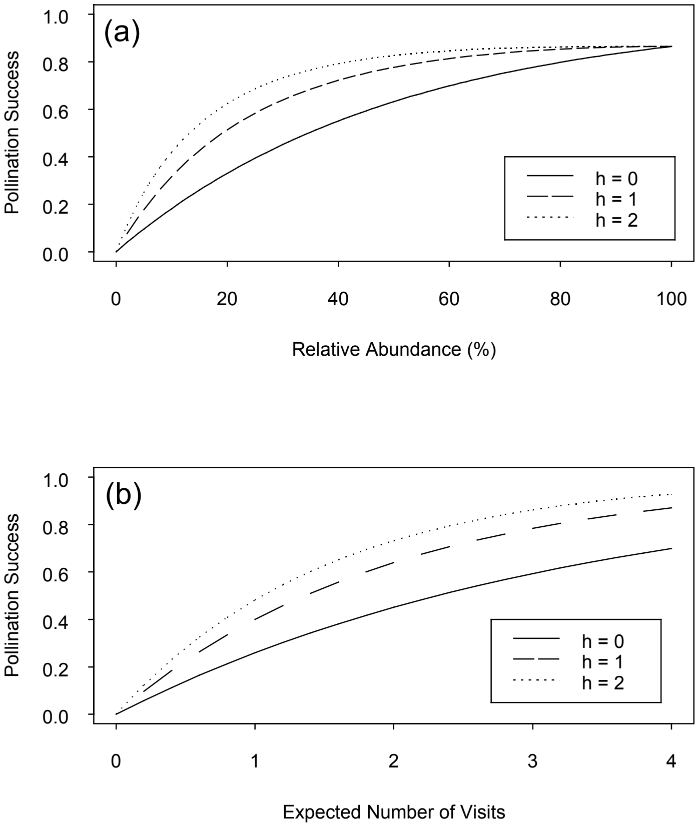

Figure 5.4 Probability of pollination success for case of no pollen carryover (h = 0), or carryover across one or two intervening flowers (h = 1,2 respectively) (**a**) as a function of relative abundance for a plant that receives an average of two visits per flower (**b**) as a function of number of expected visits for a plant that comprises 30% of community.

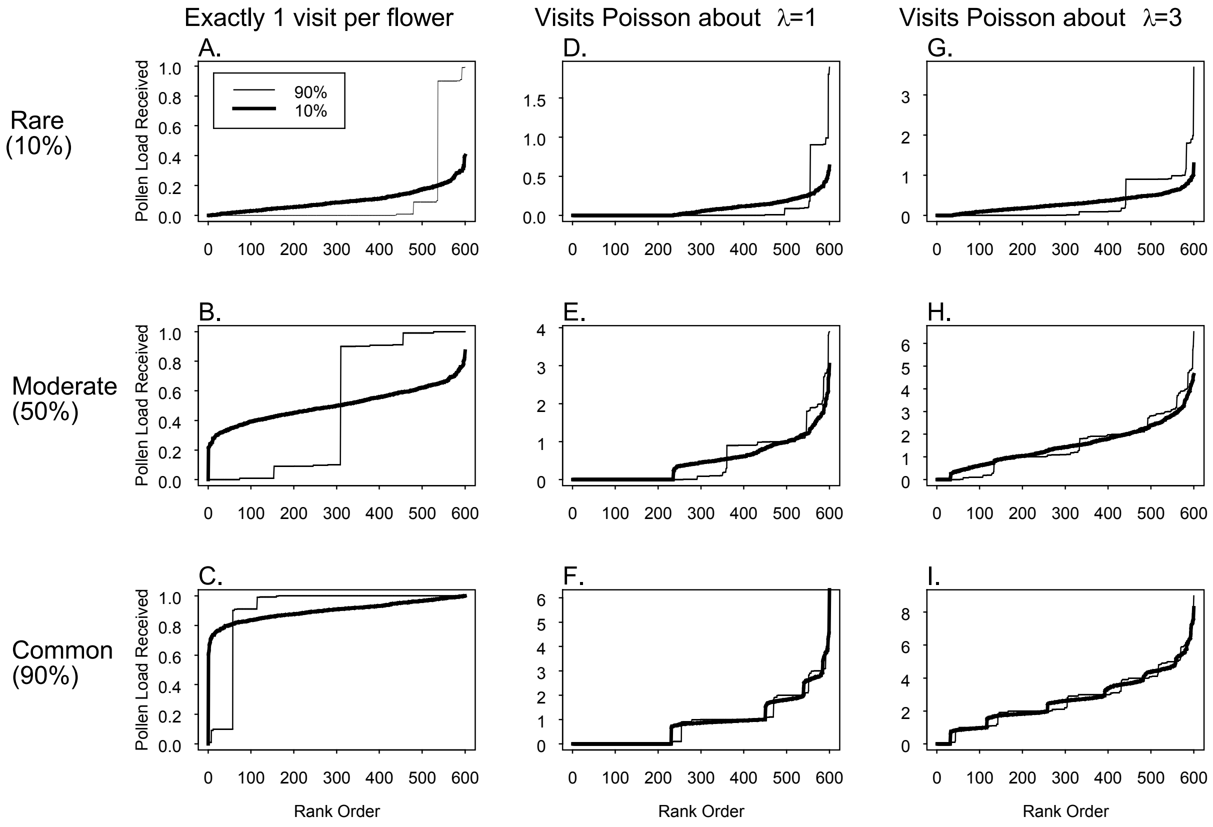

Figure 5.5 Cumulative frequency distribution of pollen loads received for 600 flowers receiving exactly one visit (A – C), a Poisson distribution of visits around a mean of 1 visit (D – F) or a Poisson distribution of visits around a mean of 3 visits (G – I), for plants with relative abundances of 10% (A, D, G), 50% (B, E, H) or 90% (C, F, I), for a 10% rate of pollen deposition (high carryover) or 90% pollen deposition (low carryover). Note variation in scale of y-axis among graphs.

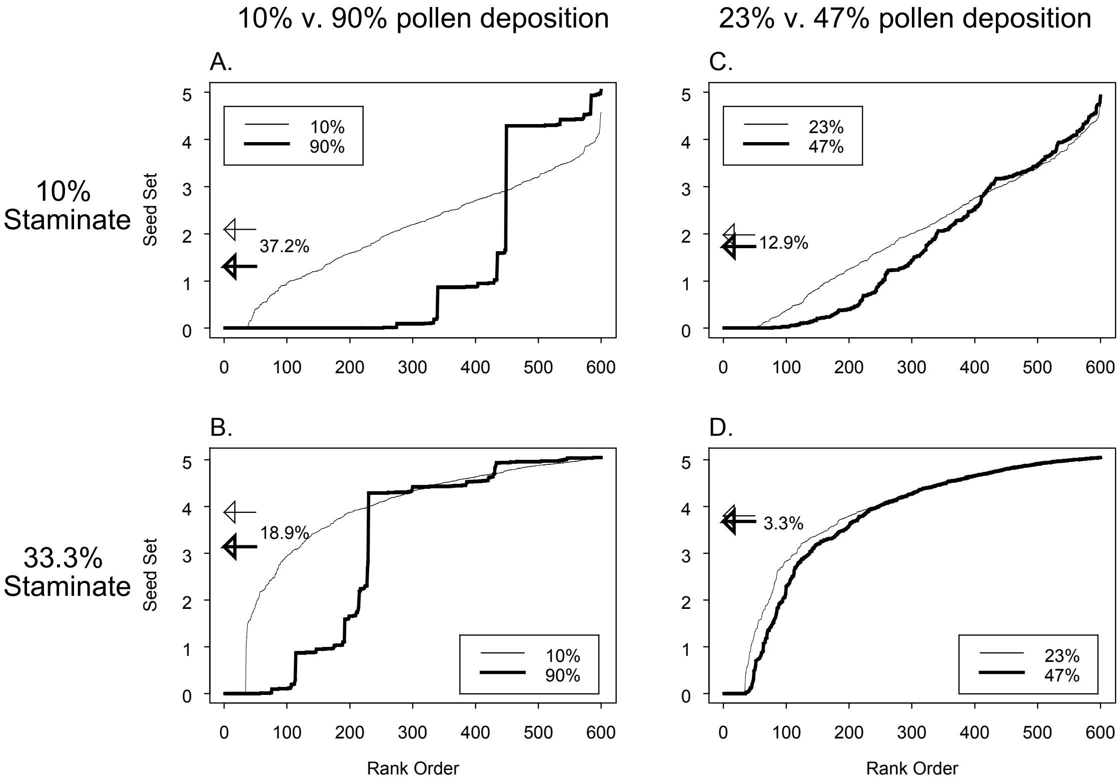

Figure 5.6 Cumulative frequency distribution of seed set for 600 flowers receiving a Poisson distribution of visits around a mean of three visits, for a species whose staminate flowers represent 10% (A, C) or 33.3% (B, D)of the floral community. More extreme deposition rates are contrasted in A and B, while moderate deposition rates, as measured for the two most common visitors to *Stellaria pubera* are contrasted in C and D. Arrows indicate average seed set for each deposition rate, and percentages indicate reduction in seed set with the higher deposition rate (reduced carryover). Deposition rates and relationship of pollen receipt to seed set are based on parameter estimates of Campbell (1986), as explained in the text.

Conclusions

The overarching goal of these studies was to develop and test hypotheses about how different floral and reproductive traits influence plants' susceptibility to experiencing interspecific competition for pollination. The first four chapters, empirically investigating competition for pollination, were designed to assay the potential for the introduced plant *Euphorbia esula* to interfere with the pollination of prairie species with a range of floral and reproductive traits. The intent of the final chapter was to refine models of pollination success in scenarios where pollen could be lost to heterospecific stigmas in order to develop hypotheses about how plant and pollinator abundance and the degree of pollen carryover affect pollination success.

The first two chapters investigated the effects of proximity to *Euphorbia* on *Sisyrinchium campestre*, a self-incompatible plant with an unrestrictive morphology, and *Viola pedatifida*, a self-compatible plant with a restrictive floral morphology. The difference in floral restrictiveness suggests that *Sisyrinchium* is more likely than *Viola* to receive *Euphorbia* pollen, but a comparison of *Euphorbia* pollen receipt rates between these studies indicates that *Viola* receives more *Euphorbia* pollen per stigma than *Sisyrinchium*. There are multiple possible explanations for this outcome. First, all three species were primarily visited by solitary bees, which would create opportunities for pollinator sharing despite the different morphologies. Additionally, *Sisyrinchium* flowers were far more abundant than *Viola*, and it is possible that as a result pollinators showed increased loyalty to *Sisyrinchium* while making more interspecific flights to *Viola*. *Viola* stigmas also persist for several days, whereas *Sisyrinchium* stigmas typically only persist for one day, and this extra time could allow more opportunities for heterospecific pollen transfer to *Viola*.

I had hypothesized that *Viola* would be more affected than *Sisyrinchium* by heterospecific pollen receipt because *Sisyrinchium*, being self-incompatible, would be expected to have adaptations minimizing deleterious effects of 'improper' (*sensu* Rathcke 1983) self-pollen, and these adaptations could also minimize effects of heterospecific pollen receipt. I hypothesized that *Viola*, being self-compatible, would be less likely to possess traits minimizing effects of improper pollen receipt. In keeping with this set of hypotheses, *Viola* experienced decreased seed set as a result of *Euphorbia* pollen receipt, whereas neither fruit nor seed set was affected for *Sisyrinchium*.

I also investigated fruit and seed set of both species either near to or approximately ten meters from *Euphorbia* for both species in order to determine whether either experiences decreased pollination success near *Euphorbia*. Neither species experienced decreased reproductive success at these distances, and for *Viola* the trend was toward increased success in close proximity to *Euphorbia*. For *Sisyrinchium*, hand-pollination failed to significantly increase fruit or seed set, indicating that pollinator service was not limiting at either distance. For *Viola*, patterns were not significantly different for chasmogamous and cleistogamous flowers, indicating that the trend toward increased success near *Euphorbia* was not attributable to differences in pollinator service. Overall, these results suggest that both species share pollinators with *Euphorbia*, but that neither species experiences reduced reproductive success as a result of interactions with *Euphorbia*.

In the next two chapters, I sought to determine whether patterns of pollen incidence and effects observed for *Sisyrinchium* and *Viola* were similar across a broader array of species. In chapter 3, I compared rates of heterospecific pollen receipt and stigma sizes across 29 species which were categorized as having unrestrictive or restrictive floral morphologies. I found that as hypothesized, but counter to the pattern observed for *Sisyrinchium* and *Viola*, flowers with restrictive morphologies received less *Euphorbia* and other heterospecific pollen than flowers with unrestrictive morphologies. This discrepancy in results illustrates the importance of investigating patterns across a diverse assemblage of species, rather than generalizing patterns from comparisons between two species. In chapter 3, I also found that average stigma size was smaller for species with restrictive than unrestrictive morphologies. This result in conjunction with the finding of greater heterospecific pollen receipt for flowers with unrestrictive morphologies is consistent with the hypothesis that a large stigma functions to decrease the effect of heterospecific pollen receipt. To my knowledge this is the first test of this hypothesis.

The final empirical chapter investigates the effect of *Euphorbia* pollen receipt for five species in addition to previously described results for *Viola* and *Sisyrinchium*, for a total of seven species. Breeding system studies and associated evidence suggests that five of the species are self-compatible whereas two (*Sisyrinchium* and *Lithospermum canescens*) are self-incompatible. Three species were classified as having restrictive morphologies, whereas four were classified as having unrestrictive morphologies. Large quantities of *Euphorbia* pollen, applied with a time delay for all species except *Viola*, were found to decrease fruit or seed set for three species, whereas the remaining four species were unaffected. There was no clear trend for the effect of *Euphorbia* pollen receipt to differ between restrictive and unrestrictive flowers. There was a suggestion that self-incompatible species are less affected by *Euphorbia* pollen receipt than self-compatible species, as neither self-incompatible species was significantly affected. However, larger sample sizes would be necessary to test this conclusion statistically.

Overall, these studies suggest that *Euphorbia* is relatively unlikely to decrease the fruit or seed set of simultaneously flowering species due to heterospecific pollen receipt. Considering the three species significantly affected, the effect of *Viola* apparently depended on the order of pollen arrival, as fruit and seed of flowers exposed to *Euphorbia* pollen after conspecific pollen was similar to flowers receiving only outcrossed pollen. The effect on *Linum* and *Zizea* of *Euphorbia* pollen receipt was significantly different from control flowers receiving just conspecific pollen only when the amount of *Euphorbia* pollen received was quite large and delivered long before conspecific pollen. For both *Linum* and *Zizea*, these conditions probably occur rarely in nature. These results suggest that application of large amounts of heterospecific pollen exaggerate the effects on fruit and seed set. This may be a useful technique to compare the relative effects of heterospecific pollen receipt among species with different traits. However, if the goal is to determine how pollination success is likely to be affected under natural conditions, then these results emphasize the importance of applying heterospecific pollen in biologically reasonable quantities and timing regimes.

The research presented here does not definitively answer whether the presence of *Euphorbia* affects the pollination of simultaneously flowering species. This study suggests that heterospecific pollen receipt in naturally occurring quantities and timing regimes is unlikely to result in reduced pollination success. However, species flowering simultaneously with *Euphorbia* could still experience loss of pollen to *Euphorbia* flowers or decreased visit rates in the presence of *Euphorbia*. A related study at a different location in fact found decreased pollinator visit rates and decreased conspecific pollen receipt for some species nearby *Euphorbia*, but fruit and seed set were not measured (Larson et al. 2006). The potential for these effects were investigated in this study only for *Sisyrinchium* and *Viola*, and for these two species plants distant from *Euphorbia* were typically still within 20 meters. Future studies would benefit from measuring fruit and seed set of multiple species nearby *Euphorbia* and at larger distances from *Euphorbia*. Such a study should also have similar native plant communities at both locations, conditions that could not be satisfied at the prairie used for this study.

In the final chapter, I developed analytical and simulation models to investigate how plant relative abundance, pollinator visit rates, and the degree of pollen carryover affect the probability of pollination success for populations susceptible to competition by loss of pollen to heterospecifics. My model demonstrates that predictions resulting from the classic model of competition by pollen loss (Levin and Anderson 1970) are accurate only for the extreme case of pollinator visit rates approaching zero. Under more realistic scenarios, the classic model would overestimate effects of competition by this mechanism. My analytical models also call into question the hypothesis that pollen carryover reduces competition by pollen loss by making clear that expected pollen receipt per stigma is unaffected by pollen carryover. This implies that pollen carryover can only reduce competition by pollen loss if the relationship between pollen receipt and pollination success is non-linear.

To address the potential importance of a non-linear relationship between pollen receipt and pollination success, I created a simulation model based closely on the assumptions of the analytical model, but allowing different functional forms of the pollen receipt – pollination success relationship. The model suggests that if a threshold of pollen grain receipt is necessary for pollination success, then for low thresholds, pollination carryover increases expected pollination success. In contrast, for high thresholds, pollen carryover decreases expected pollination success. Finally, the model indicates that if pollination success is an increasing but decelerating function of pollen receipt, then pollen carryover is expected to mitigate effects of competition by pollen loss.

In conclusion, in this research I have worked to further develop and test hypotheses about which traits affect a plant's susceptibility to experiencing effects of competition for pollination. Unlike previous studies, I have sought to investigate the importance of different traits, including floral restrictiveness, breeding system and stigma size, by investigating effects across a large range of species. I also examined the potential for competition for pollination in greater depth for a subset of two species with contrasting floral traits. Finally, I have furthered our theoretical understanding of competition for pollination by refining models of this interaction and developing hypotheses about the circumstances in which pollen carryover could affect this interaction.

Literature Cited

Agren, G. I., and T. Fagerstrom. 1980. Increased or decreased separation of flowering times - the joint effect of competition for space and pollination in plants. Oikos **35**:161-164.

Aigner, P. A. 2004. Ecological and genetic effects on demographic processes: pollination, clonality and seed production in *Dithyrea maritima*. Biological Conservation **116**:27-34.

Armbruster, W. S., M. E. Edwards, and E. M. Debevec. 1994. Floral character displacement generates assemblage structure of Western-Australian triggerplants (*Stylidium*). Ecology **75**:315-329.

Armbruster, W. S., and A. D. McGuire. 1991. Experimental assessment of reproductive interactions between sympatric *Aster* and *Erigeron* (Asteraceae) in interior Alaska. American Journal of Botany **78**:1449-1457.

Arroyo, J., and A. Dafni. 1992. Interspecific pollen transfer among cooccurring heteromorphic and homomorphic species. Israel Journal of Botany **41**:225-232.

Ashman, T. L., T. M. Knight, J. A. Steets, P. Amarasekare, M. Burd, D. R. Campbell, M. R. Dudash, M. O. Johnston, S. J. Mazer, R. J. Mitchell, M. T. Morgan, and W. G. Wilson. 2004. Pollen limitation of plant reproduction: Ecological and evolutionary causes and consequences. Ecology **85**:2408-2421.

Baker, J. D., and R. W. Cruden. 1991. Thrips-mediated self-pollination of two facultatively xenogamous wetland species. American Journal of Botany **78**:959-963.

Bakke, A. L. 1936. Leafy spurge, *Euphorbia esula* L. Iowa Agricultural Research Station Bulletin **198**:209-245.

Bateman, A. J. 1947. Contamination in seed crops III. Relation with isolation distance. Heredity **1**:303-336.

Beattie, A. J. 1969. Studies in the pollination ecology of *Viola*. I. The pollen-content of stigmatic cavities. Watsonia **7**:142-156.

Beattie, A. J. 1971. Pollination mechanisms in *Viola*. New Phytologist **70**:343-&.

Beattie, A. J. 1974. Floral evolution in *Viola*. Annals of the Missouri Botanical Garden **61**:781-793.

Belcher, J. W., and S. D. Wilson. 1989. Leafy spurge and the species composition of a mixed-grass prairie. Journal of Range Management **42**:172-175.

Bell, J. M., J. D. Karron, and R. J. Mitchell. 2005. Interspecific competition for pollination lowers seed production and outcrossing in *Mimulus ringens*. Ecology **86**:762-771.

Berg, H. 2003. Factors influencing seed: Ovule ratios and reproductive success in four cleistogamous species: A comparison between two flower types. Plant Biology **5**:194-202.

Bertin, R. I., and C. M. Newman. 1993. Dichogamy in angiosperms. Botanical Review **59**:112-152.

Bond, W. J. 1994. Do mutualisms matter? Assessing the impact of pollinator and disperser disruption on plant extinction. Philosophical Transactions of the Royal Society of London Series B **344**:83-90.

Brewbaker, J., and S. K. Majumder. 1961. Cultural studies of pollen population effect and self-incompatibility inhibition. American Journal of Botany **48**:457-464.

Brown, B. J., and R. J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia **129**:43-49.

Brown, B. J., R. J. Mitchell, and S. A. Graham. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. Ecology **83**:2328-2336.

Brown, J. H., and A. Kodric-Brown. 1979. Convergence, competition, and mimicry in a temperate community of hummingbird-pollinated flowers. Ecology **60**:1022-1035.

Burd, M. 1994. Bateman's principle and plant reproduction: the role of pollen limitation in fruit and seed set. The Botanical Review **60**:83-139.

Butler, J. L., and D. R. Cogan. 2004. Leafy spurge effects on patterns of plant species richness. Journal of Range Management **57**:305-311.

Campbell, D. R. 1985a. Pollen and gene dispersal - the influences of competition for pollination. Evolution **39**:418-431.

Campbell, D. R. 1985b. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. Ecology **66**:544-553.

Campbell, D. R. 1986. Predicting plant reproductive success from models of competition for pollination. Oikos **47**:257-266.

Campbell, D. R., and A. F. Motten. 1985. The mechanism of competition for pollination between two forest herbs. Ecology **66**:554-563.

Cane, J. H., and D. Schiffhauer. 2003. Dose-response relationships between pollination and fruiting refine pollinator comparisons for cranberry (*Vaccinium macrocarpon* Ericaceae). American Journal of Botany **90**:1425-1432.

Caruso, C. M. 1999. Pollination of *Ipomopsis aggregata* (Polemoniaceae): Effects of intra- vs. interspecific competition. American Journal of Botany **86**:663-668.

Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. Evolution **54**:1546-1557.

Caruso, C. M. 2001. Differential selection on floral traits of *Ipomopsis aggregata* growing in contrasting environments. Oikos **94**:295-302.

Caruso, C. M. 2002. Influence of plant abundance on pollination and selection on floral traits of *Ipomopsis aggregata*. Ecology **83**:241-254.

Caruso, C. M., and M. Alfaro. 2000. Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata*. Canadian Journal of Botany **78**:600-606.

Chittka, L., A. Gumbert, and J. Kunze. 1997. Foraging dynamics of bumble bees: Correlates of movements within and between plant species. Behavioral Ecology **8**:239-249.

Chittka, L., and S. Schürkens. 2001. Successful invasion of a floral market. Nature **411**:653.

Cholewa, A. F., and D. M. Henderson. 1984. Biosystematics of *Sisyrinchium* section Bermudiana (Iridaceae) of the Rocky Mountains. Brittonia **36**:342-363.

Christiansen, P., and M. s. Muller. 1999. An illustrated guide to Iowa prairie plants. Iowa City Press, Iowa City.

Colwell, R. K. 2005. EstimateS: Statistical estimation of species richness and shared species from samples. Version 7.5. Persistent URL<purlock.org/estimates>.

Cruden, R. W., and S. Millerward. 1981. Pollen-ovule ratio, pollen size, and the ratio of stigmatic area to the pollen-bearing area of the pollinator - an hypothesis. Evolution **35**:964-974.

Culley, T. M. 2000. Inbreeding depression and floral type fitness differences in *Viola canadensis* (Violaceae), a species with chasmogamous and cleistogamous flowers. Canadian Journal of Botany **78**:1420-1429.

Culley, T. M. 2002. Reproductive biology and delayed selfing in *Viola pubescens* (Violaceae), an understory herb with chasmogamous and cleistogamous flowers. International Journal of Plant Sciences **163**:113-122.

de Jong, T. J., N. M. Waser, and P. G. L. Klinkhamer. 1993. Geitonogamy - the neglected side of selfing. Trends in Ecology and Evolution **8**:321-325.

Dunn, P. H. 1985. Origins of leafy spurge in North America. Pages 7-13 *in* A. K. Watson, editor. Monograph Series of the Weed Science Society of North America. Number 3, Champaign, Illinois.

Faegri, K., and L. van Der Pijl. 1979. The principles of pollination ecology, 3d edition. Pergamon Press, New York, NY.

Feinsinger, P. 1983. Coevolution and pollination. Pages 282-310 *in* D. J. Futuyma and M. Slatkin, editors. Coevolution. Sinauer Associates Inc., Sunderland, Massachusetts.

Feinsinger, P., and W. H. Busby. 1987. Pollen carryover - Experimental comparisons between morphs of *Palicourea lasiorrachis* (Rubiaceae), a distylous, bird-pollinated, tropical treelet. Oecologia **73**:231-235.

Feinsinger, P., K. G. Murray, S. Kinsman, and W. H. Busby. 1986. Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. Ecology **67**:449-464.

Feinsinger, P., and H. M. I. Tiebout. 1991. Competition among plants sharing hummingbird pollinators: laboratory experiments on a mechanism. Ecology **72**:1946-1952.

Feinsinger, P., H. M. I. Tiebout, and B. E. Young. 1991. Do tropical bird-pollinated plants exhibit density-dependent interactions? field experiments. Ecology **72**:1953-1963.

Feldman, T. S., W. F. Morris, and W. G. Wilson. 2004. When can two plant species facilitate each other's pollination? Oikos **105**:197-207.

Fishman, L., and R. Wyatt. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution **53**:1723-1733.

Free, J. B. 1968. The flower constancy of bumblebees. Journal of Animal Ecology **39**:395-402.

Freitas, L., and M. Sazima. 2003. Floral biology and pollination mechanisms in two *Viola* species-from nectar to pollen flowers? Annals of Botany **91**:311-317.

Galen, C., and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine wildflower, *Polemonium viscosum*. Oecologia **81**:120-123.

Galen, C., and M. E. A. Newport. 1988. Pollination quality, seed set, and flower traits in *Polemonium viscosum* - complementary effects of variation in flower scent and size. American Journal of Botany **75**:900-905.

Geber, M. A. 1985. The relationship of plant size to self-pollination in *Mertensia ciliata*. Ecology **66**:762-772.

Gegear, R. J., and T. M. Laverty. 1998. How many flower types can bumble bees work at the same time? Canadian Journal of Zoology **76**:1358-1365.

Ghazoul, J. 2004. Alien abduction: Disruption of native plant-pollinator interactions by invasive species. Biotropica **36**:156-164.

Gross, C. L. 1996. Is resource overlap disadvantageous to three sympatric legumes? Australian Journal of Ecology **21**:133-143.

Gross, R. S., and P. A. Werner. 1983. Relationships among flowering phenology, insect visitors, and seed-set of individuals - experimental studies on four co-occurring species of goldenrod (*Solidago*, Compositae). Ecological Monographs **53**:95-117.

Hansen, T. F., W. S. Armbruster, and L. Antonsen. 2000. Comparative analysis of character displacement and spatial adaptations as illustrated by the evolution of *Dalechampia* blossoms. The American Naturalist **156S**:S17-S34.

Hapeman, J. R., and K. Inouye. 1997. Plant-pollinator interactions and floral radiation in *Platanthera* (Orchidaceae). Pages 433-454 *in* T. J. Givnish and K. J. Systma, editors. Molecular evolution and adaptive radiation. Cambridge University Press, Cambridge.

Harder, L. D., and S. C. H. Barrett. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. Pages 140-190 *in* D. G. Lloyd and S. C. H. Barrett, editors. Floral Biology: Studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York.

Harder, L. D., M. B. Cruzan, and J. D. Thomson. 1993. Unilateral incompatibility and the effects of interspecific pollination for *Erythronium americanum* and *Erythronium albidum* (Liliaceae). Canadian Journal of Botany **71**:353-358.

Harder, L. D., and W. G. Wilson. 1998. Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. Ecology **79**:2789-2807.

Heinrich, B. 1975a. Bee flowers - hypothesis on flower variety and blooming times. Evolution **29**:325-334.

Heinrich, B. 1975b. Energetics of pollination. Annual Review of Ecology and Systematics **6**:139-170.

Heinrich, B. 1979. Bumblebee economics. Harvard University Press, Cambridge.

Henderson, D. M. 1976. Biosystematic study of Pacific Northwestern blue-eyed grasses (*Sisyrinchium*, Iridaceae). Brittonia **28**:149-176.

Herrera, C. M. 1987. Components of pollinator "quality": comparative analysis of a diverse insect assemblage. Oikos **50**:79-90.

Huerd, S. C., and S. E. Taylor. 1998. Leafy spurge (*Euphorbia esula* L.) spread in Iowa. Journal of the Iowa Academy of Sciences **105**:27-29.

Ingram, R. 1968. Breeding barriers in some species of *Sisyrinchium*. New Phytologist **67**:197-204.

Ishii, R., and M. Higashi. 2001. Coexistence induced by pollen limitation in flowering-plant species. Proceedings of the Royal Society of London Series B-Biological Sciences **268**:579-585.

Jennersten, O., and M. M. Kwak. 1991. Competition for bumblebee visitation between *Melampyrum pratense* and *Viscaria vulgaris* with healthy and *Ustilago*-infected flowers. Oecologia **86**:88-98.

Jennings, D. L., and P. B. Topham. 1971. Some consequences of raspberry pollen dilution for its germination and for fruit development. New Phytologist **70**:371-380.

Johnson, S. D., and T. J. Edwards. 2000. The structure and function of orchid pollinaria. Plant Systematics and Evolution **222**:243-269.

Johnston, I. M. 1952. Studies in the Boraginaceae, XXIII a survey of the genus *Lithospermum*. Journal of the Arnold Arboretum **33**:299-363.

Kanchan, S., and J. Chandra. 1980. Pollen allelopathy- a new phenomenon. New Phytologist **84**:739-746.

Kasagi, T., and G. Kudo. 2005. Interspecific pollinator movements and heterospecific incompatibility: comparisons between *Phyllodoce caerulea* and *Phyllodoce aleutica* along snowmelt gradients. Evolutionary Ecology Research **7**:73-87.

Kearns, C. A., and D. W. Inouye. 1993. Techniques for Pollination Biologists. University Press of Colorado, Niwot, Colorado.

Kearns, C. A., and D. W. Inouye. 1994. Fly pollination of *Linum lewisii* (Linaceae). American Journal of Botany **81**:1091-1095.

Kephart, S. R. 1983. The paritioning of pollinators among three species of *Asclepias*. Ecology **64**:120-133.

Knuth, P. 1908. Handbook of Flower Pollination. Clarendon press, Oxford.

Kochmer, J. P., and S. N. Handel. 1986. Constraints and competition in the evolution of flowering phenology. Ecological Monographs **56**:303-325.

Kohn, J. R., and N. M. Waser. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. American Journal of Botany **72**:1144-1148.

Kunin, W., and Y. Iwasa. 1996. Pollinator foraging strategies in mixed floral arrays: Density effects and floral constancy. Theoretical Population Biology **49**:232-263.

Kunin, W. E. 1993. Sex and the single mustard: population density and pollinator behavior effects on seed-set. Ecology **74**:2145-2160.

Kunin, W. E. 1997. Population biology and rarity: on the complexity of density dependence in insect-plant interactions. *in* W. E. Kunin and K. J. Gaston, editors. The Biology of Rarity. Champan & Hall, London.

Kunin, W. E., and A. Shmida. 1997. Plant reproductive traits as a function of local, regional, and global abundance. Conservation Biology **11**:183-192.

Kwak, M. M., and O. Jennersten. 1991. Bumblebee visitation and seedset in *Melampyrum pratense* and *Viscaria vulgaris* - Heterospecific pollen and pollen limitation. Oecologia **86**:99-104.

LaRosa, R. J., D. A. Rogers, T. P. Rooney, and D. M. Waller. 2004. Does steeplebush (*Spirea tomentosa*) facilitate pollination of Virginia meadow beauty (*Rhexia virginica*)? Michigan Botanist **43**:57-62.

Larson, D. L., R. A. Royer, and M. R. Royer. 2006. Insect visitation and pollen deposition in an invaded prairie plant community. Biological Conservation **130**:148-159.

Laverty, T. M. 1992. Plant interactions for pollinator visits: a test of the magnet species effect. Oecologia **89**:502-508.

Lertzman, K. P., and C. L. Gass. 1983. Alternative models of pollen transfer. *in* C. E. Jones and R. J. Little, editors. Handbook of experimental pollination biology. Scientific and Academic Editions, New York.

Levin, D. A. 1971. Pollen exchange as a function of species proximity in *Phlox*. Evolution **26**:251-258.

Levin, D. A. 1972. Low-frequency disadvantage in exploitation of pollinators by corolla variants in phlox. American Naturalist **106**:453-460.

Levin, D. A. 1978. The origin of isolating mechanisms in flowering plants. Evolutionary Biology **11**:185-317.

Levin, D. A., and W. W. Anderson. 1970. Competition for pollinators between simultaneously flowering species. American Naturalist **104**:455-467.

Levin, D. A., and D. E. Berube. 1972. *Phlox* and *Colias* - efficiency of a pollination system. Evolution **26**:242-&.

Levin, D. A., and H. Kerster. 1969a. Density-dependent gene dispersal in *Liatris*. American Naturalist **103**:61-74.

Levin, D. A., and H. Kerster. 1969b. The dependence of bee-mediated pollen and gene dispersal upon plant density. Evolution **23**:560-571.

Lewis, A. C. 1986. Memory constraints and flower choice in *Pieris rapae*. Science **232**:863-864.

Lindsey, A. H. 1982. Floral phenology patterns and breeding systems in *Thaspium* and *Zizia* (Apiaceae). Systematic Botany **7**:1-12.

Lord, E. M. 1981. Cleistogamy - a tool for the study of floral morphogenesis, function and evolution. Botanical Review **47**:421-449.

Magurran, A. E. 1988. Ecological diversity and its measurement. Princeton University Press, Princeton, N. J.

McGuire, A. D., and W. S. Armbruster. 1991. An experimental test for reproductive interactions between two sequentially blooming *Saxifraga* species (Saxifragaceae). American Journal of Botany **78**:214-219.

McGuire, A. D., and W. S. Armbuster. 1991. An experimental test for reproductive interactions between two sequentially blooming *Saxifraga* species (Saxifragaceae). American Journal of Botany **78**:214-219.

McLernon, S. M., S. D. Murphy, and L. W. Aarssen. 1996. Heterospecific pollen transfer between sympatric species in a midsuccessional old-field community. American Journal of Botany **83**:1168-1174.

Memmott, J., and N. M. Waser. 2002. Integration of alien plants into a native flower-pollinator visitation web. Proceedings of the Royal Society of London Series B-Biological Sciences **269**:2395-2399.

Messersmith, C. G., R. G. Lym, and D. S. Galitz. 1985. Biology of leafy spurge. Pages 42-56 of 104 *in* A. K. Watson, editor. Leafy Spurge. Weed Science Society of America.

Miyake, T., and K. Inoue. 2003. Character displacement in style length between pollinator-sharing *Clerodendrum trichotomum* and *C. izuinsulare* (Verbenaceae). Plant Systematics and Evolution **243**:31-38.

Moeller, D. A. 2004. Facilitative interactions among plants via shared pollinators. Ecology **85**:3289-3301.

Molano-Flores, B. 2004. Breeding systems of plants used for prairie restorations: A review. Transactions of the Illinois State Academy of Science **97**:95-102.

Moragues, E., and A. Traveset. 2005. Effect of *Carpobrotus* spp. on the pollination success of native plant species of the Balearic Islands. Biological Conservation **122**:611-619.

Morris, W. F., M. V. Price, N. M. Waser, J. D. Thomson, B. Thomson, and D. A. Stratton. 1994. Systematic increase in pollen carryover and its consequences for geitonogamy in plant populations. Oikos **71**:431-440.

Motten, A. F. 1982. Autogamy and competition for pollinators in *Hepatica americana* (Ranunculaceae). American Journal of Botany **69**:1296-1305.

Murcia, C., and P. Feinsinger. 1996. Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. Ecology **77**:550-560.

Murphy, S. D. 2000. Field testing for pollen allelopathy - a review. Journal of Chemical Ecology **26**:2155-2172.

Murphy, S. D. 2002. Biochemical and physiological aspects of pollen allelopathy. Pages 245-260 *in* Inderjit and A. U. Mallik, editors. Chemical Ecology of Plants: Allelopathy in Aquatic and Terrestrial Ecosystems. Birkhauser (sic) Verlag, Switzerland.

Murphy, S. D., and L. W. Aarssen. 1989. Pollen allelopathy among sympatric grassland species - invitro evidence in *Phleum pratense* L. New Phytologist **112**:295-305.

Murphy, S. D., and L. W. Aarssen. 1995a. In-vitro allelopathic effects of pollen from 3 *Hieracium* species (Asteraceae) and pollen transfer to sympatric fabaceae. American Journal of Botany **82**:37-45.

Murphy, S. D., and L. W. Aarssen. 1995b. Reduced seed set in *Elytrigia repens* caused by allelopathic pollen from *Phleum pratense*. Canadian Journal of Botany **73**:1417-1422.

Murphy, S. D., and L. W. Aarssen. 1996. Cleistogamy limits reduction in seed set in *Danthonia compressa* (Poaceae) caused by allelopathic pollen from *Phleum pratense* (Poaceae). Ecoscience **3**:205-210.

Niesenbaum, R. A. 1999. The effects of pollen load size and donor diversity on pollen performance, selective abortion, and progeny vigor in *Mirabilis jalapa* (Nyctaginaceae). American Journal of Botany **86**:261-268.

Olson, B. E., and R. T. Wallander. 2002. Effects of invasive forb litter on seed germination, seedling growth and survival. Basic and Applied Ecology **3**:309-317.

Ostler, W. K., and K. T. Harper. 1978. Floral ecology in relation to plant species diversity in Wasatch Mountains of Utah and Idaho. Ecology **59**:848-861.

Piehl, M. A. 1965. The natural history and taxonomy of *Comandra* (Santalaceae). Memoirs of the Torrey Botanical Club **22**:1-93.

Pleasants, J. M. 1980. Competition for bumblebee pollinators in Rocky Mountain plant communities. Ecology **61**:1446-1145.

Plowright, R. C., and L. K. Hartling. 1981. Red clover pollination by bumble bees - a study of the dynamics of a plant-pollinator relationship. Journal of Applied Ecology **18**:639-647.

Poole, R. W., and B. J. Rathcke. 1979. Regularity, randomness, and aggregation in flowering phenologies. Science **203**:470-471.

Price, M. V., and N. M. Waser. 1982. Experimental studies of pollen carryover - hummingbirds and *Ipomopsis aggregata*. Oecologia **54**:353-358.

Pyke, G. H. 1984. Optimal foraging theory: a critical review. Annual Review of Ecology and Systematics **15**:523-575.

Rademaker, M. C. J., T. J. de Jong, and P. G. L. Klinkhamer. 1997. Pollen dynamics of bumble-bee visitation on *Echium vulgare*. Functional Ecology **11**:554-563.

Randall, J. L., and K. W. Hilu. 1990. Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). American Journal of Botany **77**:939-944.

Rasband, W. S. 2006. ImageJ. U. S. National Institutes of Health, Bethesda, Maryland, USA.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305-329 *in* L. Real, editor. Pollination Biology. Academic Press, Inc., Orlando.

Redbo-Torstensson, P., and H. Berg. 1995. Seasonal cleistogamy - a conditional strategy to provide reproductive assurance. Acta Botanica Neerlandica **44**:247-256.

Richards, A. J. 1997. Plant Breeding Systems, 2nd edition. George Allen & Unwin, London.

Robertson, A. W. 1992. The relationship between floral display size, pollen carryover and geitonogamy in *Myosotis colensoi* (Kirk) Macbride (Boraginaceae). Biological Journal of the Linnean Society **46**:333-349.

Robertson, C. 1895. The philosophy of flower seasons and the phaenological relations of the entomophilous floral and the anthophilous insect fauna. The American Naturalist **29**.

Selbo, S. M., and J. S. Carmichael. 1999. Reproductive biology of leafy spurge (*Euphorbia esula* L.): breeding system analysis. Canadian Journal of Botany **77**:1684-1688.

Selleck, G. W., C. Frankton, and R. T. Coupland. 1962. Leafy spurge in Saskatchewan. Ecological Monographs **32**:1-29.

Sprague, E. F. 1962. Pollination and evolution in *Pedicularis* (Scropulariaceae). Aliso **5**:181-209.

Steenhagen, D. A., and R. L. Zimdahl. 1979. Allelopathy of leafy spurge (*Euphorbia esula*). Weed Science **27**:1-3.

Stephenson, A. G. 1981. Flower and fruit abortion - proximate causes and ultimate functions. Annual Review of Ecology and Systematics **12**:253-279.

Stiles, F. G. 1977. Coadapted competitors: the flowering season of hummingbird-pollinated plants in a tropical forest. Science **198**:1177-1178.

Stout, J. C., J. A. Allen, and D. Goulson. 1998. The influence of relative plant density and floral morphological complexity on the behaviour of bumblebees. Oecologia **117**:543-550.

Straw, R. M. 1972. Markov model for pollinator constancy and competition. American Naturalist **106**:597-620.

Stucky, J. M. 1985. Pollination systems of sympatric *Ipomoea hederacea* and *Ipomoea purpurea* and the significance of interspecific pollen flow. American Journal of Botany **72**:32-43.

Thomson, J. D. 1978. Effects of stand composition on insect visitation in 2-species mixtures of *Hieracium*. American Midland Naturalist **100**:431-440.

Thomson, J. D., and R. C. Plowright. 1980. Pollen carryover, nectar rewards, and pollinator behavior with special reference to *Diervilla lonicera*. Oecologia **46**:68-74.

Valentine, D. H. 1962. Variation and evolution in the genus *Viola*. Preslia **34**:190-206.

Waites, A. R., and J. Aagren. 2004. Pollinator visitation, stigmatic pollen loads and among-population variation in seed set in *Lythrum salicaria*. Journal of Ecology **92**:512-526.

Wang, J. Q., and M. B. Curzan. 1998. Interspecific mating in the *Piriqueta caroliniana* (Turneraceae) complex: Effects of pollen load size and composition. American Journal of Botany **85**:1172-1179.

Waser, N. M. 1978a. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology **59**:934-944.

Waser, N. M. 1978b. Interspecific pollen transfer and competition between co-occurring plant species. Oecologia **36**:223-236.

Waser, N. M. 1982. A comparison of distances flown by different visitors to flowers of the same species. Oecologia **55**:251-257.

Waser, N. M. 1983. Competition for pollination and floral character difference among sympatric plant species: a review of the evidence. Pages 277-293 *in* C. E. Jones and R. J. Little, editors. Handbook of Experimental Pollination Ecology. Van Nostrand Reinhold, New York.

Waser, N. M. 1986. Flower constancy - definition, cause, and measurement. American Naturalist **127**:593-603.

Waser, N. M., L. Chittka, M. V. Price, N. M. Williams, and J. Ollerton. 1996. Generalization in pollination systems, and why it matters. Ecology **77**:1043-1060.

Waser, N. M., and M. L. Fugate. 1986. Pollen precedence and stigma closure - a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. Oecologia **70**:573-577.

Waser, N. M., and M. V. Price. 1983. Optimal and actual outcrossing in plants, and the nature of plant-pollinator interaction. Pages 341-359 *in* C. E. Jones and R. J. Little, editors. Handbook of Experimental Pollination Biology. Scientifica and Academic Editions, New York.

Waser, N. M., and M. V. Price. 1984. Experimental studies of pollen carryover - effects of floral variability in *Ipomopsis aggregata*. Oecologia **62**:262-268.

Watson, A. K. 1985. Introduction - The leafy spurge problem. Pages 1-7 *in* A. K. Watson, editor. Leafy Spurge. Monograph Series of the Weed Science Society of America, Number 3. Weed Science Society of America, Champaign, Illinois.