POLLINATION AND POLLEN LIMITATION IN MAYAPPLE (*PODOPHYLLUM PELTATUM* L.), A NECTARLESS SPRING EPHEMERAL

by

James E. Crants

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Doctoral Committee:

Associate Professor Beverly J. Rathcke, Chair
Professor Deborah E. Goldberg
Professor Ivette Perfecto
Professor Earl E. Werner

DEDICATION

This dissertation is dedicated to my parents, Duane and Diana, and my wife, Ginny,
all of whom have supported me and encouraged me to work hard for my dreams.
I hope to return the favor.

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ABSTRACT

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James E. Crants

Chair: Beverly J. Rathcke

Mayapple (*Podophyllum peltatum* L.) is a common clonal understory herb in temperate eastern North America. Its fecundity is pollen-limited because its flowers are nectarless, and native pollinators do not collect its pollen. I conducted field studies in southeastern Michigan to determine mayapple's compatibility system and whether neighboring plants facilitated its pollination. I tested for facilitation by correlating the degree of pollen limitation with the abundances of neighbors and measuring whether the removal of neighboring flowers increased pollen limitation of fecundity.

Mayapple populations in four sites were self-incompatible (SI), but all clones in one site were self-compatible (SC). This difference could reflect genetic differences or possibly differences in inbreeding depression due to resources. The site with SC had the highest light availability and outcross fruit set, suggesting that abortion of inbred ovules may be lower under high resources, resulting in expression of SC.

Visitation to mayapple flowers was consistently low (0.03-0.06 visits/flower/hour), and fruit set was pollen-limited (pollen supplementation increased fruit set 3 – 18-fold) in all three years of this study. Based on regressions of pollen limitation violets facilitated fruit set in 2005 and 2007 but reduced seed set in 2005. Garlic mustard and spring beauty reduced fruit set in 2005. Except for violets in 2007, co-flowering species did not affect pollen limitation in 2006 or 2007. Floral removal did not change pollination success in 2006, confirming that neighbors neither facilitated nor competed with mayapple for pollination in that year.

Neighboring plants could also reduce mayapple fecundity through interspecific pollen transfer (IPT). The addition of *Phlox divaricata* pollen did depress fruit set, but *Geranium maculatum* pollen did not. However, foreign pollen was rare on mayapple stigmas suggesting that IPT is unlikely to be important in the field.

Mayapple could also facilitate or compete with neighboring plants for pollination. However, correlations showed no effect of mayapple on the pollination success of wild geranium, and hand-pollination with mayapple pollen did not significantly depress fruit or seed set.

In a review of studies on pollination facilitation, I propose that future studies employ similar methods and measure effect sizes for comparisons and meta-analyses.

Chapter I

Introduction

Mayapple (*Podophyllum peltatum* L.) is a common forest understory herb in temperate deciduous forests of eastern North America. It has an unusual combination of traits related to sexual reproduction. Most notably, it does not reward its native pollinators. Laverty (1992) and Laverty and Plowright (1988) found that, while exotic honeybees (*Apis mellifera* L., Apidae) gather its copious pollen, native bumblebee queen (*Bombus* spp., Apidae) only probe for nectar, which it lacks. Consequently, it has a very low pollinator visitation rate, and its fruit and seed production are severely pollen-limited (Swanson and Sohmer 1976, Rust and Roth 1981, Laverty and Plowright 1988, Laverty 1992, Whisler and Snow 1992).

Because its native pollinators find it unrewarding, mayapple has been said to be pollinated by "deceit" (Laverty 1992), but I find this term, at best, marginally useful. A deceptive species is an animal-pollinated species that has evolved to provide no reward to its pollinators. In cases where it is unclear whether the lack of reward has arisen through natural selection for that trait, I believe that the term "unrewarding" is a better choice, because it suggests only that pollinators do not find in the species' flowers the rewards for which they are foraging. (It also does not imply an intention on the part of the unrewarding species; "deceit" is a morally laden term that seems inappropriate for the life-history strategies of amoral organisms.) Honeybees collect pollen from mayapple and thus find it rewarding, and it is not clear that native pollinators never collect mayapple pollen, nor that pollen did not serve as a pollinator reward prior to the introduction of honeybees to mayapple's range. I will use the terms "unrewarding" and "nectarless," not "deceptive," to describe mayapple's flowers.

Outside of the Orchidaceae, there are few species for which all flowers produced are unrewarding, probably because most species incur costs for failing to reward their pollinators both in low visitation and in high prevalence of heterospecific pollen transfer (see Renner 2006 for overview). Rewardlessness may be common in the Orchidaceae because this family possesses adaptations for increased pollen transfer efficiency (Nilsson 1992, Harder and Johnson 2008). Pollinia (sticky packets of tens or hundreds of pollen grains), in particular, can increase pollen transfer efficiency by reducing the loss of pollen to grooming by the pollinator or deposition on surfaces other than the stigmas of conspecific flowers (Harder and Johnson 2008). Many non-orchids may increase pollen transfer efficiency through bilateral symmetry and placement of nectar at the bases of spurs or tubes. This allows them to place and intercept pollen on particular parts of a pollinator's body, since the pollinator must orient itself with the flower in a particular way to reach the nectar (e.g., Stiles 1975, Waser 1978, Campbell et al. 1996). However, mayapple lacks all of these adaptations to low pollinator visitation, and therefore seems poorly adapted to having unrewarding flowers.

Animal-pollinated plants that are unattractive to pollinators have little potential to influence the local density of pollinators. Their visitation rate and pollination success may thus depend on external influences on pollinator density. According to the "magnet species hypothesis" (Thomson 1978), highly attractive flowers improve the visitation rates of nearby, less attractive flowers by drawing pollinators to their vicinity. This effect has been demonstrated to improve visitation and reproductive success for mayapple in one study system (Laverty 1992), but it is not clear whether this effect is generally relevant to mayapple's pollination success. Furthermore, while the positive effects of magnet species on the pollinator visitation rates of less attractive species have been demonstrated more than once (Thomson 1978, Johnson et al. 2003, Juillet et al. 2007), this benefit may be diminished or reversed if it results in a high frequency of interspecific pollen transfer. Given mayapple's lack of obvious adaptations to heterospecific pollen receipt, it is possible that the effects of rewarding neighbors on its visitation rate are often negated by any detrimental effects of their pollen on ovule fertilization in mayapple.

In addition to its low visitation rate, a further hindrance to successful pollination for mayapple is that it is both extensively clonal and (usually) self-incompatible (Swanson and Sohmer 1976, Rust and Roth 1981, Policansky 1983, Laverty and Plowright 1988, Whisler and Snow 1992). This ensures that much of the conspecific pollen its stigmas receive is genetically incompatible. Thus, mayapple receives very few pollinator visits, and the visits it does receive carry a high risk of improper pollen transfer (*sensu* Rathcke 1983), in the form of both heterospecific pollen transfer and self-pollination.

The mechanism of self-incompatibility in mayapple is unknown, though moist stigmas and the ability of self pollen to germinate on the stigma indicate that the genetic mechanism is gametophytic (Whisler and Snow 1992). Studies on mayapple's breeding system have demonstrated self-incompatibility based on the inability of a plant to produce seeds when self-pollinated (Swanson and Sohmer 1976, Policansky 1983, Motten 1986, Laverty and Plowright 1988, Whisler and Snow 1992), but a plant may fail to produce seeds from self-pollination either because it is genetically self-incompatible or because ovules fertilized by self pollen die before maturing into seeds due to inbreeding depression.

Plants often produce more ovules than they can mature into seeds. One mechanism proposed to explain this phenomenon is the "selective ovule abortion" hypothesis, which says that plants preferentially abort the least fit embryos when more ovules are fertilized than can be matured (Janzen 1971, Korbecka et al. 2002). The ability of an ovule with low vigor to reach may depend on a combination of the resources available to the maternal parent, the presence and abundance of ovules with greater vigor being supported by the same maternal plant, and active abscission by the maternal plant of fruits and ovules that are developing slowly. Thus, self-fertilized ovules, which may have severe inbreeding depression, would be more likely to develop into mature seeds if the maternal plant has more resources and if few or no outcross-fertilized ovules are competing with them for resources. If this is the case, then self-compatible plants with very limited resources could appear to be self-incompatible, since self-fertilized ovules borne by such plants would be less likely to be able to obtain the resources to develop into seeds. In contrast, the same plant might be demonstrably self-compatible if resources were abundant; in the absence of competing outcrossed ovules, a self-pollinated ovule may be vigorous enough to obtain resources if they were readily available within the mother plant.

In the chapters that follow, I consider the traits that make sexual reproduction in mayapple puzzling. I first investigate whether mayapples in my study system are self-compatible and whether the expression of self-compatibility depends on resource availability, as expected if resource limitation results in an increased stringency of selective ovule abortion. I then test whether the magnet species hypothesis improves mayapple's reproductive success, whether any benefit from the magnet species effect is potentially counter-balanced by the elevated rate of interspecific pollen transfer that this mechanism entails, and whether mayapple, in turn, affects the pollination success of one potential magnet species. Finally, I review the current hypotheses for facilitation of pollination in the literature.

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Chapter II

Self-incompatibility, ovule abortion, and resource availability in mayapple (*Podophyllum peltatum* L.): do self-compatible plants appear self-incompatible when resources are scarce?

Introduction

The predominance of self-incompatibility in mayapple (*Podophyllum peltatum* L.) presents a conundrum. The fruit and seed set of mayapple are strongly pollen-limited throughout much of its range, apparently because it lacks nectar, for which its native pollinators forage (Krochmal et al. 1974, Rust and Roth 1981, Laverty and Plowright 1988, Laverty 1992, Whisler and Snow 1992). This should selectively favor self-compatibility, yet mayapple is predominantly or consistently self-incompatible in most of the populations that have been examined to date (Policansky 1983, Motten 1986, Laverty and Plowright 1988, Whisler and Snow 1992). In addition, mate limitation may also be severe for mayapple, since it is extensively clonal, and many cases of pollen transfer may therefore be geitonogamous. Like pollen limitation, mate limitation favors self-compatibility, since the ovules of a self-compatible plant may be fertilized by pollen grains with which the plant shares S-alleles, which is not true of self-incompatible plants (Busch and Schoen 2008).

More recently, habitat destruction and fragmentation may have genetically isolated mayapple populations while reducing their size, imposing stronger selection for self-compatibility through the limited availability of genetically compatible mates. While this has not been demonstrated for mayapple, reduced fecundity through mate limitation has been demonstrated for other species in fragmented habitats (Wagenius et al. 2007, Busch and Schoen 2008). Even if S-allele diversity is high, self-incompatible mayapples may experience strong mate limitation because extensive clonal growth ensures that most of the neighbors of any given flower are genetically incompatible mates (Honnay and Jacquemyn 2008). If current mayapple populations frequently experience mate limitation as well as pollinator limitation, self-compatible individuals may have higher fitness than self-incompatible ones, and this difference may be particularly pronounced in small, isolated populations.

The mechanism of self-incompatibility in mayapple is unknown (Whisler and Snow 1992). Self pollen produces pollen tubes, and the stigma is moist, indicating that a gametophytic self-incompatibility mechanism is at work (Whisler and Snow 1992). However, a careful investigation of the self-incompatibility mechanism has not been conducted. Rather, self-incompatibility has been verified by determining whether outcross-pollinated and self-pollinated flowers in the same clone produce seeds. If outcrossed flowers produce seeds and selfed ones do not, the clone is taken to be self-incompatible. Mayapple is presumed self-incompatible because it consistently produces more fruits and seeds when hand-outcrossed than when hand-selfed, and it usually fails to set seed when selfed (Swanson and Sohmer 1976, Policansky 1983, Motten 1986, Laverty and Plowright 1988, Whisler and Snow 1992).

While these results almost certainly indicate that a genetic self-incompatibility mechanism is at work, testing for self-compatibility based on seed set cannot distinguish true genetic self-incompatibility from severe inbreeding depression expressed as ovule abortion. Clones that produce seeds from self-pollination exhibit clear signs of reduced seed set due to inbreeding depression. Whisler and Snow (1992) found that seed set per fruit for self-pollinated flowers was 10% as high as outcross seed set in three highly self-compatible patches, and fruit set per flower from selfing was slightly over half as high as fruit set from outcrossing. Given that overall seed set per flower for demonstrably self-compatible clones was about 6% as great for selfed flowers as for outcrossed ones, it is possible that other clones had self-compatibility coupled with inbreeding depression so severe that no self-fertilized ovules matured into seeds. Indeed, low seed set per fruit when self-pollinated is a recognized indication of “pseudo self-compatibility,” of which inbreeding-induced ovule abortion is one form (Busch and Schoen 2008).

Inbreeding depression may manifest itself during seed development, particularly in plants with multi-seeded fruits (Helenurm and Schaal 1996, Keller and Waller 2002). If developing seeds compete with each other for maternal resources, those that contain embryos (and endosperms) with low genetic fitness may be at a disadvantage and therefore more likely to die early in development, and fruits with few viable embryos may be likely to be aborted for similar reasons (Helenurm and Schaal 1996). Even if embryos do not compete for maternal resources, inbred embryos are more likely than embryos produced by outcrossing to be unviable, resulting in low seed set for self-pollinated flowers, even if the parent plant is self-compatible.

Strong inbreeding depression may be mistaken for genetic self-incompatibility. Both inbreeding depression and self-incompatibility result in reduced fruit and seed set for self-pollinated flowers relative to outcrossed flowers (Wiens 1984, Helenurm and Schaal 1996, Sage et al. 2001, Vaughton and Ramsey 2003). If inbreeding depression is a significant cause of low reproductive success in self-pollinated flowers, and if the probability that an ovule is aborted depends on both the severity of its inbreeding depression and the resources available to it through the maternal plant (Helenurm and Schaal 1996, Ågren et al. 2008), a maternal plant with more resources to mature ovules into seeds would appear to be more self-compatible than a plant with fewer resources.

In this study, I address the hypothesis that apparent self-compatibility, measured by seed set for self-pollinated flowers, can vary in response to the resources available to the maternal plant because the probability that an inbred ovule is aborted before it develops into a mature seed depends on its access to resources. I contrast the predictions of this hypothesis with those of the hypothesis that the ability to produce seeds from self-pollination is entirely dependent on the presence or absence of self-compatibility.

Two resources appear to limit mayapple reproduction in my system: as an understory herb, mayapple's growth and reproduction is likely to be limited by light, and based on high ramet senescence during summer dry spells, reproduction may be limited by soil moisture. The effect of these resources on fecundity was determined by regressing fruit and seed set for bagged, outcross-pollinated flowers on measures of light, soil moisture, and soil texture. If the maturation of inbred seeds and fruits depends on resource availability, three predictions follow: (1) the fruit and seed production of self-pollinated flowers should co-vary with the limiting resource. (2) Because the fecundity of outcross-pollinated flowers should be limited by the same resource, fruit and seed production for selfed flowers should be positively related to fruit and seed set for outcrossed flowers in the same environment. (3) Similarly, because the vegetative growth of the maternal plant should be limited by the same resource as fecundity, plants that produce more seeds from self-pollination should be taller than those that do not.

Distance to the nearest forest edge varied among the study patches because they were initially selected to test for an edge effect on pollen limitation. Proximity to the forest edge may affect fecundity and plant height both through edge effects on light and moisture and through edge effects for which I did not test, such as drift of fertilizers, herbicides, and pesticides from neighboring crops, decreased relative humidity, or increased wind speed near edges. Therefore, I also compared fecundity of selfed and outcrossed flowers between plants within 30 meters of the forest edge and those over 100 meters from the edge.

Materials and Methods

Study organism:

Mayapple is a common understory herb of deciduous forests in the eastern United States and Canada. It is extensively clonal, spreading by branching rhizomes. Ramets that are physiologically connected by their rhizomes are well-integrated, but most ramets are not physiologically connected to other ramets (Landa et al. 1992).

Vegetative ramets and flowering ramets of mayapple are morphologically distinct. The above-ground portion of a vegetative ramet is a single peltate leaf with two to eight deep lobes, and the above-ground portion of a flowering ramet is a stem with two opposite leaves. A single flower typically emerges in mid to late May, from the fork where the leaves meet the stem.

The flower is large (3-6 cm in diameter), white, fragrant, bowl-shaped, and nodding, with 6 to 10 petals, 12 to 24 stamens, and a single, unilocular pistil with 15-100 ovules (pers. obs.). The flower lacks nectar but has abundant pollen (Laverty and Plowright 1988, and pers. obs., Laverty 1992). The flowers are protandrous; the anthers often dehisce before the flower has opened, but the stigmas are generally not receptive until one to two days later (Swanson and Sohmer 1976). Stigmas remain receptive even as the stamens and petals begin to fall from the flower, approximately one to two weeks after anthesis (pers. obs.).

Study sites:

Five study sites, identified here as sites 1-5, were selected on four fragments of second-growth deciduous forest in Washtenaw County, Michigan, between 42°13.65’ N and 42°15.00’ N and between 83°54.15’ W and 83°56.45’ W. Each fragment was between 9 and 40 ha in area and had an extensive and evident history of logging; stumps and logging trails were common in each fragment. Site 3 was selectively logged in April 2005 (the first month of this study), and Sites 2 and 4 were selectively logged in April 2007. Sites 3 and 4 and a portion of Site 2 had forest canopies composed largely of oaks (*Quercus rubra* L. and *Q. velutina* Lam., Fagaceae), hickories (*Carya ovata* [Mill.] K.Koch, *C. cordiformis* [Wangenh.] K.Koch, and *C. glabra* [Mill.] Sweet., Fagaceae), and black cherries (*Prunus serotina* Ehrh., Rosaceae). These species were also present in Sites 1 and 5, but the canopy of Site 1 was dominated by basswood (*Tilia americana* L., Tilliaceae), and Site 5 had emergent oaks, hickories, cherries, and basswoods over a dense, low canopy of sugar maples (*Acer saccharum* Marshall., Aceraceae). In addition, a portion of Site 2 had a canopy of red maples (*A. rubrum* L.).

I selected ten to thirteen mayapple patches for study in each site (60 patches in total), with half of the patches within 30 meters of the forest edge and half over 100 meters from the edge. In each of these groups, half of the patches had wild geraniums (*Geranium maculatum* L., Geraniaceae) within 5 meters and half did not. These selection criteria were used because a parallel study evaluated the effects of wild geraniums and forest edges on pollen limitation in mayapple. Three patches per site were selected for the mating system experiments, with one patch selected randomly from among the forest edge patches, one from the interior patches, and one from the full set of study patches.

Mating system experiments

In 2005, in each of the three patches selected for the mating system study, I marked twelve forked (i.e., reproductive) ramets with apparently healthy buds for four treatments. Two ramets were marked to have their flower buds bagged with no other treatments (hereafter, the “autogamy group”), to determine whether mayapples in my sites were capable of self-pollination without pollinator visitation. Two were marked to be bagged and emasculated to test for agamospermy (the “agamospermy group”). Four were marked to be bagged, emasculated, and self-pollinated by hand (the “selfed group”), and four to be bagged, emasculated, and outcross-pollinated by hand (the “outcrossed group”), to measure the degree of self-compatibility for mayapples in my sites. A bag of fine nylon tulle was tied over the bud of each of these ramets.

Emasculation and pollination treatments were applied on one of two visits to each patch between 17 and 29 May 2005, the first and last days when open flowers with receptive stigmas were commonly observed in the study patches. When open flowers were found in bags, the bags were opened to remove the bud scales, regardless of treatment group. I emasculated flowers by plucking the stamens from the flower with a pair of tweezers. The stamens were large and turgid and could typically be removed cleanly at the base. Self pollinations were performed by applying three of the flower’s own anthers to the stigma. Two flowers receiving this treatment lacked healthy anthers, and these were pollinated with anthers from adjacent ramets. I performed outcross pollinations by applying three anthers from three other patches within the fragment to the stigma of the treated flower. The pollen source patches were at least 50 meters away from the patch to which the treatment was applied. Pollen could be seen as yellow streaks on the stigma, with individual grains visible through a hand lens, so successful pollination could be confirmed.

One patch marked for mating system treatments did not produce viable flowers inside any of the bags and could not be treated or used for analysis. Another produced only eight viable flowers, and only the self- and outcross-pollination treatments were applied in this patch. Several patches were missing single flowers from one or more treatment groups. In cases where flowers were missing from treatment groups or where treatment groups had to be dropped, flowers had undeveloped pistils or failed to open, or ramets were destroyed during the flowering season, after all unbagged flowers had opened.

After the flowering season, the pistils of all unbagged flowers within the patches used for this experiment were removed to reduce the likelihood of resources from self-pollinated flowers in bags being diverted to open-pollinated flowers that received outcross pollen. Thus, the probability that a self-compatible clone would produce fruits from self-pollination instead of selectively aborting them was increased, thereby increasing the probability that self-compatibility would be detected where it occurred.

Environmental measurements

To determine whether environmental factors limited the reproductive success of the flowers in any of my four treatment groups, I made environmental measurements in each patch and performed regressions of fruit and seed set on these measurements or on principal component axes derived from them (see "Data Analysis" below).

In September 2005, I measured canopy openness above each patch at approximately one meter above the ground using a spherical densiometer. I also collected a 30-cm soil core from the center of each patch for analyses of soil moisture, texture, and organic content. Soil moisture was determined within a day after collection using the gravimetric method. Soil texture was determined in June to August 2007 by the Bouyoucos hydrometer procedure (Bouyoucos 1936), and soil carbon was measured by the Walkley–Black wet combustion method (Walkley and Black 1934).

I revisited the patches in 2006, when all patches that flowered were incorporated into a study on pollination limitation. Mean ramet height, mean anther number per flower, and mean flower diameter were determined for a subset of the patches. In August, the leaf-area index was determined for each patch at 80 cm above the ground using an LAI-2000 Plant Canopy Analyzer (LI-COR Biosciences).

In 2007, the pollination limitation study was continued, and mean ramet height, mean anther number per flower, and mean flower diameter were determined for most of the patches that flowered. Data on anther number and floral diameter were missing for two and one patches, respectively, in Site 3. Site 5 could not be revisited in 2007. Leaf-area index was measured for a subset of the patches in July, and percent canopy cover was determined for all patches in sites 1-4. Measurements of flower diameter, anther number, leaf-area index, and canopy openness were found to correlate strongly and positively among years (e.g., large-flowered patches in 2005 also had large flowers in

2007). Thus, the measurements of leaf-area index and ramet height from 2006 and the measurements of anther number and flower diameter from 2007 were considered relevant to the 2005 study and were used in the data analysis.

Fruit and seed set

The presence, length (peduncle to stigma), and width (across the placentated wall) of each fruit in the study patches were determined on 6-15 June, 8-21 July, and 2-15 August 2005. Fruits were collected when ripe or abscised in July, and all remaining fruits that could be found were collected in August. Collected fruits were measured in three linear dimensions (length, width, and depth from the placentated wall to the opposite wall), weighed, and dissected for seed counts.

Several fruits disappeared between the July and August surveys. Fruits were at or near their maximum sizes in July, and some were ripe toward the end of the July survey. Few of the fruits that disappeared between the July and August surveys could have been aborted due to unsuccessful pollination, and the missing fruits should thus be included in measurements of fruit and seed set.

To account for mature fruits that disappeared between July and August, I estimated the number of seeds present in each uncollected fruit using curve estimation regression of seed counts as a function of the July length and width of all 172 fruits collected in 2005, including 22 fruits collected for this study, 102 fruits collected for a concurrent pollen limitation study (Chapter 3), and 48 fruits found in non-study patches. Fruit width proved to be a better indicator of final seed set than fruit length or the product of length and width, best fitting final seed set through the following equation:

$$S = 0.0007\, w^{2.9632} - 1, \qquad \text{(Eq. 1)}$$

where **S** is the number of seeds in the fruit and **w** is the width of the fruit. Equation 1 fit the data for the 172 collected fruits well ($p < 0.001$, $R^2 = 0.640$, df = 171). This equation was used to estimate seed number in the fruits that were not collected but were present in the July fruit survey, while actual seed numbers were used for all the collected fruits. For the purposes of the data analyses described below, "fruit set" and "seed set" refer to fruit set per flower and best-estimate seed set per fruit in July. Analyses were also performed on fruit and seed set based only on collected fruits, but these will only be reported if they are different from the results based on fruit and seed set in July.

Statistical analyses

All statistical analyses were performed using the statistical program SPSS for Windows 11.0, Graduate Pack, ©2001, SPSS Inc.

Effect of pollination treatment

The unit of replication was the individual mayapple patch. The dependent variables were fruit set per flower and seed set per fruit. The data for these variables were non-normally distributed, due to very low fruit set in all treatment groups except for the outcross pollination group. Data transformation could not rectify the non-normal distributions of fruit and seed set data for the bagged, bagged and emasculated, or bagged, emasculated, and selfed treatment groups. Therefore, I used non-parametric tests to determine the effect of treatment on reproductive success. Measurements of reproductive success were compared among the four treatments using Kruskal-Wallis tests, and pairwise between treatments using Mann-Whitney U tests. These comparisons were made for fruit presence and fruit width in June and fruit set and estimated seeds per fruit and per flower in July (table 2.1).

Effect of environmental, patch and floral characteristics

To determine which resources limited the fecundity of outcrossed flowers, I tested for the effects of environmental factors on outcross fruit set per flower and seed set per fruit. Based on Pearson correlations performed on the 15 patches included in this study and 58 patches used in a concurrent pollen-limitation study, several of these measured environmental variables were strongly correlated with others. Because of these correlations and the large number of environmental variables relative to the sample size, multiple regressions could not be performed using the raw measurements. The small sample size and large number of variables also made data reduction by factor analysis of the full set of independent variables an unviable option, as principal components analyses performed with sample sizes below 100 have a high risk of producing unrepresentative axes, particularly when many independent variables are involved (Allison 1999).

To reduce the number of variables and increase their independence while minimizing the probability of generating unrepresentative principal components, I performed principal components analyses on the most strongly correlated groups of biologically-related variables to generate composite variables for multiple regression. In each case, patches from the pollination limitation study, including those added to the study in 2006, were included to increase the probability that the axes generated were representative for mayapple patches in the region as a whole. All soil variables (soil moisture, soil carbon content, and soil clay, silt, and sand content) were combined to produce a "soil moisture" component (strongly correlated with moisture, carbon and clay) and a sand-silt component. These two components explained 84.5% of the variation in soil characteristics. Because soil texture could not be measured for two patches that occurred on peaty soils, soil moisture and carbon content were used to generate a principal component for a separate regression model. This component explained 95.4% of the variation in these two variables. Densiometer readings from 2005 and leaf-area index readings from 2006 were combined to produce a "light" component, which explained 68.4% of the variation in these components.

For outcrossed flowers, data for both fruit set per flower and seed set per fruit had distributions that did not differ significantly from normality, based on Kolmogorov-Smirnov tests ($P \geq 0.445$ for both fruit and seed set), and data transformation did not greatly improve their fit to a normal distribution. Therefore, linear regressions on principal components were performed using untransformed fruit and seed set data. Fruit set per flower and seed set per fruit were each analyzed with two regression models: (1) a model including soil moisture (including clay content), soil silt/sand, light, and proximity to the forest edge, and (2) a model including only soil moisture (not including clay content), light, and proximity to the forest edge.

In addition, the same measures of reproductive success were analyzed in a multiple regression model including mean ramet heights and numbers of vegetative and reproductive ramets in patches, to determine whether larger patches or taller ramets were indicative of greater resources for fruit and seed maturation. Finally, reproductive success variables were regressed on patch mean flower diameter and patch mean anther number per flower separately, to see whether greater investment in these floral organs comes at a cost to maximum potential female fitness.

For each multiple regression, a backward stepwise regression was performed to determine which, if any, of the environmental or patch variables most strongly determined reproductive success of outcrossed flowers.

If patches with greater resources for fruit and seed maturation are more likely to produce mature seeds and fruits from self-fertilization, then (1) outcross fruit and seed set should be higher in self-compatible patches, and (2) self-compatible patches should have vegetative and environmental characteristics that are associated with high outcross fruit and seed set. To test these predictions, outcross reproductive success, environmental characteristics, and patch characteristics were compared between self-compatible patches and self-incompatible patches. None of the variables compared was significantly non-normally distributed, either for all patches pooled or for the self-compatible and self-incompatible groups separately, based on one-sample Kolmogorov-Smirnov tests. Therefore, independent-samples t-tests were used for these analyses. However, soil moisture/carbon had a marginally non-normal distribution ($P = 0.064$; all other variables $P > 0.3$). A Mann-Whitney U test was performed with this variable to determine whether the t-test's assumption of normality affected the statistical significance of the results.

Results

Pollination experiments: self-compatibility, autogamy, and apomixis

Most flowers in all four treatments initiated fruit development. Of 151 flowers included in the experiment, 143 (94.7%) had not abscised their ovaries by the time of the early-season June survey of fruit sizes (6 to 15 June). Although the outcross treatment was the only treatment that initiated fruit on all flowers, the difference in fruit initiation among treatments was not significant overall, and fruit initiation did not differ significantly between any two groups (table 2.1). Fruit width did vary significantly with treatment overall, and outcross fruits were significantly wider than those in any of the other three groups (table 2.1), indicating that outcross fruits had higher ovule fertilization success and were maturing more ovules.

Most ovaries had abscised (i.e., were aborted) by the time of the mid-season July survey (8 to 21 July). By this time, only 21.0% of the flowers bore fruits. Fruit set per flower differed significantly with treatment, with fruit set for the outcross group being significantly greater than that for any other group (table 2.1). Similarly, the best estimate of seeds per fruit in July was significantly greater for outcrossed flowers than for selfed ones (table 2.1).

Plants produced fruits from flowers in the autogamous treatment group in only two patches (patches 9 and 11 in Site 3), with each of these patches producing one fruit from two flowers in this group. Using the allometric equation described above (Eq. 1), these fruits were estimated to be seedless, and they had abscised by the time of the August survey.

Plants bore fruits on bagged, emasculated, unpollinated flowers in just one patch (patch 10 in Site 3) in July. This patch had two fruits from two flowers in this treatment group. Both had abscised and disappeared by August, and each was estimated to have had a single seed based on its width.

Plants in three patches (patches 9, 10, and 11, all in Site 3) bore fruits on bagged, emasculated, self-pollinated flowers in July. Patch 9 bore two fruits from four selfed flowers in July, one of which was collected. The collected fruit was seedless, and the other fruit was estimated to be seedless based on its small size. Patch 10 bore one fruit from three selfed flowers in July. This fruit was collected and found to contain ten seeds. Patch 11 bore three fruits from four flowers in July. Two were collected and found to have one and three seeds. The remaining fruit was estimated to have contained twelve seeds.

Do current resources limit reproductive success of outcrossed flowers?

The regression model that included soil texture characteristics explained a marginally significant amount of variation in fruit set per hand-outcrossed flower, and the model that excluded them explained a significant amount of variation (table 2.2). In both of the regression analyses, the light component was the only variable included in the final model of the backward stepwise regression; fruit set increased significantly with light whether the two patches that occurred on peaty soils were included ($P = 0.007$, adjusted $R^2 = 0.423$, total df = 13) or not ($P = 0.022$, adjusted $R^2 = 0.367$, total df = 11). Although no other variables were included in the final model of the backward stepwise regression when all fruits present in the July survey were considered, proximity to the forest edge was retained in the final model considering only collected fruits per flower, when the patches on peaty soil were included. Proximity to the edge, by itself, explained a marginally significant amount of variation in fruit set based only on collected fruits ($P = 0.089$, adjusted $R^2 = 0.157$, total df = 13), with fruit set being higher near edges, but the difference was not significant for fruit set based on all fruits present in July ($P = 0.276$, adjusted $R^2 = 0.023$, total df = 13).

Both regression models of environmental variables failed to explain a significant amount of the variation in seed set per outcrossed fruit (table 2.2). Backward stepwise regressions did not produce a significant model. The final variable removed from the model that included soil texture was soil silt/sand; seed set per fruit tended to increase with soil silt content ($P = 0.117$, adjusted $R^2 = 0.189$, total df = 9). The last variable left in the backward regressions from the model that excluded soil texture was proximity to the forest edge, which was not significantly related to seed set ($P = 0.595$, adjusted $R^2 = -0.075$, total df = 10).

Is outcross reproduction related to patch traits or allocation to floral traits?

Mean ramet height and the numbers of reproductive and vegetative ramets explained a marginally significant amount of variation in July fruit set (table 2.2). Mean ramet height and the number of reproductive ramets in the patch were left in the final model of the backward stepwise regression, which was a statistically significant model ($P = 0.020$, adjusted $R^2 = 0.422$, total df = 13). In this model, the number of reproductive ramets was significantly related to fruit set ($P = 0.036$, beta = -0.507), while mean ramet height was marginally significantly related to fruit set ($P = 0.054$, beta = 0.457). Fruit set decreased significantly with increasing number of reproductive ramets in a univariate regression ($P = 0.041$, adjusted $R^2 = 0.246$, total df = 13) and increased marginally significantly with mean ramet height ($P = 0.065$, adjusted $R^2 = 0.194$, total df = 13). However, the negative relationship between outcross fruit set and the number of reproductive ramets in the patch was due largely to two patches with many reproductive ramets that set no outcross fruit.

Seed set per fruit was not significantly explained by mean ramet height and the numbers of reproductive and vegetative ramets (table 2.2). No variables were retained in the backward stepwise regression, and the last variable removed, mean ramet height, was far from statistically significant ($P = 0.725$, adjusted $R^2 = -0.095$, total df = 10). The full model also failed to explain significant variation in the number of seeds found in collected outcrossed fruits alone, but backward stepwise regression found that seed set increased marginally significantly with mean ramet height for collected fruits ($P = 0.091$, adjusted $R^2 = 0.262$, total df = 8).

Mean floral diameter and mean number of anthers per flower both failed to significantly explain both fruit set and seed set (table 2.2). Considering only collected fruits, seed set per fruit increased significantly with mean floral diameter ($P = 0.024$, adjusted $R^2 = 0.607$, total df = 6).

How do self-compatible patches differ from other patches?

Three patches, all in Site 3, bore fruits from self-pollinated flowers. However, only two of these patches produced seeds. The third patch bore two fruits from four self-pollinated flowers, one of which was collected and found to be empty, and the other presumed seedless based on its small size.

Because all self-compatible patches were in one site, it is not possible to determine whether patches in Site 3 were found to be self-compatible because this population has a high frequency of alleles for weakened self-incompatibility or because environmental conditions in the site were more favorable for fruit production than conditions in other sites. Environmental conditions varied more among sites than within sites, so Site 3 had both a different population and a different environment from the other sites.

Within Site 3, the patch that bore seedless fruits differed noticeably from the other two in two ways. The seedless patch had the most vegetative ramets of the three (116 versus 24 and 83), and it grew on the siltiest soil (41% versus 35% and 27% silt). Neither of these differences explained much variation in outcross fruit or seed set, and it therefore seems unlikely that they explain differences in measured self-compatibility.

Because all self-compatible patches were aggregated in one site, I compared the reproductive success of outcrossed flowers, as well as the environmental, vegetative, and floral traits of patches between Site 3 and the other four sites.

Patches in Site 3 tended to have more outcross fruits per flower, seeds per fruit, and seeds per flower than those that lacked fruits on selfed ramets (table 2.3), consistent with the hypothesis that mayapple's ability to mature inbred seeds is resource-limited. The difference was significant for seeds per flower (table 2.3). In addition, marginally significantly more fruits were collected per outcrossed flower in Site 3 than in the other sites (independent samples t-test: $P = 0.086$, $t = -1.869$, total $df = 12$).

The patches in Site 3 also scored significantly higher on the light axis than the other sites as a group (independent samples t-test: $P = 0.033$, $t = -1.2966$, total $df = 13$) and had significantly taller ramets (t-test: $P < 0.001$, $t = -5.202$, total $df = 12.970$). Site 3 was significantly brighter than sites 1, 2, and 5 in pairwise comparisons (Mann-Whitney U tests: $P < 0.005$), and marginally brighter than site 4 (Mann-Whitney U Test: $P = 0.078$). Ramets in Site 3 were significantly taller than ramets in each of the other four sites, pairwise (Mann-Whitney U tests: $P < 0.02$). Both light and ramet height were significantly related to July and final fruit set per flower (see above).

Discussion

A plant that produces seeds from outcross-pollinated flowers but not from self-pollinated flowers is assumed to be self-incompatible. However, while a flower may fail to produce seeds when self-pollinated because a self-incompatibility mechanism prevents self pollen from fertilizing the ovules, it may also fail because self-fertilized ovules do not reach maturity due to inbreeding depression. If this is the case, the ability of inbred ovules to develop into mature seeds may depend on the resources available to the maternal plant. This would be true if developing ovules must compete for resources with other structures of the maternal plant (e.g., rhizomes, roots, and other fruits). Ovules containing inbred embryos may not be able to obtain enough resources to develop unless the maternal plant has abundant resources.

The presence or absence of a genetic self-incompatibility system could not be determined in this study. However, it is possible to address the hypothesis that self-fertilized ovules are more likely to obtain the resources to mature into seeds when the maternal plant has abundant resources, and the evidence from this study is consistent with the predictions of this hypothesis. Plants that demonstrated self-compatibility had higher fruit and seed set when outcrossed, as well as taller ramets, than those that did not, indicating that they had more resources available for both growth and reproduction. They occurred in the site with the highest light availability, which points to light as the resource that limits the ability of a mayapple plant to mature self-fertilized seeds, and they occurred on sandy soils with low organic content and low soil moisture, suggesting that water availability does not restrict seed set from self-pollination.

The importance of light, rather than water, as a limiting resource is further supported by the higher fruit set of outcrossed flowers in well-lit patches and patches near the forest edge. Edge patches receive light from the nearby open habitat, which may not be accounted for in measurements of leaf-area index and openness of the canopy above the patch. Edge patches and better-lit patches also have lower soil moisture. Because the outcrossed flowers were heavily hand-pollinated with a mixture of pollen from three other genets, the probability that they produced fruit was presumably limited by resources other than pollen, and the positive relationship between outcross fruit set and both measured light availability (canopy openness and leaf-area index) and proximity to the forest edge suggests that light limits fruit set when pollen does not.

This is not the only study to find that self-fertile mayapple patches have higher fruit set from outcrossed flowers than patches that do not produce fruits when self-pollinated. I found that just 47.7% (+/- 11.1% SE) of outcrossed flowers bore fruits in July for self-incompatible patches, versus 83.3% (+/- 8.3% SE) outcross fruit set for self-compatible patches. Similarly, Whisler and Snow (1992) classified patches into three self-compatibility groups based on percent fruit set from self-pollination (0%, 1-50%, 51-100%) and found that more self-compatible categories had higher fruit set from outcrossed flowers (81%, 88%, and 94% for the three groups, respectively). The authors do not comment on this relationship, and since the range in mean outcross fruit set among self-compatibility classes was small, it is likely that the relationship was not statistically significant. Nevertheless, it may be noteworthy that patches that had higher fruit set from self pollination also had higher fruit set from outcross pollination in both studies. This suggests that patches that produce fruits from self-pollinated flowers have more resources for fruit maturation than those that do not, consistent with the hypothesis that apparent self-incompatibility in mayapple is often a result of inbreeding depression coupled with resource limitation. In contrast, if apparent self-incompatibility were perfectly equivalent with the presence of a functioning self-incompatibility mechanism, hand-pollination with a mixture of pollen from three or more mates (as used in my study and that of Whisler and Snow 1992) should not be substantially more likely to provide compatible pollen to self-compatible flowers than to self-incompatible ones, unless the population has extremely low S-allele diversity.

Nevertheless, the hypothesis that self-incompatibility in mayapple is strictly or partly genetically determined is in no way inconsistent with the results of this study. Alleles that confer weakened self-incompatibility may simply be more common in Site 3 than in the other sites, which could occur through natural selection or genetic drift, if historical habitat disturbance caused a genetic bottleneck. Self-compatible clones could have been well-represented during the bottleneck period, or self-compatible mayapples may have had a selective advantage in re-colonizing disturbed habitats, as strict outcrossers failed to set seed due to a lack of mates (Baker 1955). This historical selection for self-compatibility may not be evident under current conditions, with higher population densities and widespread infection by mayapple rust (*Puccinia podophylii*; Parker 1989) or other pathogens.

While my data do not permit a definitive conclusion on the degree to which apparent self-incompatibility depends on a true self-incompatibility mechanism versus inbreeding depression, the two possibilities are certainly distinguishable. Self-incompatibility prevents pollen from germinating or prevents pollen tubes from reaching and fertilizing ovules (Castric and Vekemans 2004), while inbreeding depression does not take effect until after fertilization has occurred. It is known that self pollen on a mayapple stigma can germinate (Whisler and Snow 1992), but it is not known whether pollen tubes from self pollen can reach the ovules. If a genetic incompatibility mechanism is at work, mayapple pistils fixed at a range of times following pollination with self or outcross pollen would show that pollen tubes from self pollen are stopped short of ovule fertilization. This would also reveal whether self-compatible patches are fully self-compatible (pollen tube growth rate and ovule fertilization probability are independent of whether the pollen is self or outcross pollen) or have "leaky" self-incompatibility (self pollen tubes have slower growth rates than outcross tubes).

Agamospermy and Autogamy

The patches in Site 3 were also the only ones to produce fruits in the pollination treatments intended to test for autogamy and apomixis. However, all of these fruits were abscised between July and August, and allometric estimates indicated that none produced more than one seed. Mayapple is protandrous, with anthers sometimes dehiscing before anthesis (Whisler and Snow 1992 and pers. obs.), and it is possible that any seeds produced in the apomixis treatment were the result of autogamous pollination that occurred before the emasculation treatment was performed. To date, only one apomictic population of mayapple has been reported, in western New York State (Bernhardt 1975, as cited by Swanson and Sohmer 1976), and the evidence that Site 3 represents the second such population is extremely weak. Given that no seeds were collected from either the autogamy or apomixis groups in Site 3, and that many of the fruits I have collected in this system in three years have proved to be barren, it is safest to assume that the patches in Site 3 are neither apomictic nor autogamous.

Geographic variability in the prevalence of self-compatibility in mayapple

If self-compatibility is favored by genetic bottlenecks due to decreased S-allele diversity (Baker 1955), it may be more prevalent near the expanding edges of a species' range or in portions of its range in which populations have a history of instability (Pannell and Barrett 1998, Busch and Schoen 2008). S-allele diversity can be lower in recently-established populations (Brennan et al. 2006), and populations near the limits of a species range can have lower S-allele diversity and higher prevalence of self-compatibility (Busch 2005). In the case of mayapple, the northern frontier may be particularly likely to harbor self-compatible populations, since populations near this limit are among the last to have been re-colonized following the most recent Pleistocene glaciation. Western populations, those in the pre-settlement transition zone from forest to savanna to prairie, may have also a high prevalence of self-compatibility, due to habitat disruption and fragmentation by anthropogenic fires since the last ice age. The southeastern limit of mayapple's range is potentially also of interest, as it is apparently less common near this boundary than it is near much of the northern limit, but no studies on mayapple's mating system have been conducted in this region. To determine whether mayapple's mating system differs between frontier and core populations, I will briefly review the studies, in addition to my own, that have attempted to determine mayapple's mating system.

The current study was conducted near the northern edge of mayapple's range, in a region that was dominated by oak-hickory forests at the time of the U.S. government's General Land Office survey of Michigan (ca 1816-1856). At the maximum of the Wisconsin glaciation (about 18,000 years BP), all of Michigan was covered in ice. The glaciers had retreated beyond Washtenaw County by about 14,000 years BP. Since that time, mayapple's range has expanded to approximately 170 km north of my study sites, but it is uncertain how long it has been present in the vicinity of my study. Perhaps the study system's location near, but not at, the northern limit of mayapple's range explains why the mating system for my populations as a whole was mixed, with self-compatibility present but less common than self-incompatibility.

Three other studies focused on populations similarly close to the northern limit of mayapple's range, and one of these was also close to the western limit. Swanson and Sohmer (1976) studied populations in southwestern Wisconsin and adjacent Minnesota, in the far northwestern limit of mayapple's range. They found that geitonogamously-pollinated flowers did not set seed, while outcross-pollinated flowers did. Laverty and Plowright (1988) worked in a population on Amherst Island at the northeastern end of Lake Ontario. They found no fruit set among twenty hand-selfed, bagged flowers in each of four patches, while all ten hand-outcrossed flowers in each patch produced fruit and seeds. Finally, Policansky (1983) detected self-compatible patches near Weston, Massachusetts, but he does not make it clear whether how many clones he tested or whether any were self-incompatible. Overall, self-incompatibility dominates near the northern and western limits of mayapple's range.

Even given that most populations that have been studied near the northern and western limits of mayapple's range have been self-incompatible, it is possible that self-compatibility is more common in these locations than closer to the center of the species' range. To date, five studies have tested for self-compatibility in mayapple at least 300 km from its northern, western, and southeastern limits. Policansky (1983) found self-compatible plants near Oak Ridge, Tennessee, while none of the autogamous pollinations he performed near Princeton, New Jersey yielded fruit. In North Carolina, Motten (1986) bagged and self-pollinated twelve mayapple flowers, none of which set fruit, while open-pollinated flowers receiving supplemental outcross pollen by hand had 31.3% fruit set (Motten 1986). In Delaware, Rust and Roth (1981) found that ten flowers that were autogamously hand-pollinated had all abscised their ovaries by five weeks later, while 20 geitonogamously-pollinated flowers gradually abscised their fruits over three months, and 22 of 34 cross-pollinated flowers retained their fruits until they were collected. Finally, Whisler and Snow (1992) found that 74% of the mayapple patches in their seven sites in central and northeastern Ohio were completely self-incompatible, while 6% were highly self-compatible (over 50% fruit set from self-pollination) and 20% were moderately self-compatible (non-zero fruit set from selfing, but less than 50%; Whisler and Snow 1992).

Overall, there is no evidence that self-compatibility is more common at the northern or western frontiers of mayapple's range than in the interior. Two of four studies conducted near the northern and western limits of mayapple's range found no self-compatible clones, while three of five studies conducted far from these boundaries found the same. This is inconsistent with the hypothesis that rapid range expansion after the last glacial maximum has selected for increased self-compatibility in mayapple populations at the northern limit of its range.

Whether sparse or recently established mayapple populations are more likely to have self-compatible patches cannot be determined from studies published to date, few of which provide details about population density or age. For the current study, I specifically selected forest fragments in which mayapple was common. Previous studies were probably similarly biased against sparse populations, since organisms are easier to study in places where they are abundant. Fruiting failure can be complete in sparse populations (pers. obs.), potentially increasing the strength of selection favoring self-compatibility in such populations. However, small, sparse populations with very limited sexual reproduction would have limited potential to evolve in response to selection, and it is therefore doubtful whether such populations are likely to evolve a greater prevalence of self-compatibility than denser populations. Indeed, given that self-compatible individuals are better at stably colonizing new habitats and expanding their populations (Baker 1955), the sparsest populations may be those that lack self-compatibility.

The bias toward dense populations may produce a bias toward relatively old populations. However, the effect of this bias on detected prevalences of self-compatibility is unclear. Older populations have had more time to evolve in response to selection, but the direction of selection on self-compatibility in such populations may depend on the diversity of S-alleles present. If diversity is low, mate limitation will more strongly favor self-compatibility, while if it is high, mate limitation is a weaker factor in reproductive success, and selection for self-incompatibility through inbreeding depression may dominate.

The paradox of self-incompatibility in the face of chronic pollen limitation

The apparent paradox of self-incompatibility in a chronically pollen-limited species may be explained in at least five ways.

(1) Pollen limitation in mayapple is an artifact of anthropogenic disruption of pollinator networks. For example, mayapple may have lost its most common pollinators in most habitats, or the phenological fit between the time mayapple's flowering time and its pollinators' pollen foraging times may have been altered by anthropogenic changes in habitat or climate. This explanation for the combination of chronic pollen limitation and self-incompatibility could also explain why mayapple flowers are fragrant and do not (to human eyes) resemble other flowers in much of mayapple's habitat. Pollinators learn to avoid deceptive flowers much more rapidly if those flowers have an odor unlike any familiar rewarding flowers (Kunze and Gumbert 2001).

This hypothesis is also compatible with mayapple's lack of morphological adaptations to reduce interspecific pollen transfer. A deceptive flower, being unable to win pollinator loyalty, can only be visited by inconstant pollinators. It will therefore both receive heterospecific pollen on its stigmas and lose pollen to heterospecific stigmas if it has no adaptations to increase pollen transfer efficiency and reduce interspecific pollen transfer. The evolution of universal deceit (that is, pollinator deceit by every individual in the species) is highly improbable for any species that is not pre-adapted for low pollinator constancy.

(2) If populations are not highly genetically structured (due, for example, to frequent long-distance seed dispersal), then biparental inbreeding may be rare. Between self-incompatibility and low biparental inbreeding, recessive, deleterious alleles may be exposed to selection too rarely to be purged from the population. This would maintain strong selection against self-compatibility because the fitness of progeny produced by selfing will be greatly reduced by inbreeding depression. This hypothesis found support from Whisler and Snow (1992), who found that mean seed set for open-pollinated flowers of self-compatible mayapples was not conspicuously higher than mean seed set for open-pollinated flowers of self-incompatible mayapple; the increase in fruit set due to self-compatibility was apparently compensated for by the loss in seed set per fruit due to

inbreeding depression. If inbreeding depression also greatly decreased the fitness of seeds produced from self-pollination, the production of similar numbers of seeds by self-compatible and self-incompatible individuals would favor self-incompatibility despite the genetic transmission advantage of self-compatibility (Fisher 1941).

(3) Mayapple's reproduction, and the reproduction of nearly any species, may not be truly pollen-limited (Haig and Westoby 1988). No studies have tested whether overall seed set is improved for mayapple patches in which every flower receives supplemental outcross pollen every year. The degree of pollen limitation may thus be inflated by re-allocation of resources from naturally-pollinated flowers to outcross-supplemented ones, which presumably receive a higher quality and quantity of pollen (Ashman et al. 2004). The minority of mayapple ramets that remain physically connected to each other are strongly physiologically integrated and draw resources from each other (Landa et al. 1992). Similarly, increased fruit and seed production in one year may mean fewer stored resources are available for future reproductive effort (Ashman et al. 2004). Consistent with this hypothesis, fruit production in mayapple in one year diminishes the probability of flower production in the following year (Sohn and Policansky 1977). Thus, a mayapple patch in which reproductive output was never limited by pollen would produce fewer flowers each year, and may not be more fit in the long term than one with chronic pollen limitation.

A proper evaluation of this hypothesis would require varying degrees of pollen supplementation. Applying supplemental pollen to every receptive flower a mayapple patch produces every year may eventually depress seed set for that plant below that observed in naturally-pollinated plants, but this would only show that it is possible for pollination success to be so high that resource limitation depresses seed set below natural levels. The same result may never be obtained if only half of the flowers received supplemental outcross pollen each year, which would indicate that pollination success truly does limit mayapple's long-term fitness.

(4) Mayapple's failure to provide the reward sought by its pollinators may be selectively favored because it encourages pollinators to leave the patch after visiting one or a few flowers, thus promoting outcrossing (Dressler 1981, Laverty 1992). By this argument, rewardlessness is an adaptive response to the combination of self-

incompatibility and clonal growth. Pollen limitation is simply a by-product of this adaptation.

This hypothesis is not particularly compelling for mayapple. For a self-incompatible species, self-pollination is simply a form of improper pollen transfer (*sensu* Rathcke 1983), like interspecific pollen transfer. Certainly, self-pollination can impose selection against increased pollinator rewards (Heinrich and Raven 1972); a balance between attracting pollinators and convincing them to leave is desirable. However, that balance generally lies somewhere above zero reward and the near-complete failure to attract pollinators that results from not rewarding them. Given that pollen is carried over across more than one flower visit, it is doubtful that any increase in self-pollination that resulted from modest nectar provisioning, plus the cost of nectar production, could not be compensated for by increased visitation and decreased interspecific pollen transfer, for some range of non-zero investments in nectar production.

(5) Selection does not strongly favor mutations that increase seed set in mayapple due to the long life of the adult stage. Mayapple is extensively clonal, and, while little is known about its potential life span, genets clearly persist for decades and possibly centuries (Bierzychudek 1982). It may be that reproduction by seed is simply not important to the fitness of such a long-lived plant. This argument depends on the fitness gains of increased seed production through the sexual production of more offspring being exceeded by the fitness costs through decreased survivorship or lower future sexual reproductive success.

For mayapple, the validity of this hypothesis is currently difficult to assess, though relevant data are accumulating. Understanding the costs and benefits of increased seed production depends on knowing the probabilities of seed germination, seedling survivorship to the stage of initiating clonal growth, transitions between the flowering and vegetative stages, and the branching of mature rhizomes. The effect of increased nectar production can only be assessed through nectar addition experiments, but it is much more difficult to estimate the cost of nectar production in a species that produces none.

Future directions for research

To date, six studies (including this one) have addressed mayapple's mating system, yet none has determined how self-incompatibility is enforced. The pollination biology of this species is of interest for several reasons: (1) It seems to be a deceptively-pollinated plant that is not well-adapted to pollination by deceit (e.g., it has loose pollen, unspecialized pollen placement, and extensive clonality). (2) Its pollination may be facilitated by rewarding neighbors (Laverty 1992; Chapter 3). (3) It is predominantly self-incompatible, yet its seed production is typically limited by pollination success (Swanson and Sohmer 1976, Rust and Roth 1981, Laverty and Plowright 1988, Laverty 1992, Whisler and Snow 1992, Chapter 3). And (4) it is a source of podophyllotoxin, which is used to synthesize effective drugs for lung cancer, various leukemias, and other solid tumors (Van Uden et al. 1989). Understanding mayapple's mating system would be both valuable and interesting, but until the mechanism of self-incompatibility is understood, it will be difficult to progress further in this direction.

The patterns of self-compatibility in mayapple should be determined across a broad range of spatial scales. As described above, it does not appear, based on the studies published to date, that mayapple is more likely to be self-compatible near the northern edge of its range than further south. Mating system tests must be performed in places where theory would predict a high prevalence of self-compatibility (where populations are small and isolated, where habitats have a history of instability, or at the extreme northern, western, and southeastern frontiers of the species' range) and where self-incompatibility should be favored (in old, undisturbed habitats with large populations near the core of the species' range). At finer spatial scales, Whisler and Snow (1992) found variations in self-compatibility within populations and across central and northeastern Ohio, and I found variation in self-compatibility among populations separated by 0.5 to 3.0 km. I found evidence that variation in self-compatibility at this scale was related to light abundance, indicating that inbreeding depression and resource limitation, not a genetic self-incompatibility mechanism, explains some of the cases of apparent self-incompatibility in this and other studies.

The most straightforward way to test the hypothesized relationship between the expression of inbreeding depression and resource availability more rigorously would be to perform reciprocal transplant or common garden experiments, so that ramets of both self-compatible and apparently self-incompatible clones co-occur in a range of different environments. If, for example, artificial mixed-genotype patches of mayapple were established under different degrees of light availability, an effect of light abundance on the probability of seed maturation from highly inbred ovules would reveal itself in a positive relationship between measured self-compatibility and light abundance. Sample sizes (number of clones) should be larger than those employed in this study, since some apparently self-incompatible clones may be truly self-incompatible; inbreeding can only affect apparent self-incompatibility for self-compatible clones.

Another prediction of the proposed relationship between expression of inbreeding depression and resource availability is that plants with severe pathogen infections should appear to be less self-compatible than those without them. Mayapple patches heavily infected with a species-specific rust (*Puccinia podophylii*) have much lower fruit and seed set and shorter ramets than uninfected plants (Zach Miller, pers. com.) and presumably have fewer resources for fruit and seed maturation. If outcrossing increases the probability that some of a plant’s offspring are resistant to the pathogen strains infecting the parent, a decreased expression of self-compatibility by infected plants would result in a smaller proportion of that plant’s maternally-produced offspring being susceptible to its own pathogens. To my knowledge, however, no relationship between self-compatibility and pathogenic (or parasitic) infection has been reported in plants.

	May	**June**		**July**			
Treatment	**Flowers**	**Fruit set**	**Fruit width**	**Fruit set**	**Fruit width**	**Seeds/fruit**	**Seeds/flower**
Autogamy	13 (25)	0.89 +/- 0.08 **a** (12)	7.42 +/- 1.06 **a**	0.08 **a** (2)	11.50 **a**	0 **a**	0 **a**
Apomixis	13 (25)	0.92 +/- 0.08 **a** (12)	7.88 +/- 1.09 **a**	0.08 **a** (1)	16.50 **ab**	1 **ab**	0.08 **a**
Selfing	14 (52)	0.89 +/- 0.07 **a** (13)	8.15 +/- 0.91 **a**	0.11 +/- 0.06 **a** (3)	21.06 +/- 4.83 **a**	5.11 +/- 2.89 **a**	0.52 +/- 0.36 **a**
Outcrossing	14 (52)	1.00 +/- 0.00 **a** (14)	17.28 +/- 0.84 **b**	0.55 +/- 0.10 **b** (11)	32.50 +/- 0.96 **b**	36.07 +/- 4.32 **b**	18.63 +/- 3.98 **b**
Significance		0.336 (3.386, 3)	**<0.001** (29.479, 3)	**<0.001** (20.423, 3)	**0.010** (11.296, 3)	**0.012** (10.970, 3)	**<0.001** (26.573, 3)

Table 2.1: Proportions of fruits retained and mean widths of fruits in each treatment in June, July, and when collected. Numbers in the "Flowers" column indicate the number of patches in the treatment group, with the total number of flowers in those patches in parentheses. The "Fruit set" columns show the average of the patch means of fruit set per flower, +/- the standard error, with the number of patches bearing fruit in parentheses. Widths are averages of patch mean fruit widths +/- standard error. The "Seeds/fruit" and "Seeds/flower" columns show the average of the patch means of these respective seed set values. The "Significance" row displays P values for Kruskal-Wallis comparisons of fruit set among all treatments, with Chi-square values and degrees of freedom in parentheses. Boldface letters indicate whether treatment groups differed significantly for each measure of reproductive success; values within a column that do not share a letter are significantly different from each other.

Model	**Fruits/flower**	**Seeds/fruit**
Moisture/clay/carbon, Sand/silt, Light, Edge	**0.078** (0.461, 12)	0.370 (0.133, 10)
Moisture/carbon, Light, Edge	**0.044** (0.400, 14)	0.922 (-0.338, 11)
Ramet height, Vegetative ramets, Reproductive ramets	**0.051** (0.381, 14)	0.990 (-0.407, 11)
Floral diameter	0.872 (-0.121, 10)	0.226 (0.087, 9)
Anthers per flower	0.300 (0.152, 9)	0.931 (-0.165, 8)

Table 2.2: Fit of linear regression models against fruit set per flower and estimated seed set per fruit in July and collected ("final") fruits per flower and seeds per collected fruit. The independent variables used in each regression model are listed in the left-most column. Each cell shows the P-value for the regression of the dependent variable (columns) against each model (rows). Significant ($P < 0.05$) and marginal ($P < 0.1$) values are shown in boldface. Numbers in parentheses are adjusted R^2 followed by the number of patches included in the regression.

Trait	**Site 5 Mean**	**Sites 3, 4, 6, 7 Mean**	**Significance**
Outcross fruits/flower	0.833 (0.083, 3)	0.477 (0.111, 11)	0.136 (12)
Self fruits/flower	0.528 (0.121, 3)	0.000 (0.000, 11)	**0.049** (12)
Outcross seeds/fruit	43.72 (10.21, 3)	32.56 (3.78, 8)	0.223 (9)
Self seeds/fruit	5.11 (2.89, 3)	NA	NA
Soil silt v sand	-0.071 (0.535, 3)	0.052 (0.318, 10)	0.854 (11)
Soil moisture/clay/carbon	-0.528 (0.266, 3)	0.344 (0.447, 10)	0.328 (11)
Soil moisture/carbon	-0.476 (0.079, 3)	-0.011 (0.231, 12)	0.348 (13), 0.233
Light	1.181 (0.348, 3)	-0.116 (0.253, 12)	**0.033** (13)
Ramet height (cm)	51.2 (0.6, 3)	42.8 (1.5, 12)	**<0.001** (13)
Vegetative ramets	74 (27, 3)	167 (50, 12)	0.386 (13)
Reproductive ramets	59 (9, 3)	135 (32, 12)	0.521 (13)

Table 2.3: Differences in environmental traits, patch traits, and reproductive success between the self-compatible patches in Site 5 and the self-incompatible patches in the other four sites. The environmental traits (soil characteristics and light) are principle components produced from multiple measures of light and soil characteristics and may be negative or positive numbers. High scores on the "soil silt v. sand" axis indicate silty, less sandy soils. High scores on the "soil moisture/clay/carbon" indicate that soils are moist and rich in clay and organic matter. Similarly, high scores on the "soil moisture/carbon" axis indicate moist soils rich in organic matter. Finally, high scores on the "light" axis indicate that leaf-area index is low and canopy openness is high, which is consistent with higher long-term-average light levels. Values following mean scores are standard errors and numbers of patches included. Significance was measured by independent-samples t-tests. P-values are shown, with significant and marginally significant values in boldface and degrees of freedom in parentheses. Two P-values are given for "soil moisture/carbon," which had a marginally significantly non-normal distribution; the second P-value (after the parentheses) was determined by a Mann-Whitney U-test.

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Chapter III

Pollinator-mediated interactions between a nectarless species (*Podophyllum peltatum* L.) and its co-flowering neighbors: a test of the benefits of having attractive neighbors

Introduction

Approximately 6% of all animal-pollinated angiosperm species provide no rewards to pollinators that visit their flowers (Renner 2006). These species typically have very low visitation rates and low reproductive success. Thus, while withholding pollinator rewards presumably allows the resources that would have gone into rewards to be used for other fitness-promoting functions (e.g., growth, survival, and seed maturation), the cost of doing so is probably more substantial than these benefits. This unfavorable cost-benefit ratio for withholding rewards explains why so few animal-pollinated plant species follow this strategy, but it begs the question: why are any animal-pollinated plant species unrewarding?

One mechanism that may weaken selection against unrewarding flowers is Thomson's (1978) "magnet species" hypothesis, which proposes that a species whose flowers fail to attract many pollinators experience greater visitation rates, and perhaps greater reproductive success, in the presence of a species that pollinators find highly attractive. Given that unrewarding species are exceptionally ineffective at attracting pollinators, it is not surprising that this hypothesis has found support in studies involving such species (Laverty and Plowright 1988, Laverty 1992, Johnson et al. 2003, Juillet et al. 2007).

To examine the magnet species phenomenon, I chose mayapple (*Podophyllum peltatum* L., Berberidaceae) as an ideal study system because it is unrewarding to its native North American pollinators, bumblebee queens (*Bombus* spp.), and accordingly, it has a low pollinator visitation rate and low fruit and seed production (Swanson and Sohmer 1976, Rust and Roth 1981, Laverty and Plowright 1988, Laverty 1992). Unlike many other unrewarding species, however, it lacks any mechanisms to reduce interspecific pollen transfer (as found in the Orchidaceae; Nilsson 1992, Harder and Johnson 2008) and has fragrant flowers, which facilitates learned avoidance in pollinators (Kunze and Gumbert 2001, Galizia et al. 2005). While the combination of the absence of pollinator rewards and the presence of floral fragrance results in a very low visitation rate, the combination of extensive clonality and self-incompatibility (Swanson and Sohmer 1976, Policansky 1983, Laverty and Plowright 1988, Whisler and Snow 1992) reduce the probability that pollen transfer among flowers results in fertilization. Thus, mayapple presents an enigma in that it does not reward its native pollinators yet appears to be poorly adapted to compensate for the low visitation rate and low pollinator constancy that result from this trait.

The magnet species effect has previously been demonstrated for this species (Laverty 1992). However, this study involved a magnet species with an exceptionally high visitation rate per flower (*Pedicularis canadensis* L., Scrophulariaceae) that does not typically occur near mayapple populations, concentrated in a narrow area within a single study site. The magnet species effect can only be relevant to the evolution of unrewarding flowers in mayapple if it mitigates the reproductive costs of withholding rewards in a wide variety of contexts, including contexts where potential magnet species are only moderately attractive to pollinators or occur in numerous, widely-distributed, small patches.

In this study, I tested for the effects of common, co-flowering, nectar-producing species on mayapple's pollination success in six study sites in southeastern Michigan. I considered four potential interactor species that commonly co-flowered with mayapple: wild geranium (*Geranium maculatum* L., Geraniaceae), violets (*Viola* spp.,Violaceae), spring beauty (*Claytonia virginica* L., Portulacaceae), and the invasive exotic herb garlic mustard (*Alliaria petiolata* [Bieb.] Cavara and Grande, Brassicaceae). I tested for effects of the abundance of each species on mayapple's pollinator visitation, fruit set per flower, and seed set per fruit, using both natural variation in floral abundance and neighbor removal experiments. To see if mayapple shared pollinators with these and other species, I collected pollinators to determine which species' pollen were found on pollinators that carried mayapple pollen. In addition, because mayapple lacks obvious adaptations to reduce interspecific pollen transfer, yet the effect of heterospecific pollen on mayapple's mating success has not been examined previously, using hand-pollinations, I tested whether the pollen of wild geranium and wild blue phlox (*Phlox divaricata* L, Polemoniaceae) on mayapple stigmas had the potential to interfere with ovule fertilization by mayapple pollen. Finally, I tested for effects of mayapple on fruit and seed set of wild geranium, and I tested whether mayapple pollen on wild geranium stigmas had the potential to interfere with ovule fertilization for this species. While several studies have investigated the effects of rewarding neighbors on the pollination of unrewarding species (e.g., Laverty and Plowright 1988, Johnson 2000, Johnson et al. 2003), few have considered the interaction in the opposite direction (Anderson and Johnson 2006). Any effect of unrewarding species on the pollination success of a rewarding species may influence the ecological and evolutionary trajectories of populations of the rewarding species (see Rathcke 1983).

In addition to these potential pollinator-mediated interactions between mayapple and its rewarding neighbors, I examined the potential for habitat edge effects on mayapple's pollination success. Forest edges are better-lit and warmer than forest interiors (Matlack 1993, Chen et al. 1995), possibly allowing pollinators with high minimum flight temperatures to operate there (Herrera 1995). They also have greater plant diversity (Chen et al. 1992, Fraver 1994), and many forest species flower in greater abundance in well-lit environments such as edges (Moore and Vankat 1986, Collins and Pickett 1988), which may attract abundant and diverse pollinators. If the magnet species effect operates because visitation to unrewarding species is simply a function of the local abundance of effective pollinators, then mayapple will have higher visitation near forest edges if edges have a higher abundance of effective pollinators than interiors.

This study addresses the following questions: Does pollen receipt limit fruit and seed production in mayapple in my system; can fruit and seed set be increased by adding outcross pollen to mayapple stigmas by hand? If so, is pollen limitation attributable to a low pollinator visitation rate, as expected if pollinators avoid flowers they find unrewarding? Does pollinator visitation increase, and does pollen limitation decrease, with increasing abundance of nectar-producing, co-flowering neighbors, as predicted by the magnet species hypothesis? Is pollination success related to floral abundances at the scale of entire study sites (hectares)? Does visitation rate increase, and does pollen limitation decrease, near forest edges or in brightly-lit patches, as expected if pollinators preferentially forage in warmer, brighter environments cool days? Do the diversity and abundance of flowers vary with distance to the forest edge? Do mayapple and its nectar-producing neighbors share individual pollinators, as predicted by the magnet species hypothesis? Does interspecific pollen transfer between mayapple and its neighbors potentially depress pollination success for either? What effect, if any, does mayapple have on its neighbors' pollinator visitation rate and pollen limitation? Do larger mayapple patches have higher pollen limitation, as expected if clonal growth promotes geitonogamous self-pollination? Do conspecific neighbors decrease pollen limitation without affecting visitation rate, as expected if mate availability limits mayapple's reproductive success?

Methods

Study species:

Mayapple (*Podpophyllum peltatum* L.) is a common herb in eastern North American temperate deciduous forests. It is self-incompatible and extensively clonal. The above ground portion of each vegetative ramet is a single peltate, lobed leaf, while reproductive ramets have two leaves emerging from one node on vertical stem, with a single large (3-7 cm), white, nodding flower at this node. The flowers have no nectar, abundant pollen, and a sweet fragrance. They are typically open for one to two weeks in mid to late May, with the total flowering period lasting about three weeks. Nectar-seeking bumblebee queens (*Bombus* sp.) are the most commonly observed visitors to mayapple flowers, though pollen-foraging honeybees (*Apis melifera*) also visit (Swanson and Sohmer 1976, Rust and Roth 1981, Laverty and Plowright 1988, and pers. obs.).

Study sites

The study was conducted in forest fragments in western Washtenaw County, Michigan, in the southeastern portion of the state. In April of 2005, I located 10 to 13 mayapple patches in each of 5 sites (identified as sites 1 through 5), totaling 60 patches. Each site was located in an upland forest fragment at least 300 meters long by 300 meters wide. The fragments were located in an agricultural matrix dominated by fields of corn, soybeans, and wheat, and sheep pastures. Each had secondary forest that had been cleared of trees at least once, and sites 2, 3, and 4 were selectively logged by their landowners during the study. The forest in each site had large (~ 30-40 m tall) oaks (mostly *Quercus rubra*), hickories (*Carya ovata*, *C. glabra*, and *C. cordiformis*), and black cherries (*Prunus serotina*). These co-occurred with similarly large basswoods (*Tilia americana*) in site 1 and with a dense subcanopy of small (~10 m tall) sugar maples (*Acer saccharum*) in site 5. Half of site 2 occurred on moist, very peaty soils under a canopy of large red maples (*A. rubrum*).

In 2006, to increase sample size and to test whether an older forest would provide a better pollination environment for mayapple, I added another site with 13 patches to the study, for a total of six sites with 73 patches. The new site was in the Nan Weston Preserve at Sharon Hollow (NWP), a 100-ha natural area owned and managed by The Nature Conservancy. The mayapple patches used in this site were in upland mesic forests dominated by beech (*Fagus grandifolia*) and sugar maple, with a high diversity of less dominant tree species. Unlike the forests of the other five sites, NWP’s forests have never been cleared, and selective logging was light even before the Conservancy acquired the property and halted logging entirely (Douglas Pearsall, pers. comm.).

Mayapple study patches

Mayapple patch characteristics

To test whether forest edges or wild geraniums facilitate mayapple pollination, in April 2005, I selected mayapple patches within each of five second-growth sites to be divided approximately evenly among four groups, determined by proximity to the edge (less than 30 meters or more than 100 meters from the edge) and the presence or absence of geranium within five meters. 21 of 32 edge patches and 16 of 28 interior patches had geranium flowers within five meters. The patches with geranium outnumbered those without it because geraniums near some patches were not detected before they began flowering. The number of reproductive and vegetative ramets and the length and width of the patch were measured. The location of the patch was determined using a Magellan SporTrak MAP GPS unit. The approximate distance of the patch from the nearest forest edge was estimated by pacing, and most distances could be checked against distances along the edge-to-interior transects used to quantify site floras in 2005 (see below), which were measured with tapes.

In 2006, reproductive and vegetative ramets were again counted and the length and width were measured for all patches. For all patches, mayapple ramets with healthy flowers were marked with individual numbers to reduce the probability of miscounting flowers. The GPS locations of the patches in NWP were determined, and the distance of each of these patches to the edge was determined by pacing, if possible. Some patches were quite far from the nearest edge, and the distance of each such patch to the edge was estimated on a map. Between 24 and 26 August, I measured the mean height of reproductive ramets in each patch to the nearest half decimeter.

In 2007, in each patch, I marked the stem of each ramet bearing an open flower with a vertical stripe, counting the flowers in the patch as I did so. This was much faster than numbering stems, while still serving to reduce miscounts. I also measured the heights, floral diameters, and anther counts for three ramets in each patch. I did not count ramets, except in four patches that had died back dramatically between years. Site 5 was eliminated from the study due to lack of access. Therefore, floral diameter and mean number of anthers per flower were not measured in this site.

Environmental characteristics

Between 24 and 25 September 2005, I measured the openness of the forest canopy over the center of each colony in Sites 1 through 5 using a spherical densiometer. I also collected a 30-centimeter-deep soil core from the center of each colony and measured its moisture content using the gravimetric method.

Between 24 and 26 August 2006, I measured the leaf-area index for each patch in all sites using an LAI-2000 (Licor Corporation). Leaf-area index and canopy openness as measured by densiometer were significantly negatively related (linear regression, $p << 0.0001$, $R^2 = 0.182$, adjusted $R^2 = 0.170$, beta = -0.426, df = 72), confirming that the two methods measure similar but not identical phenomena. The leaf-area index readings were presumed to be less subject to observer error and bias, and, therefore, these were used in the statistical analyses described below, with densiometer readings excluded to maximize the independence of factors.

In 2007, I collected 30-centimeter soil cores from the patches in Site NWP in June and determined their percentage moisture with the gravimetric method. From June to August 2007, I measured soil organic content for all 73 soil samples by the Walkley-Black wet combustion method (Walkley and Black 1934) and soil texture for 65 samples by the Bouyoucos hydrometer method (Bouyoucos 1936). Eight samples from Site 2 were collected from very peaty soils, and valid soil texture analyses could therefore not be performed on them (Donald Zak, pers. comm.).

Soil texture analysis produced three interdependent variables: percent sand, percent silt, and percent clay. Because these must sum to 100%, they are strongly correlated with each other, and including together as explanatory variables in statistical analyses would substantially diminish the apparent explanatory power of each of them. Therefore, soil texture was reduced to a single axis using a principal components analysis (PCA). This axis explained 77.2% of the variation in soil texture components and correlated positively with soil percent silt ($r = 0.902$) and clay ($r = 0.718$) and negatively with soil percent sand ($r = -0.994$).

Neighborhood floral displays

In 2005, all plants known to produce showy flowers within five meters of each patch were identified between 2 and 6 May. The abundance of each flowering species was described in qualitative terms (“few,” “many,” etc.).

Presence/absence data for geraniums and other species that co-flowered with mayapple did not explain a significant amount of the variation in pollination success among patches, and qualitative abundance categories were too ambiguous to reliably test for an effect on pollen limitation. Therefore, between 17 and 27 May 2006, all showy flowers were counted and identified (1) inside the patch, (2) 0 to 1 meters from the patch, and (3) 1 to 5 meters from the patch. The survey included all 73 patches (70 of which flowered) in all six sites.

Information on floral displays was not collected in 2007.

In the statistical analyses described below, flower abundances measured in 2006 were used for all three years, on the assumption that flower abundances were strongly correlated between consecutive years. This assumption is supported for at least some species by the positive correlations found when the subjective categories of flower abundance in 2005 were given ordinal classifications and floral abundances within 5 meters in 2006 were regressed on them. Geranium and violet abundances were particularly strongly correlated between years ($R^2 > 0.50$), while garlic mustard abundance was less consistent ($R^2 = 0.274$), and spring beauty abundance was poorly correlated ($R^2 = 0.114$).

The poor correlation for spring beauty is related to the difference in the times when floral abundances were estimated or quantified in 2005 and 2006. Peak flowering for spring beauty occurred about a week to two weeks before mayapple began flowering, at about the time when abundances were estimated in 2005. By the time floral abundances were quantified in 2006, spring beauty floral abundance had begun to decline, with more dramatic declines near some mayapple patches than others. In contrast, the violet species were at peak flower from approximately two weeks before mayapple flowered into the first week of flowering, and floral abundances of geranium and garlic mustard were estimated in 2005 based on the number of individuals with prereproductive morphologies, which was probably strongly related to the number of flowers present when mayapple flowered.

Removal of potential facilitators

To test whether correlations between abundances of nectar-producing neighbors and mayapple's pollinator visitation or pollen limitation were attributable to the presence of those neighbor's flowers, I removed the flowers on all plants within one meter of each of three mayapple patches per site. Neighboring flowers were removed twice during mayapple's flowering season, early in the first week of flowering and again early in the second week. Flowers were counted and identified before they were removed.

Floral abundance on edge-to-interior transects:

To test whether the floral environment of the forest edge differed from that of the forest interior, I surveyed the flowering displays of Sites 1 through 5 between 1 and 9 June 2005. In each site, flowers or inflorescences were counted along two 150-meter transects running from the forest edge to the forest interior. In sixteen 2 m x 2 m plots on each transect, I counted flowers for species with large, distinct flowers and inflorescences for species with small flowers in compact inflorescences. Because the goal of the survey was to create a description of the site flora that was relevant to mayapple, and because the survey was conducted in the two weeks after the end of peak mayapple flowering, fruits, flower pedicel scars, and infructescences were included in the counts. Floral displays were surveyed on herbaceous species and shrubs below two meters above the ground.

Geranium patches

In 2006, I selected 20 geranium patches in four sites (Sites 1 through 4) to test for effects of mayapple on wild geranium pollination. These were divided into two groups: patches with one of the mayapple patches used in the study within 5 meters and patches with no mayapple flowers within 15 meters. The locations of these geranium patches were determined by GPS, but they were not further characterized.

Within each geranium patch, I outcross-pollinated 5 flowers (6 in one patch) by hand, marking each with red paint on the pedicel immediately after pollination, and marked an equal number with white paint as control flowers. In 15 of the patches, I pollinated 5 flowers with mayapple pollen immediately followed by outcross pollen and marked them with yellow paint. Each flower was on a separate ramet, but multiple flowers may have been selected in the same genet in some cases. Flowers were selected that had open stigmas and still had ample pollen in at least one of their two whorls of anthers. This ensured that the stigmas were receptive but had not been open for much more than one day.

Pollen voucher slides

In 2006 and 2007, I collected flowers of 31 species that co-flowered with mayapple in my sites that were likely to be biotically pollinated, based on floral morphology. These were identified and collected during mayapple flowering, refrigerated until the end of mayapple flowering or until inclement weather prevented fieldwork, and used to make pollen voucher slides in the lab. To produce pollen voucher slides, the anthers of flowers of each collected species were blotted with a small (~ 3mm X 3mm) block of 0.2 mg/mL gelatin stained with basic fuchsin (Beattie 1971, as described in Kearns and Inouye 1993). The gelatin was melted onto glass slides and covered with a coverslip, and the edges were sealed with fingernail polish. The pollen on these slides was used to identify the pollen on collected bees and floral stigmas (described below).

Pollinators

Pollinator observations

In 2005, I observed pollinator visits to one haphazardly selected mayapple patch (1-63 flowers visible, mean = 12.67 flowers) for 10 minutes every hour in the field during the flowering season, from 17 to 31 May. As often as feasible, I alternated between patches near the forest edge and patches in the interior. A total of 97 observations were conducted in 2005.

In 2006, pollinator observations were conducted every one to two hours from 9 to 25 May. Due to the continued extremely low rate of pollinator visitation, I allocated more time to other, more data-productive field methods in 2006. A large storm system brought cool, wet weather from 11 through 16 May, further reducing the number of pollinator observations. However, in this year, during approximately half of the observation times, two field assistants observed visitation to mayapple or to neighboring flowers of other species during my observation periods. Overall, 52 observations of mayapple were conducted in 2006.

In 2007, few formal observations were conducted once it became apparent that visitation rates were comparable to those of the previous two years. Observations were conducted on 11, 18, and 19 May, with a single observation on 21 May. A total of 29 observations were conducted in 2007.

Several times each day, generally following each pollinator observation period, I determined the temperature and relative humidity using a sling psychrometer.

Pollinator collections

Bees visiting mayapple were collected whenever possible in 2006 and 2007. In addition, one beetle and two moths found on mayapple flowers (but not moving among them) were collected in 2006. Insects visiting neighbors of mayapple were collected haphazardly in 2006, as were *Bombus* queens observed searching for nest sites. In 2007, I attempted to capture all *Apis* workers and approximately half of all *Bombus* queens and workers that I observed within the study sites during the period of mayapple flowering. Capture efficiency increased through the flowering season, and the vast majority of captures occurred in the second week of mayapple flowering, but capture efficiency remained well below 50% throughout the season.

All captured insects were blotted for pollen using gelatin with basic fuchsin, and the pollen was mounted on microscope slides, as described for the pollen voucher slides above. Mayapple pollen was counted along five uniformly-spaced transects at 400X magnification on each slide, and other pollen was counted and identified to the narrowest taxonomic category possible. The coverslips were 41.5 fields of view (21 mm) across, and pollen counting transects were placed at 7, 14, 21, 28, and 35 fields of view from the top of the coverslip.

Pollen limitation

To determine whether low pollen deposition limits fruit and seed production for mayapple and whether pollen limitation of reproductive success was lower near forest edges and in the presence of wild geranium and other co-flowering plants, I added outcross pollen to a subset of the flowers in each patch, where patch size permitted. From 17 to 29 May 2005, I pollinated up to 5 flowers in 32 mayapple patches with outcross pollen, marking the remainder as controls (the full flowering season ran from 17 to 31 May). The outcrossed flowers received pollen by hand in addition to any natural pollen receipt that may have occurred. The pollen was applied with a nylon-bristled paintbrush using pollen from at least three unmarked patches at least ten meters away, and the same brush was used for all pollinations. Enough pollen was added to lend a yellow coloration to the stigmatic ridges. In each patch, at least one ramet with an apparently healthy flower was marked as a control and otherwise not manipulated. Controls were also marked in 5 patches in which no flowers received supplemental outcross pollen. (The remaining patches were used in a study on mayapple's mating system, described in Chapter 2, failed to flower, or were severely damaged after flowering.)

In 2006, all pollinations were applied from 17 to 24 May (the full flowering season ran from 9 to 25 May). Outcross pollen was added by hand to up to seven flowers per patch, but no more than half of the flowers in the patch, for 62 of the patches (the other eight patches did not produce sufficient flowers, or their flowers were too old for pollination on the day when hand pollinations were applied). Pollen was deposited on the stigmas by applying the anthers of flowers from three patches at least ten meters away directly to the stigma. This method deposited pollen much more efficiently than the paintbrush. As I pollinated each flower, I noted the number written on the ramet (see "Mayapple patch characteristics" above).

In 2007, I hand-outcrossed 3 to 5 flowers per patch (fewer than 1/2 of the flowers in the patch) in any patch with at least 7 flowers. Each flowering ramet was marked with a vertical stripe on its stem, and after crossing a flower, I added a horizontal stripe crossing the vertical one. Crosses were performed by applying three anthers from mayapple patches at least ten meters from the recipient patch. All crosses were performed from 11 to 17 May (the full flowering season ran from 11 to 22 May).

In all three years, all control and outcrossed flowers were inspected for the apparent good health of the stamens and ovaries. Flowers with pistils that seemed unviable (moldy, dark-colored, absent, or very small) were excluded from flower counts. All healthy flowers were examined without magnification for nectar, and several flowers in different patches were inspected for nectar with a 10X hand lens throughout each day.

Heterospecific pollen transfer

Because mayapple has loose pollen, unspecialized floral morphology, and unrewarding flowers, I predicted that it would have a high ratio of heterospecific pollen to outcross conspecific pollen. If heterospecific pollen interferes with the ability of mayapple pollen to fertilize ovules after reaching the stigma, this could depress fruit and seed set even below the limitation due to lack of pollinator visits. To determine whether heterospecific pollen can interfere with the successful outcross pollination of mayapple flowers, in 2006, in any mayapple patch with at least 12 flowers, I pollinated 3 to 5 flowers, but not more than one third of the patch, with heterospecific pollen followed immediately by outcross pollen. If pollen-bearing *Geranium maculatum* flowers could be found near the patch but over 5 meters away, I applied entire geranium flowers to the stigmas of the treated mayapple flowers until the stigmatic ridges became yellow. If geranium was not available, I applied pollen of *Phlox divaricata* in similar fashion, opening the floral tubes and applying the anthers within to the mayapple stigmas. Application of geranium or phlox pollen was followed by pollination with mayapple pollen using three anthers from three patches at least 10 meters away. Overall, I applied this treatment using geranium pollen in 33 patches and using phlox pollen in 8 patches.

Reproductive success

In mid to late July 2005, I measured the length and width, in millimeters, of all mayapple fruits present on ramets in the marked patches and collected all ripe, abscised fruits. In August 2005, I collected all remaining fruits. In the lab, I measured the length and width of each fruit in millimeters, weighed it (fresh) to the nearest tenth of a gram, and counted the seeds. Fruit width in July was found to be related to seed number by the equation:

$$\text{Seed number} = 0.0007 * \text{width (mm)} \wedge 2.9430 - 1. \qquad (1)$$

This equation fit the data for 173 fruits collected in 2005 well ($R^2 = 0.629$), and it was used to estimate the number of seeds in fruits that disappeared between July and August. Fruits that had continued to develop into mid July, by which time fruits were approaching their maximum volume, had presumably been successfully pollinated.

In 2006, I collected all mayapple fruits between 24 and 27 July and stored them in a refrigerator. The length, width, and depth of each fruit were measured to the nearest millimeter, and the fresh weight of each fruit was determined to the nearest tenth of a gram. Beginning in late August, approximately half of the fruits were dissected and the seeds, empty seed coats, and undeveloped ovules were counted. Thus, for these fruits, I was able to determine not only fruit set per flower and seed set per fruit, but also seed set per ovule, which may be a better measure of pollen limitation. However, because initial analyses did not indicate that seed set per ovule produced different statistical results than seed set per fruit, and because fruits began to decompose by the end of October, halfway through the seed/ovule/empty coat counts, only developed seeds were counted for the other half of the fruits.

In 2007, I collected fruits from 24 to 29 July. Only 155 fruits were found in this year, and all were dissected and their seeds counted on 30 July. Their lengths, widths, and weights were not determined because there was no obvious need for additional allometric data.

Statistical analyses

The unit of replication for each treatment group was the individual patch. One to three pollination treatment groups were represented in each patch: (1) control flowers, receiving only natural pollination service, (2) outcross-supplemented flowers, receiving outcross pollen by hand, in addition to natural pollination service, and (3) HPT flowers, receiving heterospecific pollen followed by outcross pollen by hand, in addition to natural pollination service.

All statistical tests were performed using SPSS 11.01 for Windows (SPSS Corp. © 1989-2001). The significance of each result is categorized as significant ($p < 0.05$), marginally significant ($p < 0.10$), or non-significant ($p > 0.10$). Trends are reported where for non-significant results where $p < 0.11$. Whenever $p < 0.11$, p-values are reported in the text.

Pollen limitation

Pollen limitation was assessed using an index of pollen limitation developed by Larson and Barrett (2000):

$$PL_{fruit} = 1 - F_o / F_s, \qquad (2)$$

where F_o is the percent fruit set of open-pollinated controls and F_s is the percent fruit set of outcross-supplemented flowers. This index is given a lower bound of zero, on the assumption that cases where control flowers have higher fruit set than outcross-supplemented flowers are probably the result of experimental or Type I statistical error. This assumption is not valid in populations for which pollen receipt limits fruit set only weakly or not at all; if adding supplemental outcross pollen by hand has little effect on fruit set, then control fruit set should exceed outcross fruit set about as frequently as the reverse, and rounding negative values to zero produces a bias in favor of positive pollen limitation. In this study, equation (2) produced negative values in only 6 cases out of 104, so Larson and Barrett's (2000) assumption that negative values are the result of error is both reasonable and unlikely to greatly alter the distribution of values of PL_{fruit}.

The distribution of pollen limitation values for fruit set calculated by this index did not deviate significantly from a normal distribution in any year, when neighbor-removal patches were excluded (one-sample Kolmogorov-Smirnov tests, $p = 0.148$, 0.208, 0.410, $N = 21$, 32, 16, for 2005, 2006, and 2007 respectively). When neighbor-removal patches were included, the PL_{fruit} data became marginally significantly non-normal for 2005 and 2006, but not for 2007 ($p = 0.055$, 0.097, 0.370, $N = 26$, 43, 21 for 2005, 2006, and 2007, respectively). However, no transformation of PL data substantially improved the normality of the distribution, and the untransformed index was used for analyses.

I calculated a similar pollen limitation index for seed set per fruit:

$$PL_{seed} = 1 - S_o / S_s, \qquad (3)$$

where S_o is the number of seeds per fruit for open-pollinated control flowers and S_s is the number of seeds per fruit for outcross-supplemented flowers.

For the same reason that Larson and Barrett's (2000) index may not be appropriate for populations with low pollen limitation of fruit, the analogous equation (3) for seed set per fruit would not be appropriate for populations with low pollen limitation of seeds per fruit. Fruit set may be pollen-limited, but if the relatively few naturally pollinated flowers that set fruit generally have sufficient pollen for maximum seed production, then control fruits should have higher seed set than outcross-supplemented ones about as often as not. Variation in seeds per fruit can be increased by the fact that seed production is limited not only by pollen and resources, but also by ovule number. In mayapple, ovule number is highly variable among flowers (I counted between 15 and 96 total ovules, including seeds, empty seed coats, and undeveloped ovules, in 235 fruits in 2006). Thus, even if pollen generally limited seed set, control seed set may exceed outcross seed set in some patches for reasons other than experimental or statistical error.

PL_{seed}, as calculated by equation (3), was negative in 13 of 61 cases, indicating a high risk of bias toward detecting significant pollen limitation if all negative values of pollen limitation were rounded to zero. Therefore, I modified PL_{seed} to allow for negative values. Because the index in equation (3) may have infinitely negative values if these are not rounded up to 0, I calculated a different index for negative values:

$$PL_{seed} = -(1 - S_s / S_o). \tag{4}$$

This index, which cannot fall below -1, was applied to any case where PL_{seed} fell below zero when calculated by the conventional index. When equation (3) is applied whenever PL_{seed} is positive and equation (4) whenever it is negative, PL_{seed} ranges from -1 to 1, with 0 indicating no pollen limitation. This index is also normally distributed for all three years (one-sample Kolmogorov-Smirnov tests, $p = 0.556, 0.981, 0.407$, $N = 14, 33, 14$, for 2005, 2006, and 2007, respectively; $p = 0.999, 0.962, 0.464$, $N = 12, 23, 12$, if neighbor-removal patches are excluded).

Overall pollen limitation

To assess overall pollen limitation, I conducted one-sample t-tests to determine whether the pollen limitation indices of fruit set and seed set were significantly greater than zero. Patches from which neighboring flowers were removed in 2006 were excluded from that year's analyses.

Effects of pollination date

If any of my hand-pollinations were applied before or after period when the stigma was receptive, they may have been ineffective, and this would depress the measured degree of pollen limitation. If this occurred, then pollen limitation would vary with pollination date, with greater pollen limitation near the middle of the flowering season than early or late in the season. To test for such an effect, I regressed pollen limitation of fruit and seed set on date of hand pollination for each study season.

Neighborhood floral displays and mayapple reproductive success

I performed backward and forward stepwise linear regressions to determine whether patch traits, environmental characteristics, and flowering neighbors affected pollen limitation of fruit and seed set. I used both backward and forward regression because the significance of a variable often depends on whether other variables are included in the model. For each year (2005-2007), I performed two sets of regressions for both PL_{fruit} and PL_{seed}, one for early-season flowering neighbors (slightly before and into the first week of mayapple flowering) and one for late-season flowering neighbors (in the second week of mayapple flowering). Cases with missing values were excluded listwise. The criterion for exclusion from the backward stepwise regressions was $p > 0.10$, and the criterion for inclusion in the forward stepwise regressions was $p < 0.05$.

Both sets of regressions included patch traits (mean ramet height, floral diameter, and anthers per flower, and log-transformed flowers per patch) and environmental characteristics (distance to the forest edge, leaf-area index, soil organic content, and soil texture). In addition, to control for effects of site, I included site mean pollen limitation as a factor in the analysis (using mean PL_{fruit} when testing for effects on PL_{fruit} and mean PL_{seed} when testing for effects on PL_{seed}). The effect of including site mean PL is to factor out variability in pollen limitation among sites, so that the remaining variables explain only variability within sites. This is desirable to the extent that inter-site variability is due to factors not included in the regression model, but not desirable to the extent that the factors in the model explain variation among sites. Unfortunately, there is no way of knowing what percentage of inter-site variation in PL is attributable to the variables included in the regression models. Therefore, in each case where the final model produced by forward or backward stepwise regression did not include site mean PL, I produced a model that was identical to the final model, but with the addition of site mean PL. In addition, I repeated all forward and backward stepwise regressions without site mean PL as a variable, leaving the remaining variables to explain all within- and among-site variation in PL.

In addition to site mean PL, patch traits, and environmental characteristics, the regressions for early-season floral displays included the log-transformed abundances of garlic mustard, spring beauty, geranium, and violets during the first floral survey. Because few mayapples were flowering during the early-season floral survey, few patches had flowering conspecific neighbors at this time, and the abundances of neighboring conspecific flowers were therefore not included in these regression models. Removal patches were excluded from the analyses for 2006, but not for 2005 and 2007. Neighbor removal did not affect the abundances measured in the early survey, since floral abundance was determined prior to flower removal, and removal of neighboring flowers in 2006 presumably affected the floral neighborhood in 2006 but not in 2005 or 2007.

The regressions for late-season floral displays included the log-transformed abundances of neighboring mayapples, garlic mustard, geranium, and violets. Spring beauty flowers had become too uncommon to be included in the late-season regression models. Removal patches were excluded from analysis in all three years because the floral abundances measured in the late-season survey in 2006 were influenced by the removal of neighboring flowers after the first survey (floral abundances were only quantified in 2006, and these measurements were applied to all three years).

Not all independent variables were measured for all patches. Soil texture could not be measured for eight patches in Site 2 that occurred on deep peat, and floral diameter and anther number were not measured in the ten patches of Site 5 because these variables were only measured for all study patches in 2007, when access to Site 5 could not be obtained. To determine whether the exclusion of these 18 patches affected the outcome of the regression analyses, the regressions were repeated without soil texture, floral diameter, and anther number.

Neighbor removal

ANOVAs were used to test whether the removal of neighbors after the first survey of floral displays affected the floral abundances in the second survey. The abundance of each species within one meter of the patch in the second survey was treated as the dependent variable, with first-survey abundance as a covariate and neighbor-removal treatment as a fixed effect. If the neighbor removal treatment was effective, then there should be a significant effect of the interaction between removal treatment and first-survey abundance, in a direction consistent with neighbor-removal patches having lower second-survey floral abundances than expected based on their first-survey floral abundances.

To test whether the removal of neighboring flowers depressed pollination success, I used independent-samples t-tests to compare PL_{fruit} and PL_{seed} between removal treatments in 2006.

Patches for neighbor removal were selected semi-randomly; in each site, one patch was selected at random among the forest interior patches, one from among the forest edge patches, and one from the full set. However, it is possible that the patches thus selected were not representative of the full population of study patches. If neighbor-removal patches were in naturally poor or favorable pollination environments relative to unmanipulated patches, this could affect the apparent effect of neighbor removal on pollen limitation of reproduction. To see whether these two sets of patches were in significantly different pollination environments apart from any effect of my manipulations of floral abundance, I also performed t-tests to see whether the same patches differed in pollen limitation in 2005 and 2007.

Flora of sites

Linear and curve estimation regressions had little power to determine whether species richness, species diversity, total flower number, or the abundances of flowers of particular species varied with distance to the edge. The ten-meter increments of distance along the survey transects were also not directly related to the distance classes used to select mayapple patches for study (< 30 meters and > 100 meters from the edge), so analyses based on 10-meter increments do not necessarily assess whether the habitat of the "edge" patches was floristically different from that of the "interior" patches. Therefore, to test whether floral communities varied with distance from the forest edge using a method consistent with that used to select patches, I used one-way ANOVAs to test whether species richness, diversity, total flower counts, and flower counts for each species varied significantly among three distance classes. These classes were (1) 0 to 30 meters from the forest edge (the first four plots of each transect), (2) 40 to 90 meters from the forest edge (the next six plots), and (3) 100 to 150 meters from the edge (the last six plots). In addition, independent samples t-tests were used to compare the flora of the edge distance class with that of the interior distance class, since all of the mayapple patches used in this study were in one of these two classes.

Site mean pollen limitations of fruit set per flower and seed set per fruit were regressed on the mean abundances of flowers of garlic mustard, geranium, mayapple, and violets per $4m^2$ plot in the transects for each site. Site 5 had a much higher abundance of violets than the other four sites surveyed, and regressions on violet abundance were therefore repeated with this site excluded. Regressions were performed for all three years' pooled data to determine whether any species had a consistent effect over an extended period, as well as for 2005 and 2006 separately. There were only three sites for which both mean pollen limitation and mean site floral abundances were available in 2007 because site 5 was dropped from the study and pollen limitation could not be calculated for any patches in site 1 in that year, and because NWP was not part of the study in 2005, when the transect surveys were performed. Consequently, regressions of site mean pollen limitation in 2007 on site floral abundances are not reported (though data from this year were included in the regressions on pooled data for all three years).

Heterospecific pollination of mayapple

To test for effects of pollination treatment on the probability of fruit set, I performed binary logistic regressions of fruit presence or absence within a pollination treatment group in a patch as a function of pollination treatment. If heterospecific pollen interferes with ovule fertilization and thus reduces the effectiveness of outcross pollination by hand, then fruit and seed set for HPT flowers will be lower than for outcross-supplemented flowers. If hand-pollination with heterospecific pollen rendered all outcross pollen received later ineffective at fertilizing ovules, then flowers receiving the HPT treatment would have even lower fruit and seed set than control flowers (only outcross pollen received naturally prior to hand-pollination could fertilize ovules). However, not all heterospecific pollen may be equally effective at suppressing fertilization success, and the comparison between flowers pollinated with geranium pollen and those pollinated with phlox pollen is also of interest.

Because the comparisons of interest are all comparisons between two pollination treatments, treatments were compared pairwise: HPT with geranium vs. HPT with phlox, outcross vs. HPT with geranium, outcross vs. HPT with phlox, control vs. HPT with geranium, and control vs. HPT with phlox.

For treatment groups bearing fruit, I compared arcsine-transformed fruit set and untransformed seed set between the HPT treatment with wild geranium pollen and the one with phlox pollen using t-tests. I also compared fruit and seed set between outcross-supplemented flowers and each of the two HPT groups, and between control flowers and each of the HPT groups. Patches from which neighboring flowers were removed were included in these analyses because neighbor removal was not expected to modify the effects of heterospecific pollen receipt.

Geranium patches

The effects of pollination treatment and presence or absence of mayapple flowers within five meters on geranium's fruit set per flower, seed set per fruit, and seed set per flower were analyzed using t-tests and ANOVAs. None of these response variables required transformation to achieve normality in this species. ANOVAs were performed to test for an overall effect of pollination treatment on fruit set per flower, seed set per fruit, and seed set per flower. Separate t-tests were also performed to compare control treatment groups with outcross-supplemented and HPT treatment groups and to compare the outcross-supplemented and HPT groups with each other.

T-tests were performed to compare fruit set, seed set per flower, and seed set per fruit within five meters of mayapple versus over fifteen meters from mayapple for each pollination treatment group. ANOVAs were used to test for a significant effect of the interaction between proximity to mayapple and pollination treatment on all three measures of fecundity, with control and outcross-supplemented treatments compared to test for effects of mayapple on pollen limitation, and outcross-supplemented and HPT treatments compared to test whether heterospecific pollen interfered with outcrossing success.

Insect pollen loads

Nine bumblebee queens and two honeybee workers were collected in 2005, and sixteen bumblebee queens, one bumblebee worker, and five honeybee workers were collected in 2006. For each of these insects, mayapple pollen grains were counted and the average numbers of pollen grains carried were calculated for both insect taxa and for each of the floral taxa from which they were collected (including the ground as a floral taxon). These averages were used as rough estimates of the importance of each insect taxon as a pollinator of mayapple and the strength of the interaction through pollinators between each floral taxon and mayapple.

Results

Pollen limitation

Overall pollen limitation

Flowers that received supplemental outcross pollination by hand produced more fruits than naturally-pollinated control flowers, with more seeds per fruit, in 2005, 2006, and 2007 (table 3.1). The yearly average values for PL_{fruit}, an index of pollen limitation of fruit set that ranges from 0 (no pollen limitation) to 1 (total fruiting failure due to pollen limitation), ranged from 0.60 to 0.84. PL_{fruit} was significantly greater than zero in all years (one-sample t-test, $p << 0.001$ in all years, df = 25, 31, 20 in 2005, 2006, and 2007, respectively).

PL_{seed} is an index of pollen limitation of seed set per fruit that ranges from -1 to 1, with all values at or below 0 indicating no pollen limitation. Its mean in each year ranged from 0.24 to 0.64, indicating that pollen limitation of fecundity was expressed more strongly in depressed fruit set than in depressed seed set. However, PL_{seed} was still significantly greater than zero in all three years (one-sample t-test, $p << 0.001$, = 0.039, = 0.0008, df = 13, 22, 13 in 2005, 2006, and 2007, respectively).

Effects of date of hand pollination on apparent pollen limitation

PL_{fruit} was not significantly related to pollination date or patch flower number in any year (linear regressions, p = 0.183, 0.441, 0.205, adjusted r^2 = 0.034, -0.013, 0.035, df = 25, 31, 20, in2005, 2006, and 2007, respectively).

PL_{seed} increased significantly with pollination date in 2005 (linear regression, p = 0.015, adjusted r^2 = 0.351, df = 13), indicating that the earliest hand-pollinations in that year were applied too early to be efficacious. Pollen limitation of seed set was not significantly related to pollination date in the other two years (p = 0.818, 0.455, adjusted r^2 = -0.045, -0.032, df = 22, 13, for 2006 and 2007, respectively).

Regression models for pollen limitation of fecundity

Sixteen regression models were produced for each year, including all combinations of (1) forward and backward stepwise regressions, (2) models including or excluding variables that were not measured in every patch (soil texture and floral traits), (3) models using floral abundances in the first or second week of mayapple flowering, and (4) models including or excluding site mean pollen limitation as a factor. The full models are shown in tables A1-A4 in the appendix I discuss the importance of each variable that occurred in more than 25% of the models it could have occurred in when site mean pollen limitation was excluded, noting cases where the inclusion of site mean pollen limitation changed a variable's importance.

In 2005, pollen limitation of fruit set increased with the abundances of garlic mustard and spring beauty and with increasing silt and clay content in the soil, while it decreased with the abundance of violets (table 3.1). Pollen limitation of seed set in that year increased with the abundance of violets in every model produced, and it increased with the number of flowers in the patch in two of six models and decreased with soil carbon content in three models (table 3.1).

In 2006, floral abundances had no substantial relationship to pollen limitation. The two models that included the abundance of any species' flowers were large (5 or 7 variables) relative to the sample size (21 and 19 total degrees of freedom, respectively), and their, and these models thus have low reliability. Instead, pollen limitation of fruit set in 2006 was best explained by site mean pollen limitation, ramet height (when site mean pollen limitation was included in the model), and leaf-area index (especially when site mean pollen limitation was excluded). Pollen limitation of seed set

In 2007, the abundances of neighboring flowers again appeared in few models. Spring beauty was positively related to pollen limitation of fruit set in one of four models (its flowering had waned too much by the time of the second floral survey to be included in the models based on that survey). Violet abundance was negatively related to pollen limitation of fruit set, but was excluded from all models that included site mean pollen limitation. It was also negatively related to pollen limitation of seed set. In addition to these relationships to floral abundance, pollen limitation of seed set declined with increasing leaf-area index of the forest canopy above the patch, except when site mean pollen limitation was included in the model, and pollen limitation of fruit set was significantly positively related to site mean pollen limitation in almost every model in which this variable was included.

Effects of neighbor removal on pollen limitation

For *Alliaria petiolata*, the most abundant neighbor to mayapple, abundance in the late-season survey declined in both removal and non-removal patches, with the overall decline being significant (ANOVA, $p < 0.0001$, $F_{1\ 62} = 17.902$). Abundance declined more for removal patches than for non-removal patches, resulting in a significant effect of the interaction between removal treatment and floral survey ($p = 0.030$, $F_{1\ 62} = 4.912$), consistent with successful suppression of the abundance of garlic mustard flowers. For the next most abundant species, *Geranium maculatum*, flower abundance tended to increase from the early survey to the late survey, and it increased much more for non-removal patches than for removal patches. Accordingly, there were significant effects of survey ($p = 0.017$, $F_{1\ 30} = 6.372$), removal ($p = 0.014$, $F_{1\ 30} = 6.747$), and the interaction between the two ($p = 0.043$, $F_{1\ 30} = 4.462$). Overall, floral abundance declined between surveys ($p = 0.004$, $F_{1\ 84} = 8.900$), and it declined faster for the removal patches than for the non-removal patches, resulting in a significant effect of the interaction between removal treatment and survey ($p = 0.017$, $F_{1\ 84} = 5.984$), indicating that the removal treatment was effective at reducing floral abundance overall. This effectiveness hinged largely on reductions of garlic mustard abundance in the removal patches. Considering only species other than *A. petiolata*, the overall reduction in flower number from the early to the late survey was marginally significant ($p = 0.098$, $F_{1\ 76} = 2.814$), and though this reduction was largely confined to the removal patches, the effect of the removal x survey interaction was not significant ($p = 0.204$, $F_{1\ 76} = 1.639$). This may be because blackberries (*Rubus allegheniensis*) began flowering between surveys, producing up to ~240 flowers within a meter of the nine patches in which it was present and increasing variability in floral abundance for both treatment groups.

On average, patches with neighboring flowers removed had PL_{fruit} equal to 0.71 +/- 0.29 (mean +/- S.E.), while PL_{fruit} for unmanipulated patches was 0.57 +/- 0.40. PL_{seed} was 0.33 +/- 0.44 for removal patches and 0.20 +/- 0.45 for unmanipulated patches. Thus, mean pollen limitation of both fruit set per flower and seed set per fruit was somewhat higher for the neighbor-removal patches than the unmanipulated patches in 2006. However, these differences were not significant for either fruit set (independent samples t-test, $p = 0.217$, $t = -1.266$, $df = 24.507$) or seed set ($p = 0.473$, $t = -0.726$, $df = 31$).

PL_{fruit} and PL_{seed} also did not differ between the removal patches and unmanipulated patches in 2005 or 2007, when neighbor removal treatments were not performed (all $p > 0.31$), indicating that the two groups did not differ in pollen limitation for reasons unrelated to removal treatment. As was true in 2006, PL_{fruit} was insignificantly higher in the removal patches in both 2005 and 2007, indicating that the removal treatment in 2006 had even less effect on pollen limitation of fruit set than comparison between removal and non-removal patches in that year seems to indicate. There was also no significant effect of the interaction between year and the neighbor removal treatment on PL_{fruit} (ANOVA: $p = 0.876$, $F_{2\ 84} = 0.133$). In both 2005 and 2007, PL_{seed} could only be calculated for two patches in the removal treatment group, preventing meaningful comparison between either of these years and 2006.

Nan Weston Preserve versus second-growth fragments

In 2006, neither PL_{fruit} nor PL_{seed} differed significantly between NWP, the largest, least disturbed forest fragment, and the remaining sites, all smaller, second-growth forest fragments (independent-samples t-tests: $t = -0.561, 0.229$, $df = 30, 21$, $P = 0.579, 0.821$, for PL_{fruit} and PL_{seed}, respectively). The same was true in 2007 ($t = 1.238, 0.862$, $df = 19, 12$, $P = 0.231, 0.405$).

Flora of sites

Total floral abundance did not vary significantly among the three categories of distance from the forest edge (0-30 m, 40-90m, 100-150 m; ANOVA, $p = 0.160$, $F_{2\ 164} = 1.852$), though the plots with the highest floral abundance were within 10 meters of the edge. Similarly, distance category did not explain significant variation in species richness ($p = 0.789$, $F_{2\ 164} = 0.238$) or Simpson's reciprocal diversity index ($p = 0.671$, $F_{2\ 140} = 0.401$).

Alliaria petiolata was less common in the intermediate distance class than in the other two, producing a marginally significant effect of distance on its abundance ($p = 0.053$, $F_{2\ 41} = 3.158$). *Gallium aparine*, which was most abundant within 30 meters of the edge, was also marginally significantly affected by distance class ($p = 0.076$, $F_{2\ 50} = 2.715$). There was an outlier plot in Site 4 in which there were approximately 1500 *G.*

aparine flowers (all other plots with this species had between 2 and 210 flowers). When this plot was excluded, the abundance of *G. aparine* flowers was significantly affected by distance from the edge ($p = 0.0003$, $F_{2\ 49} = 9.521$), with more flowers near the edge and fewer flowers in the intermediate class than in the interior. *Viola* abundance tended to increase with distance from the forest edge, and its abundance was significantly related to distance class ($p = 0.012$, $F_{2\ 10} = 7.071$). The abundances of the remaining species that were common enough for statistical analysis did not vary significantly with distance class, and most species were not common enough for statistical analysis.

Site mean PL_{fruit} increased with site mean abundance of garlic mustard flowers in 2005 (linear regression, $p = 0.015$, $R^2 = 0.895$, adjusted $R^2 = 0.859$, beta = 0.946, df = 4). Site mean PL_{fruit} declined significantly with mean geranium abundance in 2006 ($p = 0.047$, $R^2 = 0.780$, adjusted $R^2 = 0.706$, beta = -0.883, df = 4) and for all three years pooled ($p = 0.030$, $R^2 = 0.361$, adjusted $R^2 = 0.302$, beta = -0.601, df = 12). Site mean PL_{fruit} tended to decline with the abundance of violets in the transects in the pooled data as well, when site 5 was excluded from analysis, but this tendency was not significant ($p = 0.100$, $R^2 = 0.271$, adjusted $R^2 = 0.191$, beta = -0.0521, df = 10), and it was eliminated entirely with the inclusion of site 5 ($p = 0.689$, $R^2 = 0.015$, adjusted $R^2 = -0.074$, beta = 0.123, df = 12). All other regressions of site mean pollen limitation of fruit and seed set on site floral abundances were insignificant (all $p > 0.14$).

The abundances of garlic mustard and geranium tended to be negatively related. This relationship was far from significant when all five of the sample sites were considered (linear regression, $p = 0.500$, $R^2 = 0.163$, adjusted $R^2 = -0.115$, beta = -0.404, df = 4), but became significant when site 5, which had few flowers of either species, was excluded ($p = 0.028$, $R^2 = 0.945$, adjusted $R^2 = 0.917$, beta = -0.972, df = 3). It is likely that this relationship partially explains why PL_{fruit} increased with the abundance of garlic mustard and decreased with the abundance of geranium. It is impossible, based on this study, to determine the causal relationships among geranium abundance, garlic mustard abundance, and pollen limitation of fruit set per flower for mayapple with certainty.

Effects of heterospecific pollination of mayapple

The probability that heterospecifically-pollinated treatment groups within patches bore fruit was not significantly affected by the species used in the heterospecific treatment (logistic regression, $p = 0.644$, Nagelkerke $R^2 = 0.007$). However, among the treatment groups that bore fruit, fruit set differed significantly with the species used for heterospecific pollination (independent samples t-test: $p = 0.015$, $t_{22.011} = 2.642$), with flowers pollinated using phlox pollen having lower fruit set than those pollinated sing geranium pollen. Seed set per fruit did not differ significantly between the two groups ($p = 0.539$, $t_{26} = -0.622$).

The probability that a treatment group bore fruit did not differ significantly between the outcross-supplemented treatment and the HPT treatment using geranium pollen (logistic regression, $p = 0.8875$, Nagelkerke $R^2 = 0.0004$). Among treatment groups that bore fruit, fruit set for outcross-supplemented flowers did not differ significantly from fruit set for the flowers pollinated with wild geranium before outcrossing (independent samples t-test, $p = 0.863$, $t_{63} = -0.173$). Seed set was also not significantly different between these two groups ($p = 0.892$, $t_{63} = 0.136$).

The outcross treatment groups did not have a higher probability of bearing fruit than the HPT treatment group pollinated with phlox pollen (logistic regression, $p = 0.692$, Nagelkerke $R^2 = 0.003$). However, among treatment groups that bore fruit, fruit set was significantly lower for flowers pollinated with wild blue phlox prior to outcrossing than for flowers receiving only outcross pollen (independent samples t-test, $p = 0.007$, $t_{13.632} = 3.178$). Seed set per fruit was not significantly different between these treatment groups ($p = 0.637$, $t_{47} = -0.475$).

Treatment groups pollinated with geranium followed by outcross pollen were not more likely to bear fruit than control treatment groups (logistic regression, $p = 0.426$, Nagelkerke $R^2 = 0.009$). However, among treatment groups that bore fruit, control fruit set was lower that the HPT fruit set (independent samples t-test, $p < 0.001$, $t_{72} = -4.475$), as was control seed set ($p = 0.003$, $t_{72} = -3.116$).

The probability of fruit set for the control treatment groups was not significantly lower than that for groups of flowers heterospecifically pollinated with phlox pollen (logistic regression, $p = 0.965$, Nagelkerke $R^2 < 0.001$). Fruit set among treatment groups that set fruit did not differ significantly between the two treatments (independent samples t-test, $p = 0.381$, $t_{56} = -0.882$), but seed set per fruit was significantly lower for the control treatment ($p = 0.020$, $t_{56} = -2.397$).

Geranium maculatum pollination

Fruit set was not significantly higher for outcross-supplemented geranium flowers (66.7 +/- 6.6% fruit set, mean +/- SE) than for unmanipulated control flowers (54.7% +/- 6.7% fruit set; t-test: $P = 0.210$, $t_{38} = -1.275$). However, seed set per fruit was higher in fruits from outcross-supplemented flowers (4.49 +/- 0.13 seeds per fruit) than for fruits from control flowers (3.87 +/- 0.23 seeds per fruit; $P = 0.026$, $t_{26.77} = - 2.357$). Overall, outcross-supplemented flowers produced significantly more seeds (3.44 +/- 0.32 seeds per flower) than did control flowers (2.38 +/- 0.31 seeds per flower; $P = 0.023$, $t_{38} = - 2.379$).

Geranium flowers treated with mayapple pollen prior to outcross pollination had 56.7 % +/- 8.3% fruit set (mean +/- SE) and produced 4.26 +/- 0.17 seeds per fruit and 2.86 +/- 0.38 seeds per flower. Although these values were lower than the corresponding values for flowers that received supplemental outcross pollen without heterospecific pollen, they were not significantly so (fruit set: $p = 0.347$, $t_{33} = 0.953$; seeds per fruit: $p = 0.269$, $t_{30} = 1.126$; seeds per flower: $p = 0.258$, $t_{33} = 1.152$). Fruit and seed set for HPT flowers were also not significantly higher than they were for unmanipulated control flowers (fruit set: $p = 0.850$, $t_{33} = -0.190$; seeds per fruit: $p = 0.214$, $t_{29} = -1.271$; seeds per flower: $p = 0.324$, $t_{33} = -1.001$).

Proximity to mayapple patches had no effect on control fruit set (t-test: $p = 0.794$, $t_{18} = 0.265$), seed set per fruit ($p = 0.477$, $t_{12.71} = -0.734$), or seed set per flower ($p = 0.663$, $t_{18} = -0.443$). The same was true for outcross-supplemented flowers (fruit set: $p = 0.627$, $t_{18} = -0.494$; seeds per fruit: $p = 0.589$, $t_{17} = -0.551$; seeds per flower: $p = 0.462$, $t_{18} = -0.751$) and for HPT flowers (fruit set: $p = 0.639$, $t_{13} = 0.480$; seeds per fruit: $p = 0.106$, $t_{11} = 1.761$; seeds per flower: $p = 0.370$, $t_{13} = 0.929$).

Proximity to mayapple had no significant effect on pollen limitation of fruit set per flower (ANOVA: pollination x mayapple p = 0.595, $F_{1\ 36}$ = 0.287), seed set per fruit (p = 0.714, $F_{1\ 33}$ = 0.137), or seed set per flower (p = 0.815, $F_{1\ 36}$ = 0.056). It also did not influence the effectiveness of heterospecific pollen in suppressing fruit set (p = 494, $F_{1\ 31}$ = 0.478) or seed set per flower (p = 0.240, $F_{1\ 31}$ = 1.433). There was a trend toward a greater effect of heterospecific pollen on seed set per fruit when mayapples were within five meters (p = 0.104, $F_{1\ 28}$ = 2.816). HPT seed set was significantly lower than outcross seed set in geranium patches with mayapple flowers nearby (t-test, p = 0.046, t_{16} = 2.161), but not in patches without nearby mayapples (p = 0.678, t_{12} = 0.678).

Pollinator observations

Overall, 36 hours and 10 minutes of 10-minute floral observations (217 observations total) were conducted over the three years of the study, including all species observed. An average of 12.65 flowers were observed in each period, for a total of 457.67 flower-hours of observations. Pollinator visits were observed on 16 of the 217 total observations, with a total of 83 flower visits observed.

178 observations were conducted on mayapple, with an average of 12.67 flowers observed in each 10-minute period, for a total of 375.8 flower-hours of observations of mayapple. Insects were seen to land or rest on mayapple flowers in 7 observations over the three years of the study, with 8 insects making a total of 18 visits to individual flowers.

Two of these visitors were honeybees foraging for pollen on 26 May 2005 in Site 5. They conducted 12 of the 18 observed visits to mayapple, while each of the remaining six insects visited one flower apiece. These included a medium (~1 cm) fly, a white moth (*Tetracis cachexiata*: Geometridae), a small (~5 mm) beetle (Carabidae), a mosquito, a small (~1 cm), dark moth, and a bumblebee queen. Of these, only the bumblebee queen is large relative to the distance between the anthers and the stigma, mobile enough to visit many flowers per day, and possessed of hairs that acquire and retain pollen. She touched the stigma of a single flower in Site 4 on 21 May 2005, without gathering pollen or probing for nectar.

Overall mayapple's average visitation rate was 0.062 +/- 0.029 visits per flower per hour (mean +/- SE). When only bumblebees and honeybees are included, this rate is reduced to 0.035 +/- 0.026 visits per flower per hour, or one visit per flower per 28.6 daylight hours.

Several visits to mayapple were recorded outside of the ten-minute observation periods. In 2005, a honeybee was observed gathering pollen from several flowers at Site 5 on 26 May, the same date and site where the two honeybee foraging bouts were recorded during 10-minute observations.

In 2006, a bumblebee queen visited six flowers in a patch in Site 4 on 9 May, neither gathering pollen (the anthers had not yet dehisced) nor probing for nectar, before switching to wild geranium. A honeybee was observed gathering pollen from six flowers in Site 5 on 17 May, in the same patch where two of the foraging bouts in 2005 had been recorded. Another honeybee was captured from a study patch in Site 3 on 20 May, and a bumblebee queen briefly visited two mayapple flowers in two patches in the same site later that day. Again, the honeybee was gathering pollen, while the bumblebee queen visited flowers with indehiscent anthers, neither gathering pollen nor probing for nectar. On 23 May, a bumblebee queen foraging for nectar on geraniums visited a single mayapple flower in a study patch in Site 1, gathering pollen from it, before switching back to geranium. The following day, a honeybee worker was seen gathering pollen from three flowers in a patch in Site 2.

In 2007, a honeybee worker was captured from an unmarked mayapple patch in Site 3 after it had been observed visiting eight flowers within the patch and gathering pollen from them. This patch was across a logging trail from the patch in which a honeybee was captured in Site 3 the previous year.

In addition to honeybees and bumblebee queens, insects found in mayapple flowers outside of observation periods included numerous small carabid beetles, geometrid moths (*T. cachexiata*), several mosquitoes, a medium-sized (~1 cm) fly that had been captured by a crab spider, and an unidentified small (~1 cm) bee.

Although no insect is a definitively demonstrated pollinator of mayapple, it is likely that honeybees and bumblebees are effective pollinators of the species, as both have been previously observed visiting sequences of several mayapple flowers (Swanson and Sohmer 1976, Laverty and Plowright 1988), and both are large, highly motile, and capable of carrying large amounts of pollen on their bodies. In contrast, most of the other visitors to mayapple observed in this study used the flowers as temporary landing platforms, much as they might use a leaf or twig. The two exceptions were *T. cachexiata* and the caribid beetles. The former were only found resting inside mayapple flowers, where they were well-camouflaged; they were never detected flying or resting on another surface, and they only moved if disturbed. The latter were commonly found in mayapple and trillium flowers, where they ate pollen and floral organs. They did not leave the flowers unless disturbed, and they were not seen entering flowers. Neither species retained pollen well, based on the number of mayapple pollen grains found on specimens collected from mayapple flowers. Only nine mayapple pollen grains were found on the bodies of two *T. cachexiata*, and only eight on the body of a beetle, all of which were collected directly from mayapple flowers. In contrast, a honeybee foraging on mayapple was found to carry approximately 3600 pollen grains, and care was taken not to collect pollen from her corbiculae for this sample.

Finally, several bumblebee queens approached mayapple flowers, hovering in front of them without contacting them. This happened seven times over the three years of the study: three times on 17 May 2005, once on 26 May 2005, once on 9 May 2006, once on 17 May 2006, and once on 11 May 2007.

Overall, honeybees visited mayapple flowers on seven occasions, and bumblebees visited mayapple flowers on four occasions and approached flowers without contacting them on seven occasions. All honeybee visits occurred between 10:00 and 15:30 Eastern Standard Time, all bumblebee visits between 11:00 and 14:00, and all bumblebee approaches without contact between 11:30 and 16:30. Observers were present in the field most frequently between 8:00 and 17:30 EST, with abrupt declines in observation effort before and after those times.

The air temperature when honeybees were observed foraging on mayapple ranged from 17.5° C to 23°C. Bumblebee visits to mayapple flowers occurred from 17.5° C to 21.5°C, while approaches without visiting occurred between 19°C to 26°C (however, no temperature readings were made on 17 May 2005, when three approaches to flowers were recorded). The mean air temperature recorded in the field was 17.9°C (+/- 0.5 °C, SE).

Honeybees visited mayapples between the eight and fifteenth days of the flowering season, while bumblebees visited (i.e., contacted flowers) from the first to the fourteenth day, with the one observed case of pollen foraging by a bumblebee occurring on the fourteenth day of the 2006 mayapple flowering season. Bumblebees approached mayapple without contacting them on the first day of the season in five cases, with the other approaches occurring on the ninth and tenth days.

Between the sole occurrence of bumblebee foraging occurring on the fourteenth day of the 2006 flowering season and the occurrence of all honeybee visits between the eighth and fifteenth days of flowering, there was a significant tendency for foraging on mayapple to occur later in the season than contacts without foraging or approaches without contact (ANOVA: $p = 0.005$, $F_{2\ 15} = 7.889$). However, among bumblebees alone, this change in behavior throughout the flowering season was not statistically significant ($p = 0.113$, $F_{2\ 8} = 2.895$). Behavior at flowers was not affected by air temperature ($p = 0.624$, $F_{2\ 14} = 0.490$) but was marginally affected by time of day ($p = 0.098$, $F_{2\ 15} = 2.724$), with approaches without contact tending to occur later in the day than approaches with contact.

To see whether pollinators preferentially visited patches with certain characteristics, I tested whether patches visited by honeybees or bumblebees differed from unvisited patches in their floral neighborhoods, environments, or patch traits. The leaf-area index above visited patches was lower (4.03 +/- 0.16, mean +/- SE) than above other patches (4.35 +/- 0.07), and this difference was marginally significant (independent samples t-test, $p = 0.064$, $t_{71} = 1.885$). Patches that were visited had more conspecific neighboring flowers within five meters (14.4 +/- 4.2) than those that were not observed receiving visits (5.4 +/- 1.3), and this difference was significant ($p = 0.042$, $t_{46} = -2.094$). Not only did visited patches have more mayapple flowers nearby, they also bore more flowers themselves (67.0 +/- 12.3) than patches not observed to receive visits (32.4 +/- 3.9), and this difference was highly statistically significant ($p = 0.006$, $t_{71} = -2.825$).

To determine whether observed pollinator visitation was related to pollination success, I used t-tests to compare PL_{fruit} and PL_{seed} between patches that were visited and those that for which no visits were observed and ANOVAs to test for effects of the interaction between patch visitation (visited or not) and pollination treatment (control versus outcross-supplemented) on fruit and seed set. However, no significant effects were detected (all p > 0.12).

In 26 observations of wild geranium (2 in 2005 and 24 in 2006), the average visitation rate was 1.9300 +/- 1.6678 visits per flower per hour (mean +/- SE). Andrenid bees accounted for 49 of 51 visits observed in geranium, with a small beetle and a fly accounting for the remaining two. Andrenids visited geraniums in four of the observations, and the fly and beetle both visited in a single observation. The two observations in 2005 included the fly/beetle observation and a later observation in which 29 andrenid visits were observed. When these unusual observations are excluded, the 2006 observations showed a visitation rate of 0.2449 +/- 0.1387 visits per flower per hour, which is 5.4 times as great as mayapple's visitation rate in 2006 (0.0457 +/- 0.0390, including only a mosquito and a geometrid moth).

All observations of geraniums were conducted between 6:30 and 14:30. This was not representative of the active period of andrenid bees, as all four andrenid visits that occurred during the ten-minute observations occurred after 13:00. However, when all observations of andrenid activity are considered, whether or not they were recorded during ten-minute observation periods, activity occurred from 7:30 to 17:00.

Insect pollen loads

Only one mayapple pollen grain was detected on a bee not collected from mayapple, and only two non-mayapple pollen grains (one *Alliaria petiolata* and one *Taraxacum officinale*) were found on bees collected from mayapple (figure 3.1). Bees collected from flowers generally bore large numbers of pollen grains belonging to the species from which they were collected, but five bees caught on *Geranium maculatum* and one caught on *Elaeagnus umbellata* carried little or no pollen from these species.

The nineteen bumblebee queens caught from the ground carried few pollen grains, but these grains came from a wide variety of species. This diversity was a result of large sample size; bees on flowers bore pollen from an average of 3.9 +/- 0.5 species (mean +/- S. E., n = 14), while bees on the ground bore pollen from 3.2 +/- 0.5 species (n = 19), but bees on flowers were subdivided by the species on which they were caught, with no more than five bees being caught on a single species. The difference in pollen richness between bees caught on flowers and those caught on the ground is not significant (independent samples t-test, p = 0.345, df = 31, t = 1.003).

Bees caught on flowers carried more pollen grains per bee (586.9 +/- 233.5) than those caught on the ground (27.2 +/- 11.8), and this difference was significant (p = 0.032, df = 13.066, t = 2.394). (These pollen counts come from five transects per microscope slide covering approximately 1/6 of the slide's total area, and some pollen undoubtedly remained on the bees after blotting with gelatin to produce the slides, so the actual quantities of pollen carried were presumably at least six times as great as the numbers reported.)

Discussion

Pollen limitation, pollinator visitation, and fragment size

As found in previous studies, fruit and seed set of mayapple were strongly limited by pollen receipt in my study system. Overall seed production per flower was between 2.7 and 18 times as high in outcross-supplemented flowers as in control flowers, depending on the year. All of these values are in the range of those obtained in previous studies, conducted in Ohio (Whisler and Snow 1992), North Carolina (Motten 1986), Wisconsin and Minnesota (Swanson and Sohmer 1976), Delaware (Rust and Roth 1981), and on an island in Lake Ontario (Laverty and Plowright 1988, Laverty 1992). Based on the results of these six studies, pollen limitation is consistent and severe in mayapple, at least across the northern and eastern fringes of its range.

This severe pollen limitation reflects very low pollinator visitation rates, in my study and in earlier studies. I found that mayapple received just 0.035 +/- 0.026 visits per flower by bumblebees and honeybees. This rate is very similar to that recorded by Laverty (1992), who observed 0.037 +/- 0.012 visits per flower per hour, Laverty and Plowright (1988), who observed 0.057 +/- 0.030 visits per flower per hour. 79% of visits to mayapple in Laverty's (1992) study were conducted by *Bombus vagans* queens, but only 37% of the visitors that I observed on mayapple were bumblebee queens of any species, the rest being honeybee workers. The greater proportional visitation by honeybees in my system may be explained by the presence of commercial beehives near Site 4, but it is not clear why bumblebee queens had a lower absolute visitation rate in my system than in Laverty's (1992) system.

I predicted that pollen limitation would be lower for mayapple in larger, less disturbed forest fragments, if forest disturbance and fragmentation decrease mayapple's pollination success by disrupting natural pollination networks in which it is involved. Such an effect might explain why bumblebee visitation was higher in the fragment studied by Laverty and Plowright (1988) and Laverty (1992), which was about 75 ha in area, than in my Sites 1 through 5, which were on fragments each less than 40 ha in area. If so, pollen limitation of fruit and seed set should have been lower in NWP, 100 ha in area, than in my other study sites. This was not the case.

If natural pollinator networks in southeastern Michigan were sensitive to forest fragmentation, these networks have been similarly disrupted in both small, highly disturbed forest fragments and in slightly larger, less disturbed fragments. However, it is more likely that mayapple would not have a high pollinator visitation rate even if its habitat were pristine. Mayapple flowers remain intact and receptive for up to ten days, as found in this study and by Swanson and Sohmer (1976); species with very low visitation rates often have long-lived flowers, increasing the number of visits each flower receives in its lifetime despite low visitation per hour (Rathcke 1988, Ashman and Schoen 1994). In addition, the fitness of a mayapple plant is probably not highly sensitive to seed production. The clones are long-lived (Bierzychudek 1982), and the fitness of seeds is inhibited by limited fruit dispersal and high seedling mortality (Rust 1980). Thus, selection may simply have favored clones that invested fewer resources in pollinator rewards in favor of greater allocation to growth and survival.

Facilitation of mayapple pollination

The effects of co-flowering neighbors on mayapple's fruit and seed set were mostly insignificant, and no species had similar effects in all three years. Three taxa—garlic mustard, spring beauty, and violets—significantly affected pollen limitation in multiple regression models based on 2005 fecundity data, and only violets influenced pollen limitation in 2007. Pollen limitation was not related to the abundances of nearby flowers in 2006. Similarly, removing the flowers of neighboring plants in 2006 had no significant effect on fruit or seed set, though both fruit set and seed set tended to be more pollen-limited in the neighbor-removal patches. Garlic mustards negative effect on mayapple's pollination success and the facilitative effects of violets, based on fruit set, were also seen in whole-site surveys of floral abundance. Mayapple's fruit set was more strongly pollen-limited in sites with more garlic mustard in 2005, and it tended to be less pollen-limited in sites with more violets for all years pooled, if site 5 was excluded from analysis. Pollen limitation of fruit set also declined with site mean abundance of geranium in 2006 and for all three years pooled.

There are four likely explanations for the weak effects of heterospecific neighbors on mayapple's pollination success. (1) Positive effects of co-flowering neighbors on mayapple's visitation rate were negated by negative effects on ovule fertilization due to heterospecific pollen transfer (HPT). (2) Mayapple's visitation patterns were those of a rewarding species. (3) The magnet species in this system were not attractive enough to greatly influence mayapple's visitation rate. (4) The magnet species were too diffusely distributed to produce large variations in pollinator density, or similarly, pollinator density did not vary substantially across the spatial scales used in this study. These explanations are not mutually exclusive, and each probably contributed in reducing the significance of my results.

The results of the HPT pollination treatments in 2006 demonstrate that other species' pollen potentially inhibits ovule fertilization by outcross pollen in mayapple. Adding phlox pollen prior to outcrossing resulted in depressed fruit set relative to outcrossing alone, but it did not affect seed set, and wild geranium pollen had no effect on fruit set or seed set. Phlox pollen may have been a more effective inhibitor of fertilization because of its small diameter relative to geranium pollen. Geranium pollen grains were 60 to 100 microns in diameter, as opposed to 23 to 25 microns for phlox pollen and 30 to 33 microns for mayapple pollen. Coarser pollen may interfere less effectively with fertilization because there are larger gaps between pollen grains on a stigma if the grains are quite large or because larger grains are more likely to be brushed aside during subsequent hand-outcrossing. Consistent with the latter explanation, just 2.2 geranium pollen grains were counted per bee collected from geranium flowers, suggesting that geranium pollen does not adhere well to the bodies of bees and may also adhere poorly to mayapple stigmas.

The other three explanations for a weak magnet species effect in my system may also explain why the previous study of pollination facilitation in mayapple detected strong results in multiple years (Laverty and Plowright 1988, Laverty 1992). While the dominant visitors to mayapple flowers in my system were honeybees that foraged for pollen and found the flowers rewarding, the dominant visitors in their system were bumblebee queens that foraged for nectar and found the flowers unrewarding. The honeybees I collected from mayapple carried very little pollen from other species but heavy loads of mayapple pollen. Those observed visiting mayapple visited many flowers per patch and never switched to another species, similar to the behavior of honeybee foragers on mayapple described by Laverty and Plowright (1988). In contrast, bumblebee queens visiting mayapple flowers never visited many of them at a time, and two of them switched between mayapple and wild geranium flowers, which was also consistent with observations by Laverty and Plowright (1988), except that the bees in their system switched between mayapple and common lousewort (*Pedicularis canadensis* L., Scrophularicaceae), which was very rare in my system.

The potential magnet species in my system were either too uncommon to test for their effects on mayapple pollination (e.g., lousewort) or too rarely visited by bumblebee queens to strongly influence mayapple's visitation rate (apparently true of all four species tested). Garlic mustard and spring beauty were not expected to be effective magnets, since bumblebee queens were seen very rarely on the former (as also observed by Cruden et al. 1996) and never on the latter, but both geranium and violet were plausible potential magnets, since both are visited by bumblebee queens and both offer nectar as a reward. I observed 0.25 visits per flower per hour in wild geraniums. I did not conduct as many ten-minute observations of violets, and I observed no visits during these observations, but Laverty (1992) observed 0.71 visits per flower per hour to this taxon. Both of these visitation rates are much higher than those recorded for mayapple (0.035-0.057 visits/flower/hour), but much lower than the visitation rate for lousewort in Laverty's

(1992) study (3.53 visits/flower/hour). Also, none of the visitors recorded for wild geranium were honeybees or bumblebees (though both taxa were collected from geranium flowers), so this species may not have been visited by mayapple's visitors much more frequently than mayapple was.

The importance of magnet strength in explaining why my results were less significant than those of the previous study is further demonstrated by removal experiments conducted in this study and in Laverty's (1992) study. I found that patches from which I removed neighboring flowers within one meter had insignificantly higher pollen limitation than those with neighboring flowers left in place. In contrast, Laverty (1992) found that mayapples within 25 meters of louseworts from which he removed flowers, unlike those with intact louseworts within 25 meters, did not have significantly greater fruit set than mayapples over 50 meters from the nearest lousewort (he does not say whether removal patches had significantly lower fruit set than patches with lousewort flowers left in place within 25 meters). The great attractiveness to pollinators of lousewort flowers relative to the flowers of the potential magnets in my system probably explains the stronger effect of his removal treatments. However, given that I performed my removal treatments in the year when neighbor abundance had the least effect on pollen limitation, within just one meter of the manipulated patches, removing neighbors that had both positive and negative effects on pollen limitation in other years, it may be noteworthy that pollen limitation even showed a trend in the predicted direction. Removing only facilitator species across a larger distance in a year when neighbor abundance significantly influences mayapple pollination might have produced a significant effect of removal.

All of the potential magnet species I tested were included in my analyses because they were widespread and common, but this is also a reason to expect that they may not greatly affect pollinator densities greatly. The abundances of spring beauty and garlic mustard varied greatly among sites, so that some study patches were very far removed from flowers of these species, but the distance from any study patch to the nearest violet or geranium was rarely more than about 30 meters. Furthermore, geranium and violet abundance were not positively correlated, so many patches that were far from one of these taxa were nearby the other. In contrast, Laverty (1992) measured visitation, fruit set, and seed set for mayapples within 25 meters of large patches of lousewort to the same variables for patches over 50 meters from the nearest lousewort. The lousewort itself was concentrated in the middle of their study site, while the potential magnets in my system were scattered throughout most of the sites where they occurred.

Because mayapple's pollination success is so low, I did not expect any species to significantly depress it, yet the abundance of both garlic mustard and spring beauty were related to high pollen limitation of fruit set. Garlic mustard is rarely visited by mayapple's major pollinators (Cruden et al. 1996), and I never saw honeybees or bumblebees on spring beauty, making both competition for visits and HPT unlikely mechanisms for their interactions with mayapple. Garlic mustard may negatively affect mayapple's pollination success through its effect on the visibility of mayapple flowers to pollinators. Mayapple flowers are only visible from the side, beneath the leaf canopy of the plant, and garlic mustard may simply block this view. In contrast, short-statured plants such as violets and geraniums may facilitate mayapple pollination because their flowers are at or below the height of mayapple flowers. Pollinators approaching or departing from violets or geraniums would be well-positioned to perceive mayapple flowers, or the presence of the flowers of short-statured plants may be correlated with the absence of garlic mustard. The effect of spring beauty may be a byproduct of a positive correlation between its abundance and that of garlic mustard (linear regression, $p = 0.001$, adjusted $R^2 = 0.193$, total $df = 48$).

Conspecific neighbors were not significant facilitators of mayapple pollination in any of the regression models, at either the patch scale or the site scale. This result was unexpected, since flowers produced by conspecific neighbors were expected to be a source of compatible pollen and thus improve pollination quality. Conspecific neighbors may not have been as beneficial to fecundity because (1) many patches may have been polyclonal, providing a source of outcross pollen in patches that I assumed to be isolated from other genets, and (2) neighboring ramets that appeared to belong to different genets may have been part of genet in the study patch, thus providing self pollen when I assumed that they were providing outcross pollen. Alternatively, conspecific neighbors may have had little effect on receipt of compatible pollen. If visitation to each flower is uncommon, pollen transfer between flowers of different genets may be quite rare.

Effects of edges and light on mayapple pollination success

I predicted that pollen would be less limiting to mayapple fecundity near forest edges because forest edges are bright and warm, with high plant diversity (Chen et al. 1992, Matlack 1993, Fraver 1994), and many forest species flower in greater abundance in bright microhabitats (Moore and Vankat 1986, Collins and Pickett 1988). This may attract abundant and diverse pollinators. If so, and if the magnet species effect operates because magnet species increase the local density of pollinators, mayapple should have higher visitation near forest edges. This prediction was not supported; PL_{fruit} and PL_{seed} did not varied significantly with distance only in regression models too large to be considered meaningful. Pollen limitation of fruit and seed set declined with increasing leaf-area index (i.e., patch shadiness), so pollinators apparently preferred well-lit microenvironments, as expected. However, leaf-area index did not vary with distance to the forest edge. This may mean that edges were not better-lit than forest interiors, though leaf-area index measurements are more sensitive to light entering the understory from above than from the side. The assumption that edges would have more flowers and greater floral diversity was also not met, based on edge-to-interior floral surveys in 2005. Pollinator abundance near the forest edge versus the interior was not quantified, but while small-bodied pollinators appeared to be much more abundant near the edge and under open canopy, there was no conspicuous relationship between the density of honeybees or bumblebees and the brightness of the understory. Apparently, pollen limitation for mayapple was not a function of distance from the forest edge because mayapple’s pollinators were not more abundant near edges because edges did not have more open canopies than interiors or because honeybees and bumblebees are both somewhat endothermic and did not tend to aggregate in well-lit microenvironments as expected.

Pollinator behavior

Insects were seen to land or rest on mayapple flowers in 7 of 178 observation periods. However, these visitors included some insects that were probably not consistently recorded as visitors, such as mosquitoes and small beetles, while others that were consistently noted were probably not effective pollinators, such as moths of the species *Tetracis cachexiata* (Geometridae), a white moth occasionally found resting on the floral parts of mayapple flowers. These moths were never observed to leave a mayapple flower unless disturbed, and two specimens collected directly from mayapple flowers bore very little pollen.

Honeybees were only observed visiting mayapples late in the mayapple flowering season, after mayapple had been flowering for at least a week. In some mayapples, the anthers do not dehisce and present pollen until several days into the flowering season (pers. obs.). However, this probably does not explain the delay in pollen foraging by honeybees. In other clones, the anthers dehisce before the flowers open. Thus, pollen appears to be available on the first day of the flowering season, and increasingly so through the first week, after which pollen availability appears to decline. I did not quantify pollen availability, however, and it has not yet been determined whether the quantity, quality, or accessibility of mayapple pollen as a food source changes during the flowering season. The composition of mayapple pollen may change over time, or mayapple flowers may become more appealing to honeybees' innate aesthetics by the end of the first week of flowering. For example, mayapple flowers may become more fragrant, or their petals may produce or degrade pigments that are visible in the UV portion of the bee visual spectrum. The lag between anthesis and earliest honeybee visitation was not perfectly related to the minimum flight temperatures of honeybees. The lowest temperature at which honeybees were observed foraging on mayapple was 17.5 degrees, but in all three years, air temperatures exceeded this about a week before the first honeybee foragers were observed.

There was a strong tendency for bumblebee queens to approach flowers without contact early in the flowering season. Bumblebees contacted mayapple stigmas without landing both early and late in the flowering season, and the only bumblebee observed foraging on mayapple visited very late in the flowering season in 2006. Thus, the longer the flowering season progressed, the more closely bumblebee behavior toward mayapple flowers resembled that of honeybees foraging for pollen, though this shift was not statistically significant. This may indicate that bumblebees were increasingly likely to forage for pollen as the season progressed, either because more bumblebee queens had established nesting sites and required pollen for their brood or because mayapple pollen became more usable as a resource as it aged. This could happen if podophyllotoxin concentrations in pollen declined with pollen age, provided that bees are sensitive to this particular poison. The tendency for approaches without contact to occur later in the day than approaches with contact might be due to a learned association between unrewarding flowers and mayapple's scent (Gumbert and Kunze 2001, Kunze and Gumbert 2001), which is stronger and can be detected at a greater distance, at least to the human nose, when temperatures are warmer (pers. obs.).

Patches in which honeybee or bumblebee visits were observed had more conspecific neighboring flowers and bore more flowers themselves than did patches that were not observed to receive visits. This is consistent with the pollen foraging behavior of the honeybees (and one bumblebee), since pollinators visit resource-dense patches more frequently than resource-poor ones (Thomson 1981, Kunin 1993, Ohashi and Yahara 2002, Feldman 2006). In contrast, visitation to an unrewarding flower is expected to decline with the flower's abundance (Ferdy et al. 1998, Castillo et al. 2002). Pollen foragers represented a majority of the observed visitors to mayapple in this study, and it is therefore not surprising that the overall pattern of visitation is more consistent with what would be expected for rewarded pollinators than for unrewarded ones.

Alternatively, the greater mayapple floral abundance in and around the patches that were visited could reflect a preference of bees for well-lit environments. The canopies over patches in which visits were observed had marginally significantly lower leaf-area indices than those over patches in which honeybees and bumblebees were never observed to visit mayapple flowers. The number of flowers a patch produced was significantly negatively correlated with leaf-area index, as well. Thus, the apparent preference of pollinators for patches with many flowers could reflect a preference of pollinators for well-lit patches coupled with the tendency for better-lit patches to bear more flowers. However, this explanation does not seem likely, given that the difference in leaf-area index between visited and unvisited patches was much less statistically significant than the differences in patch flower number and the number of conspecific neighboring flowers.

A third explanation for the relationships between large numbers of mayapple flowers in the patch and within five meters, low leaf-area index, and higher probability of observing honeybee and bumblebee visits is that rewarded pollinators found patches more easily when flowers released fragrance when warmed by direct sunlight. Just as mayapple's scent may repel unrewarded pollinators, it may be used by pollen-foraging bees to locate mayapple patches and flowers.

Mayapple's effects on geranium pollination

The degree of pollen limitation for wild geranium plants was not significantly affected by whether the nearest mayapple flowers were within five meters or over ten meters away, and mayapple pollen added by hand did not significantly reduce geranium's fecundity relative to that obtained from outcross-supplementation alone. However, among only those patches that had mayapple within five meters, flowers that received hand-pollination with mayapple pollen prior to outcrossing did have significantly lower seed set per fruit than flowers that were only outcrossed. The biological significance of this difference in the effect of mayapple pollen on geranium seed set with proximity to mayapple patches is challenging to imagine. Perhaps geraniums further from mayapple were more likely to have already been pollinated naturally prior to hand-pollination. Geranium flowers selected for hand-pollination were pollinated on the day their stigmas opened, yet each of several stigmas I observed with a hand lens prior to pollination already bore geranium pollen. If geraniums near mayapple were less likely to be naturally pollinated prior to hand-pollination, pollen limitation should have been higher for these flowers than for those far removed from mayapple. While there was a trend toward greater pollen limitation of fruit set near mayapple, it was far from significant, and fruit set was not significantly pollen-limited near mayapple.

Future directions

The magnet species effect, population growth, and individual fitness

The benefits of the magnet species effect for the population growth of the beneficiary species are not understood. The effect has been documented in several studies to date (Thomson 1978, Laverty 1992, Johnson et al. 2003, Juillet et al. 2007), but few studies have evaluated whether facilitation of pollination results in an increase in population growth or in the genetic fitness of the individual plant. Increased fecundity may have some effect on population growth if the survival of early life-history stages limits population growth, but if the survival of adults is more critical for population growth, then increased fecundity will have little positive effect, and it may even decrease population growth if survival trades off against fecundity. Future studies on facilitation of pollinator visitaiton should consider the effect of improved pollinator visitaiton on outcrossing rates and adult survival and not assume that increased fecundity will mean increased fitness or population growth.

Would a mayapple plant that rewarded pollinators have higher fitness?

The cost of not rewarding pollinators seems clear; unrewarding species have low visitation rates and low mating success relative to otherwise similar rewarding species (Neiland and Wilcock 1998). However, the assumption that unrewarding species incur this cost is underlain by the assumption that unrewarding species are just like rewarding species, but without the rewards. However, rewardlessness may evolve and be maintained only when rewarding pollinators results in very little or no increase in fecundity (e.g., Ackerman 1986). A straightforward way to test whether unrewarding species currently exist in environments in which pollinator rewards do not increase fecundity is to add artificial nectar or nectaries to naturally rewardless flowers. Similarly, removing pollinator rewards from rewarding species may reveal whether rewards improve the fecundity of these species.

There is a shortcoming inherent to reward supplementation and removal experiments, which is that they assess what the effect on fitness would be of producing more or fewer pollinator rewards if doing so entailed no change in the allocation of energy or other resources. The energetic costs of nectar production have been estimated in a few species (Pleasants and Chaplin 1983, Harder and Barrett 1992), but it may be impossible to determine how nectar-producing species would re-allocate their resources if they became nectarless, how nectarless species would re-allocate resources to produce nectar, and what net effect a change in nectar production would have on adult growth and survival. While some species are polymorphic for nectar production (see Renner 2006 for review), rewarding and unrewarding morphs of these species co-occur in the same populations, and the costs and benefits of reward production must be affected in important ways by this difference in context from purely rewarding or purely unrewarding species. Only in species whose natural populations vary greatly in their ratio of rewarding to unrewarding morphs might the true costs of nectar production be estimated. For example, while cardinal flower (*Lobelia cardinalis* L., Campanulaceae) usually has nectar-rich flowers, at least one nectarless population has been found (Brown and Kodric-Brown 1979). Some insight into the costs of nectar production in this species might be gained by comparing the fitness of plants in this population to that of plants in nectar-producing populations and by conducting reciprocal transplant or common garden experiments to determine the conditions under which nectar-producing or nectarless morphs have a fitness advantage.

Is the magnet species effect a random and meaningless phenomenon?

As described above, several studies have documented clearly that unrewarding species may benefit from the magnet species effect. This is a curious phenomenon in its own right, but the relevance of the magnet species effect to anything else is not made obvious by the mere fact of its existence. Thus far, it has been neither suggested nor demonstrated that this form of facilitation is typical for unrewarding species, that it affects the reproduction of any rewarding species, or that it has any predictable influence on the evolution of a species. This may explain why, despite the existence of several empirical tests of the magnet species effect (Thomson 1978, Laverty and Plowright 1988, Laverty 1992, Gumbert and Kunze 2001, Johnson et al. 2003, Juillet et al. 2007), the theory behind it has not yet developed beyond Thomson's (1978) verbal model. To date, the magnet species effect seems to have been taken as something that occurs sometimes when a highly attractive species and a less attractive species happen to co-flower in close proximity. Perhaps it is time to ask not only whether the magnet species effect occurs, but when it can be expected to occur and how it can be expected to influence the evolutionary trajectories of the species involved.

Predicting the occurrence of the magnet species effect would be trivial were it not for the fact that it was initially proposed to explain the behavior of pollinators on two rewarding species of *Hieracium* (Thomson 1978). Every subsequent demonstration of this effect has involved an unrewarding species and one or more rewarding species (Laverty and Plowright 1988, Laverty 1992, Johnson et al. 2003, Juillet et al. 2007), including the present study, which might suggest that it is relevant only to unrewarding species. If, however, a highly attractive species of *Hieracium* can increase the visitation rate of a slightly less attractive species of *Hieracium* that co-flowers with it, then the magnet species effect apparently occurs in at least some cases where asymmetrical competition for pollinator visits might be predicted instead. What determines whether facilitation or competition occurs? Relevant factors can be proposed, of course. Perhaps facilitation is more likely if the species in question have similar colors in the visual systems of their pollinators (Gumbert and Kunze 2001, Kunze and Gumbert 2001, Galizia et al. 2005). Perhaps competition for pollinator visits is more likely when the relevant pollinators are relatively scarce (Tepedino and Stanton 1981, Klein et al. 2003) or when population densities are high (Kunin 1997). However, until the theory and empirical study of the magnet species effect proceeds beyond its absence or presence in a system, the occurrence of the magnet species effect will remain unpredictable, beyond its likely relevance to any unrewarding flowering plant species.

This leaves the question of the evolutionary implications of the magnet species effect. For unrewarding species, there is one obvious implication, namely that facilitation of pollination by rewarding neighbors reduces the costs of being unrewarding and therefore reduces selection for reward provisioning. Similarly, selection may favor mimicry of rewarding neighbors by unrewarding species. However, many species lack the morphological variation to evolve mimicry of their rewarding neighbors, and the direction of selection may be too variable to result in resemblance to any one model. How facilitation of visitation by rewarding neighbors affects the evolution of unrewarding species undoubtedly depends on the facilitating species and their similarities to each other and to the unrewarding species. The question of the evolutionary implications of the magnet species effect is further complicated by its occurrence in rewarding species. In general, selection favors a degree of distinctiveness in rewarding species to promote pollinator constancy (Kunze and Gumbert 2001); does this rule of thumb break down when one rewarding species' pollinator visitation is promoted by another species?

Theory has not come so far as to speculate how the magnet species effect might favor the evolution of rewardlessness. I would argue that the presence of abundant neighbors with rich pollinator rewards may favor evolution toward rewardlessness in a less-rewarding species that does not compete effectively with these neighbors for pollinator service. Individuals of a species that consistently loses in competition for pollination may sacrifice little fecundity by investing less in rewards and gain little by investing more, within the range of the species' variation in reward provisioning strategies. If the fitness costs of allocating resources to reward provisioning change more rapidly with changes in allocation to rewards than do the benefits, selection will favor diminished reward provisioning. Thus, interactions with superior competitors for pollinator attention potentially promote and maintain rewardlessness as a reproductive strategy.

Is mayapple truly and consistently unrewarding?

It is possible that mayapple is not consistently unrewarding to bumblebee queens. Honeybees make regular use of its pollen (Laverty and Plowright 1988, and personal observation). I observed one bumblebee queen gathering mayapple pollen from a single flower, interrupting a foraging bout on wild geranium. Two other queens contacted several mayapple flowers that had indehiscent anthers, briefly grappling the stigma with their forelegs, without attempting to gather nectar or pollen. These queens may have simply proceeded further than others I observed along the behavioral pathway for nectar gathering before recognizing the unrewarding flower, or they may have been seeking pollen and been unable to determine that it was absent before contacting the flowers. Most of the bumblebee queens I observed were not foraging on flowers, but searching for nest sites. Bumblebee queens do not require pollen until they have a nest; adults do not consume pollen, but it is an important nutrient source for developing brood.

Alternatively, rewardlessness in mayapple may be favored due to its effects on the frequency of outcross pollination relative to geitonogamy. Mayapple lacks the adaptations to low pollinator constancy that are evident in the Orchidaceae (though it does have long-lived flowers), but since it is both extensively clonal and self-incompatible, it may be advantageous for a mayapple genet to encourage bees to fly to other patches without visiting many flowers. A bee that finds mayapple rewarding will visit many flowers, but much of the pollen it deposits on stigmas will be self pollen. In contrast, an unrewarded pollinator will engage in long inter-floral flight distances promoting outcrossing (Laverty and Plowright 1988). One test of the hypothesis that rewardlessness is favored in mayapple because it reduces geitonogamy would be a nectar addition experiment, as suggested above. A patch with artificial nectar in its flowers should have lower fruit and seed set, or lower overall offspring fitness, than an unmanipulated patch.

Other effects on pollinator abundance and, hence, pollination success

The magnet species hypothesis proposes that highly attractive flowering plants increase the visitation rate of their less attractive neighbors by drawing a greater number of pollinators to their vicinity than would otherwise congregate there (Thomson 1978), but rewarding flowers are not alone in promoting the aggregation of pollinators. Among the motivations of this study was the hypothesis that, all else being equal, pollinators in forest habitats in cool weather would preferentially forage in well-lit microhabitats to minimize the risk of being forced to cease foraging by becoming too cold to fly. This hypothesis was not supported for mayapple's pollinators, but andrenid and halictid bees and bombylid flies were conspicuously more active in well-lit patches of *Claytonia virginica*, *Geranium maculatum*, and *Alliariai petiolata* than in shady patches. Pollinators may also aggregate near water sources, nesting sites, breeding grounds, or aggregations of prey species (some plants are pollinated by wasps). Pollination of carrion-mimicking flowers may be related to the local abundance of carrion, in a manner very analogous to the magnet species effect.

In many cases, the local abundance of non-floral resources may be more difficult to determine than the abundance of floral resources. Bumblebee nests are notably difficult to locate, partly because of the bees' aversion to foraging very near the nest (Dramstad et al. 2003). Nests of solitary bees that do not have highly aggregated nesting sites could be even more difficult to locate, since only one bee uses the nest, and many solitary bees construct multiple nests in a season (Krombein 1967, Wcislo and Cane 1996). Certainly, potential nest sites must be even more difficult to identify, as would be potential breeding grounds. However, very large aggregations of nesting sites for solitary bees are sometimes quite easy to locate (Wcislo and Cane 1996), and larger aggregations are more likely to have detectable effects on the pollination success of plants. Thus, a preliminary study of the effects of nest sites, or anything else that causes pollinators to aggregate, on pollination success should begin by locating pollinator aggregations and then proceed to testing for effects of distance from the aggregations on pollen limitation, competition among pollinators, or other phenomena of interest.

Conclusions

In contrast to a previous study on pollination facilitation in mayapple, I found that the effects of mayapple's neighbors on its pollination success were weak in most years and inconsistent from year to year. Accordingly, neighbor removal experiments had no significant effect on the pollen limitation of mayapple patches. However, based on the relationship between pollen limitation of fruit set and the floral abundances of co-flowering species both within five meters of my study patches and along edge-to-interior sampling transects for entire study sites, garlic mustard has a negative effect on mayapple's pollination success, while violets have a positive effect. Wild geranium had a weak positive effect, and spring beauty had a weak negative effect, but both species' effects are potentially due to correlations between their abundances and the abundance of garlic mustard. Pollination facilitation may have been weaker in this study than in a previously published study because the magnet species in this system were less attractive and more diffusely distributed, because positive effects through increased visitation were negated by negative effects through interspecific pollen transfer, or because most visitors to mayapple foraged for pollen, moving strictly within the species instead of moving between neighboring flowers and mayapple flowers.

Contrary to expectation, mayapple's pollination success was not related to the number of conspecific flowers present on neighbors within five meters of a patch. This may be due to difficulties in determining whether nearby ramets belonged to different genets, or it may be that mayapple's low visitation rate prevents nearby compatible mates from promoting its reproductive success substantially.

It is possible that heterospecific pollen receipt negatively affects the ability of mayapple pollen to fertilize ovules. Wild geranium pollen did not interfere with outcross pollination success, but hand-pollination with phlox pollen prior to outcrossing depressed outcross fruit set.

Very few studies on pollination facilitation consider interactions in two directions, though an understanding of the two-way interaction is necessary to understand the ecological and evolutionary implications. I tested whether mayapple affected the pollination success of wild geranium and whether mayapple pollen potentially interferes with the ability of geranium pollen to fertilize ovules. Pollen limitation for geranium was insignificantly higher within five meters of a mayapple patch than over ten meters from the nearest one. Hand-pollination with mayapple pollen did not significantly depress fruit or seed set for wild geranium overall, but it did depress fruit set for geraniums growing within five meters of a mayapple patch, possibly because these geranium flowers were less likely to have been naturally pollinated prior to hand-pollination.

I predicted that pollinators would be more abundant near forest edges and that mayapple's fecundity would therefore be less pollen-limited near edges, but I did not find this to be the case. This may be because mayapple's pollinators were not highly sensitive to the difference in air temperature between forest edges and forest interiors.

Future studies on the magnet species effect should investigate its relevance to rewarding species and to the evolution of rewardlessness. It is possible that mayapple in my system did not benefit greatly from the magnet species effect precisely because it was rewarding to most of its visitors. Whether pollinators find mayapple unrewarding in most systems in most years should be investigated more thoroughly. In addition, the effects of other factors that influence pollinator density should be investigated for their effects on the pollination success of unrewarding plants.

Factor	**2005**		**2006**		**2007**	
	Fruit	**Seed**	**Fruit**	**Seed**	**Fruit**	**Seed**
Alliaria	+ 6 (5)			(+ 1)		+ 1 / 6
Claytonia	+ 2 / 4	(+ 1) / 3		(– 1)	+ 1 / 4	
Geranium	+ 1 (2)			(– 1)	– 1 / 7	(+ 1) / 6
Viola	– 3	+ 6 / 6	(+ 1)		– 3 (0) / 7	– 2 / 6
Podophyllum in	(– 1)	+ 2 / 6		(+ 1)	– 6 (3) / 7	– 1 / 6
Podophyllum out						
Ramet height	+ 1 (0)	– 1 / 6	(– 5)			
Flower diameter				(– 1) / 4		
Anther number					+ 1 / 4	
Edge	(– 1)		+ 1	(– 1)	+ 1 (0) / 7	
LAI		– 1 / 6	+ 8 (4)	– 1 (0)		– 3 (1) / 6
Soil carbon	– 1	– 3 / 6			+ 1 (0) / 7	(– 1) / 6
Soil texture	+ 3 / 4		+ 1 / 4	(+ 1) / 4		
Site mean PL	(+ 1)		(+ 8)		(+ 6) / 7	(– 1) / 6

Table 3.1: Number of final models from backward and forward stepwise regressions that included each variable (rows) to explain variation in pollen limitation of fruit set and seed set in each year (columns). In each cell, the direction of the effect of each variable on pollen limitation is indicated by + or –. The number of models that included that variable when site mean pollen limitation was not part of the initial regression model follows. If this number was different when site mean pollen limitation was included, it is followed by the number of models in which the variable was included when site mean pollen limitation was included, in parentheses (e.g., "– 6 (3)"). In most cases, a variable could be included in up to eight models. If the maximum possible number of models was less than eight, this is indicated by a slash and the maximum number of models (e.g., "– 3 / 6")

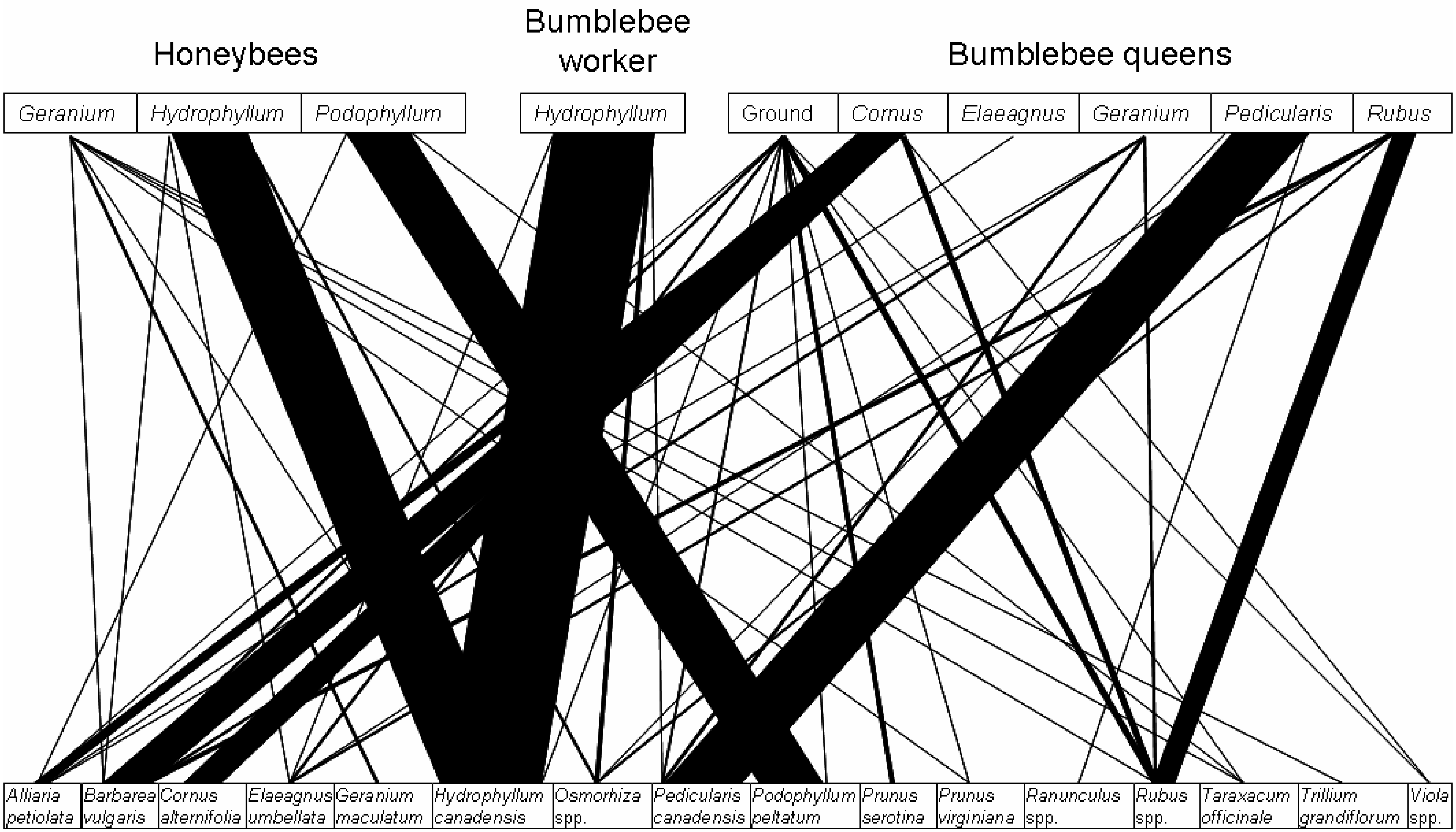

Figure 3.1: Pollen transport web based on honeybees and bumblebees collected in 2006 and 2007. Taxa listed across the bottom are plant species whose pollen was found on the collected bees. Taxa across the top (including "Ground") are taxa from which bees were collected. The width of each bar is proportional to the square root of the average number of pollen grains of each plant taxon (bottom) found on honeybees, bumblebee queens, or a bumblebee worker collected from each taxon or the ground (top).

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Appendix to Chapter 3

Tables of regression models

Tables A.1 through A.4 below show the results of backward and forward stepwise regression models of PL_{fruit} (pollen limitation of fruit set per flower) and PL_{seed} (pollen limitation of seed set per fruit) as functions of neighborhood floral abundances, environmental variables, and the vegetative and floral traits of the patches.

The methods behind the models are explained in detail, and their results summarized, in Chapter III, but I present brief explanations here and in the legends of the tables.. For each year, models were generated for early- and late-season floral abundances, including and excluding variables that were not measured in all patches, and including and excluding site mean pollen limitation as a factor. Only sites 1 – 5 were studied in 2005. The Nan Weston Preserve at Sharon Hollow was added in 2006. In 2007, site 5 was removed from the study.

Season	2005			2006			2007		
	Model	β	P	Model	β	P	Model	β	P
Early-season survey, full model, backward stepwise	Soil carbon Less sandy soil Claytonia Geranium Viola $R^2 = 0.621$ (0.503)	-0.452 0.460 0.662 0.542 -0.835	0.034 0.019 0.002 0.014 0.0004	Ramet height Site mean PL $R^2 = 0.512$ (0.468)	-0.276 0.603	0.084 0.0007	Patch flowers Claytonia Anthers per flower $R^2 = 0.564$ (0.455)	-0.773 0.467 0.415	0.003 0.065 0.082
With site mean PL added to final model	Soil carbon Less sandy soil Claytonia Geranium Viola Site mean PL $R^2 = 0.632$ (0.475)	-0.458 0.416 0.642 0.541 -0.869 0.034	0.056 0.036 0.005 0.019 0.0015 0.877	N/A	N/A	N/A	Patch flowers Claytonia Anthers per flower Site mean PL $R^2 = 0.623$ (0.486)	-0.566 0.382 0.371 0.309	0.048 0.128 0.113 0.218
Forward stepwise	Alliaria $R^2 = 0.273$ (0.237)	0.523	0.013	Site mean PL $R^2 = 0.439$ (0.415)	0.663	0.0003	Site mean PL $R^2 = 0.404$ (0.361)	0.635	0.008
With site mean PL added to final model	Alliaria Site mean PL $R^2 = 0.287$ (0.212)	0.582 0.100	0.074 0.546	N/A	N/A	N/A	N/A	N/A	N/A
Total df	21			24			15		
Early-season survey, reduced model, backward stepwise	Claytonia Viola $R^2 = 0.249$ (0.183)	0.358 -0.539	0.088 0.014	Ramet height Site mean PL $R^2 = 0.516$ (0.483)	-0.291 0.637	0.032 <0.0001	Site mean PL $R^2 = 0.430$ (0.400)	0.656	0.0012
With site mean PL added to final model	Claytonia Viola Site mean PL $R^2 = 0.273$ (0.174)	0.269 -0.447 0.183	0.250 0.064 0.401	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Alliaria $R^2 = 0.191$ (0.157)	0.437	0.026	Same results as backward stepwise	N/A	N/A	Same results as backward stepwise	N/A	N/A
With site mean PL added to final model	Alliaria Site mean PL $R^2 = 0.206$ (0.137)	0.327 0.165	0.202 0.515	N/A	N/A	N/A	N/A	N/A	N/A
Total df	25			31			20		

Late-season survey, full model, backward stepwise	Less sandy soil Alliaria R^2 = 0.553 (0.493)	0.539 0.670	0.009 0.002	Site mean PL R^2 = 0.439 (0.415)	0.663	0.0003	--	--	--
With site mean PL added to final model	Less sandy soil Alliaria Site mean PL R^2 = 0.572 (0.480)	0.531 0.561 0.174	0.0011 0.028 0.445	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Same results as backward stepwise	N/A	N/A	Same results as backward stepwise	0.663	0.0003	Patch flowers R^2 = 0.571 (0.532)	-0.756	0.003
With site mean PL added to final model	Same results as backward stepwise	N/A	N/A	N/A	N/A	N/A	Patch flowers Site mean PL R^2 = 0.609 (0.531)	-0.618 0.238	0.029 0.350
Total df	17			24			12		
Late-season survey, reduced model, backward stepwise	Distance to edge Geranium Viola Patch flowers Site mean PL R^2 = 0.541 (0.388)	-0.552 0.417 -0.369 -0.508 0.631	0.015 0.052 0.069 0.033 0.009	Ramet height Site mean PL Viola R^2 = 0.562 (0.515)	-0.358 0.643 0.224	0.011 <0.0001 0.099	Site mean PL Patch flowers Geranium R^2 = 0.670 (0.588)	0.471 -0.506 -0.380	0.018 0.013 0.046
With site mean PL added to final model	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Alliaria R^2 = 0.190 (0.147)	0.435	0.049	Ramet height Site mean PL R^2 = 0.516 (0.483)	-0.291 0.637	0.032 <0.0001	Site mean PL R^2 = 0.367 (0.322)	0.606	0.013
With site mean PL added to final model	Alliaria Site mean PL R^2 = 0.203 (0.115)	0.313 0.169	0.319 0.586	N/A	N/A	N/A	N/A	N/A	N/A
Total df	20			31			15		

Table 3.A.1: Models produced by backward and forward stepwise regressions of PL_{fruit} on patch, environmental, and neighborhood floral characteristics. "Reduced models" included site mean PL_{fruit}, mean ramet height, log-transformed number of flowers in the patch, distance to the nearest forest edge, soil carbon content, and log-transformed abundances of neighboring flowers within five meters. "Full models" included all of these variables plus soil texture, mean number of anthers per flower, and mean floral diameter. Because soil texture could not be measured for eight patches in site 2 found on deep peat, and floral traits were not measured in site 5, sample size was larger for the reduced models. For "early-season" models, neighbor removal patches were included for 2005 and 2007, but not 2006. For "late-season" models, neighbor removal patches were excluded for all three years because the removal treatment altered floral abundances in the late survey, since the removal and the survey were both performed in 2006. If site mean PL_{fruit} was not included in the final model, a new model that included this variable was produced. Sample sizes are smaller than the number of patches in the study because PL_{fruit} could only be measured if the outcross treatment group produced at least one fruit. "--" = no model produced. "N/A" = not applicable.

Season	2005			2006			2007		
	Model	β	P	Model	β	P	Model	β	P
Early-season survey, full model, backward stepwise	--	--	--	Anthers per flower Leaf-area index Alliaria Claytonia Geranium Patch flowers Site mean PL $R^2 = 0.626$ (0.408)	-0.481 -0.496 0.394 -1.008 -0.595 0.785 0.509	0.031 0.031 0.098 0.005 0.023 0.004 0.041	--	--	--
With site mean PL added to final model	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Viola $R^2 = 0.555$ (0.514)	0.745	0.003	No significant variables	N/A	N/A	Alliaria Flowers in patch $R^2 = 0.751$ (0.680)	0.695 -0.563	0.008 0.020
With site mean PL added to final model	Viola Site mean PL $R^2 = 0.615$ (0.538)	0.519 0.333	0.080 0.240	N/A	N/A	N/A	Alliaria Flowers in patch Site mean PL $R^2 = 0.754$ (0.630)	0.639 -0.545 0.075	0.089 0.046 0.824
Total df	12			19			9		
Early-season survey, reduced model, backward stepwise	Ramet height Leaf-area index Claytonia Viola $R^2 = 0.737$ (0.620)	-0.782 -0.371 0.518 1.010	0.046 0.081 0.058 0.003	No significant variables	N/A	N/A	Leaf-area index Soil carbon Site mean PL Geranium Viola $R^2 = 0.768$ (0.622)	-1.241 -1.839 -1.503 0.427 -1.653	0.004 0.005 0.014 0.079 0.008
With site mean PL added to final model	Ramet height Leaf-area index Claytonia Viola Site mean PL $R^2 = 0.761$ (0.611)	-0.746 -0.314 0.454 0.873 .224	0.062 0.159 0.108 0.020 0.398	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Viola $R^2 = 0.513$ (0.472)	0.716	0.004	No significant variables	N/A	N/A	No significant variables	N/A	N/A
With site mean PL added to final model	Viola Site mean PL $R^2 = 0.594$ (0.521)	0.468 0.378	0.093 0.166	N/A	N/A	N/A	N/A	N/A	N/A
Total df	13			22			13		

Late-season survey, full model, backward stepwise	--	--	--	No significant variables	N/A	N/A	--	--	--
With site mean PL added to final model	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Forward stepwise	Viola Soil carbon R^2 = 0.802 (0.752)	0.810 -0.536	0.0010 0.010	No significant variables	N/A	N/A	Viola R^2 = 0.629 (0.576)	-0.793	0.011
With site mean PL added to final model	Viola Soil carbon Site mean PL R^2 = 0.814 (0.734)	0.883 -0.674 -0.181	0.003 0.038 0.527	N/A	N/A	N/A	Viola Site mean PL R^2 = 0.632 (0.510)	-0.737 0.079	0.080 0.830
Total df	10			19			8		
Late-season survey, reduced model, backward stepwise	Viola Soil carbon Patch flowers R^2 = 0.855 (0.801)	0.988 -0.525 0.395	0.0003 0.006 0.043	No significant variables	N/A	N/A	Leaf-area index R^2 = 0.268 (0.195)	-0.518	0.085
With site mean PL added to final model	Viola Soil carbon Patch flowers Site mean PL R^2 = 0.863 (0.784)	1.045 -0.638 0.383 -0.143	0.0010 0.031 0.062 0.561	N/A	N/A	N/A	Leaf-area index Site mean PL R^2 = 0.311 (0.158)	-0.408 0.235	0.226 0.473
Forward stepwise	Same results as backward stepwise	N/A	N/A	No significant variables	N/A	N/A	No significant variables	N/A	N/A
With site mean PL added to final model	Same results as backward stepwise	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Total df	11			22			11		

Table 3.A.2: Final models produced by backward and forward stepwise regressions of PL_{seed} on measurements of patch, environmental, and neighborhood floral characteristics. See legend of Table A.1 for a full explanation. Sample sizes for PL_{seed} are smaller because this variable could only be measured on the subset of patches included in Table A.1 that produced fruits in both the outcross-supplemented and unmanipulated control treatment groups.

Season	2005			2006			2007		
	Model	β	P	Model	β	P	Model	β	P
Early-season survey, full model, backward stepwise	Soil carbon Less sandy soil Claytonia Geranium Viola $R^2 = 0.621$ (0.503)	-0.452 0.460 0.662 0.542 -0.835	0.034 0.019 0.002 0.014 0.0004	Distance to edge Leaf-area index Less sandy soil $R^2 = 0.464$ (0.388)	0.317 0.520 0.395	0.079 0.004 0.031	Patch flowers Claytonia Anthers per flower $R^2 = 0.564$ (0.455)	-0.773 0.467 0.415	0.003 0.065 0.082
Forward stepwise	Alliaria $R^2 = 0.273$ (0.237)	0.523	0.013	Leaf-area index $R^2 = 0.295$ (0.265)	0.544	0.005	Patch flowers $R^2 = 0.375$ (0.331)	-0.613	0.012
Total df	21			24			15		
Early-season survey, reduced model, backward stepwise	Claytonia Viola $R^2 = 0.249$ (0.183)	0.358 -0.539	0.088 0.014	Leaf-area index $R^2 = 0.252$ (0.227)	0.502	0.003	Patch flowers Viola $R^2 = 0.333$ (0.259)	-0.363 -0.448	0.076 0.032
Forward stepwise	Alliaria $R^2 = 0.191$ (0.157)	0.437	0.026	Same results as backward stepwise	N/A	N/A	Viola $R^2 = 0.202$ (0.160)	-0.449	0.041
Total df	25			31			20		
Late-season survey, full model, backward stepwise	Ramet height Less sandy soil Alliaria Viola $R^2 = 0.716$ (0.629)	0.401 0.743 0.816 -0.390	0.061 0.0012 0.0005 0.030	Leaf-area index $R^2 = 0.295$ (0.265)	0.544	0.005	--	--	--
Forward stepwise	Less sandy soil Alliaria $R^2 = 0.553$ (0.493)	0.539 0.670	0.009 0.002	Same results as backward stepwise	N/A	N/A	Patch flowers $R^2 = 0.571$ (0.532)	-0.756	0.003
Total df	17			24			12		
Late-season survey, reduced model, backward stepwise	Alliaria $R^2 = 0.190$ (0.147)	0.435	0.049	Leaf-area index $R^2 = 0.252$ (0.227)	0.502	0.003	Distance to edge Patch flowers Geranium Viola $R^2 = 0.641$ (0.511)	0.401 -0.685 -0.511 -0.448	0.096 0.005 0.021 0.054
Forward stepwise	Same results as backward stepwise	N/A	N/A	Same results as backward stepwise	N/A	N/A	Patch flowers Soil carbon $R^2 = 0.532$ (0.460)	-0.756 0.547	0.003 0.021
Total df	20			31			15		

Table 3.A.3: Final models produced by backward and forward stepwise regressions of PL_{fruit} on measurements of patch, environmental, and neighborhood floral characteristics, excluding site mean PL_{fruit}. See legend of Table A.1 for a full explanation, and see Table A.1 for comparison with results when site mean PL_{fruit} is included in the initial model.

Season	2005			2006			2007		
	Model	β	P	Model	β	P	Model	β	P
Early-season survey, full model, backward stepwise	--	--	--	Soil carbon R^2 = 0.165 (0.118)	-0.406	0.076	--	--	--
Forward stepwise	Viola R^2 = 0.555 (0.514)	0.745	0.003	No significant variables	N/A	N/A	Alliaria Flowers in patch R^2 = 0.751 (0.680)	0.695 -0.563	0.008 0.020
Total df	12			19			9		
Early-season survey, reduced model, backward stepwise	Ramet height Leaf-area index Claytonia Viola R^2 = 0.737 (0.620)	-0.782 -0.371 0.518 1.010	0.046 0.081 0.058 0.003	No significant variables	N/A	N/A	Leaf-area index R^2 = 0.223 (0.158)	-0.472	0.088
Forward stepwise	Viola R^2 = 0.513 (0.472)	0.716	0.004	No significant variables	N/A	N/A	No significant variables	N/A	N/A
Total df	13			22			13		
Late-season survey, full model, backward stepwise	--	--	--	No significant variables	N/A	N/A	--	--	--
Forward stepwise	Viola Soil carbon R^2 = 0.802 (0.752)	0.810 -0.536	0.0010 0.010	No significant variables	N/A	N/A	Viola R^2 = 0.629 (0.576)	-0.793	0.011
Total df	10			19			8		
Late-season survey, reduced model, backward stepwise	Viola Soil carbon Patch flowers R^2 = 0.855 (0.801)	0.988 -0.525 0.395	0.0003 0.006 0.043	No significant variables	N/A	N/A	Leaf-area index R^2 = 0.268 (0.195)	-0.518	0.085
Forward stepwise	Same results as backward stepwise	N/A	N/A	No significant variables	N/A	N/A	No significant variables	N/A	N/A
Total df	11			22			11		

Table 3.A.4: Final models produced by backward and forward stepwise regressions of PL_{seed} on measurements of patch, environmental, and neighborhood floral characteristics, excluding site mean PL_{seed}. See legend of Table A.1 for a full explanation, and see Table A.2 for comparison with results when site mean PL_{seed} is included in the initial model.

Chapter IV

Facilitation of pollination among pollinator-sharing plants: overview and prescriptions for future study

Plant-plant interactions in pollination networks

Highly specialized plant-pollinator interactions, as exemplified by interactions between figs and fig wasps or yuccas and yucca moths, are among the most fascinating phenomena in pollination biology and common examples in popular accounts of both pollination biology and coevolution. Yet such specialization is quite rare among plant-pollinator interactions. Generalization is the rule, and even plant or pollinator species that specialize on one partner typically specialize on a highly generalized partner (Bascompte et al. 2003, Ashworth et al. 2004). Thus, plant-pollinator interactions occur in extended networks much more often than in simple two-partner mutualisms (e.g., Memmott et al. 2004).

Indirect interactions are inherent to interaction networks. In food webs, for example, two species may compete by consuming the same prey species (exploitation competition), or one species may indirectly support another by consuming its predators (trophic cascade). Similarly, in pollination networks, two plant species may compete for the services of the same pollinators, decrease each other's reproductive success through improper pollen transfer, or facilitate each other's pollination success.

Rathcke (1983) reviewed the literature on interactions between plants mediated by pollinators. At that time, competition for pollination had been repeatedly invoked as a mechanism behind observed variation in flowering time or floral morphology, but rigorous tests for the occurrence of competition for pollination were unusual. Studies on facilitation of pollination were much less common. Since then, many studies have examined competitive interactions between plants mediated by their pollinators, but studies on facilitation remain relatively rare. Nevertheless, tremendous progress has been made in elucidating some aspects of facilitation of pollination, particularly as it applies to food-deceptive species and to conspecific interactions within rewarding species.

Here, I review the literature on facilitative interactions between plants, mediated by their pollinators. I particularly emphasize interactions among rewarding plants, as an excellent review of the pollination of rewardless species has been published quite recently (Renner 2006). I begin by providing my definitions of important technical terms that have multiple definitions in the literature. I then review the evidence for eight recognized hypotheses for facilitation of pollination. Finally, I make recommendations for future studies that I believe would accelerate progress in understanding the patterns and processes of pollinator-mediated interactions among plants, including both competition for pollination and facilitation of pollination.

Definitions

Target and associate species

Following Goldberg et al. (1999), I use the term “target” to refer to the individual or species whose response is being measured, while a species or individual expected to induce a response is an “associate.” Studies on competition and facilitation in pollination systems are functionally similar to studies on these same interactions mediated by other limited resources. Both kinds of studies involve a focal species to which focal individuals belong. A variable that is expected to change in response to interactions with other organisms is measured on target individuals that are exposed to different abundances of individuals of one or more other species (the associates). In contrast to studies on interactions for abiotic resources, however, studies on interactions for pollination continue to have no established response variables or methods for manipulating the abundances of associates. Perhaps a more explicit recognition of the parallels will promote greater standardization in measurements and methods in the field of pollination, facilitating comparisons among studies.

Facilitation of pollination

Most studies define facilitation of pollination loosely, and often not explicitly, as a positive effect of one species' flowers on the pollinator visitation, pollination success, or reproductive success of another species with which it shares pollinators, a definition I model after Waser's (1978) definition of competition for pollination (below). Ideally, this interaction would be measured in both directions, and the effect of the interaction on demography would be measured, but studies are usually limited to one direction of interaction and do not usually test for effects on fitness beyond pollinator visitation, fruit set, or seed set. Studies vary in the response variable measured, the method for measuring it, and the methods for measuring and manipulating the abundances of associates, which also prevents the use of a more precise definition. For the purposes of this review, I will use the loose definition above, specifying which component of reproductive success has been used as a response variable and how the influence of associates has been manipulated, as necessary.

Facilitation of pollination is assumed to occur through increased pollinator visitation to the target species due to the presence of the flowers of the associate species (though I describe some exceptions). Heterospecific neighbors are not expected to improve the quality of the pollen that arrives on a plant's stigmas. Nevertheless, I reserve terms such as "facilitation of visitation" or "facilitation of pollinator visits" for cases in which this mechanism is specified.

Competition for pollination

Although competition for pollination is not a focus of this study, it frequently occurs in the same systems as facilitation, and some mention of it is therefore unavoidable. As with facilitation of pollination, competition for pollination cannot be defined narrowly without greatly limiting the number of studies that can be said to test the phenomenon. I therefore follow the definition of Waser (1978): "If at least 1 of 2 or more co-occurring species suffers a reproductive loss as a result of sharing a pollinator, competition for pollination can be said to occur." Competition for pollination can take the form of competition for pollinator visits or improper pollen transfer (defined below).

Competition for pollinator visits

Competition for pollination has been divided into "exploitation competition" and "interference competition (e.g., Brown and Kodric-Brown 1979, Pleasants 1980)." In this terminology, exploitation competition for pollination is competition for pollinator visits, by analogy with the broader definition of exploitation competition as competition in which individuals or populations negatively affect each other by depleting a shared resource. The attention of pollinators is a finite resource that synchronously flowering plants may deplete. However, pollinators are not abiotic resources, but foraging organisms that exhibit a preference for highly rewarding plants and a freedom to leave them. This important difference is largely responsible for the phenomenon of facilitation of pollination; if pollinators were not more abundant near attractive flowers, there would be no potential for the flowers of one species to facilitate visitation to the flowers of another species. For this reason, I follow Waser (1983) and Rathcke (1983) in avoiding the term "exploitation competition" in this context. Instead, I will use the terms "competition for pollinator visits," "competition for pollinators," or "competition for visitation."

Interspecific pollen transfer and improper pollen transfer

The term "interference competition" has been used synonymously with interspecific pollen transfer (Waser 1983, and references therein), which is itself a special case of improper pollen transfer (IPT; Rathcke 1983). IPT includes any transfer of incompatible pollen to stigmas, including both heterospecific pollen and genetically incompatible conspecific pollen. IPT is sometimes equated with interference competition because it bears some relationship to the animal behaviors (e.g. territoriality) that the term "interference competition" was intended to describe. Incompatible pollen may interfere with compatible pollen by physically preventing it from reaching the stigmatic surface and germinating, fertilizing ovules that would otherwise have been fertilized by compatible pollen (e.g., Fishman and Wyatt 1999), or suppressing the germination of compatible pollen through allelopathy (e.g., Murphy 2000). Despite these similarities between IPT and interference competition, I will use the term "IPT" wherever applicable, as "interference competition" can be applied to interactions that have very different mechanisms from IPT, such as if mixed-species displays were less attractive to pollinators than displays of either species alone (Rathcke 1983).

Heterospecific pollen transfer

Most studies of plant-plant interactions through pollination that involve more than one species consider the effect of the interaction for a single target species, ignoring the effect on any associate species. This being the case, it is useful to have a term that specifically refers to pollen transfer between a target species and other species. Following Murphy (1995), I use the term "heterospecific pollen transfer" (HPT) for this purpose. I use "heterospecific pollen receipt" to refer to the receipt of other species' pollen.

Methods

I searched ISI Web of Knowledge for papers containing the phrase "facilitation of pollination." To find articles relating to conspecific facilitation (e.g., as seen when Allee effects are relevant) I then searched for papers that cited Sih and Baltus (1987), the earliest paper in the previous search for which a list of "cited by" papers could be generated. Rathcke (1983)'s chapter of facilitation and competition was the earliest article in the list, but neither the references that cite it nor the references cited in it were available. However, I did find every reference that Rathcke cited in reference to facilitation, as well as every reference those references cited on the subject, and so on, in an effort to both find every relevant article and trace the origin of the idea that facilitation of pollination could occur. I also searched for "facilitate* AND pollinat*."

Hypotheses for facilitation of pollination among rewarding species

1. Pollinator support

The assumption that competition for pollinators is a likely interaction between two co-flowering species that share pollinators is based on a view of pollinator services as a finite resource. However, as mentioned in the definitions above, pollinators are organisms, not abiotic resources. As organisms, their interaction with flowering plants is commonly recognized as a mutualism; the plant receives assistance in mating with others of its species, and in exchange, the pollinator receives nectar, pollen, or some other reward. If a plant species has a positive effect on a pollinator species, and that pollinator species has a positive effect on another plant species, the intuitive indirect effect of the first plant species on the second one is facilitation. This is essentially the logic behind the pollinator support hypothesis, proposed by Rathcke (1983); the flowering plant species in a community collectively support pollinators in greater abundance and diversity than any one plant species could alone.

Sequential flowering—In general, competition between two species for any resource is expected to result in selection for traits that reduce the negative fitness consequences of the competitive interaction. Flowering plants may reduce competition for pollination by diverging in their flowering times, as first suggested by Robertson (1895). Beginning in the 1970s, numerous researchers began to find sequential flowering of pollinator-sharing species wherever they looked (Mosquin 1971, Frankie et al. 1974, Heithaus 1974, Heinrich 1975, Reader 1975, Stiles 1975, 1977). These cases of sequential flowering with minimal overlap in flowering times were based on sorting species' flowering times from earliest to latest in a season and deciding whether they looked uniformly staggered, and many may not stand up to more rigorous scrutiny (Poole and Rathcke 1979). However, it is likely that competition for pollinators has driven divergences in flowering times in at least some communities, and there is solid evidence that flowering time can evolve in response to selection against interspecific pollen transfer (e.g., Waser 1978a).

It is clear that multiple plant species are in flower throughout the active seasons of pollinators in at least most communities. To some extent, this pattern is probably the result of a simple rule of community assembly: species will not persist where they cannot complete their life cycles. Many long-lived pollinators require a series of plants coming into and fading out of bloom if they are to remain in a community (Baker 1963, Stiles 1975). The population stability of non-migratory pollinators, in particular, may be dependent on the abundance of floral resources throughout the growing season, and their populations may vary within and between seasons in response to variations in floral rewards (Tepedino and Stanton 1981).

Sequential mutualism—The flowering period of a single species within a season is frequently shorter than the active foraging periods of its pollinators. Consequently, pollinators rely on sequential flowering of different species throughout the season. If floral resources become scarce in a community at some point during the flowering season, the local pollinator population may decline due to increased death from starvation, decreased reproduction, or increased migration out of the community. Thus, the species that flower at any given time in the active period of their pollinators effectively facilitate the pollination of later-flowering species with which they share pollinators, a phenomenon that has been labeled "sequential mutualism" (Waser and Real 1979). This is one form of facilitation through mutual pollinator support.

Waser and Real (1979) found evidence for sequential mutualism of *Ipomopsis aggregata* pollination by *Delphinium nuttallianium* in the Colorado Rockies. In their study site, *D. nuttallianum* flowered before *I. aggregata*, with little overlap. Both species were pollinated by broad-tail hummingbirds (*Selasphorus platycercus*), while only *I. aggregata* was pollinated by rufous hummingbirds (*S. rufus*), which typically arrived in the community after *D. nuttallianum* was finished flowering. In years when *D. nuttallianum* produced relatively few flowers, the abundance of broad-tail hummingbirds was low, and *I. aggregata* experienced low seed set, while rufous hummingbird abundance was unrelated to the floral density of *D. nuttallianum* (Waser and Real 1979). These facts strongly suggest that *D. nuttallianum* promoted the seed set of *I. aggregata* by supporting one of its major pollinators prior to *I. aggregata*'s flowering season.

Less direct evidence for this mechanism of facilitation has been found for *Clarkia xantiana* ssp. *xantiana* and its congeners in the southern Sierra Nevada range (Moeller 2004). Moeller (2004) found that *C. x. xantiana* populations with more congeners present had higher pollinator visitation per plant and lower or equal pollen limitation of seed set than populations without congeners. *Clarkia* specialist pollinators were more sensitive to *Clarkia* diversity than were generalists (Moeller 2004, 2005). These results may be due to the long lifespan of the specialist pollinators relative to the flowering time of any one *Clarkia* species, combined with some degree of staggered flowering times among the species (Moeller 2004).

Community stability—In theory, facilitation through pollinator support is not limited to sequentially flowering species. If coflowering species experience asynchronous changes in floral abundances from year to year, they may sustain larger, more diverse, or more stable pollinator communities as a group than any one species could sustain alone. More diverse pollinator communities will provide more reliable service if the populations of different pollinator species fluctuate asynchronously (Potts et al. 2001). This form of facilitation through pollinator support would be apparent as (1) positive relationships between floral functional diversity, pollinator functional diversity, and community stability and (2) shifts in floral dominance rank-orders among years, with pollinators shifting their preferences accordingly. Such studies are unlikely to be connected to the literature on facilitation of pollination, and it is likely that there are many findings along these lines of which I am not aware.

There is evidence that functional diversity in pollination networks can lead to greater stability. Fontaine et al. (2006) manipulated the functional diversity of plants and pollinators in caged experimental communities. Plants had two functional groups (open flowers and tubular flowers), as did pollinators (long-tongued and short-tongued). For communities that contained both open and tubular flowers, species richness and total plant abundance was higher after two years for the communities that contained both long- and short-tongued pollinators than for communities that contained just one functional group of pollinators (Fontaine et al. 2006). The experiment was not designed to test whether floral functional diversity promotes the diversity and abundance of pollinators, which would be a necessary step in facilitation through pollinator support.

2. Magnet species

Thomson (1978) tested the hypothesis that pollinator preferences between two species of *Hieracium* (Asteraceae) were positively density-dependent within several patches that ranged from dominance by *H. auranticum* to dominance by *H. florentinum*. The per-head visitation to each species within a patch was expected to increase with that species' relative abundance within the patch. This prediction was borne out for *H. auranticum*, but not for *H. florentinum*. Visitation to *H. florentinum* conformed to the prediction except that the patch that was most heavily dominated by this species was the one in which it received the fewest visits per flower. Visitation was higher overall for *H. auranticum*. He proposed that patches with at least a moderate proportion of *H. auranticum* were more attractive to pollinators than stands of nearly pure *H. florentinum*, but that pollinators switched between the two species readily within a patch, possibly because *H. auranticum* heads became "crowded."

Thomson (1978) called the greater visitation to a less attractive species in patches that contain a more attractive species the "'magnet species' phenomenon." Of course, it need not refer to systems in which the two interacting species occur in discrete, mixed-species patches. In general, pollinator abundance can be expected to be higher near highly rewarding flowers, and nearby flowers of less rewarding species may receive more visits than flowers of the same species further from the rewarding display. It should also be noted that this mechanism of facilitation may occur in systems where the overall effect of the magnet species on pollinator visitation to the target species is competitive. The more attractive species may out-compete the target species for pollinator visits, so that the target's visitation would be higher if the magnet species were completely absent from the community, yet visitation for the target species may be higher in close proximity to the magnet species than it is several meters distant.

The study in which Thomson (1978) first proposed the magnet species hypothesis seems to be the only published study to explicitly invoke this mechanism to explain interactions between two rewarding species. It is perhaps significant that *Hieracium* is apomictic. Thus, while Thomson (1978) observed that pollinators collected from each species bore mixed pollen loads and that pollinators collected from *H. florentinum* were more likely to bear *H. auranticum* pollen than vice-versa, any interspecific pollen transfer that might have occurred would have had no effect on the reproductive success of either species.

However, several studies have detected facilitative effects of highly rewarding species on the visitation of less rewarding species in a manner consistent with the magnet species hypothesis. For example, Bartomeus et al. (2008) found that, for 70% of native plants in Mediterranean shrublands in northeastern Spain, mean visitation rate was higher in 50m x 50m plots invaded by *Carpobrotus* of probable hybrid origin (Aizoaceae) than in paired uninvaded plots. In contrast, visitation was lower for 60% of native plants in plots invaded by *Opuntia stricta* (Cactaceae). There were more total visits in plots with *Carpobrotus* than in uninvaded plots, while plots with *Opuntia* did not have more total visits than uninvaded plots. Thus, while *Carpobrotus* evidently drew additional pollinators to the plots it invaded and thereby facilitated the pollination of several native species, *Opuntia* simply competed with the natives for pollinator visits without increasing the number of pollinators in the plot (Bartomeus et al. 2008). Whether the effects of the two invaders on pollinator visitation translated into effects on reproductive success or fitness was not tested, but the conditions for facilitation through shared pollinators may be quite strict if the negative effects of IPT and competition for other resources are considered (Feldman et al. 2004). In addition, while *Carpobrotus* increased visitation for most native species, it decreased visitation for "a few specialized species with zygomorphic flowers (Bartomeus et al. 2008)." The authors do not speculate on why such species should have lower visitation in invaded plots when other species have higher visitation. They indicate that flower-visiting beetles were especially common in plots invaded by *Carpobrotus* (Bartomeus et al. 2008); perhaps this species facilitated the visitation of plants visited by beetles, but competed for bee visits with species with zygomorphic flowers without attracting substantially more bees to the plot.

Moragues and Traveset (2005) studied the effect of *Carpobrotus* on the pollination of native species on Mallorca, the largest of the Balearic Islands, Spain, in the Mediterranean Sea. Like Bartomeus et al. (2008), they compared invaded plots with nearby uninvaded plots. They conducted observations of pollinator visits for each of four native species, noting the number of insect visits, the identities of the visitors, and the number of flowers present in groups of fifteen observed plants of one species in each observation. Though these data are sufficient to calculate visitation per flower per hour, they provide data only on the total number of insect visits to the group and the number of flowers receiving visits. They found increased pollinator visitation to *Anthyllus cytisoides* (Fabaceae) and *Cistus salviifolius* (Cistaceae) in invaded plots and decreased visitation to *Lotus cytisoides* (Fabaceae) in the invaded plots, by at least one measure of visitation in at least one of two years. The flowers of the *Cistus* species were actinomorphic, while both legumes had zygomorphic flowers. They found no effect of plot type on *Cistus monspeliensis* visitation by either measure in either year. The study was conducted in two sites with two different species of *Carpobrotus*; *C. acinaciformis* was associated with *A. cytisoides* and *C. monspeliensis*, while *C. edulis* was associated with *L. cytisoides* and *L. salviifolius*. Differences in fruit or seed set between invaded and uninvaded plots were not determined. However, IPT was addressed for the *Cistus* species; negligible *Carpobrotus* pollen was found on native stigmas, and supplemental hand-pollination with *Carpobrotus* pollen, alone or in mixture with conspecific outcross pollen, did not significantly depress seed set relative to unmanipulated control flowers. Thus, at least for the two *Cistus* species, it is unlikely that heterospecific pollen transfer results in a competitive effect of *Carpobrotus*.

Lopezaraiza-Mikel et al. (2007) also found facilitation of pollinator visits by an invasive species with highly rewarding flowers, based on a removal experiment in semi-natural vegetation in Bristol, England. They collected a greater number of floral visitors on native plants in plots where the *Impatiens glandulifera* flowers were left in place than in plots where they were removed. This difference was significant even when total floral abundance (which was higher in control plots than in removal plots) was included as a covariate. Coleoptera, Diptera, Hymenoptera, and Hemiptera all responded similarly to *I. glandulifera*, indicating that the difference in visitor abundance between treatments was not due to the response of any single order (unlike *Carpobrotus*, which had a particularly positive effect on Coleoptera abundance; Bartomeus et al. 2008). Apparently as a result of the greater visitor abundance, visitor diversity was also higher in plots with *I. glandulifera* flowers. However, *I. glandulifera* pollen dominated the pollen transport network of both plot types, and it is quite possible that IPT reduces, eliminates, or even reverses the positive effect of this species on visitation to native species (Lopezaraiza-Mikel et al. 2007).

Rewarding flowers may not be all that may serve as magnet species for pollinators. Roy (1996) found that pseudoflowers of the rust *Puccinia monoica*, which infects *Arabis holboelii* (Brassicaceae), promoted pollinator visitation to flowers of *Anemone patens* (Ranunculaceae) in sagebrush-dominated alpine habitats in the Colorado Rockies. The study was intended to test the visitation-density curve proposed by Rathcke (1983; below). The flowers of *A. patens* and the pseudoflowers of *P. monoica* both provide sugary liquids as rewards, making the magnet species effect a less obvious mechanism than it is in the case of deceptive species. Thus, visitation to both species was expected to increase in mixed plots relative to monospecific plots at low density, with the combined displays of the two species attracting more pollinators than the display of either species alone, while competition was expected at high density. *A. pratens* experienced higher visitation by flies in the presence of the pseudoflowers, regardless of total floral density, but the presence of the flower had no effect on fly visitation to *P. monoica* pseudoflowers. Thus, *P. monoica* appears to be a magnet species facilitating visitation by flies to *A. patens*. In contrast, visitation per flower per hour by halictid bees declined with increasing patch density for both species, but no significant effect of plot composition was detected. Thus, interactions among flowers through halictid visitors appeared to be competitive.

Facilitation by the magnet species effect depends on the tendency of generalist pollinators to visit unfamiliar flowers to assess their value. Individual pollinators rarely show perfect fidelity to a single species, but in many pollinator species, they do show a strong preference for one species once they have identified it as a rewarding species (e.g., Heinrich 1979). Visitation to flowers outside of the preferred species is sometimes referred to as "mistake pollination," (Baker 1976) particularly when the new flower belongs to an unrewarding species. However, this term discourages recognition of the fact that it is adaptive for pollinators to occasionally visit unfamiliar flowers (Renner 2006). Pollinator inconstancy might better be regarded as "sampling," as it allows pollinators to periodically assess whether the flower type they are currently exploiting is the most rewarding one available (Renner 2006).

Deviations from perfect floral fidelity may be beneficial to a highly rewarding plant species because they make it possible to compete with other species for the loyalties of pollinators. They also benefit unrewarding species by providing them with floral visits by pollinators that have not learned or have forgotten that they are unrewarding. Sampling visits can directly transfer pollen among conspecific flowers of a species that offers little or no reward only if the pollinator visits a series of flowers of this species, which may occur in a large proportion of sampling visits (e.g. Laverty and Plowright 1989, Laverty 1992). Sequential visits to a species that initially proves unrewarding is beneficial for pollinators, since rewards may vary among flowers within a species for many reasons unrelated to the average reward offered by flowers of that species. Abandoning a new species after visiting a single unrewarding flower could cause a pollinator to fail to exploit a rich resource, if it happened to visit a flower that had recently been visited by another pollinator or one that was too young or too old to produce rewards (Renner 2006). Thus, inconstancy will tend to benefit the less rewarding species of a community, as well as highly rewarding species that have recently begun to flower or to produce rewards. It cannot be expected to benefit the species to which a pollinator was loyal before sampling other species, as members of the formerly preferred species lose visits and pollen to the sampled species, and may receive heterospecific pollen if the pollinator reverts to its original preference.

The magnet species hypothesis applies only to facilitation of pollinator visitation. Because it involves pollinators switching between the plants that attract them from a distance and the heterospecific neighbors of those plants, it is also likely to involve IPT. The net effect may be a decrease in fitness relative to individuals located further from the display (Waser 1978b, a, Campbell 1985, Campbell and Motten 1985).

Alternatively, if a plant places its pollen precisely and in a different location than the preferred species, pollen transfer may occur between visits to the less rewarding species even if many visits to the preferred species (and others) intervene. About 1/3 of all orchid species are nectarless and thought to be pollinated “by deceit” (Cozzolino and Widmer 2005). It is probably not coincidental that orchids often package their pollen in pollinia, which may be placed precisely on the pollinator’s body with sticky pollinaria, reducing losses to grooming, pollinivory, and loss to heterospecific stigmas, as well as heterospecific pollen deposition on stigmas. These plants seem to be adapted (or preadapted) to take advantage of occasional mistakes or sampling forays.

3. Density-visitation / abundance-visitation curve

When two rewarding species co-flower and share pollinators, the resulting interactions may range from facilitation through neutrality to competition for pollination, and all of these outcomes have been detected in various studies. There must be some mechanism or set of mechanisms that determine what sort of interaction will occur. One such mechanism was proposed by Rathcke (1983), who proposed that per-flower visitation may increase, peak, and then decline as the floral density of a local display increased. This may happen if (1) pollinators preferentially forage in patches with high floral densities, but (2) the flowers within a patch must compete for the attentions of any pollinators that they collectively attract. The model was developed specifically to refer to patch density, not the number of plants or flowers in the patch, on the theory that greater floral density would lead to decreased inter-floral flight distances within patches, so that a dense patch would provide a higher net reward (after foraging costs are considered) than one with an equal number of flowers more sparsely arranged. Effects of floral abundance alone must be attributed to another mechanism and will be discussed below, under “Total display size.”

There are multiple ways by which the assumptions of the density-visitation model may be met. Quite often, individual pollinators preferentially visit larger displays but visit a smaller proportion of the flowers in large displays than in small displays, a counter-intuitive phenomenon that recent optimal foraging models have sought to explain (Goulson 2001, Ohashi and Yahara 2002). More obviously, any community has a finite supply of pollinators that a flower patch may attract, and this firm limit on the number that can be attracted is presumably approached with a saturating curve of patch visitation rate as a function of patch density. Finally, the effect of increased patch density on patch attractiveness may decline as patch density increases. A patch with ten flowers may be far more visible, rewarding, or innately appealing to pollinators than one with five, but an additional doubling to twenty flowers might increase visibility, likely rewards, or innate attractiveness to a smaller degree. Goulson (2000) suggested that pollinators may visit a smaller proportion of the flowers in larger patches because their movement rules for avoiding revisiting flowers become ineffective at high density. This not only explains why individuals visit a smaller proportion of the flowers in a denser patch, but also might explain why a patch with twice the density of another patch may not be twice as attractive; if a pollinator cannot expect to collect twice the rewards from a patch with twice as many flowers, it should not be twice as attracted to that patch.

The density-visitatation model has been tested in a small number of two-species studies. Ghazoul (2006) found that visitation to 50 *Raphanus raphanistrum* (Brassicaceae) flowers in study plots first increased with the density of flowering heads of *Cirsium arvense* (Asteraceae), and then declined when more than 32 heads were present (Ghazoul 2006). In contrast, Feldman (2006) found that pollinator visits to patches of *Brassica rapa* (Brassicaceae) increased as a linear or saturating function of the density of flowers in the patch, as did the number of plants visited by a pollinator per visit to a patch. He argued, based on an earlier model (Feldman et al. 2004), that facilitation of pollination by the density-visitation model would occur if only if visitation increased as a sigmoid function of density, so that the number of pollinator visits the patch receives increases as an accelerating function of patch density at low densities. Because neither the aggregative response (visits to the patch as a function of patch density) nor the functional response (number of plants visited per patch visit as a function of patch density) was sigmoid, he concluded that facilitation of pollinator visitation is not occurring at low density (Feldman 2006). This conclusion was supported by a decline in the number of visits per plant per hour as patch density increased (Feldman 2006). Notably, however, seed set per fruit and per flower increased with patch density, indicating that, while facilitation of visitation was not occurring, facilitation of pollination through pollination quality (below) did take place (Feldman 2006). Finally, Bosch and Waser (1999) found that visitation and seed set were higher for denser arrays of *Aconitum columbianum* (Ranunculaceae), with plant number held constant.

<u>Total display size</u>—Independent of patch density, a larger flower patch will generally attract more pollinators than a small patch. If pollinator attraction increases faster than patch size, then any additional flowers will facilitate the visitation of the flowers already in the patch. A larger patch may attract more pollinators because it offers

more rewards, or it may simply be more visible. For honeybees, for example, an object must occupy approximately 5 degrees of the pollinator's field of view to be visible, and 15 degrees before its color can be determined (Lehrer et al. 1990, Spaethe et al. 2001). For honeybees, which recruit nest-mates to highly rewarding floral resources, a larger patch may be easier for recruits to locate.

The effect of display size can be expected to resemble the effect of density because the mechanism of facilitation (or competition) is essentially the same; more rewarding patches attract more pollinators. As such, the mechanisms by which facilitation may occur at small display sizes, gradually disappearing and becoming competition at large display sizes, are essentially the same as those described for the density-visitation model.

For example, Sih and Baltus (1987) found that pollen limitation declined with patch size in *Nepeta cataria* (Lamiaceae). More pollinators were attracted to large patches, and individual pollinators were less likely to leave a large patch immediately upon encountering it. Patch size had different effects on individual behavior in different bee taxa. Individual honeybees visited more flowers, but a smaller proportion of them, in larger patches. Bumblebees visited more flowers and a larger proportion of them, and solitary bees visited fewer flowers in larger patches. A multiple regression of pollen limitation on visitation by the three categories of bees explained 67% of the variation in pollen limitation. The authors therefore concluded that the lower pollen limitation of large patches was due mostly to the effect of patch size on pollinator visitation (Sih and Baltus 1987). It is possible that higher mean pollen quality in large patches, due to the availability of more potential mates, may have contributed to lower pollen limitation in these patches, which would be consistent with the Allee effect hypothesis (below). However, the high explanatory power of pollinator visitation strongly suggests that total display size is among the relevant mechanisms behind this pattern.

Ågren et al. (2008) found that the frequency of fruit initiation per flower in populations of *Vincetoxicum hirundinaria* (Asclepiadaceae) was higher in larger populations, but they could not determine whether this was due to higher pollination success in large populations. Similarly, Brys et al. (2007) found higher fruit set per plant and per flower and higher seed set per ovule and per plant in larger potted populations of

Primula vulgaris (Primulaceae), while population density had no effect on these variables. More isolated populations had lower seed set per ovule and per plant. Because the plants were potted, any differences in environmental variables among populations were not causally related to population size, and the differences in reproductive success may reasonably be attributed to pollination.

4. Reward complementarity

Rathcke (1983) proposed that two species that share pollinators but provide different rewards may attract or support more pollinators, and therefore experience higher visitation rates, than either species in isolation. This mutual facilitation of visitation would be an emergent property because the effect of each species on the other's visitation rate would not be predictable from information about each species' density or its relative attractiveness to pollinators (Rathcke 1983).

To date, this hypothesis has received little attention. In part, this may be due to its apparently limited sphere of relevance. Pollinators that forage on flowers for only one resource (e.g., hummingbirds, butterflies, moths, most wasps, most flies, all of which forage on flowers only for nectar) are not amenable to this mechanism of facilitation, and flowers that offer both pollen and nectar are intuitively less likely to benefit from sharing pollinators with flowers that offer one or both of these rewards.

However, it is possible for facilitation to occur between two species that both offer pollen and nectar, and reward complementarity is at least a possible mechanism for this. Ghazoul (2006) observed that bees in his system visited the target species (*Raphanus raphanistrum*: Brassicaceae) for pollen and an associate species (*Cirsium arvense*: Asteraceae) for nectar, though both species produce both rewards. *R. raphanistrum* is poor in nectar but rich in pollen relative to *C. arvense*. He also found that groups of 50 *R. raphanistrum* plants received significantly more pollinator visits in arrays with 24 *C. arvense* plants than in arrays with 24 conspecific plants (Ghazoul 2006), while seed set per fruit was insignificantly higher in the arrays that contained both species. Furthermore, visitation to *R. raphanistrum* flowers in plots containing mixtures 50 flowers of this species and varying numbers of heads of *C. arvense* increased with the abundance of *C. arvense* heads in the plot, up to 32 heads. After this point, visitation declined with increasing abundance of *C. arvense* heads (Ghazoul 2006). Thus, in at least one system, there is good reason to believe that the complementary rewards hypothesis is relevant even in two species that both offer pollen and nectar rewards.

Facilitation of pollinator visitation by the reward complementarity mechanism may often occur between plants that provide both nectar and pollen, for two reasons. First, almost every flower that produces nectar also produces pollen, with the obvious exception of unisexual female flowers. Thus, even if facilitation through complementary rewards always involved a species that offered only pollen, the nectar-providing species with which it interacts will generally have pollen as well as nectar, even if it has relatively little pollen or the pollen is of low nutritional quality for the pollinator. Second, it may not be uncommon for flowers that serve primarily as pollen sources to provide a small quantity of nectar, as is apparently true of *R. raphanistrum* (Ghazoul 2006). Thus, flowers may be "cryptic" pollen flowers or nectar flowers, in the sense that common methods for assessing available floral rewards may lead a researcher to believe that both nectar and pollen serve as rewards when, in practice, pollinators collect one or the other reward, exclusively or nearly so, from the species in question.

5. Mate availability

At very low abundance or density, populations may decline due to the failure of individuals to find compatible mates or to inbreeding depression caused by low population genetic variability (Allee 1931, 1951), a phenomenon known as the Allee effect (e.g., Hackney and McGraw 2001, Fischer et al. 2003, Davis et al. 2004). A corollary of this effect is that, as population size or density increases from very low levels, the fecundity of individuals and the survival and fecundity of their offspring may increase, reducing, halting, or reversing the population decline seen at lower abundances. By the definition of facilitation used in this review, this is essentially a form of conspecific facilitation.

This basic mechanism need not apply only to populations at such low abundances or densities that deterministic extinction is a danger. A similar phenomenon may be seen in the populations of many common plants. Isolated individuals or those in small or sparse patches may experience more frequent geitonogamy than those with more potential mates nearby. Thus, even if pollinator visitation is high in small patches, fecundity may be low due to inbreeding depression or low diversity of self-incompatibility alleles.

Tests of the relationship between floral density and pollination success should preferably be conducted using experimental arrays, not natural variation in floral density. Floral density in natural populations may reflect the suitability of microsites for plant growth and reproduction, which may, in turn, influence fruit and seed set, as well as the provisioning of pollinator rewards. Thus, plants on poor sites may produce fewer flowers and provide less abundant rewards per flower, resulting in low visitation and fruit and seed set, and low plant vigor may limit fruit and seed set even if pollinator visits do not.

The potential for microsite variation to confound experimental results was realized in a study by Bosch and Waser (1999). These authors found that visitation and pollen receipt in natural populations of *Delphinium nuttallianum* Pritzel and *Aconitum columbianum* Nutt. (both Ranunculaceae) were not related to the number of flowers per square meter, while seed set was much lower in sparse populations. Based on these results, it is possible that the pollen received by flowers in denser populations was of higher quality, or it may be that denser populations occurred on better microsites, which also resulted in higher seed set per flower. To test this, they manipulated densities in arrays of potted plants for each of the two species (Bosch and Waser 2001). They found that both visitation and seed set were independent of the density of the array in *D. nuttallianum*, as expected if microsite quality explained variation in seed set in natural populations. Thus, the authors would have been mistaken had they assumed that differences in pollen quality between dense and sparse populations had explained their earlier results.

In addition to finding support for the display size hypothesis, Brys et al. (2007) found evidence for the mate availability hypothesis. *Primula vulgaris* is distylous; pin flowers, with long styles and short stamen filaments, can only cross-pollinate with thrum flowers, with short styles and long filaments, and vice-versa. In their potted populations, they found that pollen limitation of fruit set was greater for the more common of the two morphs, with increasing pollen limitation for that morph the more biased the population was. Thus, the more common morph in a population experienced increased pollen limitation due to a lack of compatible mates.

Campbell and Husband (2007) found that mate availability declined in small populations of *Hymenoxys herbacea* (Asteraceae), while per-head visitation increased. However, pollen did not limit seed set per floret. Thus, while the mechanisms behind the mate availability hypothesis of facilitation and the display size or density-visitation hypothesis were in effect, neither facilitation nor competition for pollination occurred, as measured by seed set.

6. Müllerian mimicry

It has been argued that rewarding species sometimes facilitate each other's pollination by mutual mimicry (Macior 1970, Brown and Kodric-Brown 1979, Schemske 1981), making it more likely that the same effective pollinator uses them all ("Müllerian mimicry"). For the species, the advantage of mutual mimicry is that a pollinator is more likely to visit the flowers of a species if they resemble the flowers of other species it knows to be rewarding. However, this benefit comes at a cost of increased IPT if pollinators visit co-flowering species indiscriminately as a result of their similar floral morphologies.

Generally, it is argued that Müllerian mimicry among species with rewarding flowers is beneficial if flower visitation is positively density-dependent. However, as discussed above, visitation is often negatively density-dependent (Sih and Baltus 1987, Goulson 2000, Ohashi and Yahara 2002). It is conceivable that rare species may benefit from mutual mimicry, given that a very rare floral morph is unlikely to win pollinator loyalty (Ackerman 1986). If two rare species are able to win loyalty by converging on a similar floral morphs, they may experience higher visitation and reproductive success as a result (Bobisud and Neuhaus 1975). For most species, however, convergence on a common morphology cannot be expected to promote per-flower or per-plant visitation.

Müllerian mimicry, if it occurs in pollinator-sharing plants, should be favored by the use of indiscriminate pollinators. Producing flowers that are morphologically distinct from those of other species will not be advantageous if it decreases pollinator visitation without increasing pollinator constancy, relative to producing flowers similar to those of other species in the community.

Müllerian mimicry may also be more likely to benefit species with deep, zygomorphic flowers. By controlling how pollinators approach the flower, zygomorphy facilitates the precise placement of pollen on a pollinator's body. This allows several pollinator-sharing species to deposit and intercept pollen from different parts of the pollinator's body, a form of niche partitioning, which, in turn, reduces the cost of sharing inconstant pollinators due to IPT.

Finally, Müllerian mimicry is more likely to be beneficial if pollinators have large foraging ranges and long memories, such that a pollinator is likely to encounter many different species and remember a search image even when the original model has not been encountered in several days. Grant and Grant (1968) proposed that hummingbird-pollinated flowers in the United States and Canada may often be red in part because hummingbirds learn to associate red flowers with copious nectar rewards throughout their migratory ranges. Insect pollinators are also capable of retaining search images for extended periods (Heinrich 1976, Gegear and Laverty 2001), and Müllerian mimicry may therefore be effective in some systems with insect pollinators. Bierzychudek (1981) found that the hypothesis of facilitation of pollinator visitation due to greater apparent population density not supported for the tropical butterfly-pollinated plants *Asclepias curassavica* (Asclepiadaceae) and *Lantana camara* (Verbenaceae). However, she proposed that mimicry may be beneficial across a broader spatial scale, so that butterflies respond to the abundance of yellow and red flowers throughout their entire foraging range rather than within flower patches.

For the reasons outlined above, Müllerian mimicry may be likely to occur in hummingbird-pollinated flowers. Hummingbirds are long-lived, notoriously indiscriminate floral visitors (Brown and Kodric-Brown 1979, Borgella et al. 2001) that generally forage on zygomorphic flowers with deep tubes, and many species are migratory or have broad foraging ranges (Grant and Grant 1968, Feinsinger 1976).

In short, Müllerian mimicry should be favored when pollinators have low constancy and when IPT can be minimized by precise placement of pollen or differences in flowering phenology. Müllerian mimicry is neither commonly accepted nor commonly studied (Roy and Widmer 1999).

7. Competitor-free space for pollinators

Just as flowering plants sometimes compete for pollinator visits, pollinators sometimes compete for floral resources (Steffan-Dewenter and Tscharntke 2000, Goulson 2003, Thomson 2004). When this occurs, some competitors may be displaced to inferior floral resources (Gross and Mackay 1998, Goulson 2003). Flowers of less preferred species may thus benefit from the presence of strong or aggressive competitors among the pollinator fauna, and those that occur closest to patches of the preferred species may benefit the most, as suggested by Ghazoul (2006). Additionally, the preferred species may benefit from the presence of the other plant species because the “competitor-free space” that they provide maintains the populations of the inferior competitors among the pollinators, providing reproductive assurance for the preferred floral resource in years when the strongest competitors among the pollinators are not abundant (Ghazoul 2006).

This hypothesis appears to be original to Ghazoul (2006), but the premise was anticipated by earlier theoretical papers. For example, ideal free distribution theory predicts that foragers gather more abundantly, but not exclusively, in resource-rich patches (Fretwell and Lucas 1970, Stebbins 1970). By this model, foragers are expected to deplete the richest resources most intensely, reducing the rewards per forager in each patch to the same level. If additional foragers are added, additional, poorer resources may be exploited, consistent with Ghazoul’s (2006) competitor-free space hypothesis of facilitation. Similarly, Goulson’s (1994) model of competition for pollinator fidelity between two plants shows that the less abundant plant may win a share of the pollinator visits, even if it is less rewarding, if pollinators are so numerous that the flowers of the more abundant plant are typically depleted of reward.

To my knowledge, the competitor-free space hypothesis has not yet been tested, and Ghazoul (2006) did not indicate that he believed it applied to his study system. However, there is evidence that pollinators will shift to less rewarding floral resources when they lose access to their preferred resources. Rathcke (1988) found that, when *Ilex* *opaca* (Aquifoliaceae) stopped flowering, bumblebees switched to foraging on *Kalmia latifolia* and *Gaylussacia frondosa* (both Ericaceae) in greater abundance. *Ilex* produced more nectar per flower than *Kalmia*, and it produced flowers at much greater densities than *Gaylussacia* (Rathcke 1988). If inferior competitors for pollinator visits can receive increased visitation when a superior competitor stops flowering, it is logical that they may also receive more visits when a competitively dominant pollinator enters the system.

The competitor-free space hypothesis is not clearly distinct from the magnet species hypothesis, except that Ghazoul (2006) suggests that aggressive displacement of competitors from rich floral resources (true interference competition) may be involved. Aggressive displacement occurs in territorial hummingbird species (Grant and Grant 1968) and perhaps some other vertebrate pollinators, but it may not be common among even reputedly highly competitive invertebrate pollinators (Butz Huryn 1997). If displacement occurs only through exploitation competition, then this hypothesis essentially says that pollinators aggregate near rich floral resources and are more likely to visit less rewarding species if they happen to grow near those resources; this is the magnet species hypothesis, essentially as Thomson (1978) originally described it.

8. Complementary displays

Ghazoul (2006) also discussed a "complementary attraction" hypothesis that similar to the pollinator support hypothesis. If different plant species attract different generalist pollinators, then their combined display will attract a broad spectrum of generalists (Rathcke 1988, Moeller 2004), which benefits all plant species involved by reducing variability in total pollinator abundance and overall pollination service (Ghazoul 2006). This mechanism is distinct from the pollinator support hypothesis in that it involves attraction to a mixed display rather than long-term support of pollinator populations (Ghazoul 2006), and I therefore consider it a separate hypothesis. The complementary attraction hypothesis would predict that floral patches with greater plant species diversity would have more stable pollinator service and greater pollinator species diversity.

It is not clear from Ghazoul's (2006) limited exposition of the hypothesis whether pollinator visitation should be expected to increase for all or most species in more diverse patches. If it is simplistically assumed that each species is highly appealing to one generalist pollinator and less attractive to other generalists, then each species in a diverse patch could attract many visits by its "own" pollinator and also benefit from a magnet species effect, receiving extra visits from the pollinators of the other species. In addition, if one species' primary pollinator was not abundant in one year, it would be ensured a certain minimum level of pollinator service by being part of a multi-species display. Whether either of these predictions is borne out in real pollination systems has not been tested, but no real pollination system would meet my simplifying assumptions, and it is entirely possible that any individual pollinator with a floral preference would be no more likely to visit a diverse patch or to visit the other species in that patch.

Pollination by deceit:

The magnet species hypothesis has been tested most often with deceptively pollinated species, which provide no rewards but depend on pollinators to facilitate mating. Such tests have found that unrewarding flowers that are close to rewarding neighbors receive more visits and have higher male and female fitness than those that have few rewarding neighbors (Laverty and Plowright 1988, Laverty 1992, Alexandersson and Ågren 1996, Johnson et al. 2003, Juillet et al. 2007), apparently benefitting from the higher concentration of pollinators near rewarding plants. When the magnet species effect is not detected for a deceptive species (Gumbert and Kunze 2001), this may be attributable to the spatial scale at which the effect of rewarding plants is tested (Johnson et al. 2003). The magnet species effect may be detected within areas on the order of tens of square meters (Johnson et al. 2003), hundreds of square meters (Laverty and Plowright 1988, Laverty 1992, Juillet et al. 2007), or hectares (Alexandersson and Ågren 1996, Johnson et al. 2003). However, pollination success for deceptive species has not been found to be related to the abundances of rewarding flowers within square-meter plots (Gumbert and Kunze 2001, Johnson et al. 2003).

This mechanism is particularly relevant where the unrewarding flower does not closely resemble the rewarding one; a pollinator is presumably decreasingly likely to travel far to investigate an unfamiliar flower (and insect pollinators are unlikely to see flowers more than a few meters away; Spaethe et al. 2001). However, even in cases of Batesian mimicry (see below), the magnet species effect is likely to apply. Pollinators are able to learn the spatial locations of rewarding and unrewarding flowers that are morphologically indistinguishable (Makino and Sakai 2007), and it thus benefits a Batesian mimic to flower in close proximity to its model (Gumbert and Kunze 2001).

The density-visitation model of facilitation is only likely to be relevant to deceptive species when densities are so low that genetic isolation becomes a significant problem. As population density decreases, there must be a point below which the probability of cross-pollination declines due to very low encounter rates between individual pollinators and the flowering plants of a deceptive species. At higher densities, per-flower visitation is expected to decrease with increasing patch density for deceptive species (Alexandersson and Ågren 1996, Castillo et al. 2002, Pellegrino et al. 2005).

Most deceptive species are in the Orchidaceae, of which approximately one in three are deceptive, and many of the remaining deceptive species have unisexual flowers with one deceptive sex that mimics the other, rewarding one (Cozzolino and Widmer 2005, Renner 2006). The prevalence of deception among the orchids is probably not coincidental. Rather, bilateral symmetry, the packaging of large numbers of pollen grains in pollinia, and the presence of a column, a reproductive structure made of fused stamens and styles, may combine to make for efficient pollen placement on a particular part of a pollinator's body, rendering deception less costly for orchids than for other angiosperm families (Nilsson 1992). A deceptive species is unable to rely on pollinator constancy to minimize HPT, but only orchids appear to have a pre-adaptation for reducing HPT without obtaining pollinator constancy with rewards. Deceptive non-orchids may benefit from facilitation of pollinator visits by rewarding neighbors (Laverty and Plowright 1988, Laverty 1992), but if deceit increases the risk of HPT too greatly, the net effect of rewarding neighbors may be negative.

Perhaps the only combination of mechanisms by which universal deceit (i.e., deception by all flowers in the species) could evolve in a species lacking adaptations to minimize HPT is through reduction in self-pollination combined with reduced resource costs of reward production (Laverty and Plowright 1988). Pollinators fly further after visiting an unrewarding flower than a rewarding one (e.g., Burd 1995, Johnson and Nilsson 1999, Smithson 2002, Johnson et al. 2004b). If the reproductive costs of low visitation and HPT for deceptive members of a species are smaller than the costs of self-pollination and reward production for the rewarding members, natural selection will favor an increasing frequency of pollinator deception.

However, universal deceit seems to be a less likely outcome than reward polymorphism in non-orchids (e.g., Golubov et al. 1999, Castillo et al. 2002), if the relative frequencies of the two phenomena are an indication (Renner 2006). A model by Smithson and Gigord (2003) provides a possible explanation for this. They found that the optimal strategy for a foraging pollinator when unrewarding plants were rare in a population was to forage indiscriminately but abandon the unrewarding inflorescences more quickly than the rewarding ones. If the unrewarding morph was moderately common, however, it became more advantageous to selectively avoid the deceptive plants. An empirical test of this model found that bumblebees followed the predicted strategies (Smithson and Gigord 2003). If a large enough percentage of the individuals in a species were deceptive, the best strategy must be to avoid the species altogether. However, as deceit spreads in a population, it is the transition from the strategy of indiscriminate visitation with rapid abandonment of unrewarding displays to discrimination against unrewarding plants that is most likely to inhibit a further increase in the prevalence of deceit in the plant population. Rapid abandonment of unrewarding inflorescences can result in reduced geitonogamy (Johnson et al. 2004b), but avoidance of unrewarding plants at a higher prevalence of deceit may more than counter-balance this advantage. Golubov et al. (1999) found evidence inconsistent with this prediction; nectarless honey mesquites (*Prosopis glandulosa* var. *torreyana*, Fabaceae) experienced much lower visitation than nectarful individuals, but their female reproductive success was similar and their male success was higher. In other systems, however, rewardlessness presumably carries a penalty of the sort suggested by Smithson and Gigord's (2003) model.

Batesian mimicry

One aspect of facilitation of pollination in deceptive species bears special mention is Batesian mimicry, or the selectively-favored mimicry of a rewarding model flower by the flowers of a deceptive species. For Batesian mimicry by deceptive species to be effective, three requirements must be met (Dafni 1984, Ackerman 1986, Johnson 1994) (1) the mimic must be rare relative to the model, (2) the mimic and model must be difficult or impossible for the pollinator distinguish from each other, and (3) the mimic's range must be a subset of the model's range (unless pollinators have long memories; Waldbauer 1988).

Batesian mimics essentially benefit from facilitation by the magnet species effect, but with the important difference that, rather than receiving exploratory visits from pollinators that recognize them as different species from the magnet species, they receive visits from pollinators that mistake them for their rewarding model (Dafni 1984, Johnson 1994, Gumbert and Kunze 2001). This difference in morphological similarity to the magnet species, in turn, results in two differences in pollinator behavior. First, while pollinators that have recently encountered non-mimicking deceptive species are generally less likely to visit them than naïve pollinators (Makino and Sakai 2007), pollinators that have recently encountered a Batesian mimic are more likely to visit than are pollinators that have not (Gumbert and Kunze 2001, Johnson et al. 2003). Pollinators foraging on the model of a Batesian mimic may learn to distinguish the two, but they are still much more likely to mistake the mimic for their forage species than are pollinators foraging on species that do not closely resemble the mimic (Gumbert and Kunze 2001) . Second, if the pollinator is an insect, because foragers on the model species can learn to recognize the mimic, but their eyes lack the spatial resolution to recognize the mimic at a distance, they may frequently approach the mimic closely without landing on it (Gumbert and Kunze 2001). In contrast, if the pollinator's forage plant does not resemble the deceptive species, the pollinator can avoid the mimick without approaching closely (Kunze and Gumbert 2001, Makino and Sakai 2007).

Numerous possible cases of Batesian mimicry have been described (e.g., Dafni and Ivri 1981, Dafni 1983, Dafni and Calder 1987, Johnson 1994, 2000, Gumbert and Kunze 2001, Gigord et al. 2002). However, few of these have tested whether a putative mimic meets the requirements outlined above (Johnson 1994, 2000, Galizia et al. 2005).

Future directions for research on facilitation of pollination

There are standard protocols that researchers of abiotically-mediated competition and facilitation have come to follow for measuring the direction and strength of plant-plant interactions, greatly facilitating comparisons among studies in different systems (e.g., Goldberg et al. 1999). In principle, studies of competition and facilitation mediated by pollinators are no different than studies of abiotically-mediated interactions. Though the resources involved behave quite differently (ions, photons, e.g., versus living, pollinating organisms), the essential questions are quite similar. The goals of research on pollinator-mediated interactions include determining: (1) how sensitive one species' pollination success is to the presence, abundance, and density of another species, (2) how a species' pollination success is related to its own abundance and density, and (3) what traits of the plant species involved determine how they interact through shared pollinators.

Unfortunately, virtually every researcher who chooses to address one of these questions follows a unique method of doing so. Researchers may use natural or artificial populations. Possible effect variables include: abundance or density of target plants or flowers, patch area, patch isolation, abundance or density of associate plants or flowers, intermixing of target and associate plants, abundance or density of target plants or flowers relative to associate flowers, types of rewards provided by target or associate plants, and quantities of rewards provided by target or associate plants. Densities and abundances may or may not be manipulated experimentally, and the areas within which they are measured or manipulated range from 1-m^2 plots to hectares. Response variables include: pollinator visits per flower, inflorescence, plant, or patch per hour, fruit set per flower, inflorescence, or plant, seed set per ovule, fruit, inflorescence, or plant, pollen removal or deposition per flower, and pollen limitation of fruit set per flower, seed set per ovule, or seed set per fruit. Pollen limitation may be estimated by comparing unmanipulated, open-pollinated flowers to open-pollinated flowers to which supplemental outcross pollen is applied by hand, or the hand-pollinated flowers may be bagged to exclude pollinators, and, if so, bagged, unmanipulated flowers may also be involved in calculating pollen limitation. Results are reported in widely varying ways, so that a study on eight flower patches in a single population (Anderson and Beare 1983) may provide more data points for comparison than a study on many patches in three populations (Johnson et al. 2004a). Empirical research rarely attempts to explicitly test models from theoretical work (Feldman 2006 being a notable exception) Thus, while many studies on competition and facilitation of pollination have been published since Rathcke's (1983) review of these phenomena, almost no progress has been made in synthesizing these diverse studies or understanding what determines whether pollinator-sharing, co-flowering plants interact positively, negatively, or not at all.

The one area of great progress in the field of pollinator-mediated interactions among plants is in the study of deceptively pollinated species. Although research in this area suffers from the same lack of standardized methods that has hindered the study of interactions between rewarding species, the potential outcomes of interactions with deceptive species are reduced because (1) the deceptive species will either benefit from rewarding neighbors are show no response to them, since it has little capacity to experience reduced pollination success, and (2) the deceptive species is always the target species. The effect of deceptive species on other species has only been tested in my own study of the effect of mayapple (*Podophyllum peltatum*, Berberidaceae) on the pollination of wild geranium (*Geranium maculatum*, Geraniaceae). Geraniums were neither more nor less pollen limited within five meters of mayapple flowers than they were more than fifteen meters away. This result was predictable; deceptive species interact so rarely with pollinators that any pollinator-mediated effects they have on other species will almost certainly be miniscule. While this may be an effective argument against investing limited resources on investigating the effects of deceptive species on the pollination success of their neighbors, the lack research into these effects reflects the overwhelming tendency for pollinator-mediated interactions to be studied in only one direction and is probably not indicative of any well-reasoned avoidance of the question.

It is a sign of poor focus in a field of study when progress in understanding the fundamental patterns and processes occurs only where the possible outcomes of an interaction are limited. I strongly recommend that future studies on pollinator-mediated plant-plant interactions follow the model set out by studies on abiotically-mediated interactions. Specifically, for each interaction, in each direction, the target species should be exposed to environments in which the associate is present and environments in which it is absent. For an individual study, it may be valuable to include a range of densities or abundances of targets or associates, and abundances may be manipulated by arranging a variety of different artificial arrays or by transplantation or removal in natural populations, but at a minimum, response variables must be measured on the target in the presence and absence of the associate.

In addition, the use of a certain minimum set of response variables would be a tremendous boon to the enterprise of synthesizing the results of multiple studies. Visitation per flower (or head) per hour is a valuable metric for assessing pollinator responses to differences in plant abundances and densities. Fruit set per flower and seed set per fruit are usually easy to measure, and while they are imperfect surrogates for individual fitness, (Ashman et al. 2004, Knight et al. 2005), they are, nevertheless, commonly accepted surrogates. Beyond this, measures of the degree of pollen limitation of these variables are useful. There is a standard measure of pollen limitation of fruit set: PL = 1 – open-pollinated-control fruit set / open-pollinated-outcross-supplemented fruit set, with negative values of PL rounded up to zero (Larson and Barrett 2000). The same basic equation can generally be applied to seed set, though some alternative may be advisable if negative values of PL are frequent.

Ecological systems are enormously complex, and there is only so much that can be accomplished using reductionist approaches. Nevertheless, it should be recognized that such approaches are often very useful in testing specific mechanisms, bringing suggestive patterns into clearer focus, and determining how much a mechanism or model can explain. For example, Feldman (2006) performed a field test of a mathematically explicit model by Feldman et al. (2004). The model predicted how a particular response variable (visits to a patch per unit of time) should change with a particular effect variable (plant density within the patch) in order for facilitation by the density-visitation curve hypothesis to occur, and the field experiment tested for that relationship (Feldman 2006). He did not find facilitation by this mechanism, (although he did find results consistent with the mate availability hypothesis), but the results were clear, as was the mechanism being tested.

Because ecological systems are extremely complex, reductionist methods of inquiry may never be sufficient for understanding them. There is a certain value in being able to demonstrate that, in a wide range of natural systems, plants do interact with each other through their shared pollinators. Nevertheless, reductionist methods do have an important role to play in ecological research. Simplified, controlled systems allow the researcher to select a particular hypothesis for evaluation, determine how its predictions differ from those of other hypotheses, and put it to the test. The system or replicates of it can then be altered to perform the test again and probe the range of outcomes that the hypothesis in question can explain.

The density-visitation curve hypothesis would seem to be highly amenable to testing by this method. Although it is not mathematically explicit, it does predict a clear qualitative pattern of interaction between two quantifiable variables. Visitation per flower per hour should increase with increasing patch density, level off, then decline. This is not a prediction about reproductive success, which may be confounded by IPT or the mate availability effect (though data on reproductive success as a function of patch density would certainly be valuable). It is not a prediction of visitation per plant or patch. It is therefore a hypothesis that has almost been tested many times over (e.g., Anderson and Beare 1983, Alexandersson and Ågren 1996, Feldman 2006, Zorn-Arnold and Howe 2007) but truly tested on few occasions (e.g., Kunin 1997, Bosch and Waser 2001, Steven et al. 2003, Ghazoul 2006). Other hypotheses may be similarly amenable to reductionist inquiry (the magnet species effect, complementary displays, reward complementarity, and possibly mate availability), while others depend on whole ecological systems and are unlikely to function in simplified model systems (pollinator support, Müllerian mimicry, competitor-free space).

Finally, interactions should be evaluated in two directions, when feasible. Different mechanisms of pollination make different predictions about two-way interactions, but many make very similar predictions in one direction. After all, each of these hypotheses is intended to explain the relatively narrow range of phenomena that can be labeled “facilitation of pollination.” If a positive result is consistent with multiple hypotheses, its power to test any one of them is diminished. Although any study that has the power to disprove a hypothesis has scientific value, its value is enhanced if it is able to disprove all but one of several alternative hypotheses. In studies on facilitation of pollination, distinguishing the magnet species hypothesis from the density-visitation curve hypothesis and the reward complementarity hypothesis depends on testing interactions in two directions.

Each of the hypotheses presented in this review is testable, though some are clearly more tractable than others. However, resolving which hypotheses have true explanatory power depends on studies that are clear in defining the hypotheses they are testing and consistent enough in their methods and measurements to facilitate comparisons of greater sophistication than narrative reviews or vote-counting procedures. Studies designed to test a variety of hypotheses at once and leave no more than one of them standing would be extremely helpful, though the difficulties in designing such experiments are considerable. The field of pollinator-mediated interactions among plants is highly complex, but a hypothesis-driven approach, in which certain experiments are conducted and certain measurements made in each study, has tremendous potential to clarify how plants interact via shared pollinators and, with any luck, how plant-pollinator interactions contribute to the structure and function of ecological communities.

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Chapter V

Conclusions

*Chapter II: Is self-compatibility in mayapple (Podophyllum peltatum L.) determined by environmental conditions***?**

The results of my surveys of self-compatibility, maternal plant size, and environmental conditions were consistent with the hypothesis that the stringency of selective ovule abortion is greater in plants with more limited resources. All of the patches in Site 3 produced fruits when self-pollinated (though one may not have produced any seeds), while no patches in any other site did.

Environmental measurements suggested that light may be the resource that limits the expression of self-compatibility in mayapple. Site 3 had the most open canopy of the five sites used in this study. Thus, self-compatible patches were found under brighter light conditions than self-incompatible patches. If selective ovule abortion is more stringent when light is less available (i.e., when the maternal parent has less stored carbohydrate available for seed maturation), then self-compatible plants in shadier sites may abort all self-fertilized ovules and thus appear to be self-incompatible. The presence of detectable self-compatibility only in the best-lit site is consistent with this prediction. In contrast, water did not apparently limit the ability of inbred ovules to develop, since Site 3 had the driest, sandiest soils with the lowest organic content of the five sites.

A role of resource availability in the expression of self-compatibility was further supported by data on outcross fruit set and mean ramet height. If self-compatibility is expressed only in plants with abundant resources, the high availability of resources in these plants should also be evident in other ways. Fruit and seed set for hand-outcrossed fruits is generally expected to be limited by resources (since pollen receipt does not limit ovule fertilization). As expected, Site 3 had the highest mean outcross fruit set and seed set of the five sites. Similarly, greater resource availability in mayapple should be expressed in greater vegetative growth, through more rapid clonal expansion and taller mean ramet height. While clonal expansion was not monitored, Site 3 was found to have the tallest ramets of any site. Thus, self-compatible patches in my study system occurred in brighter conditions and had higher fecundity from outcrossed flowers and taller ramets than self-incompatible patches.

All of the results of this study are consistent with the hypothesis that the probability of an inbred ovule reaching maturity depends on the resources available to it from the maternal plant. However, particularly because all self-compatible patches were in one site, it is impossible to conclude that variation in apparent self-incompatibility (the inability to produce seeds from self-pollination) does not simply reflect variation in true self-incompatibility (the presence of an effective mechanism to prevent ovule fertilization by self pollen). Mayapple's clonal growth should prove useful in separating these possibilities. If patches of mayapple containing ramets from each clone in the study were established in each site, or under a range of light intensities in a common garden, the extent to which apparent self-incompatibility depends on genotype versus resource availability should become clear in breeding system experiments performed after the plants have had several years to respond to their new environmental conditions.

*Chapter III: Pollinator-mediated interactions between mayapple (*Podophyllum peltatum *L.) and co-flowering neighbors: a test of the benefits of having attractive neighbors*

Pollen limitation of fruit set for mayapple was inconsistently affected by the local abundance of co-flowering rewarding plants. Three species (garlic mustard, spring beauty, and violets) significantly affected pollen limitation of fruit or seed set in 2005, but the only effect of heterospecific neighbors on pollen limitation in 2006 or 2007 was an effect of violet on pollen limitation of fruit set in 2007. Overall, garlic mustard and spring beauty depressed pollination success for mayapple in 2005 based on fruit set data. Violets facilitated pollination based on fruit set data, but depressed pollination success based on seed set. Removing neighbors within a meter of a subset of the mayapple patches in 2006 had no effect on pollen limitation. Pollination quality was not significantly better in a large reserve with a history of light disturbance (NWP) than in smaller fragments with histories of heavy disturbance. This suggests that habitat disruption does not explain mayapple's low pollination success or that the pollinator network in NWP is disrupted as badly as the networks in the other five sites. Conspecific flower density also had no effect on pollination success, in contrast to results of other studies on deceptive species.

It is possible that any positive effect of neighboring flowers on mayapple's visitation rate was negated by negative effects of heterospecific pollen receipt. Mayapple flowers that were hand-pollinated with wild geranium pollen prior to hand-pollination with outcross pollen (in addition to natural pollination) did not have significantly lower fruit or seed set than flowers receiving only the supplemental outcross pollination treatment. However, by the same test, the pollen of woodland phlox significantly depressed fruit set per flower. Thus, there is some potential for heterospecific pollen to interfere with mayapple's pollination success. Phlox pollen may have had a more significant effect than geranium pollen because it is smaller, which would leave smaller pore spaces for the subsequently applied mayapple pollen to contact the stigma and may allow it to adhere more firmly to the stigma. If phlox pollen is more effective in interfering with ovule fertilization than geranium pollen due to its smaller size, this would suggest that most species have greater potential to interfere with fertilization than geranium does. Geranium pollen grains were found to be 82 microns in diameter, on average, while the pollen grains of most other species collected ranged from 16 to 45 microns (spring beauty was the one exception, averaging 72 microns).

Very few studies on pollinator-mediated interactions between plants consider the interaction in two directions. In the case of unrewarding species, this unidirectional perspective assumes that the unrewarding species has no effect on the pollination success of its neighbors. However, if pollinators perceive patch quality to be lower when unrewarding species are present, or if pollen from the unrewarding species interferes with ovule fertilization in the flowers of its heterospecific neighbors, the species is a resource parasite (*sensu* Rathcke 1983, citing personal communication from B. A. Hazlett) rather than a commensalist. Mayapple did not significantly influence the pollination success of wild geranium, and adding mayapple pollen to geranium stigmas by hand did not significantly interfere with the effect of adding outcross pollen by hand. However, the difference in fruit set between outcross-supplemented flowers and HPT flowers was significant for geraniums within five meters of mayapple patches. The mechanism by which heterospecific pollination by hand could be effective only when the pollen source is within five meters is unclear.

Observed pollinators approaching mayapple, taken as a group, shifted from approaching flowers without contact or contacting flowers without seeking rewards to gathering pollen from flowers as the season progressed. However, most of this shift was because all observations of honeybee visits occurred in the second week of mayapple flowering. Pollinators that approached or visited mayapple were seen in patches under canopies with low leaf-area indices, in patches with more flowers, and in patches with more conspecific neighboring flowers within five meters. This is consistent with the behavior of rewarded pollinators. Rewarded pollinators may use mayapple's strong scent to locate flowers and patches, while deceived pollinators may use it to avoid them, as indicated by the tendency for approaches without contact to occur later in the day (when it is warmer and the scent of mayapple flowers stronger) than approaches with contact but without foraging attempts.

Mayapple was not less pollen limited near forest edges than it was far from them. This may be because the assumptions that edges would be better lit and have greater floral abundance and diversity than interiors were not met. Pollen limitation of fruit was lower in patches under canopies with low leaf-area indices, and patches that received observed pollinator visits had lower leaf-area indices than those that did not, suggesting that the assumption that better-lit environments would be better environments for pollination was valid.

Future research should evaluate when the magnet species effect is likely to be relevant, particularly where deceptive species are not involved, at what spatial scales it can be detected, how it affects the magnet species, and what effect it has on the evolution and stability of reward provisioning for both the magnet species and the beneficiary of the effect. Furthermore, the effect of facilitation of pollinator visits on heterospecific pollen transfer and the net result of neighboring co-flowering plants' effects on each other's visitation and probability of HPT need to be evaluated.

Chapter IV: Facilitation of pollination among pollinator-sharing plants: overview and prescriptions for future study

There are at least eight mechanisms by which one group of plants may facilitate another group's pollinator visitation or pollination success. Each mechanism has clear predictions and limitations, but it is rare that two studies test the same mechanism in the same way. Studies use different response variables and manipulate the abundances of target and associate species in different ways. The resultant variation in research methodologies and data presentation greatly complicates comparison among studies and synthesis of data from multiple sources. As a result, progress in the field of facilitation of pollination in the past 25 years has come largely in the form of the accumulation of studies documenting its occurrence, as well as the addition of a few new hypotheses. Most of the hypotheses presented have been shown to work somewhere, and some have been shown not to work somewhere, but determining why a hypothesis works in some systems but does not work in other, similar systems has remained elusive.

The one area in which definite progress has been made is that of the facilitation of pollinator visitation for species that deceive their pollinators. Because these species have very low visitation rates and fecundities, the effect of rewarding, co-flowering neighbors on these traits are unlikely to be negative. The lack of potential for competition eliminates the need to determine what factors might determine whether the interaction is facilitative or competitive.

In contrast, progress in defining the boundary between competition and facilitation is badly needed for interactions between rewarding plants, but the challenges of synthesizing studies using widely divergent methodologies and measurements have prevented the development of any clearer idea of what determines whether a pollinator-mediated plant-plant interaction is facilitative, competitive, or parasitic.

Researchers should look to studies of facilitation and competition among plants outside of the realm of pollination as models on which to pattern future studies. Although the reductionist methods used in such studies have their limitations, the dearth of powerful theories of indirect interactions in pollination biology indicates that reductionist methods also have their advantages.

Interactions must also be tested in two directions. The predictions of some of the facilitation hypotheses are indistinguishable in one direction: the presence of species B is expected to increase visitation (or fruit set or seed set) for species A through their interactions with shared pollinators.

Most of the hypotheses presented in Chapter IV are amenable to testing by reductionist methods; they make clear predictions about pairwise interactions at a spatial scale at which manipulations of target species and associate species abundances are feasible.

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Appendix

Details on the natural history of mayapple and my study sites

Overview of the natural history of mayapple

Mayapple is a common understory herb of deciduous forests in the eastern United States and Canada. It is extensively clonal, spreading by branching rhizomes. Rhizome segments disintegrate at approximately 6-12 years of age and have, very roughly, a 50% chance of branching in each year, with the probability of branching being lower when the rhizome makes a vegetative ramet or a fruit-bearing sexual ramet than if it makes a non-fruiting sexual ramet (Sohn and Policansky 1977). Ramets that are physiologically connected by their rhizomes are well-integrated; of radiolabeled carbon fixed in a mayapple shoot, 5 to 10% may be translocated to a connected shoot through the rhizomes, at a distance of up to a meter, and physiological integration of mineral nutrients and water occurs over greater distances (Landa et al. 1992). However, Landa et al. (1992) found that almost 90% of rhizome systems they harvested were composed of a single ramet at the tip of a chain of rhizome segments. Thus, a mayapple clone can be considered, to a fair approximation, to be a patch of physiologically independent plants that are genetically identical, though the few ramets that are connected are well-integrated (Landa et al. 1992), and as many as 8 ramets may be connected to the same rhizome network (Sohn and Policansky 1977). Clonal patches can extend up to ten or more meters in diameter, though many patches of such size might be polyclonal. Stands of mayapple may extend over one hundred meters, but the genotypic composition of such stands has never been determined; they may be single clones of great age or multiple clones that have grown together into a single patch. In most of the second-growth fragments used in this study, where patches are rarely as much as ten meters in diameter, dozens of patches were typically found within any hectare, making it highly unlikely that any stand spanning as much as fifty meters in diameter could be a single clone.

Vegetative ramets and flowering ramets of mayapple are morphologically distinct. The above-ground portion of a vegetative ramet is a single peltate leaf with two to eight deep lobes, larger leaves having more lobes. Below ground, a dormant bud can be found in what appears to be a node on the rhizome at the base of the leaf (this node is actually several nodes and short internodes; Landa et al. 1992). The above-ground portion of a flowering ramet is a stem with two opposite leaves that resemble the leaf of a vegetative ramet, except that they are cordate rather than peltate. A single flower typically emerges from the fork where the leaves meet the stem. Sexual ramets sometimes have three leaves, one leaf, or no leaves, and they rarely have two or three small flowers instead of a single large one. Sexual ramets do not have a dormant bud on the node at the base of the shoot.

The flower is large (3-6 cm in diameter), white (pink in some subspecies), fragrant, bowl-shaped, and nodding, with 6 to 10 petals, 12 to 24 stamens, and a single, unilocular pistil with 15-100 ovules (pers. obs.). The flower lacks nectar but has abundant pollen (Laverty and Plowright 1988, and pers. obs., Laverty 1992). Flowers open in May, usually in the second half of the month, shedding their three to six sepals in the process. The flowers within a clone generally open over one to three days, and a large percentage of the flowers in a population may open in a single warm (> 18°C) day (pers. obs.). The flowers are protandrous; the anthers often dehisce before the flower has opened, but the stigmas are generally not receptive until one to two days later (Swanson and Sohmer 1976). Stigmas remain receptive even as the stamens and petals begin to fall from the flower, approximately one to two weeks after anthesis (pers. obs.). A population's total flowering period lasts two to three weeks (Whisler and Snow 1992). Bumblebee queens (*Bombus* sp.) are the most commonly observed pollinators, though honeybees (*Apis melifera*) have also been observed to visit (Swanson and Sohmer 1976, Rust and Roth 1981, Laverty and Plowright 1988).

Immature fruits are green and poisonous, due to the presence of podophyllotoxin (Osweiler 1996), which is apparently unique to Podophyllum and a few closely related genera (Peng et al. 2006). They ripen in August, becoming yellow, edible, and sweet smelling. The leaves, stems, rhizomes, and roots contain the same toxins found in the unripe fruits, with the highest concentration of toxin in the rhizomes (Osweiler 1996). The toxicity of mayapple may, in part, explain its commonness, even in habitats that experience intense herbivory by white-tailed deer (*Odocoileus virginianus*). The seeds are known to be dispersed by eastern box turtles (*Terrapene carolina*; Rust and Roth 1981, Braun and Brooks 1987), but are probably also dispersed by a variety of mammals (Rust and Roth 1981), including white-tailed deer, which eat the fruits (pers. obs.).

Study sites

I conducted my research in six study sites in five forest fragments in western Washtenaw County, Michigan. Five of these study sites were established in 2005 in four forest fragments. The fragments on which sites 4 and 6 were located were owned by a single family, while the remaining fragments were each divide among several families. This multiple ownership, combined with changes in land management over time, resulted in patchwork forests, with late-successional trees over a shady, herbaceous understory abutting heavily thinned early-successional trees over dense shrubs and saplings. This variability was enhanced by topographic and edaphic variability, since each fragment was centered on rough or wet terrain (terrain that could not be farmed efficiently).

Site 1 was centered at 42°14.95’ N and 83°56.42’ W. It was bordered to the west and northeast by crops (corn, *Zea mays* L., and soybean, *Glycine max* [L.] Merr.), to the northwest by a closely mown road and a small creek, to the southwest by a powerline corridor, and to the southeast and east by the remainder of the forest fragment, which was approximately 35-40 ha in area. The soil was sandy loam. The forest canopy over this site was dominated by large (~20 m) basswoods (*Tilia americana* L.), hickories (shagbark, *Carya ovata* [Mill.] K.Koch, bitternut, *C. cordiformis* [Wangenh.] K.Koch, and pignut, *C. glabra* [Mill.] Sweet.), and oaks (red, *Quercus rubra* L., and black, *Q. velutina* Lam.). Basswood, in particular, formed a thick canopy, and the site was deeply shaded by the end of mayapple’s flowering period. Mayapple (*Podophyllum peltatum* L.) was common, as were wild geranium (*Geranium maculatum* L.), wood phlox (*Phlox divaricata* L.), and garlic mustard (*Alliaria petiolata* [M. Bieb.] Cavara & Grande).

Violets (*Viola* spp.) were more common in this site than in most of the others. The site was centered on an ephemeral pond that lost all standing water in June, after canopy leaf-out. Two of the five interior patches were in damp soil near the pond, while the rest were on slopes to its south and northeast. All soils were loam or sandy loam. Four edge patches were located near the south-facing edge adjacent to the powerline corridor, and another for were near the west-facing edge adjacent to cropland. A low ridge paralleled the south edge, with the study patches on the south slope of the ridge. On the west edge, the terrain sloped down from a ridge of fieldstones at the forest-field border.

Site 2 had two clusters of patches in a fragment ~ 25 ha in area. The first cluster had eight patches centered at 42°14.76' N and 83° 54.18' W. Only two of these patches were within 30 meters of the forest edge, as only two could be found so close to the edge in this portion of the site. One of the edge patches was adjacent to a gravel road bordering corn and soybean fields, and the other was near a particularly open portion of a broad, ephemeral pond that ran through the middle of the cluster. Both edges faced south. The soil in the cluster was moist and composed entirely of peat to a depth of at least one meter. The forest canopy was dominated by large (~ 25 m) red maples (*Acer rubrum* L.) with scattered black cherries (*Prunus serotina* Ehrh.). In 2005 and 2006, the maple canopy was thick and summer shade was deep. However, beginning in late summer 2006, strong storms toppled several large trees, and many more were selectively cut by the landowner in early spring of 2007, so that some of the patches in the forest interior were well-lit. In addition to mayapple, mayflower (*Maianthemum canadensis* L.) and blackberry (*Rubus allegheniensis* L.) were common understory flowers. Garlic mustard was common near the edge patches. A second cluster of five sites was centered at 42°14.86' N and 83°54.45' W, with three patches near a west-facing edge adjacent to a cornfield. This portion of the site had uneven topography, with moderately steep slopes from the level of the adjacent farm fields down to a permanent pond in the forest interior. The soil was loam or sandy loam, with a thick layer of leaf litter. Large (~ 25-30 m) oaks, hickories, and black cherries dominated the canopy. Spring beauty (*Claytonia virginica* L.), wild geranium, and garlic mustard were the most abundant flowers on the forest floor.

Site 3 was centered at 42°14.81’ N and 83°56.01’ W, at the opposite end of the same fragment in which Site 1 was located. All seven edge patches were near an east-facing edge separated from a cornfield by a closely mown road. The five interior patches were due west of the edge patches. The terrain was level through most of the site, with occasional ephemeral ponds, but the northernmost patches were on a slight downward slope toward the northwest. The forest was dominated by oaks, hickories, and black cherries ranging from ~ 15 to 30 m tall. It was selectively logged in April 2005 and thus had the most open canopy of any of the study sites. In addition to mayapple, the most abundant herbs were garlic mustard, wild geranium, and spring beauty. Violets were relatively abundant, as were prickly gooseberries (*Ribes cynosbati* L.). Multiflora rose (*Rosa multiflora* Thunb.) was abundant in the site, but not in the vicinity of the study patches. The soil was sandy loam under a thick layer of leaf litter.

Site 4 was centered at 42°13.70’ N and 83°55.87’ W, in a 9-ha forest fragment centered on a permanent pond. The six edge patches and two interior patches were at the same elevation as the adjacent cornfield to the north, while the other four interior patches were approximately 10 meters lower in elevation, near a wooded swamp on the north side of the pond. The higher-elevation forest had a canopy of small (~10-15 m tall) black cherries, American elms (*Ulmus americana* L.), hickories, and oaks, with scattered oaks and hickories emerging from the canopy (to ~ 30 m). This part of the forest had evidently been cleared more recently than the forest near the pond and on the slope rising up from it. This older portion of the forest had tall (~ 25-30 m) red maples, hickories, oaks, and black cherries, with smaller (~ 15 m) elms and black cherries common on the slopes. Mayapple, wild geranium, and garlic mustard were abundant throughout the site. Jack-in-the-pulpit (*Arisaema triphyllum* [L.] Schott.) was much more common in this site than in any other. Prickly gooseberry was also common. The soil was loam or sandy loam, with higher soil organic content and a thinner leaf litter layer in the lower-elevation patches.

Site 5 was centered at 42°13.71’ N and 83°55.00’ W, at the southeastern end of an elongated 30-ha forest fragment. The five edge patches were located near an east-facing edge adjacent to a sheep pasture, while the five interior patches were northwest of these. The terrain was quite hilly, ranging from a broad but ephemeral pond/swamp along the northeast edge of the fragment to a ridge approximately 20 m higher in the forest interior. One interior patch was at the top of this ridge, with the rest at its base. The forest throughout the site was composed of a low canopy (~10-15 m tall) of sugar maples (*Acer saccharum* Marshall.) with scattered emergent (~25-30 m tall) hickories, basswoods, sugar maples, oaks, and, near the ponds, red maples. The sugar maple canopy cast a very deep shade, and the understory was sparse far from edges. Mayapples were highly abundant, but discrete patches suitable for study were widely scattered. Instead, fields of ramets spreading tens of meters, with no clear clonal boundaries, were typical. Wood phlox, toothwort (*Cardamine concatenata* [Michx.] O. Schwarz.), anise root (*Osmorhiza longistylis* [Torr.] DC.), and violets were all common. Multiflora rose was highly abundant in places, but, as with site 5, it was not common near the study patches. It was not apparent that multiflora rose and mayapple occupied different habitats, except that the rose was apparently less shade-tolerant, but mayapple was rarely found near large rose bushes in either of these sites. Soil was sandy loam with a thin layer of leaf litter.

Fruit and seed production in mayapple was found to be highly pollen-limited in 2005. It was hypothesized that the severe pollen limitation detected could be a result of disruptions of plant-pollinator interactions caused by habitat destruction and forest fragmentation. To determine whether mayapple pollination would be improved in a less disturbed habitat, a site was added in a large, old-growth fragment approximately 15 km southwest of the original five sites.

The Nan Weston Preserve at Sharon Hollow (NWP) is a 100-ha natural area owned and managed by The Nature Conservancy. It is centered on wet forest in a drainage that flows into the Raisin River in southwestern Washtenaw County, but roughly half of the preserve is upland mesic forest, where the mayapple patches used in this study were located. The study patches were widespread through the preserve, but were centered at 42°10.96' N and 84°6.79' W. The five edge patches were at the north end of the preserve, with one patch near a north-facing edge bordering a gravel road, two near a south-facing edge bordering a powerline corridor, and two near the north-facing edge on the other side of the corridor. The eight interior patches were approximately 200 to 400 meters southwest of the edge patches, but were often closer to other open habitats (large ponds or fields) than they were to the powerline corridor. The forest around the study patches was dominated by large (~30 m) beech (*Fagus grandifolia*) and sugar maple, with a high diversity of less dominant tree species. Unlike the forests of the other five sites, NWP's forests have never been cleared, and selective logging was probably light even before the Conservancy acquired the property and halted timber extraction entirely (Douglas Pearsall, pers. comm.). Herbaceous species were highly diverse. In addition to mayapple, anise root, sweet cicely (*Osmorhiza claytonia* [Michx.] C. B.), wild geranium, wood phlox, rue anemone (*Anemonella thalictroides* [L.] Spach.), large-flowered trillium (*Trillium grandiflorum* [Michx.] Salisb.), hairy waterleaf (*Hydrophyllum macrophyllum* Nutt.), and Dutchman's breeches (*Dicentra cucullaria* [L.] Bernh.) were all common in the site. Discrete mayapple clones were difficult to find. Rather, as with site 7, most mayapples occurred in fields of ramets spread across tens of meters, with no clear clonal boundaries. Soils were much sandier and poorer in organic matter than in the other sites.