The ecology and evolution of pollinator-mediated interactions among spring flowering plants

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Abstract

Pollinator sharing in mixed species communities is expected to significantly contribute to mating patterns in contemporary populations but may also affect the evolutionary trajectory of traits associated with plant mating. In this thesis, I considered how the spring environment and pollinator sharing may contribute to the widespread convergence in traits among spring flowering species using comparative biology. The proposed correlation between a spring flowering phenology and white or light floral colour, fleshy fruits, woody growth forms and understory occupation is confirmed. In addition, I examined the effects of pollinator responses to community and population traits to determine the relative importance of inter- and intraspecific interactions in pollinator mediated reproductive success of a spring flowering species, *Trillium grandiflorum*. In this study, the reproductive success of *T. grandiflorum* was pollen limited. However, the magnitude of pollen limitation was influenced only by intraspecific density and varied independently of community diversity. The results of this thesis contribute significantly to our understanding of pollinator-mediated interactions in spring flowering communities but also highlight future avenues of investigation.

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Chapter One: Introduction

1.1 Overview of plant mating

Stochasticity in pollinator services is thought to be responsible for some of the major evolutionary transitions of the angiosperms. For example, self-pollination (Stebbins 1970) and wind-pollination (Linder and Rudall 2005) are derived conditions from a hermaphroditic outcrossing ancestor. Both are hypothesized to have resulted via selection for decreased reliance on biotic pollinators (Stebbins 1970, Linder and Rudall 2005). Pollen limitation, or, a reduction in reproductive success attributable solely to a deficiency in pollination service, is a nearly ubiquitous feature of plant mating (Knight et al. 2005). While pollen limitation has been extensively studied in a variety of environments, only a few studies have considered both the spatial and temporal dynamics of pollen limitation (Knight et al. 2005). Both inter-annual variance in pollinator services as the result of climatic conditions (i.e. Macior 1978a) and spatial variation in the structure of pollinator communities (i.e. Moeller 2005) can significantly affect pollinator-mediated reproduction in flowering plants. Moreover, characteristics of the plant communities themselves can influence pollinator foraging behaviour and effect the degree of pollen limitation in flowering plant populations.

1.2 Inter- and intraspecific effects on plant mating

In flowering plants, intraspecific interactions can influence both the reproductive success of individuals and the persistence of populations through their effects on pollinator foraging behaviour (Forsyth 2003, Moeller 2004, Feldman 2008). Through manipulative experiments and observational studies we have gained considerable insight into how intraspecific variation in traits associated with pollinator attraction (i.e. density,

presences of rewards) affects pollinator-mediated reproduction. However, flowering plants rarely form single species populations, more frequently they co-occur and flower simultaneously with other species generating mixed species communities. The characteristics of each species in a community can affect pollinator responses to plant communities and in turn those effects mediate changes in pollinator-mediated reproduction in flowering plants. Similar to pollinator responses to variation in intraspecific traits, pollinators will also respond to variation in interspecific traits in mixed species communities. Pollinators are known to exhibit changes in their functional (Feldman 2006), numerical (Moeller 2005) and aggregative (Grindeland et al. 2005) responses associated with changes in community-level traits such as species richness and density. Moreover, a recent meta-analysis suggests that the number of species in a community is positively correlated with the degree of pollen limitation in that community (Vamosi et al. 2006).

1.3 Pollinator-mediated interactions and community dynamics

Many outstanding questions in pollination biology arise from large gaps in our knowledge of how pollinators respond to changes in community traits, such as species density and diversity, in mixed species communities. However, we can make significant and meaningful predictions about the role of community if we understand how pollinators respond to community traits. For example, if co-flowering species experience a reduction in reproductive success as the result of pollinator-mediated competition, we expect selection for a divergence in plant mating traits to alleviate competitive interactions. Indeed, co-flowering species frequently display character divergence in traits that may

partition pollinator use among co-flowering species (i.e. flowering phenology - Armbruster 1985, and floral morphology -Muchhala and Potts 2007).

Alternatively, if co-flowering enhances reproductive success as the result of pollinator-mediated facilitation, we expect selection to enhance traits that promote facilitative interactions among co-flowering species. For example, community-level convergence in traits associated with pollinator attraction, such as morphology or colour, has been suggested as a mechanism by which pollinator-mediated facilitation may occur (Schemske 1981). Mechanistically, pollinator visitation is expected to increase in communities displaying convergent traits as the result of an increase in the community-level floral display (Dejong et al. 1992). In spite of historical recognition of the potential for pollinator-mediated facilitation (Rathcke 1983, Waser 1983), evidence is generally limited to morphologically similar congeners and non-rewarding species that benefit from the presence of a rewarding species (Laverty 1992, Johnson et al. 2003, Moeller 2004)

Importantly, both competition for, and the facilitation of pollination likely represent extremes on a spectrum of possible pollinator-mediated interactions among co-flowering species. Indeed, many studies fail to find any effect of pollinator sharing or co-flowering on the reproductive success of associated species (Macior 1978a, Schemske 1981, Bauer 1983, Motten 1986, Rathcke 1988). Understanding how both community and population characteristics affect pollinator-mediated interactions among community members is important for our understanding of the ecology and evolution of plant-pollinator interactions. Unfortunately, most studies to date have focused only on interactions among one plant species and one pollinator species, or on interactions between two plant species mediated by a single pollinator. Integrating both population

and community ecology are paramount to our standing of pollination as an ecological process. A primary goal of my thesis was to integrate these approaches.

1.4 Research objectives

My thesis applies a variety of approaches, including comparative biology, population surveys and a manipulative field experiment. I focus on deciduous forest spring flowering communities located within Southeastern Ontario and Western Quebec and in particular the reproductive ecology of the spring flowering perennial, *Trillium grandiflorum*. Extensive literature exists on the reproductive ecology of spring flowering communities (e.g. Macior 1978a, Schemske et al. 1978, Motten et al. 1981, Motten 1986, Kudo et al. 2008), highlighting a high degree of synchronicity in flowering phenology among spring blooming species, convergence in floral morphology and colour and pollinator sharing among co-flowering species. These traits increase the likelihood of pollinator-mediated interactions among co-flowering species, making spring flowering communities a useful system for studying interspecific interactions.

In chapter two I employ comparative approaches to address community-level trait convergence in spring flowering communities. Specifically, I test for correlated evolution between a spring flowering phenology and a suite of reproductive, vegetative and ecological traits for North American taxa. The correlation between a spring flowering phenology and three traits is confirmed; spring flowering species generally have white flowers, woody growth forms and occupy the understory layer of forests. These results are discussed in light of the spring pollination environment and pollinator-mediated interactions among co-flowering species.

In chapter three I present the results of an experimental study where I examined how population and community characteristics can affect the reproductive success of *T. grandiflorum*. I explicitly test the relative importance of intra- and interspecific interactions on pollinator-mediated reproduction. Specifically, I answer the following question: how do focal species density and community diversity affect pollinator-meditated reproductive success in *Trillium grandiflorum*? In a factorial design, I experimentally manipulated both focal species density and community diversity and found that intraspecific interactions explain a significant amount of variation in pollinator-mediated reproduction while interspecific interactions did not.

Finally, I discuss the broad implications of my research and future avenues of exploration in chapter 4.

Chapter Two: Phylogenetic analysis of trait convergence in the spring flora

2.1 Introduction

The timing of reproduction is a key component of an organism's life history. Indeed, phenotypic variation in the timing of reproduction has been found to influence fitness for a variety of animal and plant species (Visser et al. 1998, Walther et al. 2002, Frick et al. 2010). In animal-pollinated plants, mating success can vary with the timing of reproduction because of temporal variation in mate availability, pollinator availability, and the suitability of the environment for breeding (Brody 1997, Sargent and Roitberg 2000, Houle 2002, Inouye 2008, Ehrlen and Munzbergova 2009, Thomson 2010). Selection on the timing of reproduction likely explains some fascinating patterns in nature, such as synchronous mast flowering in the aseasonal forests of Southeast Asia, where over 80% of the canopy members from distinct families such as Burseraceae, Dipterocarpaceae, Fabaceae, Myristicaceae, and Polygalaceae flower simultaneously over a period of a few weeks to a few months (Ashton et al. 1988).

In temperate deciduous forests, flowering in spring blooming species is initiated by patterns of snowmelt and the accumulation of suitable climatic conditions (Kudo et al. 2008). Spring in temperate deciduous forests is marked by the sudden onset of multiple understory species flowering simultaneously, which results in synchronous, community-level flowering (Macior 1978a, Schemske et al. 1978, Motten 1986, Kudo et al. 2008). For these species, mating is constrained to the period of suitably warm conditions prior to canopy closure (Schemske 1977, Macior 1978a, Schemske et al. 1978, Motten 1986, Kudo et al. 2008). With canopy closure comes a sudden decline in the abundance of understory species in flower that is as remarkable as the onset of the spring bloom.

Throughout spring, the earliest flowering species experience a dramatic drop in the availability of light as canopy closure occurs (Ida and Kudo 2008, Kudo et al. 2008). It has frequently been noted that many species that share a spring flowering phenological strategy also exhibit a shared set of unique life-history traits, including similarities in flower colour and symmetry, fruit type, reproductive schedule, and seed dispersal mechanisms (Schemske et al. 1978, Bierzychudek 1982, Motten 1986, Jolls 2003, Whigham 2004). While a few authors have put forward adaptive hypotheses (i.e. Macior 1978a, Schemske et al. 1978, Motten 1986), the observation that spring flowering plants share several life history traits remains, for the most part, remains anecdotal and unexplored.

The pattern of similar life history traits among the spring flowering plants may be the result of common evolutionary history, or, alternatively, it may signify adaptation by disparately related taxa to the spring flowering environment. Traits are often shared among species within genera and families as the result of common ancestry, and if a spring flowering phenology evolves more frequently within certain clades, or, within a clade that diversified rapidly subsequent to adopting the strategy, the apparent correlation may be an artefact of a shared evolutionary history. As a result, using a phylogenetically-controlled analysis is critical (Harvey and Pagel 1991). Taking phylogenetic relationships into account, I am interested in determining if evidence exists for correlated evolution among flowering phenology and other reproductive, vegetative, and ecological traits for species that occur in the temperate deciduous forests of eastern North America. In the following sections I review the reproductive traits under examination and discuss the

adaptive mechanisms that could explain their apparent correlation with a spring flowering phenology.

Reproductive Schedule

Fewer than 10% of forest herbs display an annual reproductive schedule (Struik 1965, Jolls 2003). Instead, most species are iteroparous, i.e., perennials that reproduce many times throughout their life span (Struik 1965, Bierzychudek 1982, Jolls 2003, Whigham 2004). In cool temperate climates, flowers of the earliest blooming entomophilous species may experience pollinator-limited reproductive success as the result of variation in early spring climatic conditions. Indeed, this is case for species of both the temperate deciduous forests (Schemske et al. 1978, Kudo et al. 2004) and temperate boreal forests (Barrett and Helenurm 1987). Annual variation in pollination services can be mitigated by flowering over several years (Barrett and Helenurm 1987), which could lead to a correlation between spring flowering and iteroparity.

Corolla Colour

It has frequently been noted that during peak understory spring flowering periods, many co-flowering species exhibit white, or light-coloured flowers (Schemske et al. 1978, Motten 1986), suggesting that selection for a spring flowering phenology may be correlated with the evolution of white or lightly coloured flowers, or vice-versa. One potential explanation for this phenomenon is that there is an adaptive advantage to having white or lightly coloured flowers in the spring environment. Schemske and colleagues (1978) suggest that white may be the most conspicuous colour against the brown leaf litter background of the forest floor (but see Endler 1993). High visibility to potential

pollinators is likely to be an important trait in the variable pollination environment of the spring.

Fruit type

Biotic interactions can affect reproductive success in the spring flora through their influence on pollination, seed production, and seed dispersal. For spring flowering species, ant-mediated seed dispersal (myrmecochory) occurs in approximately 30% of species compared to an occurrence rate of less than 5% in all angiosperm species (Lanza et al. 1992, Lengyel et al. 2010). Elaisome-bearing seeds (i.e., those with an ant-specific food reward) are well suited to ant-mediated seed dispersal and are often packaged within fleshy fruits prior to dispersal (Dunn et al. 2007). While many forest plants display adaptations to animal fruit dispersal, ant-mediated seed dispersal may be particularly common in spring blooming species because of their numerical dominance and seasonal patterns in the foraging behaviour of frugivorous birds (Thompson 1981).

Growth form and Forest strata layer

Most canopy forming species in the temperate deciduous forests of Eastern North America are wind pollinated, while most spring flowering species that occupy the understory of the forest rely on a biotic pollen vector (Ulyshen et al. 2010 and references within). In temperate deciduous forests, insects are the most abundant potential animal pollinators in early spring conditions (Macior 1978a, b, Schemske et al. 1978, Motten et al. 1981, Motten 1986, Hannan and Prucher 1996) and many studies have noted that insect diversity is greatest near the ground level (Ulyshen and Hanula 2007, Hirao et al. 2009). Moreover, as described above, the spring flora may be experiencing selection to jointly attract potential pollinators in the variable spring climate. As a result, spring

blooming species may be converging in stature to jointly attract more potential pollinators to spring flowering communities than single species flowering in solitude. Selection to increase fitness in the variable spring environment could lead to community-level convergence to both a herbaceous growth form and understory layer occupation.

Using literature surveys I have compiled data on plant mating traits for 414 species within 98 genera and 45 angiosperm families. Here, using a recent molecular phylogeny of the angiosperms (Bremer et al. 2009), I investigate the proposed correlation among the presence of a spring flowering phenology and other reproductive, vegetative and ecological traits. Specifically, I investigated whether reproductive schedule (iteroparous versus semelparous), white floral colour (white versus non-white) or light floral colour (light versus non-light), fruit type (fleshy versus dry dehiscent), growth form (woody versus herbaceous), and forest strata layer (understory versus overstory) have evolved in a correlated fashion with a spring flowering phenology. Finally, I discuss the implications of my findings in light of the adaptive hypotheses outlined above.

2.2 Methods

2.2.1 Literature survey

I performed a literature search using the Flora for the Northeastern United States and the adjacent regions of Canada (Gleason and Cronquist 1991), systematically searching the entries for angiosperm species for which flowering phenology was reported as "spring", "summer", "fall" or "autumn". The survey was limited to one source to ensure that data were limited to species occurring in the temperate deciduous forest region. This method also recognizes the fact that information gathered from different floras may not represent independent data points, as floras likely obtain species

description information from the same sources. Families with pollination syndromes described as “anemophilous” (wind-pollinated) and/or “hydrophilous” (water pollinated) were excluded. A summary of the plant families included in the analysis can be found in Table S2.1.

Once I had identified all families for which at least one species had flowering phenology described as a season, data describing reproductive schedule, flower colour, fruit type, growth form, and forest strata layer were gathered from the flora. A summary of the traits and their associated character states used in the analysis, and as reported in Gleason and Cronquist (1991) can be found in Figure 2.1 and Table S2.2, respectively. If multiple states were reported for a trait in the species description, the species was recorded as having the most frequent trait state for that species. Subspecies and varieties were included when they were reported to have different trait states; if the subspecies or variety had the same trait states as the species then it was excluded from the analysis. In total, only two species had multiple subspecies or varieties (for a total of six taxa); all were included in the analyses.

2.2.2 Phylogeny re-construction

I used the software package Phylomatic, (Webb et al. 2008), to create a phylogeny based upon the megatree of the Angiosperm Phylogeny Group (APG; Bremer et al. 2009). Phylomatic places the study species on the backbone of the APG megatree and prunes the remaining taxa from the tree. The megatree is primarily a family-level resolution of angiosperm evolutionary history. As is the case in most comparative studies, a certain amount of phylogenetic uncertainty was present in the tree (i.e. polytomies). The branch

lengths published in the tree file were retained and used within the correlated evolution tests.

2.2.3 Testing for correlated evolution

To test for correlated evolution among flowering phenology and the reproductive, vegetative, and ecological traits of the species in the dataset, I used the *Discrete* module within the comparative phylogenetic analysis software package BayesTraits (Pagel and Meade 2006). *Discrete* (as described in Pagel 1994), investigates correlated evolution between two discrete binary traits by fitting two continuous-time Markov models to the character state data. The first model of evolution allows the traits to evolve independently of one another within the phylogeny. The independent model estimates two transition rates per trait, and calculates a likelihood for both character states of each trait, for every node within the phylogeny. The second model of evolution allows the traits to evolve in a correlated fashion, where the transition rates for each character state of a trait depends in part on the character state of the other trait at that node in the phylogeny. The dependent model of evolution estimates eight character state transition rates, and calculates a likelihood for the four possible character state combinations of the dependent model for each node in the phylogeny (see Figure 2.1 for a graphical depiction of the character states, transition rates and possible evolutionary pathways in the models of evolution).

To test for correlated evolution I conducted 6 pair-wise comparisons of flowering phenology with traits described in the Introduction. To assess statistical significance, and determine if traits evolve in a correlated pattern, a Likelihood Ratio Test was used to compare the likelihood estimates of the independent and dependent models of evolution (Pagel 1994). The likelihood ratio for these tests approximates the χ^2 distribution with the

degrees of freedom being equal to the difference between the number of parameters estimated for the dependent and independent model of evolution (Pagel 1994). Support for a model of correlated evolution is indicated if the likelihood ratio statistic exceeds the χ^2 critical value, indicating a significantly better fit of the dependent model of evolution. To account for potential error associated with the resolution of polytomies, I used the APE package in the R-program (Paradis et al. 2004) to create a sample of 100 randomly resolved, dichotomously branching trees (see Figure 2.2 for an example phylogeny used in the analysis). Standard errors of the test statistics are based on this sample.

2.3 Results

2.3.1 Traits correlated with a spring flowering phenology

First, I considered whether the reproductive, vegetative and ecological traits examined were correlated with flowering phenology irrespective of phylogenetic considerations. I found that three of the four reproductive traits were significantly correlated with a spring flowering phenology, as were the vegetative and ecological traits under investigation (Table 2.1). Specifically, floral colour (both white and light), fruit type, growth form and forest strata layer were significantly correlated with flowering phenology while reproductive schedule was not.

When phylogenetic relationships were taken into consideration, the likelihood ratio tests indicated that a correlated model of evolution fit the data significantly better for three of the four reproductive traits, as well as for the vegetative and ecological traits examined (Table 2.2). Specifically, floral colour (white and light), fruit type, and growth form and forest strata layer evolve in a correlated manner with flowering phenology, while phenology and reproductive schedule evolve independently.

Reproductive schedule

My results indicate that flowering phenology and reproductive schedule (i.e., perennial versus annual life history) evolve independently. The dependent model of evolution fit the character state data better than independent model of evolution, however, the likelihood ratio statistic was not statistically significant (Table 2.2). The distribution of iteroparous and semelparous species was nearly identical for spring and non-spring flowering species. Within phenological groups, approximately one third of the species were semelparous while the remaining two thirds were iteroparous in their reproductive schedule.

Floral colour

Both light and white floral colour do not evolve independently of flowering phenology; the dependent models of evolution fit the character state data significantly better than the independent models of evolution (Table 2.2). Among the 41 spring flowering species for which floral colour data were available, the majority have white or lightly coloured flowers (75% and 90% respectively; Table 2.1).

Fruit type

A remarkable difference was observed in the distribution of fruit type among the spring and non-spring flowering species. While the vast majority of spring flowering species had fleshy fruits (89%) only a small proportion of non-spring flowering species had fleshy fruits (8%). Again, this correlation between a spring flowering phenology and the presence of fleshy fruits was highly significant for the 100-tree sample (Table 2.2), however, the broad range of likelihood ratio statistics indicate that the analysis is somewhat sensitive to the phylogenetic hypothesis being used.

Growth form

Of the spring flowering species, nearly three quarters were reported as having a woody growth form compared to only three species for the non-spring flowering taxa (Table 2.1). This notable difference in the distribution of herbaceous and woody species among the phenological groups was statistically significant, indicating that flowering phenology and growth form evolve in a correlated fashion (Table 2.2). However, similar to fruit type, the broad range of likelihood ratio statistics indicate that the correlation between a woody growth form and a spring phenology is somewhat sensitive to the phylogenetic hypothesis being used.

Forest strata layer

Finally, forest strata layer and flowering phenology do not evolve independently; the dependent model of evolution fit the character state data significantly better than the independent model of evolution. Nearly 60% of all the spring flowering species occupied the forest understory layer while all the non-spring flowering species were reported to occupy the forest understory.

2.4 Discussion

Here I show that, for a sample of 414 plant taxa from Eastern North America, a spring flowering phenology has evolved in a correlated fashion with floral colour (light and white), fruit type (fleshy), growth form (woody) and forest strata layer (understory). In contrast, only flowering phenology and reproductive schedule appear to evolve independently. I now discuss the potential adaptive mechanisms for the trait associations, how community-level convergence in traits may affect community dynamics, the

limitations of large-scale comparative analyses and potential avenues of future investigation.

2.4.1 Adaptive mechanisms

I found no evidence of correlated evolution among flowering phenology and reproductive schedule (i.e., annual versus perennial life history; Table 2.2). This result may appear to challenge the validity of the analysis given that I was able to detect strong correlated evolution between growth form (woody versus herbaceous) and flowering phenology (Table 2.2), given that perenniality and woodiness are known to frequently co-occur. My inability to detect correlated evolution between flowering phenology and reproductive schedule in spite of strong correlated evolution between a spring phenology and woody growth form may highlight a limitation of my data set. Of the 45 spring flowering species for which I was able to collect data, reproductive schedule was reported for only 11 taxa; there simply may have not been enough data to detect any patterns with my analytical method.

In contrast, I did find evidence for correlated evolution among flowering phenology and floral colour (Table 2.2). I found that flowering phenology evolved in a correlated fashion with both light and white floral colour. However, the strength of the correlation between white floral colour and a spring flowering phenology appears to be much stronger than the correlation between light floral colour and a spring flowering phenology. This result suggests that the spring flora may in fact be converging on a single unique floral colour (white) instead of a variety of similarly lightly coloured forms (green, white and yellow).

In multi-species communities, convergence in floral colour may increase fitness in a variable pollination environment (Schemske 1981), however, the convergence to white coloured flowers remains to be explained. The presence of white coloured flowers may confer an adaptive advantage in the spring environment, such that white coloured flowers may be more easily detected in early spring light conditions (Schemske et al. 1978). Indeed, prevailing light conditions can affect a flowering plants' pollination environment either directly through resource availability for the development of floral structures (Bloom et al. 1985) or indirectly by determining floral display size and consequently pollinator foraging behaviour (Galloway et al. 2002, Ishii 2006, Kilkenny and Galloway 2008). However, the importance of the prevailing light conditions on pollinator-mediated selection for floral perceptibility may be negligible in spring flowering communities. Understory light conditions prior to canopy closure may be analogous to open light conditions, where no colour is expected to be more perceivable because of the white light conditions (Endler 1993), and as a result, pollinator-mediated selection on floral colour for increased perceptibility may be negligible.

Most spring flowering species have concurrent periods of growth and reproduction during the short period of high light availability prior to canopy closure (Rothstein and Zak 2001, Kudo et al. 2008), however, as canopy closure occurs, light conditions that favour plant growth and reproduction deteriorate rapidly. Indeed, many studies have noted that reproduction in spring flowering communities is highly constrained by the seasonal decline in light availability (Schemske 1977, Macior 1978a, Schemske et al. 1978, Motten 1986, Kudo et al. 2008). For example, individuals of *Claytonia virginica* that flower later in the spring are more resource-limited in their

reproductive success and have higher seed abortion rates than individuals that flower either prior to or during peak flowering (Schemske 1977, Motten et al. 1981). As the canopy closes, short wavelength light conditions prevail, resulting in a degradation of the quality of light which can inhibit the production of photosynthetic pigments and chloroplasts (Endler 1993 and references within). Resource constraints may limit the evolution of floral pigmentation instead of pollinator-mediated selection for floral perceptibility in spring flowering communities.

Frequently, chromatic floral pigments, such as anthocyanin, are also costly secondary metabolites (e.g. Snell et al. 2009). While studies that quantify the metabolic cost of producing these floral pigmentation are lacking, it has been show that the evolution of floral colour can be under strong non-pollinator mediated selection (Strauss and Whittall 2006, Rausher 2008). Furthermore, floral pigments likely have a significant function in vegetative tissues unrelated to pollinator attraction (Grotewold 2006). For example, the production of chromatic floral pigments has been shown to vary positively with environmental stress (Dick et al. 2009). Together these results highlight the potential importance of non-pollinator mediated selection on floral colour in the spring flowering community. To test the adaptive significance of white coloured flowers in spring environments, other temperate regions could be examined to determine if a similar correlation exists between flower colour and flowering phenology.

Though white flowers appear to be a prominent trait of the spring flora to the human eye, significant differences in the visual spectra of humans and insect pollinators may lead to differences in the perception of spring flowers. For the vast majority of insects the visible light spectrum extends into the ultraviolet radiation region (Kevan et

al. 1996). White flowers appear achromatic to humans because they evenly reflect light wavelengths across our visible spectrum, while almost always absorbing UV light (Kevan et al. 1996). As a result, floral color perceived as white by the human eye may appear chromatic to the eye of an insect pollinator (e.g. Utech and Kawano 1975). Indeed, white flowers of some spring blooming species have been shown to be chromatic to insect pollinators (Macior 1978a). However, the degree to which differences in the absorbance of UV light between species affects the visual perception of spring flowers by insect pollinators remains to be determined.

Flowering phenology was also found to be highly correlated with fruit type; by and large spring flowering species produced fleshy fruits (Table 2.2). While this analysis directly tests whether flowering phenology and fruit type evolve in a correlated fashion, the adaptive explanation may involve the predominance of ant-mediated seed dispersal (myrmecochory) in spring wildflower communities. In early summer ants are the most abundant seed dispersers and predators, and during this period plants in the spring flora mature fruits that frequently contain elaisome-bearing seeds (Thompson 1981). While forest plants display a variety of adaptations well suited to both vertebrate and invertebrate seed dispersal, elaisome-bearing seeds are particularly well suited for myrmecochory. Moreover, spring flowering species have a comparatively high incidence of myrmecochory relative to the rest of the angiosperms (Lanza et al. 1992, Lengyel et al. 2010).

In general, seed dispersal may provide many selective advantages, however, ant-mediated seed dispersal (myrmecochory) has two unique benefits assuming, as is usually the case, seeds are deposited in a midden. The deposition of seeds in waste piles may (i)

protect them from environmental heterogeneity and (ii) promote seedling growth and development because of the abundance of limiting nutrients in deposition sites (Kearns et al. 1998, Lengyel et al. 2010). Indeed, mutualisms between forest plants and ants can affect seed dispersal, germination success and seedling survival (Levey and Byrne 1993, Kalisz et al. 1999). A recent comparative study by Dunn and colleagues (2007) found that the rise in abundance of ants during the late Eocene likely drove elaisome evolution in the Monocots. For example, in the Monocot genus *Trillium,* which has many spring flowering species, it has been suggested that myrmecochory has been derived from bird-mediated seed dispersal to minimize early season seed predation by ants when frugivorous birds are both low in abundance and foraging for protein-rich food (Thompson 1981 and references within). Similar to the strong selection that frugivorous birds exert on fleshy fruited taxa (e.g. Jordano 1995), seed predation by ants may generate strong selection to utilize ant-mediated seed dispersal. Fleshy fruited taxa may be more likely to evolve a spring phenology to ensure early maturation of fruits to minimize seed predation and maximize the benefits of myrmecochory.

Moreover, the strong evidence of correlated evolution between flowering phenology and fruit type may indicate that flowering phenology affects resource allocation to fruit production. Although few long term studies have been conducted, many perennial forest herbs rarely flower in consecutive years, suggesting that there is a cost associated with sexual reproduction (Nault and Gagnon 1993, Jolls 2003). The concurrent periods of vegetative growth and sexual reproduction that characterize the spring flora may allow this phenological group of forest plants to acquire enough resources to produce large, fleshy fruits. Conversely, the light-limiting conditions of

summer in the temperate forests may generate selection to reduce investment in fruit production, leading to the observed correlation between non-spring flowering and dry dehiscent fruits.

Similarly, resource constraints may provide clues as to the adaptive mechanisms leading to the correlation between flowering phenology and growth form. Many spring flowering plants produce floral buds the preceding autumn that develop into reproductive structures with the onset of suitable environmental conditions in the spring (Bierzychudek 1982). Woody plants may be better able to mitigate the cost of reproduction than herbaceous plants because of their ability to store resources not only within below-ground structures but also in above-ground tissues.

Lastly, I found support for correlated evolution between forest strata layer and flowering phenology (Table 2.2). While many studies note the remarkable diversity of biotically pollinated plants in the understory layer (e.g. Ricklefs 2008), I am unaware of any studies that speculate why biotically pollinated taxa are less common in the canopy layer of temperate deciduous forests. Canopy closure marks the end of the spring flowering period, and Ulyshen and colleagues (2010) noted a subsequent decline in floral resource availability in the forest understory, corresponding to a vertical displacement of pollinator taxa into the canopy layer of the forest. Seasonal patterns in pollinator availability between forest strata may act synergistically with the previously outlined abiotic (e.g. light availability) and biotic (e.g. pollinator perception) constraints on reproductive success to generate selection for community-level convergence to occupy the forest understory in spring flowering species (Helenurm and Barrett 1987, Moeller 2004).

2.4.2 Caveats

In this study I conducted six separate comparisons to examine how flowering phenology evolved with one other trait. However, it is likely that each trait examined is actually just one component of a suite of traits the contributes to the "spring" identity. Furthermore, it is also likely that the traits investigated are not independent of one another and may in fact be correlated. For example, when Vamosi and colleagues (2003) examined the ecological correlates of dioecy they found a significant correlation between woody growth forms and the presence of fleshy fruits. Unfortunately, I am unaware of any analytical method that would allow me to investigate how all of these traits have evolved with flowering phenology simultaneously within my sample of phylogenetic trees.

In addition, ancestral state reconstruction may be compromised when the traits in question are correlated with faster or slower diversification (Schluter et al. 1997). In particular, traits associated with low diversification tend to be located on the terminal points of long branches, which frequently results in traits associated with low diversification rates appearing to evolve prior to traits associated with faster diversification. Previously, woody growth forms have been associated with a reduced diversification rate (Dodd et al. 1999), consequently results involving this trait must be interpreted with caution. Furthermore, ancestral state reconstruction can be sensitive to the number of observations of each character state observed. The most common state is more likely to be the reconstructed as the ancestral state when parsimony methods are used, however, *Discrete* should minimize this because it considers all possible ancestral

states (Vamosi et al. 2003). Regardless, my analysis may be sensitive to this potential bias because spring was a rare character state in all of my analyses.

2.4.3 Future directions

There are most likely other traits associated with a spring flowering phenology that would be interesting to investigate. However, my use of a single local floral limited the type of data that I could collect. Other traits that could be assessed include nectar availability (presence/absence), mechanism of seed dispersal (assisted/passive or ant-mediated/non-ant-mediated), mating system (self-compatible/outcrossing), and floral longevity (long/short) to name a few. Including these traits may increase our understanding of the relative roles of resource limitation and environmental heterogeneity in shaping the spring flora.

There are many advantages to using a maximum likelihood framework in comparative studies. In particular, *Discrete* allows estimated transition rate parameters to vary within the phylogeny and uses branch lengths to inform the probability of change within a branch (Pagel 1994). However, my analysis approximates a species-level analysis on a family-level tree. The base tree used to generate the 100-tree sample was a family-level resolution, and the addition of genera and species to the base tree resulted in polytomies below the family-level. In order to use the correlated evolution method in *Discrete,* the polytomies were randomly resolved using R, and as a result, evolutionary transitions below the family-level cannot be detected in this analysis. Applying comparative approaches, such as the one I have used here, to well-resolved phylogenies could further our understanding of what traits promote the evolution of a spring phenology and what traits are adaptations to the spring environment. In the future,

resources such as GenBank, could be used to generate a more well-resolved phylogeny of temperate region flowering plants.

2.5 Conclusion

The repeated evolution of a trait in a given environment is frequently taken as evidence of adaptation by evolutionary biologists. Here, I observe that spring flowering species frequently have white or light flowers, fleshy fruits, woody growth forms and occupy the understory of the forest. And while comparative studies can illustrate these general patterns, experimental investigations into the ecological, behavioural and genetic factors that affect the evolutionary processes in spring flowering communities are required to more thoroughly understand the contemporary patterns we observe. By linking macroevolutionary patterns and microevolutionary processes we gain additional insight into both the contemporary and historical factors structuring spring flowering community dynamics.

Table 2.1 Distribution of species among the flowering phenologies and the states for the traits. Species that had more than one state for a trait were categorized according to the most frequent state of the trait according to Gleason and Cronquist (1991) or the first state of the trait mentioned if the primary state was not explicitly mentioned.

	Flowering Phenology				
Trait and state	Spring	Non-spring	Total	χ^{2a}	P – value
Reproductive schedule:					
Iteroparous	7	227			
Semelparous	4	108	346	0.09	0.774
Total	11	335			
Floral colour:					
White	31	82			
Non-white	10	251	374	45.01	<0.001
Total	41	333			
Floral colour:					
Light (Green, Yellow and White)	37	210			
Non-light	4	123	374	12.03	<0.001
Total	41	333			
Fruit type:					
Fleshy	39	27			
Dry dehiscent	5	330	401	187.25	<0.001
Total	44	357			
Growth form:					
Woody	33	3			
Herbaceous	12	339	387	247.45	<0.001
Total	45	342			
Forest strata layer:					
Understory	26	342			
Overstorey	19	0	387	159.91	<0.001
Total	45	342			

Notes:
a - the χ^2 is compared to the χ^2 distribution with 1 degrees of freedom to determine whether the distribution of species among the states for each trait is associated with flowering phenology.

Table 2.2 Phylogenetic tests for correlated evolution between flowering phenology and reproductive, vegetative and ecological traits in a 100-tree sample of 414 species using the *Discrete* module of BayesTraits.

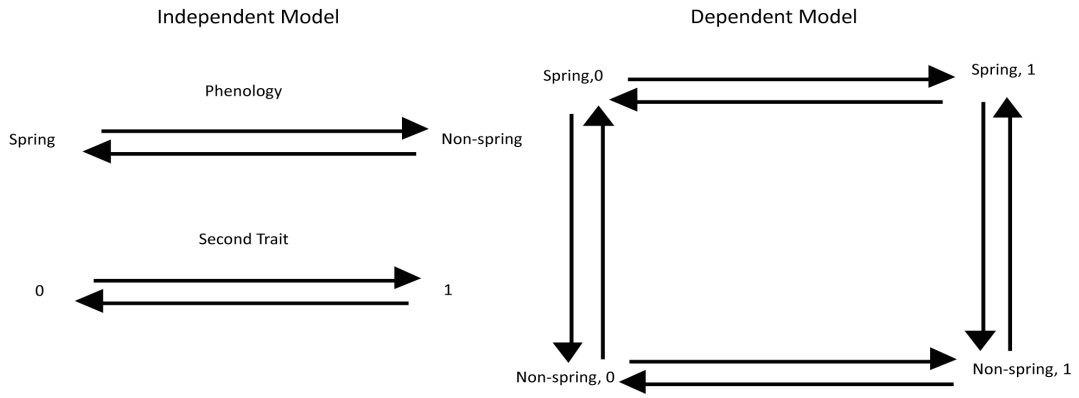
Comparison	Mean Likelihood[a] ratio statistic (±SE, p-value)	Likelihood ratio range for a 100-tree sample (p-value range[b])
Spring, reproductive schedule	3.333 (±0.023, 0.504)	1.127 – 3.364 (0.890 – 0.499)
Spring, white	18.644 (±0.0001, <0.001)	18.554– 18.647 (<0.001[c])
Spring, light	10.837 (±0.051, 0.028)	9.506 – 12.485 (0.050 – 0.014)
Spring, fruit type	33.530 (±0.127, <0.001)	30.545 – 38.873 (<0.001[c])
Spring, growth form	34.640 (±0.063, <0.001)	32.436 – 35.352 (<0.001[c])
Spring, forest strata layer	11.239 (±0.029, 0.024)	10.060 – 11.688 (0.039 – 0.020)

Notes:

[a] -For each pair of traits two models were compared to calculate the likelihood ratio statistic, a model where the traits evolve independently of one another (independent model) and a dependent model where transitions among character states in one trait depends on the character state of the other trait. The likelihood ratio statistic is compared to the χ^2 distribution with 4 degrees of freedom.

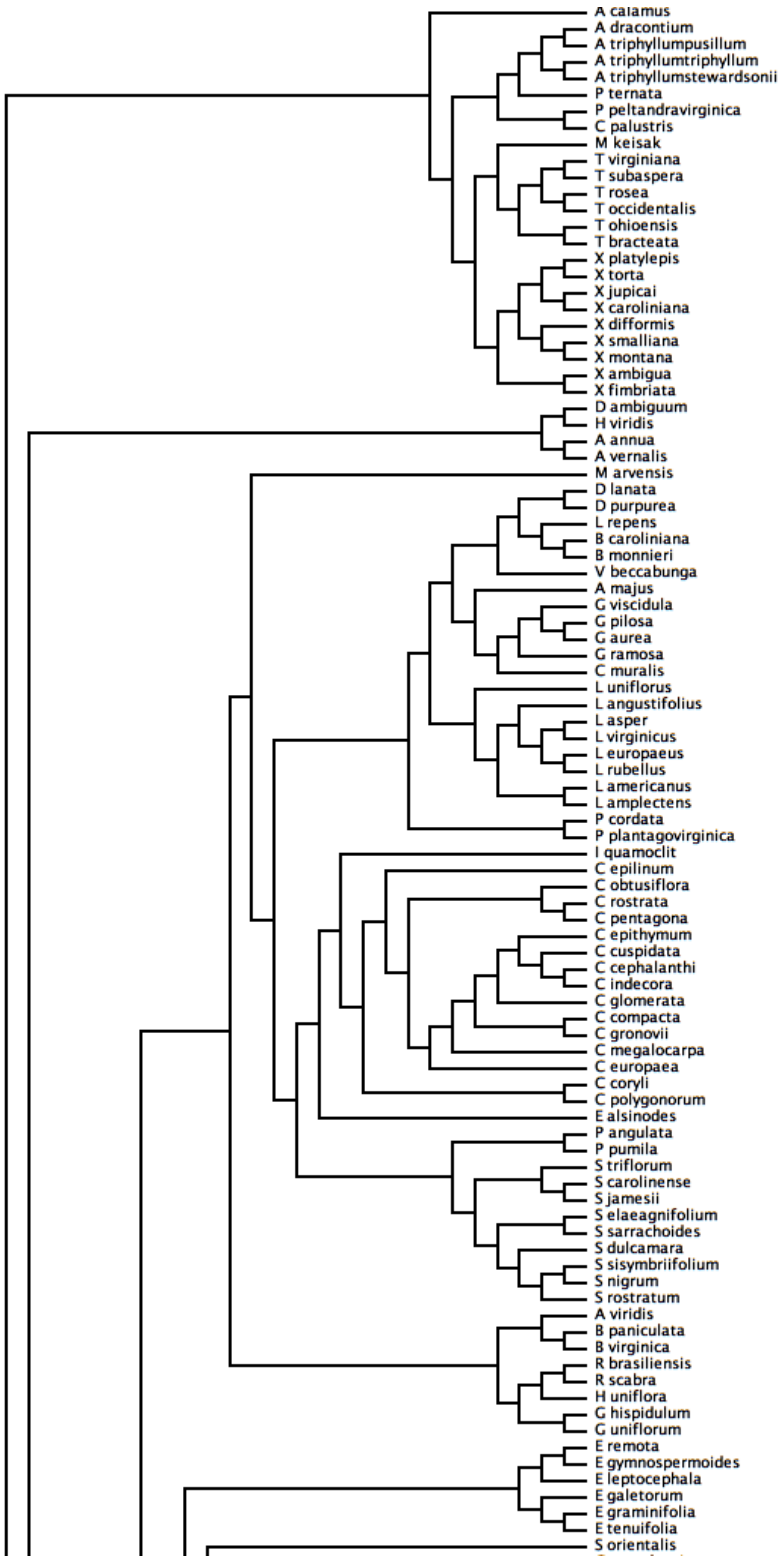
[b] -The range of p-values associated with the range of likelihood ratio statistics for the 100-tree sample.

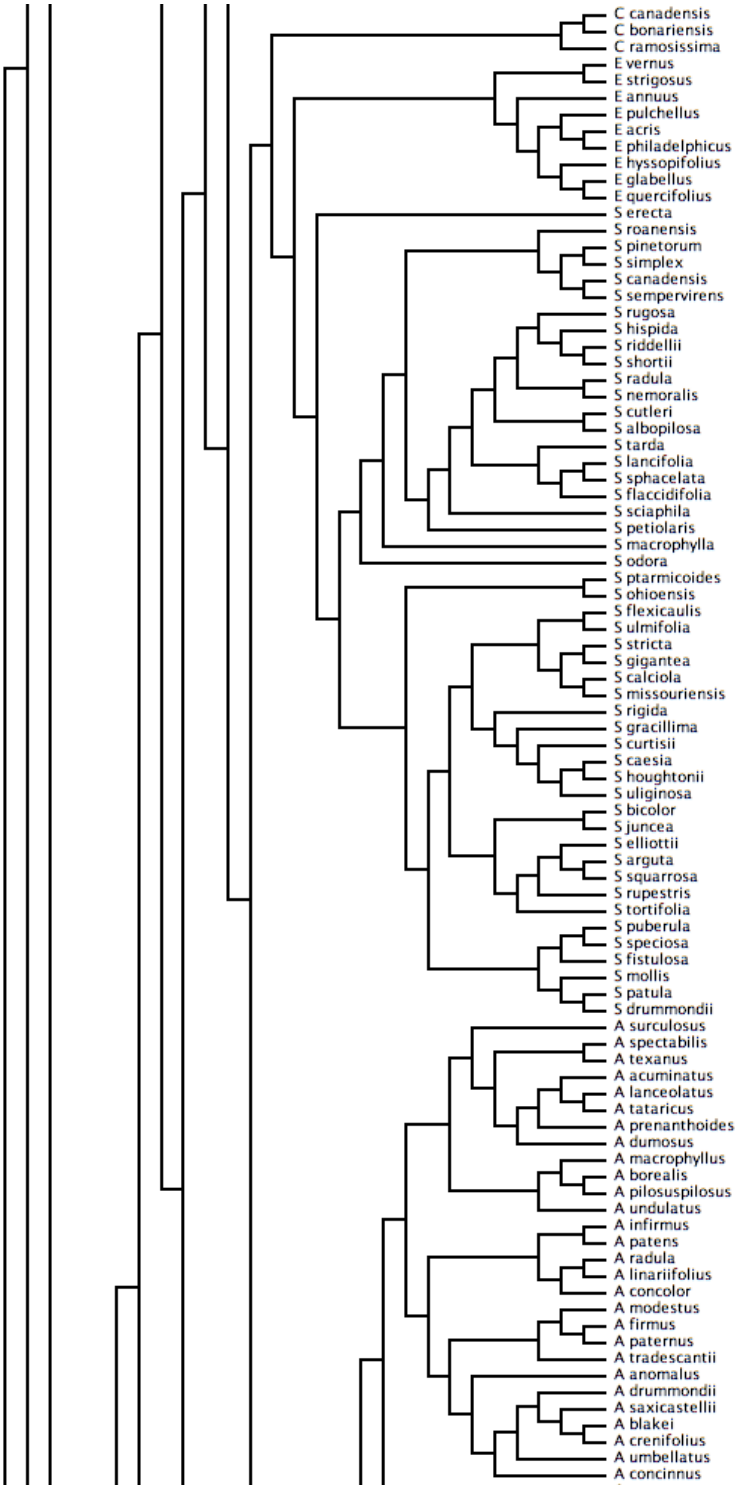
[c] -The calculated likelihood ratio statistics were significant at less than 0.001 over the entire 100-tree sample

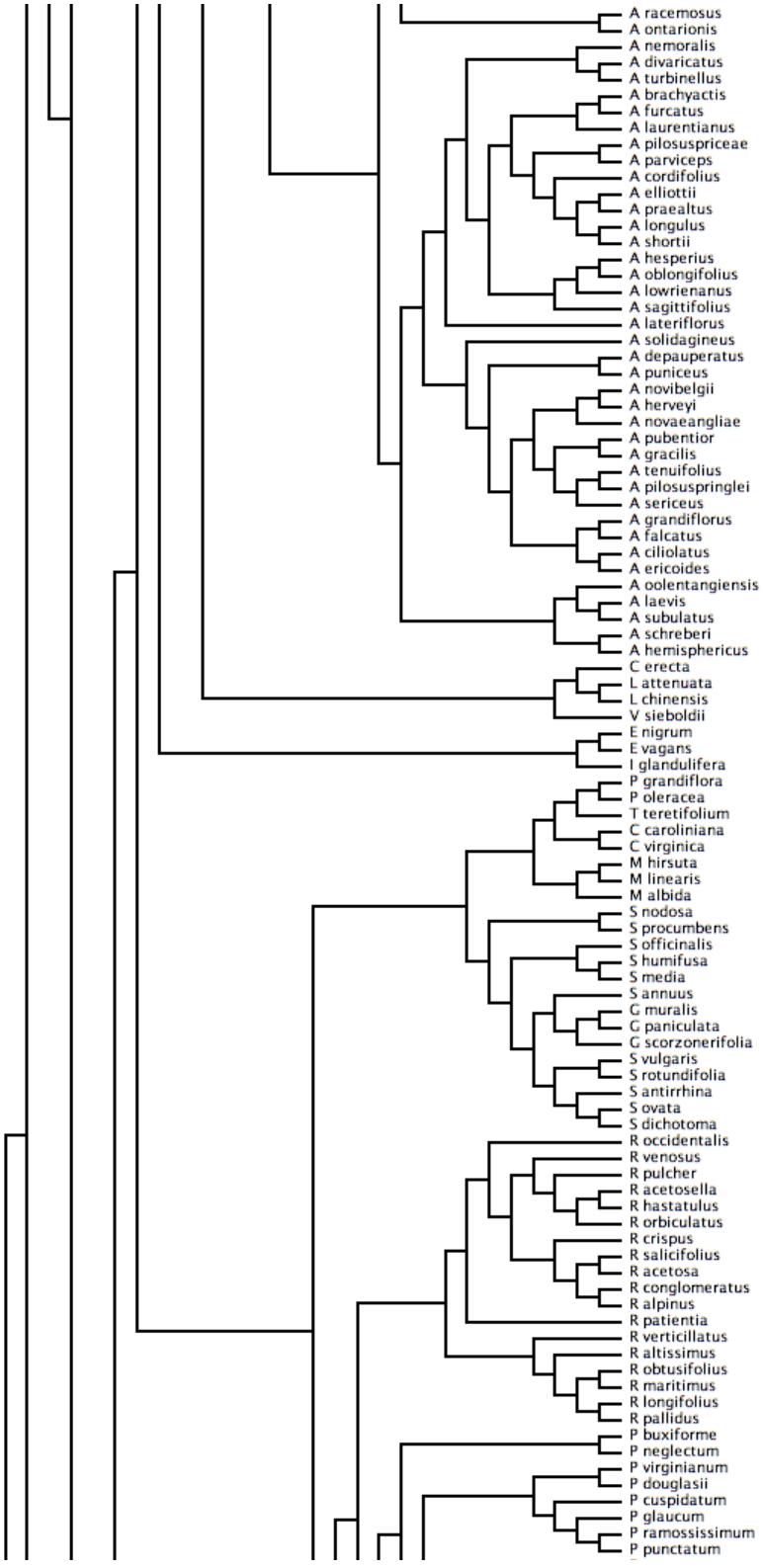


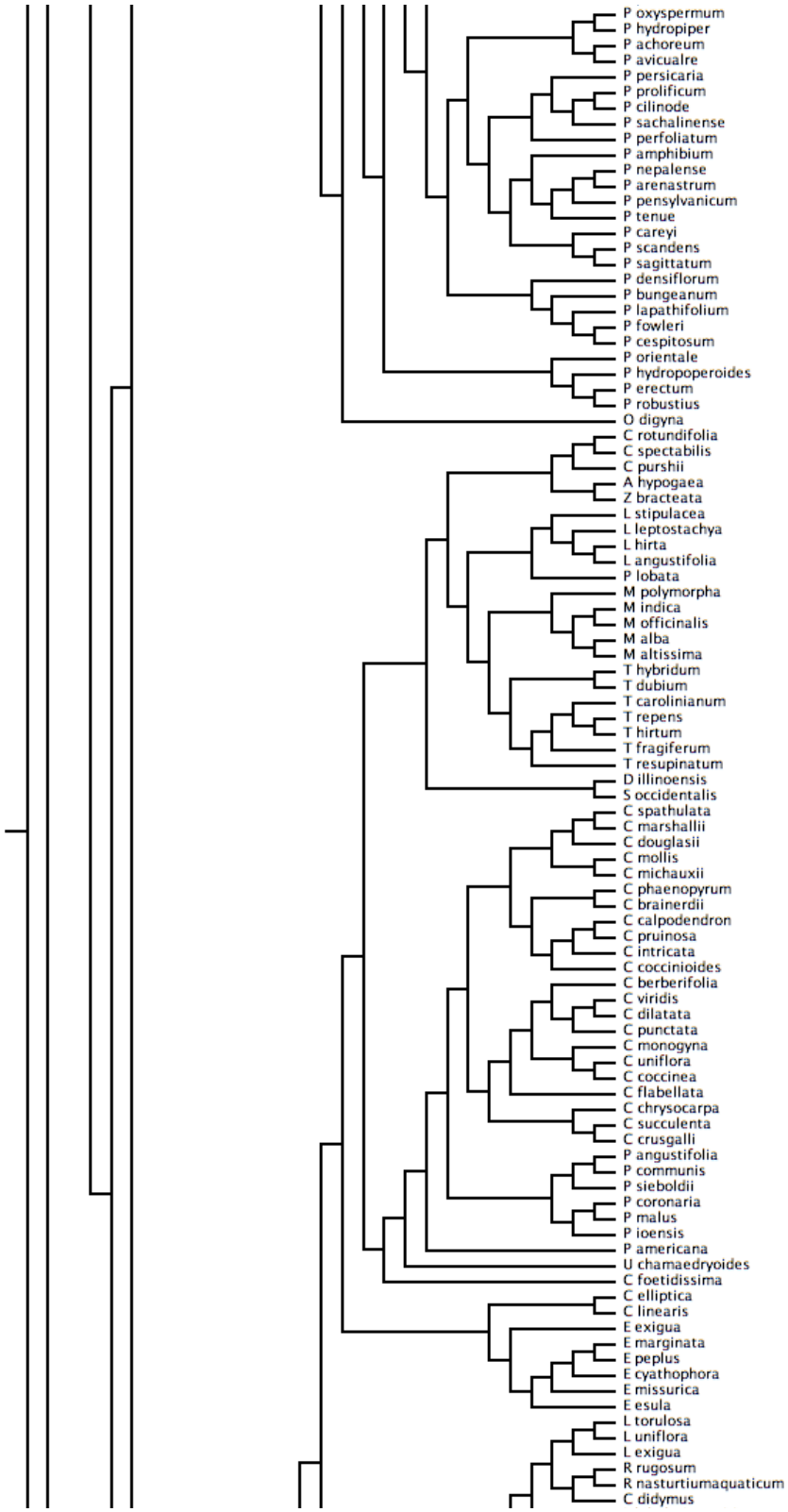
Trait	States
Flowering phenology	0 = spring, 1 = non-spring
Reproductive schedule	0 = iteroparous, 1 = semelparous
Flower colour	0 = white, 1 = non-white
	0 = light, 1 = non-light
Fruit type	0 = fleshy, 1 = dry dehiscent
Growth form	0 = woody, 1 = herbaceous
Forest strata layer	0 = understory, 1 = overstorey

Figure 2.1 Character states, estimated model transition rates and possible evolutionary pathways in the independent and dependent models of evolution.


A calamus
A dracontium
A triphyllumpusillum
A triphyllumtriphyllum
A triphyllumstewardsonii
P ternata
P peltandravirginica
C palustris
M keisak
T virginiana
T subaspera
T rosea
T occidentalis
T ohioensis
T bracteata
X platylepis
X torta
X jupicai
X caroliniana
X difformis
X smalliana
X montana
X ambigua
X fimbriata
D ambiguum
H viridis
A annua
A vernalis
M arvensis
D lanata
D purpurea
L repens
B caroliniana
B monnieri
V beccabunga
A majus
G viscidula
G pilosa
G aurea
G ramosa
C muralis
L uniflorus
L angustifolius
L asper
L virginicus
L europaeus
L rubellus
L americanus
L amplectens
P cordata
P plantagovirginica
I quamoclit
C epilinum
C obtusiflora
C rostrata
C pentagona
C epithymum
C cuspidata
C cephalanthi
C indecora
C glomerata
C compacta
C gronovii
C megalocarpa
C europaea
C coryli
C polygonorum
E alsinodes
P angulata
P pumila
S triflorum
S carolinense
S jamesii
S elaeagnifolium
S sarrachoides
S dulcamara
S sisymbriifolium
S nigrum
S rostratum
A viridis
B paniculata
B virginica
R brasiliensis
R scabra
H uniflora
G hispidulum
G uniflorum
E remota
E gymnospermoides
E leptocephala
E galetorum
E graminifolia
E tenuifolia
S orientalis


C canadensis
C bonariensis
C ramosissima
E vernus
E strigosus
E annuus
E pulchellus
E acris
E philadelphicus
E hyssopifolius
E glabellus
E quercifolius
S erecta
S roanensis
S pinetorum
S simplex
S canadensis
S sempervirens
S rugosa
S hispida
S riddellii
S shortii
S radula
S nemoralis
S cutleri
S albopilosa
S tarda
S lancifolia
S sphacelata
S flaccidifolia
S sciaphila
S petiolaris
S macrophylla
S odora
S ptarmicoides
S ohioensis
S flexicaulis
S ulmifolia
S stricta
S gigantea
S calciola
S missouriensis
S rigida
S gracillima
S curtisii
S caesia
S houghtonii
S uliginosa
S bicolor
S juncea
S elliottii
S arguta
S squarrosa
S rupestris
S tortifolia
S puberula
S speciosa
S fistulosa
S mollis
S patula
S drummondii
A surculosus
A spectabilis
A texanus
A acuminatus
A lanceolatus
A tataricus
A prenanthoides
A dumosus
A macrophyllus
A borealis
A pilosuspilosus
A undulatus
A infirmus
A patens
A radula
A linariifolius
A concolor
A modestus
A firmus
A paternus
A tradescantii
A anomalus
A drummondii
A saxicastellii
A blakei
A crenifolius
A umbellatus
A concinnus


A racemosus
A ontarionis
A nemoralis
A divaricatus
A turbinellus
A brachyactis
A furcatus
A laurentianus
A pilosuspriceae
A parviceps
A cordifolius
A elliottii
A praealtus
A longulus
A shortii
A hesperius
A oblongifolius
A lowrienanus
A sagittifolius
A lateriflorus
A solidagineus
A depauperatus
A puniceus
A novibelgii
A herveyi
A novaeangliae
A pubentior
A gracilis
A tenuifolius
A pilosuspringlei
A sericeus
A grandiflorus
A falcatus
A ciliolatus
A ericoides
A oolentangiensis
A laevis
A subulatus
A schreberi
A hemisphericus
C erecta
L attenuata
L chinensis
V sieboldii
E nigrum
E vagans
I glandulifera
P grandiflora
P oleracea
T teretifolium
C caroliniana
C virginica
M hirsuta
M linearis
M albida
S nodosa
S procumbens
S officinalis
S humifusa
S media
S annuus
G muralis
G paniculata
G scorzonerifolia
S vulgaris
S rotundifolia
S antirrhina
S ovata
S dichotoma
R occidentalis
R venosus
R pulcher
R acetosella
R hastatulus
R orbiculatus
R crispus
R salicifolius
R acetosa
R conglomeratus
R alpinus
R patientia
R verticillatus
R altissimus
R obtusifolius
R maritimus
R longifolius
R pallidus
P buxiforme
P neglectum
P virginianum
P douglasii
P cuspidatum
P glaucum
P ramossissimum
P punctatum


P oxyspermum
P hydropiper
P achoreum
P avicualre
P persicaria
P prolificum
P cilinode
P sachalinense
P perfoliatum
P amphibium
P nepalense
P arenastrum
P pensylvanicum
P tenue
P careyi
P scandens
P sagittatum
P densiflorum
P bungeanum
P lapathifolium
P fowleri
P cespitosum
P orientale
P hydropoperoides
P erectum
P robustius
O digyna
C rotundifolia
C spectabilis
C purshii
A hypogaea
Z bracteata
L stipulacea
L leptostachya
L hirta
L angustifolia
P lobata
M polymorpha
M indica
M officinalis
M alba
M altissima
T hybridum
T dubium
T carolinianum
T repens
T hirtum
T fragiferum
T resupinatum
D illinoensis
S occidentalis
C spathulata
C marshallii
C douglasii
C mollis
C michauxii
C phaenopyrum
C brainerdii
C calpodendron
C pruinosa
C intricata
C coccinioides
C berberifolia
C viridis
C dilatata
C punctata
C monogyna
C uniflora
C coccinea
C flabellata
C chrysocarpa
C succulenta
C crusgalli
P angustifolia
P communis
P sieboldii
P coronaria
P malus
P ioensis
P americana
U chamaedryoides
C foetidissima
C elliptica
C linearis
E exigua
E marginata
E peplus
E cyathophora
E missurica
E esula
L torulosa
L uniflora
L exigua
R rugosum
R nasturtiumaquaticum
C didymus

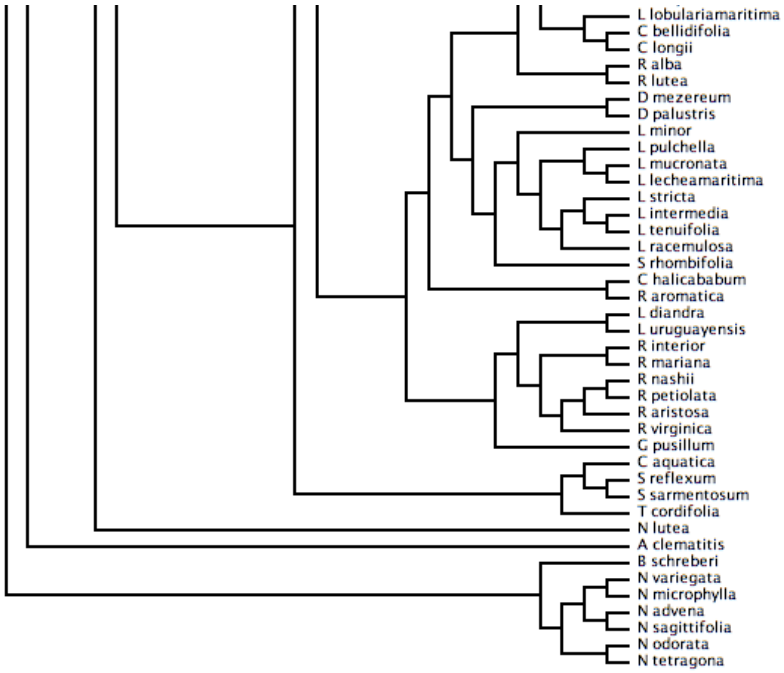


Figure 2.2 Example phylogeny from the 100-tree sample used to test for correlated evolution between flowering phenology and a suite of ecological, reproductive, and vegetative traits. The unresolved phylogeny of taxa was adapted from Bremer and colleagues (2009) and all polytomies in the unresolved tree were randomly resolved using the APE package of R (Paradis et al. 2004). Character state data for all taxa included in this study were collected from Gleason and Cronquist (1991).

2.6 Appendix

Table S2.1 List of APG-corrected families, number of genera, and the number of species within each family from Gleason and Cronquist (1991) for which flowering phenology was reported as a season and that was included in the study.

Family	Number of genera	Number of species
Acoraceae	1	1
Anacardiaceae	1	1
Apiaceae	2	3
Apocynaceae	1	1
Araceae	4	7
Aristolochiaceae	1	1
Asteraceae	6	136
Balsaminaceae	1	1
Boraginaceae	1	1
Brassicaceae	6	9
Cabombaceae	1	1
Caprifoliaceae	1	1
Caryophyllaceae	6	14
Cistaceae	1	8
Commelinaceae	2	7
Convolvulaceae	3	17
Crassulaceae	2	3
Cucurbitaceae	1	1
Ericaceae	2	2
Euphorbiaceae	2	8
Fabaceae	10	24
Gentianaceae	1	2
Geraniaceae	1	1
Lamiaceae	1	8

Lythraceae	1	1
Malvaceae	1	1
Melastomataceae	1	6
Nelumbonaceae	1	1
Nyctaginaceae	1	3
Nymphaeaceae	2	6
Onagraceae	1	1
Plantaginaceae	1	2
Polygonaceae	3	53
Portulacaceae	3	5
Ranunculaceae	3	4
Resedaceae	1	2
Rosaceae	3	29
Rubiaceae	3	5
Sapindaceae	1	1
Saxifragaceae	1	1
Scrophulariaceae	7	12
Solanaceae	2	11
Thymelaeaceae	2	2
Urticaceae	1	1
Xyridaceae	1	9
TOTAL: 45	98	414

Table S2.2 Summary of Traits and associated character states as used in the study and as reported in Gleason and Cronquist (1991)**.**

Trait	Character state coding	Reported character state from Gleason and Cronquist (1991)
Flowering phenology	Spring	Spring
	Non-spring	All other seasons
Reproductive schedule	Iteroparous	Perennials
	Semelparous	Annual or biennial
Flower colour	White	White
	Non-White	All remaining colours
	Light	White, yellow, or green
	Non-light	All remaining colours
Fruit type	Fleshy	Berry, drupe, follicle, pepo and pome fruits
	Dry dehiscent	Achene, capsule, legume, nut or nutlet, pod, schizocarp, silicle or silique fruits
Growth form	Woody	Shrub or tree growth forms
	Herbaceous	Herb growth form
Forest strata layer	Understory	Herb or shrub growth form
	Overstorey	Tall shrub, low tree or tree growth form

Chapter Three: The role of intraspecific density and community diversity on pollen limitation in *Trillium grandiflorum*

3.1 Introduction

The vast majority of flowering plants require animal pollinators to reproduce sexually (Ollerton et al. 2011), which can lead to an individual's reproductive success being limited by pollinator services - a phenomenon termed 'pollen limitation'. Indeed, a recent meta-analysis has established that upwards of 62% of flowering plants experience a reduction in fruit set as a consequence of inadequate pollination (Knight et al. 2005). Interestingly, for the most part, the factors that contribute to widespread pollen limitation are poorly understood (Ashman et al. 2004).

Both inter and intraspecific interactions have separately been demonstrated to influence pollen limitation, reproductive success and therefore the persistence of populations (Groom 1998, Forsyth 2003, Moeller 2004, Feldman 2008). For example, population density can affect pollinator foraging behaviour and at low population densities both pollinator visitation rate and visit quality may limit an individual's reproductive success (Kunin 1997). Foraging pollinators may be less likely to encounter low density patches (Steffan-Dewenter and Tscharntke 1999), may be more attracted to high density patches, and may visit more flowers within high density patches (Kunin 1997). Conversely, at high population densities, pollination may be limited by competition (Rathcke 1983). Similarly, interspecific interactions among co-flowering species have been demonstrated to significantly influence the reproductive success of individual species. For example, co-flowering species may compete for pollinator services through either exploitative competition, where one species suffers a reduced

visitation rate when in association with other species' or through interference competition, when heterospecific pollen deposition occurs as the result of pollinator sharing (Waser 1978a, 1978b). Alternatively, co-flowering species may facilitate each other's reproductive success by increasing the overall attractiveness of a patch to pollinators, or by providing temporal stability in food resources (for a recent review of mechanisms see Ghazoul 2006). While it is clear that both inter and intraspecific interactions can have important implications for plant reproductive success, few studies have attempted to examine how these two factors may operate in concert.

Here, I address the relationship between reproductive success (measured as pollen limitation) and both intraspecific density *and* community diversity in *Trillium grandiflorum* (Melanthiaceae), a common spring flowering species, using a pollen supplementation experiment. I experimentally manipulated both focal species density and flowering plant community diversity in a factorial design to answer the following question: how do focal species density and community diversity affect pollinator-meditated reproductive success in *Trillium grandiflorum*?

Spring flowering communities in temperate deciduous forests of Eastern North America are an ideal system to study the impacts of inter and intraspecific interactions on pollinator-mediated reproduction. Spring blooming species flower prior to other forest understory plants and display a remarkable amount of synchronicity in flowering phenology (Macior 1978a, Schemske et al. 1978, Motten 1986). Moreover, it has been noted that many traits are common to the spring flora. For example, many co-flowering species lack specific floral mechanisms, such as differences in floral colour or morphology, to partition pollinator use within a community. As a result, co-flowering

species frequently share generalist pollinators, such as solitary bees and flies, which are abundant in the spring environment (Macior 1978a, b, Schemske et al. 1978, Motten et al. 1981, Motten 1986, Hannan and Prucher 1996). Previous studies have shown that reproductive success of *T. grandiflorum* can be limited by pollination services and is influenced by local plant density (Smith 1998, Kalisz et al. 1999, Irwin 2000, Irwin 2001, Griffin and Barrett 2002, Knight 2003), making it a practical study system for this question. Furthermore, *T. grandiflorum* co-flowers with a large number of spring flowering species, is bee pollinated, and can be successfully transplanted into experimental plots (Smith 1998, Knight 2003).

3.2 Methods

3.2.1 Study system

Trillium grandiflorum (Michaux) Salisbury is an animal-pollinated, perennial plant found in deciduous forests of temperate North America (Lubbers and Lechowicz 1989, Gleason and Cronquist 1991). It ranges from Atlantic Canada to North Carolina and extends westward beyond the Great Lakes (Case and Case 1997). Individuals produce a single perfect terminal white flower which is subtended by three large green bracts. Flowering occurs from mid-April through June prior to canopy closure and a variety of floral visitors have been noted, including bumble bees, flies and solitary bees (Smith 1998 L.E. Hensel pers. obs). Floral longevity of *T. grandiflorum* is approximately 21 days (Ashman and Schoen 1996, Sage et al. 2001). When fruit maturation is complete, the ripe fruit will fall to the ground and the seeds are exposed at the base of the capsule. Ants remove seeds from the capsule and forage on the elaisome (Kalisz et al. 1999). Outcrossing is promoted through the presence of floral rewards, increased floral

longevity in the absence of pollen deposition, and, in some populations, self-incompatibility (Ashman and Schoen 1996, Broyles et al. 1997, Sage et al. 2001, Griffin and Barrett 2002, Steven et al. 2003). *Trillium grandiflorum* is frequently found in sympatry with many different species, including closely related congeners as well as more distantly related taxa (Smith 1998). Frequently, co-flowering sympatric species also provide floral rewards and use the same pollinator taxa as *T. grandiflorum*. In spring flowering communities, co-flowering species coexist over small spatial scales increasing the likelihood that co-flowering species do in fact engage in pollinator-mediated interactions.

In the spring of 2010 I performed a manipulative field experiment in order to gain insight into how community diversity and population density might operate in concert to affect the reproductive success of *T. grandiflorum.*

3.2.2 Site selection and plot set-up

In early April 2010 I set up four 4 m X 4 m experimental plots at six sites in the Fairmont Kenauk Ecological Reserve near Montebello, Quebec (45^{o} 42'43.97" N, 74^{o} 53'07.23" W; Figure 3.1). Study sites were chosen based on high non-*Trillium* flowering plant diversity and were separated from each other by at least 300 m (Figure 3.1). Within a site, plots were established on the basis of containing three different spring flowering species and being situated approximately 100 m from the nearest plot. Care was taken to ensure that all plots were located in ecologically similar locations; for example, if a site was sloped all plots had the same aspect, similar gradient and similar forest canopy. Experimental sites did not initially contain any *T. grandiflorum*. I used a large local population of *T. grandiflorum* as the source of experimental plants. Figure 3.1 illustrates

the location of the donor population relative to the experimental sites. Individual plants were transplanted into manipulated communities with the goal of determining how community diversity and floral density affect the reproductive success of *T. grandiflorum*.

3.2.3 Experimental design

At each of six sites two plots were randomly assigned to a low density treatment of 0.125 plants/m^2 and two were assigned to a high density treatment of 0.5 plants/m^2. A total of 20 *T. grandiflorum* individuals in floral bud stage were transplanted among the four plots (2 per low density plot and 8 per high density plot) in a grid pattern that maximized nearest neighbour distance (Figure 3.2). Transplanted individuals were watered three times during the experiment, once at the time of transplantation and twice within the first three days, at which point it snowed for two days and no further watering was performed. Post-transplant rates of deer herbivory and trampling were high. Replacement individuals were transplanted until no individuals in floral bud stage were present in the donor population. To prevent further herbivory and trampling, all transplanted individuals were covered in a flexible wire cage (see Figure 3.3). The cage had approximately 5cm holes evenly spaced throughout. The holes were sufficiently large for pollinators to pass through easily and were therefore not expected to inhibit pollinator visitation.

Once the density plots were established, one plot from each density treatment per site was randomly assigned to either a low or high diversity treatment. Because it would have been difficult to experimentally increase site diversity, all diversity manipulations

were performed through species removal. All floral buds and flowers of co-occurring species were removed at least once per week in the low diversity treatment. The number of open flowers and stems of each co-occurring species for the high diversity treatment were counted at least once per week for the duration of the experiment. All plots were centrally located within a circle with a diameter of 20 m (Figure 3.2). All flowers were removed from the exterior of the plot that were located within the diameter of this circle regardless of community diversity treatment level. This was done to ensure that treatment differences were perceivable to potential pollinators. Diversity treatments and community removal of flowers was continued until no *T. grandiflorum* individuals within the plot were flowering.

3.2.4 Pollen supplementation

In order to determine how floral density and community diversity influence the pollinator-mediated reproductive success of *T. grandiflorum*, I performed a pollen supplementation experiment (Burd 1994). Within each plot, individuals were randomly assigned to a pollination treatment: half of the individuals in each plot received supplemental hand pollination and the remaining plants were left as unmanipulated controls. Regardless of pollination treatment, all plants were subsequently exposed to natural pollination conditions. Plants that received supplemental pollination were hand pollinated once with two freshly dehisced anthers from two different donor plants from the same donor population. The magnitude of pollen limitation for each treatment was determined by comparing the reproductive success of plants that received supplemental pollen to those that were unmanipulated (control plants).

3.2.5 Fruit and seed collection

Once the experimental plants had completed flowering, a small bridal veil sac was placed over the maturing capsule to prevent the loss of seeds as the result of foraging ants (Figure 3.4). Capsules were only removed from the plant once it was fully matured and had separated but not fallen from the maternal plant. For individuals that failed to set fruit, capsules were collected when it appeared that the individual's leaves had senesced (i.e. turned brown in colour). Capsules that failed to set fruit were left on the plant as long as possible because there is little observable difference between a capsule that fails to set fruit (no seeds produced) and one that matures only a small faction of fertilized ovules into seeds (L. Hensel pers. obs.).

In addition, plant height to the base of the peduncle and largest leaf length were recorded for each *T. grandiflorum* individual as indicators of plant size. These covariates were collected to account for differences in reproductive success that may be attributed to plant size. It has previously been shown that reproductive success of *T. grandiflorum* varies positively with plant size (i.e. Griffin and Barrett 2002). Figure 3.2 summarizes the experimental design I employed to test the effects of focal species density, community diversity and supplemental-hand pollination on the reproductive success of *T. grandiflorum*.

3.2.6 Statistical Analysis

The magnitude of pollen limitation within each population was determined by comparing average fruit set per plot and the average number of seeds produced per plot between open and supplementally pollinated plants. A plant was defined as having set

fruit if at least one seed was produced within the capsule. Plants that failed to set fruit were assigned 0 for the number of seeds produced and were included in the average number of seeds produced per plot. Both average fruit set per plot (Shapiro-Wilk test, $W = 0.6895$, $p < 0.001$) and average number of seeds per plot (Shapiro-Wilk test, $W = 0.5798$, $p < 0.001$) were not normally distributed and displayed heteroscedasticity (assessed visually), as such generalized linear mixed effects models were used to examine the effects of focal species density, community diversity, and supplemental pollination on the reproductive success of *T. grandiflorum*, using the GLIMMIX procedure in SAS (SAS Institute 2009).

Multicollinearity between the community diversity measures, mean species richness and the mean floral density of co-occurring species, was high. To account for the correlated nature of these covariates a principal components analysis was performed. The first component (PC1) explained 96% of the variance and was included as the measure of community diversity in the generalized linear mixed effects models examining the effects of focal species density and community diversity on fruit set and number of seeds per fruit. Component 2 explained approximately 3% of the variance and was not considered in any further analysis. The relationship between mean species richness and mean floral density of co-occurring species is illustrated in Figure 3.5. The results of the principal components analysis are presented in Table 3.1.

For the analysis of both response variables, focal species density and pollination treatment were modeled as fixed effects, while site was included as a random effect. The significance of the random effect was assessed with a Likelihood Ratio Test. In both models, diversity (PC1) was included in the analysis as a continuous factor. Both models

were optimized using the Dual Quasi – Newton technique and model parameters were estimated using the Residual Pseudo-Likelihood technique (Littell et al. 2006). Denominator degrees of freedom for tests of fixed effects were estimated using the Kenward – Roger method (Littell et al. 2006). The negative binomial distribution was used to model both the average fruit set per plot and average number of seeds per plot analyses. The ratio of the generalized chi-square statistic and its degrees of freedom where used to evaluate each model. When the ratio is close to one the data have been modeled correctly and there is no residual over dispersion (Schabenberger 2005). The ratios for the average fruit set and average seed number models were 0.81 and 1.14, respectively. While neither of these values is particularly close to 1.0, a number of distributions were examined and the negative binomial resulted in the least amount of residual over dispersion. Accordingly, the natural logarithm link function was used in both models. Principal component one represents a linear combination of both species richness and species density within the models presented. While not presented in this thesis, it is possible to back calculate partial regression slopes of the standardized original factors from the partial regression slopes in the models presented. The back calculated partial regression slopes on the standardized factors would increase the interpretability of the effects of species richness and species density on the response variables considered when all other factors are held constant.

3.3 Results

Across all sites, 95% (114/120) of the transplanted *T. grandiflorum* individuals survived transplantation and flowered. A total of six transplanted individuals, 2 open and 4 supplemental pollinated plants, died as the result of either herbivory, animal trampling

or transplant stress, and were not replaced. At two sites, Trail 2 and Whitefish 2, almost all (100% and 95%, respectively) individuals failed to set fruit. Accordingly, these two sites were removed from the statistical analysis. The lack of fruit set is likely because herbivory was initially high and replacement individuals were transplanted into the experimental plots ca. 4-5 days after transplantation occurred at other sites. While these late transplants survived, they were not included in the analysis because of the notable differences in the measured response variables between the included four sites and the excluded two, which I suspect was exacerbated by the timing with which they were put into the plots.

In the high diversity plots the total number of co-flowering species observed ranged from one to 12 species (mean = 3.75 species), while the average number of species observed to be co-flowering during each census day ranged from one to six species (mean = 2.6 species). Likewise, average floral density of co-flowering species in the high diversity plots ranged from 0.04 to 7.3 flowers per square meter (mean = 2.6 flowers/m^2). The application of the low diversity treatment resulted in no co-flowering species occurring in the low diversity plots.

3.3.1 Fruit set

Overall, fruit set was low: only 33 of the 80 plants included in the analysis produced at least one seed in their capsule. For comparison, in a similar experiment, Knight (2003), over 93% open pollinated and 98% of plant receiving supplemental pollination set fruit. Across all populations, supplemental hand-pollination resulted in a 6-fold increase in average fruit set per plot when compared to open pollinated plants (Figure 3.6B), suggesting that pollinators were indeed limiting at my sites. However,

significant variation in average fruit set per plot likely resulted in this difference between supplemental and open pollinated plant average fruit set being non-significant (Table 3.2). In total only 8 open pollinated plants set fruit compared to 25 plants that received supplemental pollination.

Average fruit set per plot increased with focal species density (Figure 3.6A); across all populations average fruit set per plot was nearly twice as high in high density plots as compared to low density plots. In total, 29 of the 33 individuals that set fruit were from high density plots, however, this difference in the proportion of individuals that set fruit proved to be statistically insignificant (Table 3.2).

While over 96% of the variation between the community diversity measures of mean species richness and mean floral density of co-occurring species can be explained by the community diversity PC1, this variable did not explain any significant variation in average fruit set per plot in my study.(Table 3.2).

3.3.2 Seed Set

Similar to fruit set, pollen limitation of average number of seeds produced per plot declined dramatically with supplemental hand pollination. (Figure 3.6 panels B and D). Supplemental hand-pollination resulted in a four-fold increase in the average number of seeds per plot when compared to open pollinated plants. This difference was found to be statistically significant (Table 3.3 and Figure 3.6D).

Focal species density had a significant effect on the average number of seeds per plot (Table 3.3). The average number of seeds produced per plot was over 20 times greater in high density plots than in low density plots (Figure 3.6C).

Similar to fruit set, there was no detectable relationship between the average number of seeds produced per plot and community diversity PC1.(Table 3.3).

3.4 Discussion

Suboptimal fecundity is a common theme among studies that examine the factors that affect reproductive success in flowering plants (Burd 1994, Ashman et al. 2004, Knight et al. 2005). This phenomenon is exceedingly common among biotically-pollinated, primarily outcrossing perennials such as *T. grandiflorum* (Burd 1994, Vamosi et al. 2006). Similar to previous studies that have reported low seed to ovule ratios and/or evidence for pollen limitation in *T. grandiflorum* (Lubbers and Lechowicz 1989, Smith 1998, Kalisz et al. 1999, Irwin 2000, Griffin and Barrett 2002, Knight 2003), the effect of the supplemental pollination treatment on the average number of seeds per plot in my experiment (Figure 3.5 D) confirms that this species is frequently pollen limited.

Both population and community characteristics can influence the degree to which adequate deposition of compatible pollen limits reproductive success (Kunin 1997, Knight 2003, Moeller and Geber 2005). The primary goal of this study was to explore the impact of population and community characteristics on pollinator-mediated reproductive success (measured as pollen limitation). Assessing differences in reproductive success (i.e. average fruit set and average number of seeds per fruit) among treatment levels illustrated that reproductive success appears to be positively related to focal species density, while diversity (as measured by PC1) had no appreciable effect on either average fruit set or the average number of seeds per plot in this study.

Importance of pollen limited reproduction

It has been repeatedly demonstrated that inadequate pollination frequently limits the reproductive success of *T. grandiflorum* (Lubbers and Lechowicz 1989, Smith 1998, Irwin 2000, Griffin and Barrett 2002, Knight 2003). However, pollen limitation does not necessarily affect each component (fruit set or seed set) of an individual's fitness equally. For example, in this study, pollen supplementation increased both average fruit set and average number of seeds per plot (Figure 3.6B and D), however, only the increase in the average number of seeds proved to be statistically significant (Table 3.2 and Table 3.3). In contrast, previous studies that have reported pollen limitation of both fruit and seed set *T. grandiflorum* (Lubbers and Lechowicz 1989, Smith 1998, Irwin 2000, Griffin and Barrett 2002, Knight 2003). In addition, many studies have noted that climatic variability can cause significant daily and inter-annual variation in pollinator availability in spring flowering communities (Macior 1978a, Motten 1986, Barrett and Helenurm 1987, Lubbers and Lechowicz 1989, Broyles et al. 1997, Smith 1998). In this study, supplemental pollination represents an estimate of maximum fertility, and while supplemental pollination did increase average fruit set and average number of seeds per plot, both measures of reproductive success were still remarkably low, relative to previous studies (e.g. Knight 2003). Similarly, numerous studies of *T. grandiflorum* have reported relatively higher seed set of both supplemental and open pollinated individuals than the average values reported in this study (i.e. Lubbers and Lechowicz 1989, Smith 1998, Griffin and Barrett 2002, Knight 2003). Together the low levels of fruit and seed set I observed suggest that factors other that pollen deposition are also limiting the fecundity of the transplanted individuals in my study (discussed in more detail below).

Effects of intraspecific density on pollen limited reproduction

Pollen limitation of fruit set and seed number decreased substantially with increased intraspecific density, confirming the results of a previous study by Knight (2003). If pollinator foraging behaviour changes in response to increased plant density as a decelerating function, pollinators will visit proportionally fewer flowers in high density patches. This could lead to higher intraspecific competition in high density populations (Feldman 2006). In contrast, an extensive population survey and a pollen supplementation experiment examining density effects within large naturally occurring populations of *T. grandiflorum* noted no significant relationship between population density and the magnitude of pollen limitation (Smith 1998), suggesting that density does not universally impact reproduction in this species.

Furthermore, the relationship between population density and population size may contribute significantly to the variation observed in the reproductive success of *T. grandiflorum.* In addition to its effects on pollinator visitation rates, low intra-specific density in naturally occurring populations is often correlated with a reduction in the potential for genetically compatible mates, particularly for self-incompatible species (Baker 1955, Stebbins 1957). The genetic structure of small, sparse populations is unlikely to be present in this study because all plants and pollen donors were sourced from a single, large population. Although not significant for both measures of reproductive success, the reduction in fitness associated with low density that I observed likely reflects differences in the probability that a pollinator carries compatible pollen due to differences in the number of available mates within the experimental plots. As a result, in the present study, the effects of density were potentially confounded with the effects of

population size. A better test of the effects of density would have been to vary the size of plots (changing density) while holding the total number of individuals within each plot constant. The experiment performed here may be better described as a test of the impacts of population size, rather than plant density, per se.

Variation in average fruit set and average number of seeds per plot was fairly high (Figure 3.6), suggesting that pollen deposition was quite variable among plots regardless of treatment. A number of factors of could have contributed to this variation. Logistics constrained the number of experimental sites and plots that could be established and maintained for the duration of the experiment. As a result, small sample size likely contributed to this variation. Second, the sample size for high density plots was larger, increasing the strength of the estimated treatment effects in those plots. An experiment that follows the established plots for numerous growing seasons may increase the strength of the estimated treatment effects and contribute significantly to our understanding of temporal variation in the factors that affect reproductive success.

Effects of diversity on pollen limited reproduction

Spring flowering communities represent a considerable component of the diversity of temperate deciduous forests (Jolls 2003). The lack of a significant influence of diversity on pollen limitation in my study is somewhat surprising given that community species richness has been shown to be positively correlated with the magnitude of pollen limitation in other studies (Vamosi et al. 2006). The lack of either a competitive or a facilitative effect of community diversity in my study suggests that the effects of pollinator sharing are neutral on the reproductive success of *T. grandiflorum*, at least in the context of this study. Many studies examining the reproductive ecology of

spring flowering species have also failed to find effects of pollinator sharing (i.e. Macior 1978a, Schemske et al. 1978, Motten 1986), indicating that neutral interactions may dominate. In fact, pollinator limited reproduction was found to be a less significant factor in limiting reproductive success of spring flowering species than the environmental constraints of early spring climatic conditions (Motten 1986), which is also consistent with my findings. In addition, where pollinator behaviour does limit reproductive success, pollinator constancy, or, the likelihood that a foraging pollinator will continue to forage on the same species, appears to mitigate competitive interactions (Schemske et al. 1978). Alternatively, this study may not have had sufficient power to detect diversity effects.

It is possible that potential pollinators were unable to perceive the different levels of the diversity treatment. The significant increase in the average number of seeds in high density plots indicates that the density treatment affected either pollinator perception of the experimental plots or pollinator foraging behaviour within the plots. As such, it is also likely that potential pollinators did perceive a difference between the experimental plots and the surrounding spring flowering community. A recent review that summarizes the limitations of floral recognition by pollinators suggests that a flower must have a diameter of 26cm to be visible to a bee from one meter using colour contrast (Chittka and Raine 2006). The poor resolution of the bee visual system suggests that many floral signals obvious to humans may be undetectable to potential pollinators (Chittka and Raine 2006). However, the area cleared around the experimental plots may have had detrimental effects on pollinator perception of the plots. Future studies that utilize similar

experimental plots could potentially benefit from reducing the cleared area surrounding the plots to increase the potential for pollinators to recognize the experimental plants.

Resource limitation of reproduction

In addition to pollen limitation, resource limitation may have contributed to depressed fruit and seed set in my study. The discordance between the proportion of individuals that set fruit within the transplant experiment and the levels reported by Knight (2003; greater than 90%), suggests that resource limitation may have limited my ability to detect the effects of intraspecific density and community diversity on both fruit set and the number of seeds per fruit. Interestingly, resource limitation may have mainly affected fruit set in my study, as the number of seeds per fruit (data not shown) was well within the range of previous studies (e.g. Lubbers and Lechowicz 1989, Smith 1998).

Synthesis

Spring climatic conditions in temperate forests can significantly limit pollinator foraging (Macior 1978a, Schemske et al. 1978, Motten 1986, Barrett and Helenurm 1987, Lubbers and Lechowicz 1989, Broyles et al. 1997, Smith 1998), increasing the likelihood of reproductive failure of spring flowering plants. The unpredictable nature of pollination in the spring environment has lead to the evolution of alternative mating strategies, such as facultative autogamy, to provide reproductive assurance in spring flowering species (Macior 1978a, Schemske et al. 1978, Motten 1986). Pollen limitation in *T. grandiflorum* is probably the result of low pollinator visitation rates (Smith 1998). Unlike other spring flowering species, *T. grandiflorum* has relatively high rates of outcrossing (Broyles et al. 1997, Smith 1998), demonstrated self-incompatibility (Sage et al. 2001) and does not

reproduce asexually (Lubbers and Lechowicz 1989), emphasizing the fact that pollinator attraction is crucial for successful sexual reproduction and population viability.

The lack of detectable competitive effects in this study further supports the idea that the number of adequate pollinator visits may be limiting the reproductive success of *T. grandiflorum*. When pollinator activity is stochastic and results in pollen limitation, selection on traits, such as increased floral longevity and pollen viability, that increase the probability of a successful pollinator visit, may be strong. Indeed, increased longevity and pollination-induced senescence of flowers (Ashman and Schoen 1996), increased longevity in the viability of pollen (Griffin and Barrett 2002) and the ability to produce a full complement of seeds after a single pollination (Griffin and Barrett 2002) are all traits *T. grandiflorum* likely evolved to alleviate pollen limitation. Furthermore, synchronous flowering of spring blooming species may alleviate the constraints of stochastic pollination services (Macior 1978a).

3.4.1 Future directions

Here, I used a pollen supplementation experiment to test for the impact of population and community characteristics on pollen limitation in *T. grandiflorum*. Similar to other studies, I was able to detect pollen limited reproductive success and an intraspecific density effect on seed set. My inability to detect diversity effects was likely at least partially due to low levels of fruit set and high variation in seed number, although, further studies would be necessary to fully elucidate the relationship (if any) among community diversity and pollinator-mediated reproductive success. Here I summarize several potential next steps for improving our understanding of pollen limitation in this system.

First, reliably estimating the population and community effects on pollinator foraging behaviour are necessary to understand the effects of community characteristics on pollen limitation. A study could be designed to detect the mechanism by which spring flowering species reproduction is influenced by pollinator sharing. For example, in a study of pollen limitation of seed set in two montane herbs, Bosch and Waser (1999) found a significant decrease in seed set associated with low density, however, density had no effect on pollinator visitation rates or pollen deposition. In a subsequent study they examined whether resource differences or the quality of pollination services contributed to low density effects and noted that abiotic resource availability most likely limited reproductive success because detailed pollinator observations did not show a decrease in the quality of pollinator visits (Bosch and Waser 2001). Such observations in my system could shed light on the relationship between pollinator visitation, intraspecific density, community diversity, and reproductive success.

Second, increasing the number of experimental sites and plots would have improved my ability to detect intraspecific density and community diversity effects as well as interactions between these factors. It is important to note that the variation in fruit set observed in this study is considerably greater than what has previously been reported in the literature (Lubbers and Lechowicz 1989, Smith 1998, Knight 2003). It is possible that the dynamics at my experimental sites were sufficiently different from those of the previous studies and that more sites and plots would have allowed me to detect treatment effects on fruit set. Interestingly, experimental manipulation of intraspecific density appears to be a practical method for examining the effects of density on seed set (e.g. this study, Knight 2003 compared to Smith 1998). Similarly, diversity treatments might have

benefited from a diversity gradient, where the co-flowering community was experimentally manipulated within the intact plots.. In addition, using naturally occurring populations and densities of the focal species, rather than transplants, could have alleviated the apparently poor pollinator response to my 'experimental' communities. In my study, *T. grandiflorum* was not initially present in any of my experimental plots or within the vicinity, which may have confounded my treatment effects because it has been shown that pollinators will avoid unfamiliar floral resources (Forrest and Thomson 2009). In retrospect, monitoring naturally pollinated plants in the donor population could have added insight into the reproductive success of the experimentally transplanted individuals. For example, the low levels of fruit set I observed may have been the result of spring climatic conditions and not transplant effects if fruit set was similarly depressed in the donor population.

Finally, it has previously been suggested that resource limitation in *T. grandiflorum* may constrain the number of reproductive events (i.e. how many years throughout the life-span of an individual will it flower) in an individual's lifetime, but not necessarily reproductive success within a reproductive season (Griffin and Barrett 2002). This suggests that multi-year studies at a variety of localities are required before general conclusions regarding resource limitation can be made for *T. grandiflorum*.

3.5 Conclusions

Employing experimental methods that manipulate attributes of communities within the context of a pollen supplementation experiment should help illustrate how intraspecific and interspecific interactions effect pollen limitation. In this study, intraspecific density in T. grandiflorum was positively related to reproductive success

while community diversity was not. My results suggest that T. grandiflorum pollinator-mediated reproductive success is highly variable and while density may be an important contributing factor, it only explains a small portion of the variation. Because of the high variability of T. grandiflorum fruit set, a larger scale, multi-year study is likely necessary to fully elucidate the effects of density and diversity on reproductive success in T. grandiflorum.

Table 3.1 Principal components analysis between mean species richness and mean floral density of co-flowering species of the diversity treatment

Component	Eigenvalue	Percentage variance
1	8.627	96.52
2	0.311	3.48

Notes:
Component 1 was used in the analysis of focal species density and community diversity as the diversity component of the models testing effects on fruit set and number of seeds per fruit in *Trillium grandiflorum*. Component 2 did not explain a significant proportion of the variance as a result was excluded from all subsequent analyses.

Table 3.2 Generalized linear mixed effects model for effects on average fruit set of *Trillium grandiflorum* per plot

Source	df	F or χ^2	P
Fixed			
Focal species density	1, 27	0.63	0.572
Pollination treatment	1, 27	4.30	0.286
Community diversity principal component 1	1, 27	0.06	0.843
Random			
Site	1	0.00	1.000

Notes:
Denominator degrees of freedom for fixed effects were estimated using the Kenward-Roger approximation. The significance of site as a random effect was tested using a likelihood ratio test. Significant effects are shown in bold typeface.

Table 3.3 Generalized linear mixed effects model for effects on average number of seeds per fruit of *Trillium grandiflorum* per plot

Source	Df	F or χ^2	P
Fixed			
Focal species density	1, 27	27.83	**<0.001**
Pollination treatment	1, 12.83	14.34	**0.002**
Community diversity principal component 1	1, 11.05	0.45	0.514
Random			
Site	1	1.66	0.099

Notes:
Denominator degrees of freedom for fixed effects were estimated using the Kenward-Roger approximation. The significance of site as a random effect was tested using a likelihood ratio test. Significant effects are shown in bold typeface.

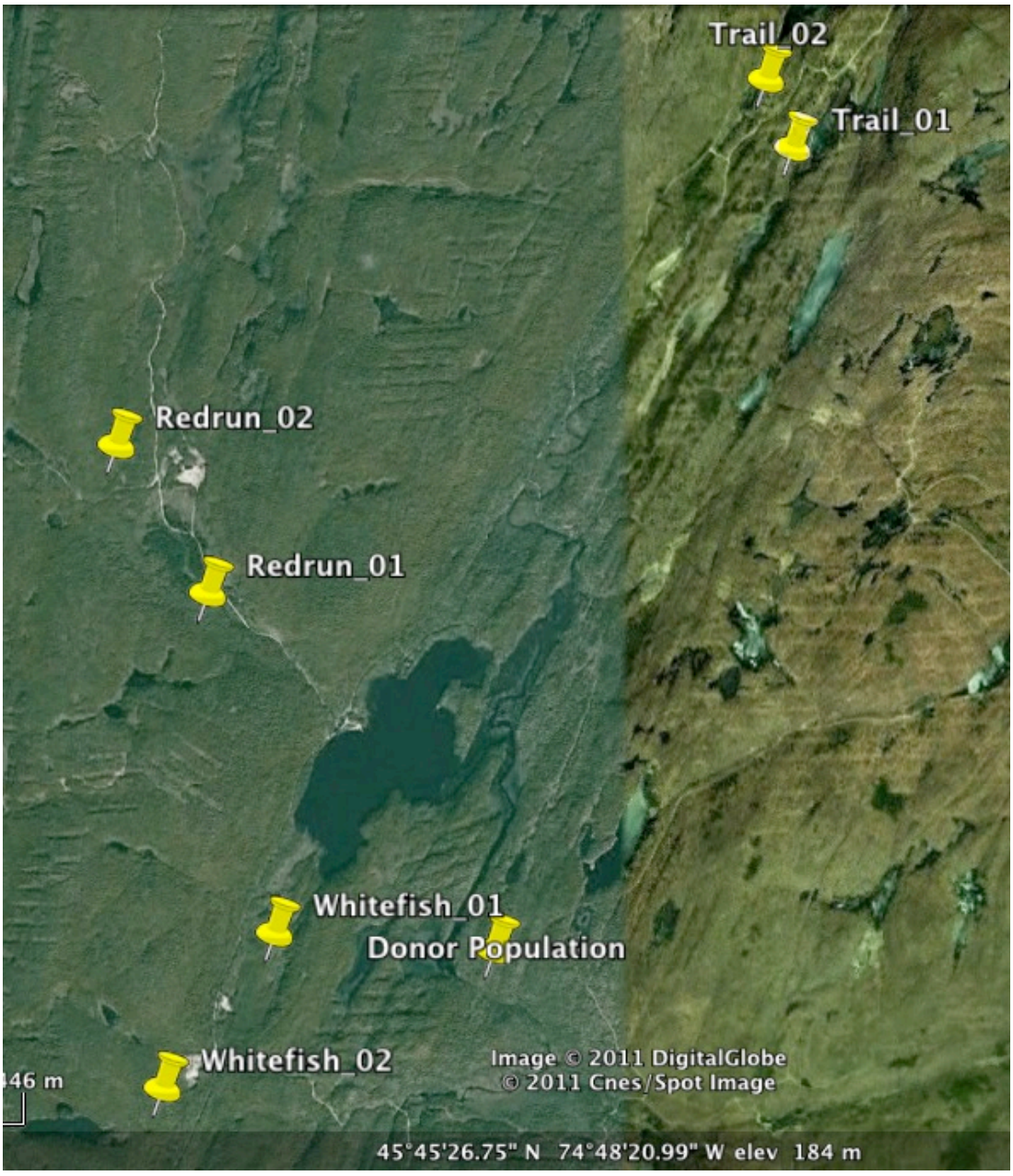


Figure 3.1 Location of experimental sites and donor population of *Trillium grandiflorum* in the Fairmont Kenauk ecological reserve near Montebello, Quebec.

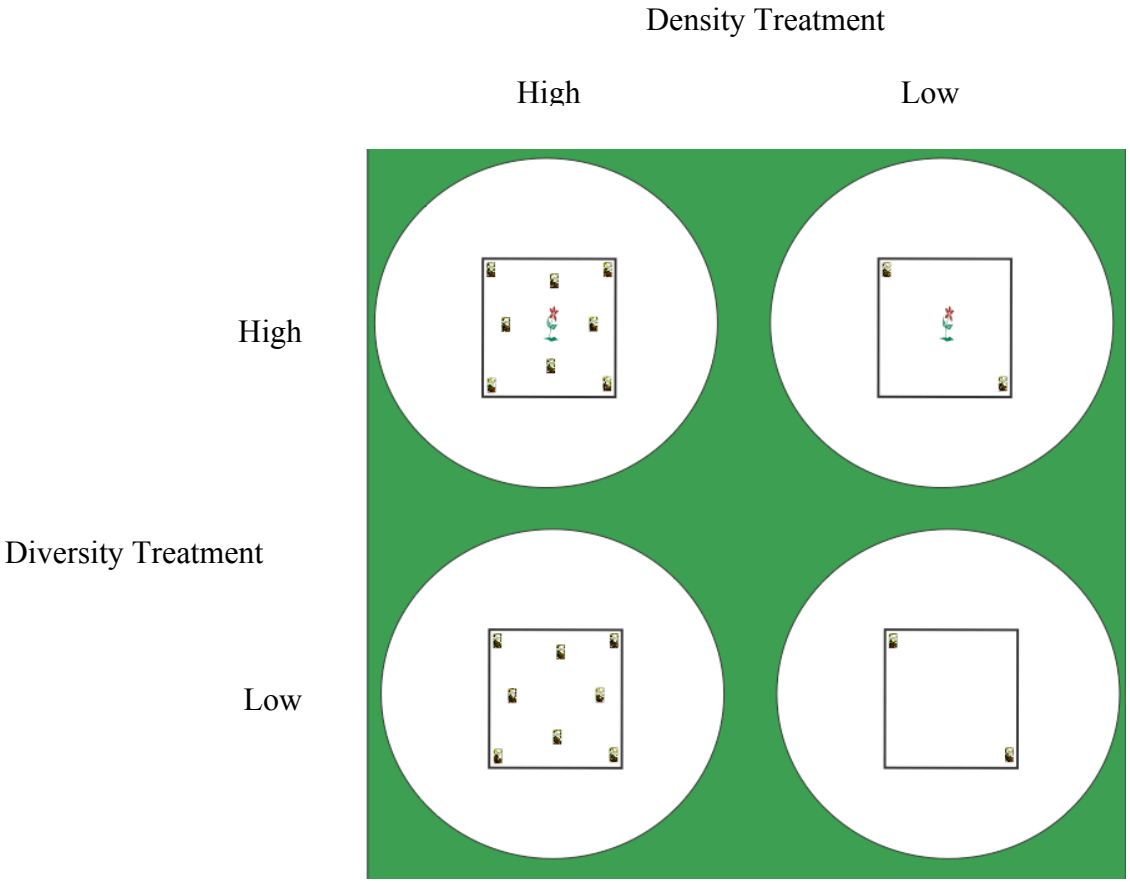


Figure 3.2 Summary of experimental design and factor levels. Levels of the diversity treatment are illustrated in the rows of the schematic while the levels of the density treatment are illustrated in the columns. The spatial arrangement of the white flowered plants in the density treatment illustrates the realized planting arrangement of *Trillium grandiflorum* individuals in the experimental plots. The red flower symbolizes the presence or absence of the co-flowering spring blooming community. The white circle represents the area of co-flowering species that was removed around each experimental plot regardless of diversity treatment. Refer to text for specifics regarding experimental plot size, spacing of plots and the diameter of the community clearing performed.



Figure 3.3 Photograph illustrating the flexible wire cages placed over transplanted *Trillium grandiflorum* individuals to prevent herbivory and trampling.



Figure 3.4 Photograph illustrating the bridal veil sacs placed over the developing capsule of *Trillium grandiflorum* individuals to prevent the loss of seeds by foraging ants.

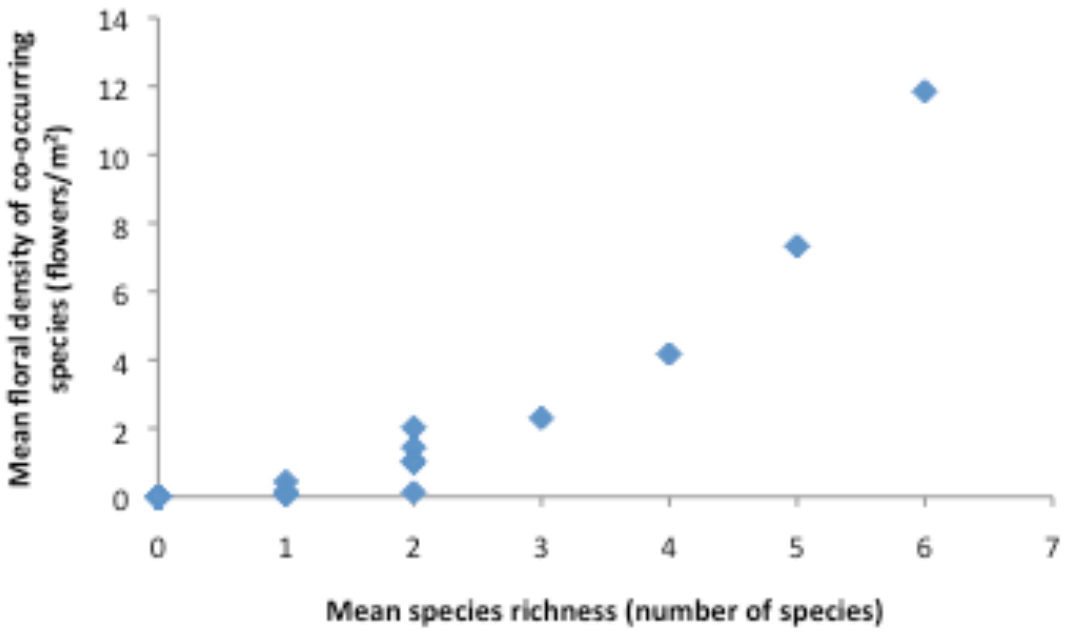


Figure 3.5 Relationship between mean species richness and mean floral density of co-occurring species (flowers/m^2) for six experimental sites located in the Fairmont Kenauk ecological reserve near Montebello, Quebec. At each site there were four experimental plots. A mean species richness of 0 represents the experimental plots assigned to the low community diversity treatment.

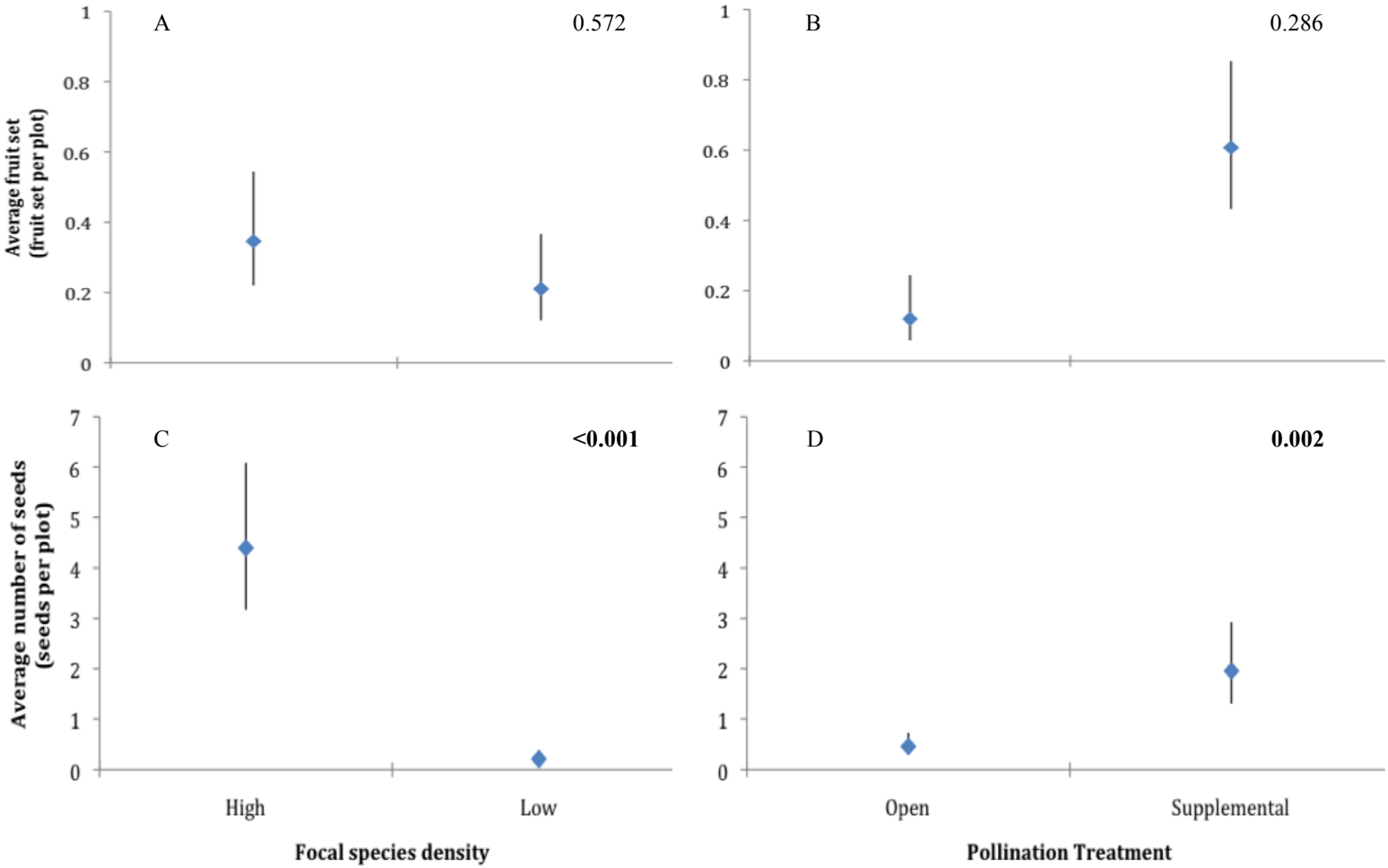


Figure 3.6 Back-transformed least square means (± SE) for log-transformed average fruit set per plot (yes (1) / no (0)) and average number of seeds per plot (number of seeds per fruit per plot) in relation to focal species density and pollination treatment for *Trillium grandiflorum* at four sites in the ecological reserve of the Fairmont Kenauk near Montebello, Quebec. Overall effects are shown in the upper right hand corner, and statistically significant effects are shown in bold typeface.

Chapter Four: Conclusions and future directions

4.1 Conclusions

In this thesis I have applied techniques from comparative biology to explore species level patterns in spring flowering communities that may have evolved as the result of pollinator-mediated interactions (Chapter 2) and undertaken an experiment to examine the importance of inter- and intraspecific pollinator-mediated interactions in spring flowering communities (Chapter 3). In doing so, I have examined both the contemporary effects of pollinator-sharing in mixed species communities and the evolutionary consequences of such.

While no chapter in this thesis directly supports either competition or facilitation of reproduction in spring flowering communities, Chapter 2 provides support for convergent evolution of traits that are associated with partitioning pollinator use in multi-species communities and, Chapter 3 highlights the importance of the local community on plant reproductive success. Together these results suggest that co-flowering and pollinator sharing in spring flowering communities have the potential to influence population dynamics and floral trait evolution.

The comparative study described in Chapter 2 confirms anecdotal evidence of correlated evolution among a spring flowering phenology and white or light floral colour, fleshy fruits, woody growth forms and understory occupation (Schemske et al. 1978, Bierzychudek 1982, Motten 1986, Jolls 2003, Whigham 2004). Evidence of adaption is frequently inferred from the repeated evolution of traits in given environment. Convergent evolution in spring flowering communities across disparately related taxa

suggest these correlated traits are in fact adaptations to the spring environment. However, it is also possible that the evolution of the correlated traits increases the likelihood of evolving a spring flowering phenology. Indeed, a more fully resolved phylogeny will prove useful in trying to distinguish the order of trait acquisition in spring flowering species. If correlated evolution among spring flowering species in their floral traits occurred as the result of pollinator sharing, this strongly suggests that the competitive paradigm (Mitchell et al. 2009) through which pollinator-mediated interactions are viewed may not always be ecologically appropriate, especially on evolutionary timescales.

My Chapter 3 results are both in agreement with and unique from what has previously been presented in the literature. While my experimental results underscore variation in reproductive success in *T. grandiflorum*, I was able to pinpoint population density (although not diversity) as an important influence on reproductive success in this system. My findings are contrary to those reported by Vamosi and colleagues (2006), which suggest that competition for pollination services drives pollen limitation in species rich communities. However, as Moeller (2004) describes, both the negative and positive effects of density may be exacerbated by interspecific interactions in species rich communities, and pollen limitation is likely influenced by both intraspecific species traits and those of the surrounding community. Whereas the importance of diversity has been illustrated in a variety of systems, my ability to detect a relationship between diversity and the reproductive success of *T. grandiflorum* may have been limited by small sample size. To further understand the relative importance of inter- and intraspecific interactions in pollinator-mediated reproduction, species addition experiments may prove useful.

Furthermore, to better understand the mechanisms underlying pollinator-mediated interactions studies examining pollinator foraging behaviour in experimental arrays would be helpful. Finally, linking changes in foraging behaviour to associated changes in plant reproductive success would be a significant contribution of our understanding of the role of inter- and intraspecific interactions in community ecology.

To date, mine is the first study attempting to account for the both number and abundance of species and how these two components of community diversity (Magurran 2004) can affect pollen limitation in mixed species communities. As such, this thesis is a novel contribution to the field of pollination biology for its integrated approach and will provide a good foundation for the future exploration of pollinator responses to population and community traits and their associated consequences on plant fitness.

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