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THE ECOLOGY OF POLLEN LIMITATION: COMPARATIVE AND EXPERIMENTAL APPROACHES

BY

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THE ECOLOGY OF POLLEN LIMITATION: COMPARATIVE AND EXPERIMENTAL APPROACHES

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ABSTRACT

Comparative and experimental approaches were used to explore factors contributing to pollen limitation in flowering plants. First, a comparative analysis of 224 species was conducted to test predictions concerning the association between eight floral and ecological traits and the intensity of pollen limitation. Whether or not the phylogenetic relationships among taxa were considered, the capacity to self-fertilize, short plant life spans, the presence of nectar and occurrence in temperate regions were associated with reduced pollen limitation. Second, intense pollen limitation was shown in Ontario populations of *Rhexia virginica* (Melastomataceae). Its poricidal anthers and low bumblebee visitation rates contributed to limited pollen removal from anthers, which provided a mechanism for levels of pollen limitation observed. Lastly, aspects of the reproductive biology of distylous *Primula mistassinica* (Primulaceae) were compared in island and mainland populations located on Lake Huron shorelines. Insularity had less influence on reproductive biology than proximate ecological factors within populations.

"But because being here is much, and because all this that's here, so fleeting, seems to require us and strangely concerns us. Us the most fleeting of all ..."

Rainer Maria Rilke, *The Ninth Elegy*, 1922

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CHAPTER ONE

GENERAL INTRODUCTION

"We emphasize the need for more broad-scale investigations
of the factors that limit plant fecundity."
S.D. Johnson and W.J. Bond (1997)

The majority of the world's flowering plant species depend on animal pollinators for sexual reproduction. In turn, these animals often depend on flowers as a source of energy and resources in the form of nectar and pollen. Both partners benefit from this association, but pollination is merely an incidental outcome of animal visits to flowers to procure resources. Plant fertility thus depends on the vagaries of mutualistic partners whose motivation for visiting flowers is not the efficient transfer of pollen between plants. This disparity between the requirements of plants and their pollinators has given rise to a diversity of floral adaptations for attracting pollinators and increasing the likelihood of cross-pollination. However, these adaptations cannot always guarantee pollinator visits, so the fertility of plants is often limited as a result of their reliance on animals as sexual agents.

The interdependence of plants and their pollinators has fascinated biologists since the time of Darwin, who published three books on plant reproductive biology (Darwin 1876, 1877, 1890). This fascination has motivated continued research that informs our general understanding of mutualistic interactions, which are prevalent in nature. Mutualisms are complex, because they involve a community of organisms interacting in a stochastic environmental context (Bronstein 1994). For this reason, pollination biology provides a rich area of enquiry into the general evolutionary mechanisms promoting the spread and maintenance of reproductive traits, and the current ecological processes that impinge upon their functioning (Lloyd and Barrett 1996).

Research into plant reproductive biology also serves two applied roles. First, it improves our ability to determine which pollination systems are most susceptible to disruption because of anthropogenic activities (Bond 1994; Kearns and Inouye 1997). A current theme in conservation biology is that the loss of ecological interactions such as pollination may be one of the greatest threats to biodiversity (Janzen 1974). Second, this research has implications for agronomic and forestry practices involving many domesticated plant species (Buchmann and Nabhan 1996; Kearns and Inouye 1997). The productivity of some crops can be greatly improved by an understanding of their

pollination biology, which can be used both to ensure adequate pollinator visitation and to select on floral traits that increase fertility.

Despite these motivations, our understanding of the reproductive biology of most flowering plants is sparse. We lack basic knowledge of the identity of pollinators for most flowering plants (Kearns and Inouye 1997). We have limited comprehension of the prevalence of generalized pollination systems, where the mutualism is obligate for neither plant nor pollinator, versus specialized systems, where the relationship is tightly co-evolved (Soulé and Kohm 1989; Waser *et al.* 1996). This restricts our ability to understand the remarkable diversity of plant sexual systems, despite active investigation of factors influencing their origin and current function (Lloyd and Barrett 1996). Furthermore, we lack general theories accounting for fertility in different plant species and different environments. This is largely the result of the stochasticity inherent in pollination systems. Pollinator visitation is inhibited by a variety of short-term ecological factors, which has obvious implications for plants that depend on pollinators for pollen transfer.

FACTORS LIMITING PLANT FERTILITY

Over evolutionary time, a variety of plant adaptations have arisen that affect the likelihood of animal pollination. Examples include traits that make flowers more noticeable, such as odours and colours that are attractive to pollinators, and the production of rewards such as nectar (Proctor *et al.* 1996). Despite these adaptations the fertility of plants is often sub-maximal. Many flowers do not produce fruits and many ovules are not fertilized to give seeds (Stephenson 1981; Sutherland 1986). In some cases, the production of flowers and ovules that are unlikely to be fertilized may be adaptive. They may indirectly contribute to either male (siring seeds) or female (setting seeds) fitness of other flowers on the plant. For example, if there are 'extra' flowers and ovules, (i) the inflorescence is larger and this may increase the probability of pollinator visits and hence fertility (pollinator attraction hypothesis - reviewed in Harder and Barrett 1996), (ii) more fruits and seeds can be matured during periods when pollinator visitation is unusually high (bet-hedging hypothesis - Ehrlén 1991; Burd 1995), (iii) male fitness

may be increased, even if female fertility plateaus when there are fewer flowers and ovules (male function hypothesis - Emms *et al.* 1997), and (iv) poor-quality fruits and seeds can sometimes be aborted to increase the overall quality of remaining fruits and seeds (selective abortion hypothesis - Stephenson and Winsor 1986; Rocha and Stephenson 1991).

There are also two proximate mechanisms that account for low fertility in flowering plants. Pollen limitation of fertility reflects inadequate pollen delivery by pollinators, and is the theme of this thesis. Resource limitation of fertility indicates that there are insufficient resources available for all fruits and seeds to be matured. It is likely that both of these concurrently limit plant fertility (Haig and Westoby 1988; Campbell and Halama 1993; Burd 1994; Wilson *et al.* 1994). The empirical requirements for differentiating the two are therefore demanding (Zimmerman and Pyke 1988), especially given that pollen limitation may be assessed at various structural (ovule, flower, plant, and population) and temporal (year and lifetime) scales. Historically, pollen limitation was demonstrated when supplementation of pollen delivery to a flower increased its fertility (fruit or seed set) relative to an open-pollinated control. However, if pollen is added to only one or a few flowers per plant, an increase in fertility may occur at the expense of other flowers on the plant. To assess whether whole-plant fertility is pollen-limited, pollen can be added to all flowers on treatment plants (Horvitz and Schemske 1988; Johnston 1991). Although this treatment can be used to demonstrate annual pollen limitation, an increase in fertility may result from utilization of resources that could otherwise have been used in subsequent years (Janzen *et al.* 1980; Bierzychudek 1981). Assessing lifetime pollen limitation in long-lived plants is intractable empirically because many details of life history must be known to fully assess the effect of pollen supplementation on fitness and population growth (Calvo and Horvitz 1990; Calvo 1993; Ehrlén and Eriksson 1995). These considerations illustrate that many previous studies were inadequate for assessing lifetime pollen limitation. Nonetheless, pollen limitation commonly limits fertility at least at the level of individual flowers (Burd 1994).

Pollen limitation likely results from interactions between features of a plant's life history and levels of pollinator activity. For example, floral traits to some extent represent adaptations to ancestral pollinator visitation rates. However, this does not guarantee maximal fertility because fluctuation of ecological factors influences visitation rates on a short-term scale (Burd 1995). This stochasticity limits fertility, even if floral traits reflect the long-term average visitation rate. Furthermore, adaptation of floral traits to prevailing pollinator visitation rates may to some extent be phylogenetically and genetically constrained (but see Galen 1996).

Pollinators may limit plant fertility in two main ways. Typically, pollen limitation occurs because pollinator visits are infrequent, so the quantity of pollen transferred limits fertility (insufficient pollen transfer *sensu* Harder and Barrett 1996). Infrequent visitation may result from either poor weather that affects the ability of pollinators to forage or simply because pollinators are uncommon. Pollinators may be uncommon because of annual variation in their population sizes, or in association with certain habitats (McCall and Primack 1992; Johnson and Bond 1997) or disruptive human activities (Powell and Powell 1987; Jennersten 1988; Aizen and Feinsinger 1994a, b; Kearns and Inouye 1997). Pollinator visitation may also be infrequent when plant population sizes are small (Sih and Baltus 1987; Lamont *et al.* 1993). Alternatively, plant fertility may be limited by the quality of pollen deposited on stigmas (inefficient pollen transfer *sensu* Harder and Barrett 1996). Some species can self-fertilize autonomously in the absence of pollinators, but this may result in reduced fitness because of inbreeding depression. Similarly, pollen transfer within flowers, between flowers on a plant or between ramets of a clone may allow some fruit and seed set in self-compatible species, but this may be offset by subsequent inbreeding depression. The quality of pollen deposited may also limit the fertility of self-incompatible species. For example, if pollen grains that are deposited have an *S* allele in common with the stigma in species with multiallelic sporophytic incompatibility systems, fertility may be low despite sufficient pollen deposition (Les *et al.* 1991; Aspinwall and Christian 1992; DeMauro 1993; Byers 1995).

THESIS OBJECTIVES

In this thesis, both comparative and experimental approaches are used to explore the relation between pollen delivery by pollinators and the fertility of flowering plants. In particular, factors that contribute to pollen limitation are investigated. We are presently unable to predict the occurrence or intensity of pollen limitation in flowering plants. For this reason, I consider the contribution of both floral features and ecological conditions to pollen limitation. We also have limited knowledge of the mechanistic linkage between pollinator visitation, pollen transport and pollen limitation. Therefore, a specific mechanism accounting for pollen limitation is investigated in Chapter 3.

The main questions addressed in the three chapters that follow are:

Chapter 2: *Do life history and ecological features affect the intensity of pollen limitation among species of flowering plants?* This question has not previously been investigated using comparative methods that account for phylogenetic relationships among taxa. I investigated this problem by analyzing whether the degree of pollen limitation in flowering plant species was influenced by their character state for four floral traits, two life history attributes, and two contrasting ecological conditions.

Chapter 3: *Does restricted pollen removal provide a potential mechanism for pollen limitation in flowering plants?* Pollen limitation is typically viewed from the female perspective of inadequate pollen deposition. The possibility that pollen removal *per se* limits fertility, which is an explicitly male perspective, has not previously been investigated empirically. To address this question, experiments were conducted within populations of *Rhexia virginica* (Melastomataceae) in the Muskoka region of Ontario. These populations provided a suitable model system because the pollination of *R. virginica* is relatively specialized and the species is at its range limit in Ontario. The floral biology, pollination biology, and prevalence of pollen limitation in this system were documented. This allowed patterns of pollen limitation to be related to potential mechanisms accounting for inadequate pollen transfer.

Chapter 4: *Do ecological differences between mainland and nearshore island populations of flowering plants cause divergence in their reproductive biology?* Colonization of islands and environmental differences between islands and the adjacent mainland are likely to influence floral traits (reviewed in Barrett 1996). Comparison of plant fertility in mainland and island populations thus contributes to our understanding of how ecological conditions affect fertility. Aspects of the reproductive ecology of populations of *Primula mistassinica* (Primulaceae) on mainland Bruce Peninsula, Ontario and the adjacent Tobermory Islands were investigated. *Primula mistassinica* is a suitable model system because of its reproductive biology and presence on many of the Tobermory Islands. The species is self-incompatible and therefore dependent on pollinators for fruit set. Furthermore, its distylous floral syndrome allows style-morph ratios to be used as a metric of the frequency of self-fertilization in mainland and island populations.

Chapters 2-4 were written as research papers for publication in the primary scientific literature. Hence, a certain amount of repetition occurs among them.

CHAPTER TWO

A COMPARATIVE ANALYSIS OF

POLLEN LIMITATION IN ANGIOSPERMS

"Future studies ... will allow us to discover whether the degree of pollen limitation varies with mating system, pollinator type, habitat marginality, and time."

M.O. Johnston (1991)

ABSTRACT

A comparative analysis was conducted to determine whether there are floral and ecological correlates of the intensity of pollen limitation among flowering plant species. Pollen limitation in 224 species previously studied was characterized by an index equal to 1 - (percent fruit set of open-pollinated control plants / percent fruit set of treatment plants that received supplemental cross-pollen). To test eight predictions concerning the occurrence of pollen limitation, each species was classified categorically for four floral traits (self-compatible vs. self-incompatible, autogamous vs. non-autogamous, specialized vs. unspecialized floral morphology, nectariferous vs. nectarless), two life history attributes (monocarpic vs. polycarpic, herbaceous vs. woody) and two ecological conditions (occurrence in open vs. forested habitats and temperate vs. tropical biomes). Pollen limitation indices of species in contrasting categories were compared using non-parametric tests (TIPs analysis). This analysis could be misleading because species share evolutionary history and are not entirely independent. Therefore, phylogenetically independent contrasts (PICs), based on the Chase *et al.* (1993) *rbc*L phylogeny of the angiosperms, were also calculated for each comparison. PICs compared pollen limitation indices of pairs of taxa that differed in the trait under investigation and were phylogenetically independent of other pairs. The results of TIPs and PICs analyses were generally congruent, suggesting that the influence of phylogenetic history on degree of pollen limitation was not severe. In both analyses, pollen limitation was less intense in species that were self-compatible, autogamous and monocarpic. TIPs analysis demonstrated that herbaceous, nectariferous, and temperate species were less likely to be pollen-limited, but using PICs this could only be corroborated among self-incompatible species. Species of open and forested habitats, and with specialized vs. unspecialized floral morphology, did not differ markedly in degree of pollen limitation using either method. None of the traits were singularly unambiguous predictors of pollen limitation, possibly reflecting its stochasticity. Potential explanations for the observed patterns, limitations of the analyses, and alternative predictors of the intensity of pollen limitation among flowering plant species are also discussed.

INTRODUCTION

Angiosperms commonly mature many fewer fruits and seeds than the flowers and ovules they produce. Two proximate ecological mechanisms that may account for this pattern are insufficient pollen delivery and limited resources for maturation of fruits and seeds (reviewed in Burd 1994). Flowers and ovules that remain unfertilized may also indirectly increase the fertility of other flowers on the plant (reviewed in Sutherland 1986). For example, unsuccessful flowers still serve an attractive function, which may augment pollinator visitation to the inflorescence as a whole, and hence increase plant fertility. These and other explanations have been used to account for patterns of fruit and seed set within populations of a particular species, but we still have limited ability to predict which species in which environments will have low fertility (Johnston 1991; Johnson and Bond 1997). In this chapter, comparative methods are used to identify floral and ecological correlates of the degree of pollen limitation in a survey of angiosperm species.

Pollen limitation occurs when the female fertility of a plant is limited by insufficient pollen delivery. This is demonstrated empirically when supplemental hand pollination of flowers increases their fertility compared to open-pollinated controls. In 62% of species that have been studied experimentally, pollen supplementation increased fertility (Burd 1994). Resource limitation has historically been considered an alternative explanation for this result (Bateman 1948), but it is likely that both pollen and resource limitation concurrently mediate fertility (Haig and Westoby 1988; Campbell and Halama 1993; Burd 1994; Wilson *et al.* 1994). For example, when seed set is the outcome of a trade-off between the cost of structures needed to attract pollinators and resources needed to mature fruits and seeds, pollen acquisition and resources might be expected to equally limit reproductive success (Haig and Westoby 1988). Recognition that both pollen delivery and resources limit fertility has led to methods for teasing apart the two (Zimmerman and Pyke 1988; Johnston 1991; Lawrence 1993; Ehrlén and Eriksson 1995). These methods acknowledge that pollen limitation can be interpreted at various structural

(ovule, flower, plant and population) and temporal (year and lifetime) scales, owing to the modular construction of plants.

One approach to predicting the occurrence of pollen limitation is to investigate whether there are interspecific patterns in its occurrence. There have been two previous comparative analyses of fertility in angiosperms. Ecological and life history traits affecting fruit set were examined in a survey of 447 species by Sutherland (1986), who proposed several adaptive explanations for the observed patterns. More recently, Burd (1994) has reviewed the results of experimental studies of pollen limitation conducted on 258 species. He concluded that pollen limitation is frequent, but spatially and temporally variable. In common with much earlier comparative ecological research, these two surveys did not account for the phylogenetic relationships among the taxa considered.

Species are linked by their phylogenetic history, so it may be improper to treat them independently for statistical analyses. An association between traits X and Y among a group of species may simply be an artifact of common ancestry (Harvey and Pagel 1991). Until recently, there was no objective method to account for the phylogenetic relationships among flowering plants. The recent publication of a family-level phylogeny for the angiosperms (Chase *et al.* 1993) allows phylogenetically independent contrasts (hereafter PICs) to be used to reconcile the non-independence of species (Felsenstein 1985; Harvey and Pagel 1991; Purvis and Rambaut 1995). This method uses a phylogenetic tree to ensure that only divergences between sister taxa contribute to statistical tests. Trait differences between these taxa (contrasts) have evolved since their common origin and in concert with any change in the trait of interest. PICs are therefore independent of one another, so *P*-values can be used to test assertions that changes in one variable are associated with changes in another (Silvertown and Dodd 1996).

There is currently debate, however, about whether data for individual species (hereafter TIPs) or PICs should be used to test for correlated trait evolution (Harvey *et al.* 1995; Westoby *et al.* 1995). Although TIPs analyses may inflate statistical degrees of freedom and increase the likelihood of Type I error, PICs decrease statistical power, make assumptions about evolutionary history, and assume that phylogeny has a greater

influence on trait expression than selection in current environments (Westoby *et al.* 1995, 1996; Barrett *et al.* 1996). In the present context, this reduces to the question of whether pollen limitation is determined more by membership within a particular clade than by the environmental milieu that a species occupies. If one is interested in whether an association is a common selective outcome that has evolved independently multiple times, then it is important to account for phylogeny. On the other hand, if one assumes that the degree of pollen limitation is influenced by contemporary ecological conditions, phylogenetic correction may be less important. In fact, these alternatives are not strictly dichotomous because related species are more likely to inhabit similar environments (phylogenetic niche conservatism; Harvey and Pagel 1991; Leishman *et al.* 1995; Barrett *et al.* 1996). That this is common is supported by meta-analyses of comparative studies in vertebrates that have shown a strong correlation between results obtained using TIPs and PICs (Ricklefs and Starck 1996; Price 1997). These reviews advocate use of both types of analysis because differences between them may provide insight into the tempo of evolutionary change in traits under consideration.

In this chapter, I use both TIPs and PICs to test predictions from several hypotheses concerning pollen limitation in angiosperms. Data on pollen limitation in 224 species from 64 families are used to test these predictions.

Hypothesis 1: Species with the capacity to self-fertilize will exhibit less pollen limitation than species that cannot self-fertilize. The first prediction is that self-compatible species will exhibit less pollen limitation than those that are self-incompatible because an additional source of pollen, self-pollen, can potentially contribute to their fertility. Burd's (1994) analysis supported this prediction. In the absence of pollinators some self-compatible species cannot self-fertilize. Self-compatibility only provides reproductive assurance if plants can self-fertilize autonomously (autogamous self-fertilization) (Lloyd 1992; Lloyd and Schoen 1992). Autogamy has often been proposed as adaptive where pollinator visitation is unreliable (Hagerup 1951; Baker 1955; Kevan 1972; Motten 1986; Bond 1996). Therefore, the second prediction is that autogamous

species will exhibit less pollen limitation than self-compatible species incapable of autogamy.

Hypothesis 2: Species with short life spans will exhibit less pollen limitation than longer-lived species. Pollen limitation may greatly reduce lifetime fitness of short-lived species, so selection should be stronger on traits minimizing pollen limitation. In contrast, fitness is accrued over multiple years in longer-lived plants so reduced fertility in one year may not lower fitness to the same extent as in short-lived species (Calvo and Horvitz 1990; Primack and Hall 1990; Zhang and Wang 1994). Two predictions derived from these considerations are that (i) monocarpic species will exhibit less pollen limitation than polycarpic species, and (ii) herbaceous species will exhibit less pollen limitation than woody species. The latter prediction assumes that most herbs have shorter life spans than woody species.

Hypothesis 3: Species with flowers that are visited more frequently and by a greater diversity of pollinators will exhibit less pollen limitation than those less likely to be visited. Two predictions following from this hypothesis are that (i) species with "unspecialized" floral morphology will exhibit less pollen limitation than those with "specialized" flowers, and (ii) nectariferous species will be less pollen-limited than nectarless ones. The former premise is supported by higher insect visitation rates to open versus tubular flowers in deciduous woodland and alpine tundra in North America and the fynbos of South Africa (McCall and Primack 1992). The latter premise is supported by higher visitation rates to nectariferous vs. nectarless species blooming in the spring in North Carolina (Motten 1986) and the higher pollination success (percentage of flowers with pollen on stigmas) of nectariferous orchids in the Cape region of South Africa compared to orchids that produce no nectar (Johnson and Bond 1997).

Hypothesis 4. Species that occur in contrasting ecological conditions will exhibit different levels of pollen limitation. The first prediction is that species occurring in open habitats will be less pollen-limited than those of forested habitats. Insect thermoregulatory capacity is higher in open habitats than shaded ones so visitation rates to flowers may be greater (Heinrich 1979b). Plants that occur in tropical regions

generally occur at lower densities than those in temperate regions (Baker 1959; Fedorov 1966). If one assumes that plants occurring at low densities are visited less often than those at higher densities (Antonovics and Levin 1980), one might predict that species occurring in temperate biomes will exhibit less pollen limitation than those in tropical biomes.

At the outset of this study, it was recognized that these comparative analyses and their interpretation are likely to be rudimentary. Pollen limitation has been investigated in a relatively small number of species and the methods used to detect it have varied extensively. Hypotheses and predictions other than those above could easily be proposed, and some of these are considered in the discussion. Nevertheless, a comparative analysis at this time was considered valuable if only to stimulate additional future research on the correlates of pollen limitation.

MATERIALS AND METHODS

POLLEN LIMITATION INDEX

An index of pollen limitation was calculated from experimental data in the literature, to characterize pollen limitation in flowering plants that have been studied. The index was equal to 1 – (the percent fruit set of open-pollinated controls relative to plants that received supplemental cross-pollen). An index of 0.0 indicates that there was no pollen limitation in the population under study. Most data used to calculate the indices were obtained from the survey in Burd (1994), and involved data on pollen limitation of fruit set for 207 species in 70 families. Indices based on data published since his review were calculated for an additional 34 species, including four new families (Appendix 1). Pollen limitation has been investigated in more than one year for 31 species and at more than one location for 29. These data were pooled to give a single index of pollen limitation for each species. The index was given a lower bound of zero because negative indices (greater fertility in controls than after outcrossing) likely result from experimental or Type I statistical error (Young and Young 1992; Burd 1994) and are not informative in

the current context. Percent fruit set was used as the measure of fertility because pollen limitation has most often been measured in terms of fruit set. The next largest dataset, seeds per fruit, was too small for meaningful comparative tests. Nevertheless, pollen limitation indices of percent fruit set and seeds per fruit for species analyzed were significantly correlated ($N = 83$, $r = 0.37$, $P < 0.001$).

Trait Categorization

The self-compatibility of each species was classified categorically according to information in the original papers. In some cases the authors simply stated whether or not a species was self-compatible, and this categorization was used for the analyses. Otherwise, a self-compatibility index equal to the ratio of fruit set after selfing to that after cross-pollination was calculated. For most analyses, self-incompatible species were defined as those having self-compatibility indices less than 0.05 (following Burd 1994). To validate this cut-off value, a separate analysis was performed in which self-incompatible species were defined as those with self-compatibility indices less than 0.50.

Each species was coded categorically for the other traits under investigation based on information in the original papers or Mabberley (1987). It was not possible to code the potential for autogamy, the presence or absence of nectar, and habitat type for every species. Autogamous species were defined as self-compatible species having greater than 20% fruit set in bagged relative to outcrossed treatments. *Agave*, *Argyroxiphium* and palms are not truly woody, so they were excluded from the herbaceous-woody comparison. Temperate species were broadly defined to include those in the Mediterranean, the Cape of South Africa, New South Wales in Australia, and a few montane and arctic species. Floral specialization was classified according to whether floral morphology restricts pollinator access to floral rewards. "Specialized" flowers were defined as those with narrow tubular flowers or complex morphologies (e.g., Orchidaceae) that can only be accessed by a subset of the pollinator fauna. "Unspecialized" flowers were those with broad open tubes, bowl-shaped flowers or small clusters of flowers (e.g., Asteraceae) that could potentially be visited by a wide range of

insect groups. The pollen limitation index and trait codings for each species in the analysis are presented in Appendix 2.

COMPARATIVE ANALYSIS USING TIPS

A comparative analysis was first conducted in which each species was treated independently. The pollen limitation indices for each trait were plotted as histograms and analyzed for uniformity using χ^2 tests. The predictions outlined in the introduction were tested by pairwise comparisons using non-parametric Wilcoxon two-sample tests (Sokal and Rohlf 1995). Different types of tests were used depending on the degree of certainty in the predictions. In most cases, one-tailed tests were used to increase power. The expectations for tests of herbaceous vs. woody species, tropical vs. temperate species and species occurring in forested vs. open habitat were less certain. In these cases, asymmetrical critical regions and directed tests were used (Rice and Gaines 1994). Directed tests provide much of the increased power of a one-tailed test, but allow detection of significant results in the "unexpected" direction. All statistical analyses were conducted using JMP (Version 3.0.2, SAS Institute, 1994).

Pollen limitation is likely influenced by several traits acting simultaneously. To investigate the relative contribution of the traits considered here to variation in the pollen limitation index, a multi-factor ANOVA was conducted. The six main effects were self-compatibility, herbaceousness, specialization of floral morphology, presence of nectar, biome type and habitat type. Only second-order interactions were included in the model, because the data were too unbalanced for calculation of higher-order interactions. Calculation of *P*-values requires the assumption that data are independent (Sokal and Rohlf 1995), but they are determined here for heuristic purposes.

PHYLOGENETIC PLACEMENT OF TAXA

To compute PICs, the phylogenetic relationships among species in the database were determined (Purvis and Rambaut 1995). Taxa were coded phylogenetically by first positioning families according to the Chase *et al.* (1993) *rbcL* phylogeny of seed plants, as used in several recent comparative analyses (Barrett *et al.* 1996; Rees 1996; Silvertown

and Dodd 1996). Ten families in the Burd database (Cactaceae, Cistaceae, Cochlospermaceae, Guttiferae (Clusiaceae), Hippocastanaceae, Hippocrateaceae, Loasaceae, Salicaceae, Staphyleaceae, and Thymelaeaceae) were not located on the tree, so they were excluded, leaving 224 species. Species placed in the Vivianaceae, Lobeliaceae, and Amaryllidaceae (*Alstroemeria*) by Burd (1994) were instead placed in the Geraniaceae, Campanulaceae, and Alstroemeriaceae, respectively, to correspond with nomenclature used for the Chase *et al.* phylogeny (Cronquist 1981). Some families were polyphyletic, but genera from the database were consistently placed in one clade either because they were present on the Chase *et al.* (1993) tree or were closely allied to a genus that was (Mabberley 1987; J. E. Eckenwalder pers. comm.). This was only problematic in Liliaceae, so for this family the tree was modified by grafting the Chase *et al.* (1995) *rbc*L phylogeny of Lilianae onto the original tree to facilitate placement of families in this group. In particular, genera of Liliaceae *s. l.* were split into Alliaceae, Convallariaceae, Liliaceae, Melanthiaceae, Trilliaceae and two clades of the polyphyletic Uvulariaceae.

Given the familial tree, relationships between species were coded to minimize the number of polytomies. PICs can be calculated despite polytomies, but the power of tests is reduced (Purvis and Rambaut 1995). Resolution of the phylogeny was facilitated by recently published phylogenies for the Amaryllidaceae (Meerow 1995), Asteraceae (Bremer 1994), Fabaceae (Doyle 1995), and Orchidaceae (Dressler 1993). Phylogenies were unavailable for most families, but placement of taxa according to traditional tribes (from Dahlgren *et al.* 1985; Mabberley 1987) and genera provided additional structure. Most families contained few taxa, so the final tree had no polytomies containing more than four genera.

Comparative Analysis using PICs

A comparative analysis was conducted using PICs based on the inferred tree. PICs were computed using the computer program CAIC (Purvis and Rambaut 1995). Branch lengths were set equal, which assumes a punctuational mode of evolution that generally performs better in simulations than other models (Purvis and Rambaut 1995). Branch lengths reported in the Chase *et al.* (1993) phylogeny were not used because *rbc*L

evolution occurs at different rates among angiosperm lineages (Clegg 1993). Because the pollen limitation index was bounded at zero and one, analyses with continuous independent variables did not meet assumptions of the independent contrasts method regardless of transformations (Garland *et al.* 1992; Díaz-Uriarte and Garland 1996). Analyses with categorical predictor variables ("Brunch" option) did meet these assumptions and were used herein.

With categorical variables, the independent contrasts method compares the pollen limitation indices of pairs of taxa that differ in the predictor variable and are phylogenetically independent of other pairs (see Purvis and Rambaut 1995 for details). Consistently higher indices in taxa with one character state indicate that evolution of this character is associated with increased pollen limitation. The distributions of the PICs were normal (Wilk-Shapiro $P > 0.05$) so a *t*-test on their mean was used to detect a significant deviation from zero (Purvis and Rambaut 1995). This deviation would indicate that changes from one character state to another are accompanied by a consistent shift in the degree of pollen limitation.

RESULTS

COMPARATIVE ANALYSIS USING TIPS

The mean pollen limitation index of species in the analysis was 0.40 (SE = 0.022). The TIPs analysis of pollen limitation in angiosperms supported seven of the eight predictions outlined in the introduction. Species that were self-compatible, autogamous, monocarpic, herbaceous, nectariferous or that occurred in open habitats or temperate regions were less likely to exhibit pollen limitation than species with contrasting character states (Table 2.1; Figures 2.1 and 2.2). Pollen limitation indices of species with specialized flowers did not differ from those with unspecialized flowers ($P > 0.30$). There was extensive variation in the degree of pollen limitation in all traits considered (Figures 2.1 and 2.2). This indicates that single traits explain only a small proportion of the variation in pollen limitation.

Table 2.1. Mean pollen limitation index, standard errors and sample sizes for traits considered in the TIPs analysis. Pollen limitation is expected to be lower in the trait listed first for each hypothesis. Distributions of the index for each class of species are given in Figures 2.1 and 2.2.

Hypothesis	Trait	Mean pollen limitation index	Standard error	Sample size
1a.	Self-compatible	0.31	0.03	135
	Self-incompatible	0.59	0.04	66
1b.	Autogamous	0.16	0.04	35
	Non-autogamous	0.38	0.03	97
2a.	Monocarpic	0.16	0.05	16
	Polycarpic	0.42	0.02	208
2b.	Herbaceous	0.32	0.03	133
	Woody	0.52	0.04	86
3a.	Unspecialized flowers	0.38	0.03	130
	Specialized flowers	0.42	0.03	94
3b.	Nectariferous	0.35	0.03	91
	Nectarless	0.47	0.05	42
4a.	Temperate	0.30	0.02	139
	Tropical	0.56	0.04	85
4b.	Open habitats	0.33	0.03	82
	Forested habitats	0.42	0.03	108

Figure 2.1. Frequency distributions of pollen limitation indices for species (TIPs) in different classes of plants: A) self-compatible vs. self-incompatible; B) autogamous vs. non-autogamous; C) monocarpic vs. polycarpic and D) herbaceous vs. woody. Sample sizes are given in Table 2.1. Each distribution is non-uniform ($P < 0.05$) based on χ^2 tests with four degrees of freedom. Pollen limitation is less severe (lower indices) in the classes indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$) based on one-tailed and directed (herb-woody comparison) Wilcoxon two-sample tests.

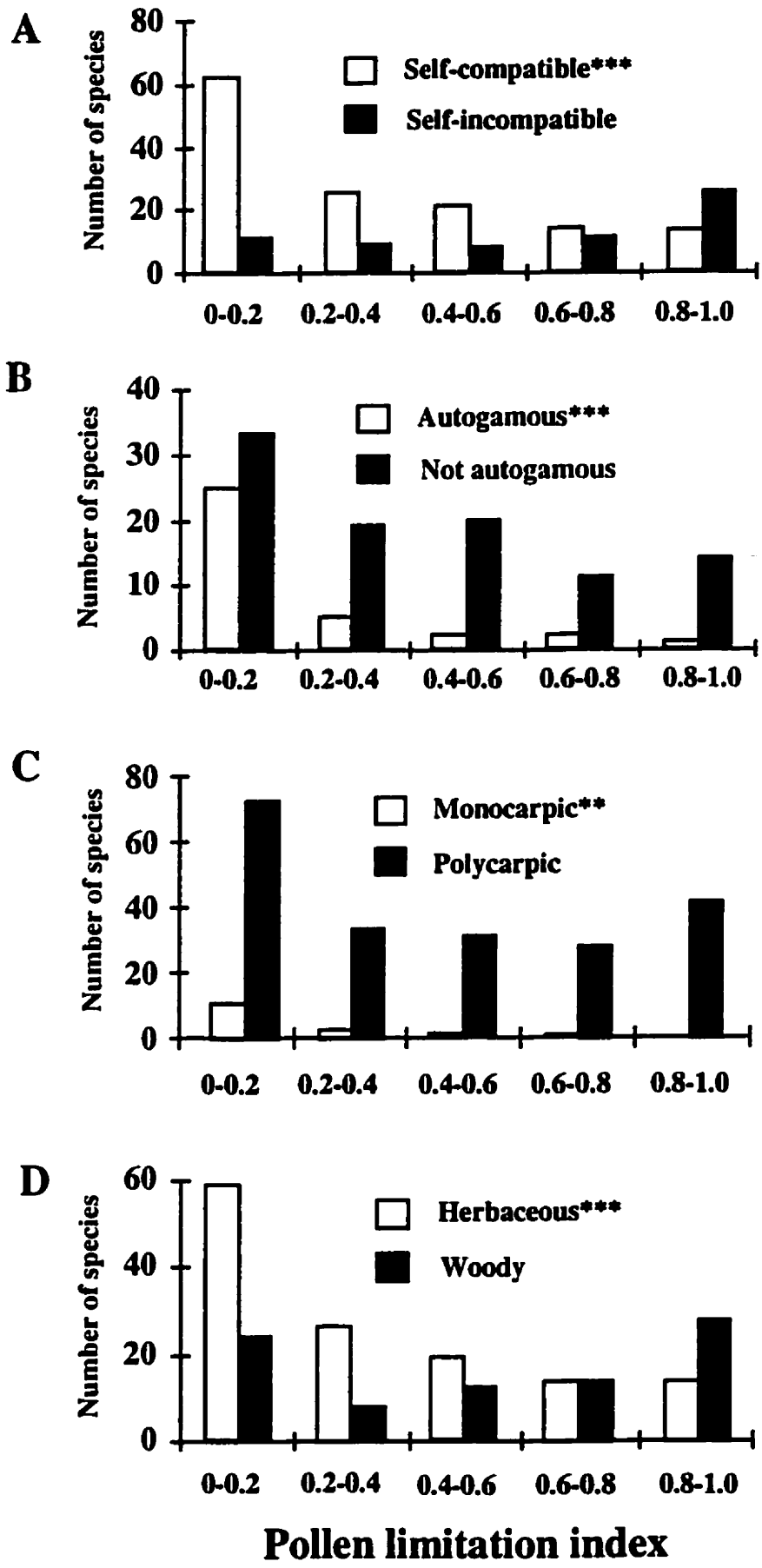
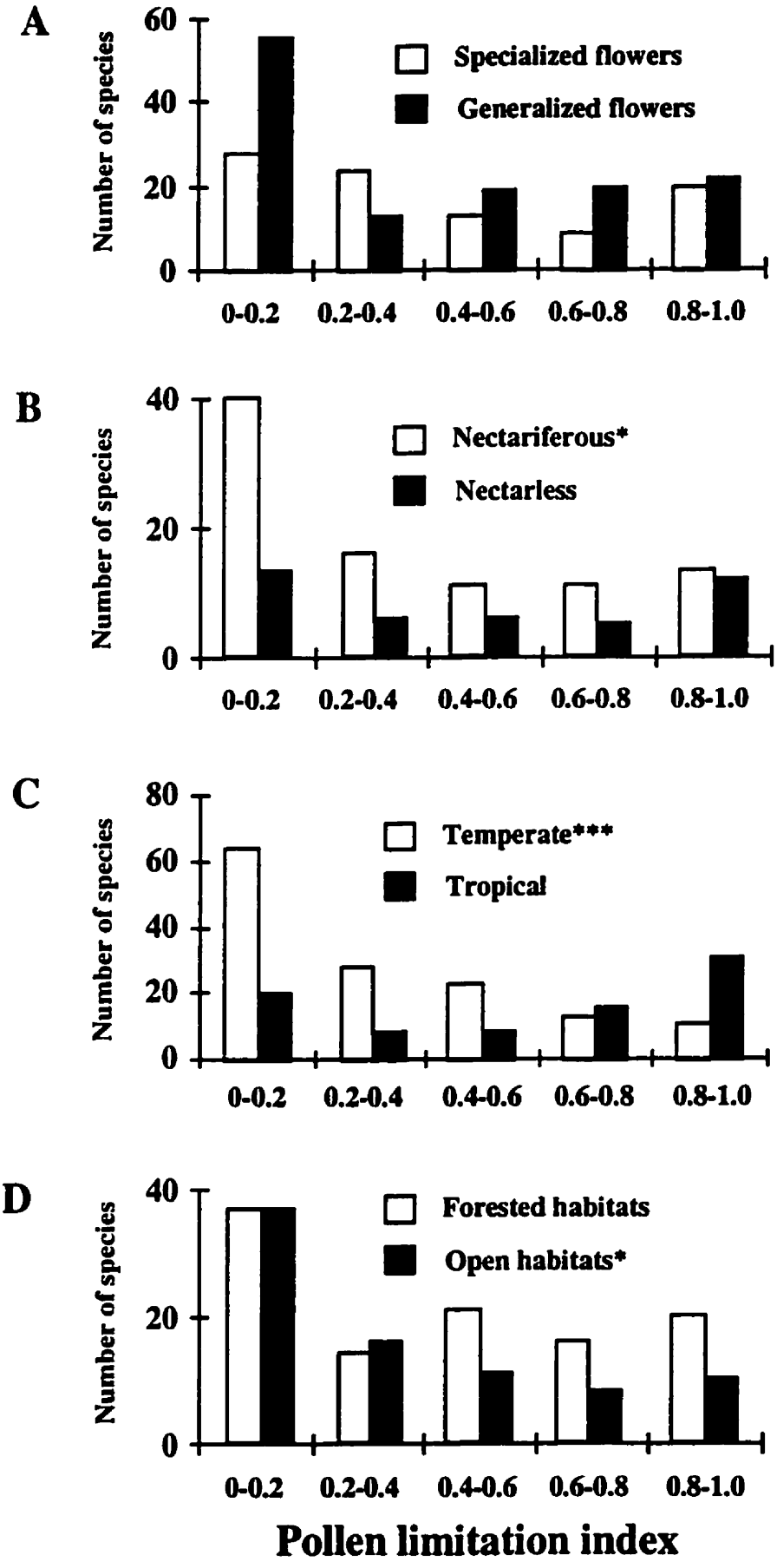

A
Number of species
80
60
40
20
0
Self-compatible***
Self-incompatible
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
B
Number of species
40
30
20
10
0
Autogamous***
Not autogamous
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
C
Number of species
80
60
40
20
0
Monocarpic**
Polycarpic
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
D
Number of species
60
40
20
0
Herbaceous***
Woody
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
Pollen limitation index

Figure 2.2. Frequency distributions of pollen limitation indices for species (TIPs) in different classes of plants: A) species with specialized flowers vs. those with generalized flowers; B) nectariferous vs. nectarless; C) temperate vs. tropical; and D) species occurring in forested habitats vs. those occurring in open habitats. Sample sizes are given in Table 2.1. Each of the distributions, except the one for nectarless species, is non-uniform ($P < 0.05$) based on χ^2 tests with four degrees of freedom. Pollen limitation is less severe (lower indices) in the classes indicated by asterisks (*** $P < 0.0001$, * $P < 0.05$) based on one-tailed and directed (temperate-tropical and forested-open habitat comparisons) Wilcoxon two-sample tests.


A
Number of species
60
40
20
0
Specialized flowers
Generalized flowers
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
B
Number of species
40
20
0
Nectariferous*
Nectarless
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
C
Number of species
80
60
40
20
0
Temperate***
Tropical
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
D
Number of species
40
20
0
Forested habitats
Open habitats*
0-0.2
0.2-0.4
0.4-0.6
0.6-0.8
0.8-1.0
Pollen limitation index

The multifactorial model accounted for 51.6% of the variation in degree of pollen limitation among species that could be classed for each of the six characters (Table 2.2). The only significant main effects were compatibility, presence of nectar and biome type. None of the second-order interactions were significant ($P > 0.10$).

COMPARATIVE ANALYSIS USING PICS

PICs confirmed four of the seven patterns found in the comparative analysis based on TIPs. There was a lower likelihood of pollen limitation in self-compatible, monocarpic and herbaceous species (Table 2.3). Pollen limitation was marginally less frequent in autogamous than non-autogamous species ($N = 26$, $P < 0.057$). Results were robust to changes in the cut-off value defining self-compatibility. A less stringent definition of self-incompatibility (self-compatibility index less than 0.50) confirmed the finding that self-compatible species were less likely to be pollen-limited (37 contrasts, $\overline{X}$ ($\pm$ SE) = 0.086 ($\pm$ 0.026), $t = 3.26$, $P_{1\text{-tailed}} < 0.001$). None of the other comparisons were significant, but in all cases trends were in the predicted direction (Table 2.3). In general, the results using PICs paralleled those using TIPs and the differences observed likely reflect loss of statistical power.

Further consideration of these results indicates that the predictive value of the traits considered was not greatly strengthened by the use of PICs. First, the mean value of significant PICs ranged from 0.06-0.14. Given that the average magnitude of the pollen limitation indices for the traits considered was about 0.50, this difference is relatively small. Second, the variances of the contrasts were relatively large, with 2-tailed confidence limits typically spanning zero. Lastly, the percentage of PICs that were in the wrong direction ranged from 29% in the herbaceous-woody comparison to 40% in the comparisons of self-compatible with self-incompatible and monocarpic with polycarpic species. Each of these observations indicates that none of the traits used in this study are unambiguous predictors of pollen limitation. This suggests that pollen limitation is too stochastic to be adequately predicted or that PICs do not adequately account for unmeasured variables.

Table 2.2. The contribution of six traits to sums of squares of variation in the pollen limitation index for 103 species. It was not possible to calculate third-order interactions. No second-order interactions contributed significantly to the final model ($P > 0.10$). When second-order interactions were included the whole model $r^2 = 0.516$.

Source of variation	df	Sum of squares	*P*
Compatibility	1	0.285	0.04
Herbaceousness	1	0.046	0.41
Floral specialization	1	0.073	0.30
Presence of nectar	1	0.762	0.001
Biome type	1	0.495	0.008
Habitat type	1	0.157	0.13

Table 2.3. Results of tests using PICs of eight predictions concerning traits associated with pollen limitation. The number and mean value (± SE) of contrasts are presented, as well as *t*-statistics and *P*-values for tests of the predictions. Positive contrasts support the stated predictions. See text and Figure 2.1 for tests of hypotheses 2b, 3b, and 4a for self-incompatible species only.

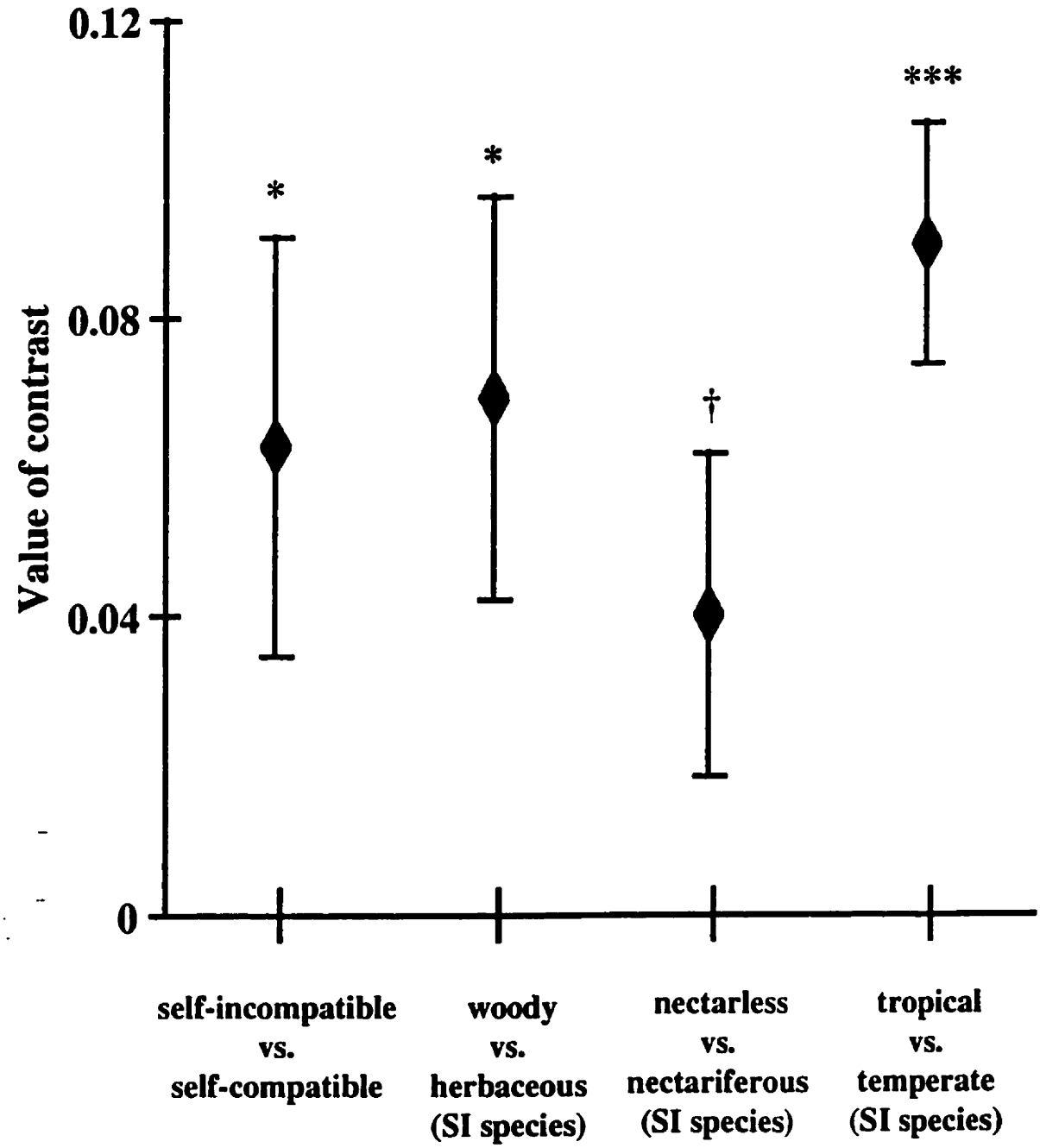
Hypothesis	Prediction for intensity of pollen limitation	Number of contrasts	Value of contrast (mean ± SE)	*t*-statistic	*P*-value	Hypothesis confirmed?
1a.	Self-incompatible > self-compatible	40	0.063 ± 0.028	2.227	0.016	Yes
1b.	Non-autogamous > autogamous	26	0.038 ± 0.023	1.637	0.057	Marginal
2a.	Polycarpic > monocarpic	10	0.118 ± 0.062	1.919	0.044	Yes
2b.	Woody > herbaceous	24	0.087 ± 0.033	2.656	$P_{dir} = 0.009$	Yes
3a.	Specialized > unspecialized flowers	23	0.010 ± 0.023	0.443	0.331	No
3b.	Nectarless > nectariferous	24	0.004 ± 0.026	0.171	0.433	No
4a.	Tropical > temperate	28	0.047 ± 0.030	1.576	$P_{dir} = 0.081$	No
4b.	Forested > open habitats	33	0.043 ± 0.027	1.603	$P_{dir} = 0.074$	No

To investigate this possibility further, PICs were conducted using subsets of the data to provide better control over confounding variables. For example, among the species surveyed there was an association between self-compatibility and both herbaceousness and occurrence in temperate habitats (for herbs, $\chi^2 = 21.61$, $P < 0.0001$; for temperate species, $\chi^2 = 18.45$, $P < 0.0001$; based on *G*-tests of independence). To remove the conflationary influence of self-compatibility, PICs were conducted for these classes using only self-incompatible species. Although PICs between self-compatible herbs and woody species were insignificant (20 contrasts, $t = 0.891$), they were significant for self-incompatible species (11 contrasts, $\overline{X}$ ($\pm$ SE) = 0.069 ($\pm$ 0.027), $t = 2.60$, $P_{dir} <$ 0.02) (Figure 2.3). This result corroborates the lowered pollen limitation of herbs relative to woody species *de facto*, and not just as a reflection of their tendency to be self-compatible. Similarly, there was less pollen limitation in self-incompatible temperate than tropical species (12 contrasts, $\overline{X}$ ($\pm$ SE) = 0.090 ($\pm$ 0.016), $t = 5.61$, $P_{dir} < 0.0001$) (Figure 2.3). Although there was no direct association between self-compatibility and the presence of nectar (for species data, $\chi^2 = 0.004$), self-incompatible nectariferous species were also less likely to be pollen-limited than nectarless species (8 contrasts, $\overline{X}$ ($\pm$ SE) = 0.040 ($\pm$ 0.022), $t = 1.80$, $P_{1\text{-tailed}} < 0.06$).

DISCUSSION

The results of this comparative analysis have revealed several predictors of pollen limitation in angiosperms. Self-compatibility and autogamy were associated with reduced pollen limitation presumably because the capacity for self-fertilization decreases reliance on cross-pollination by pollinators. Monocarpic species exhibited less pollen limitation than polycarpic species. The tendency for herbaceous, nectariferous and temperate species to be less pollen-limited is compelling because the results held only for self-incompatible species. Below, I discuss these results, then compare the TIPs and PICs analyses, drawing attention to their limitations, and finally, suggest other traits that may be informative for predicting pollen limitation among flowering plant species.

Figure 2.3. Mean (± SE) value of PICs of the degree of pollen limitation in paired classes of plants. Positive contrasts indicate that the class listed first for each comparison has more intense pollen limitation (greater pollen limitation index) than the other class. The latter three comparisons are for self-incompatible (SI) species only. See Table 2.3 and text for number of contrasts for each comparison. *** $P < 0.0001$, * $P < 0.05$, † $P < 0.06$, based on *t*-tests for a difference from zero.


0.12
0.08
0.04
0
Value of contrast
*
*
†
***
self-incompatible vs. self-compatible
woody vs. herbaceous (SI species)
nectarless vs. nectariferous (SI species)
tropical vs. temperate (SI species)

FACTORS INFLUENCING POLLEN LIMITATION

The hypothesis that pollen limitation is reduced in species capable of self-fertilization was strongly supported by both TIPs and PICs. Self-fertilization in plants can be mediated by insects or it can occur autogamously (Lloyd and Schoen 1992). The reduced pollen limitation in self-compatible species confirms that self-fertilization increases plant fertility by minimizing their dependence on insects for pollen transfer between plants. Autogamous species have less dependence on animal pollinators, and were therefore less likely to be pollen-limited than non-autogamous self-compatible species. This conforms with the well-appreciated benefit of autogamy as a means of reproductive assurance when pollination is uncertain (Baker 1955; Lloyd 1980).

Self-compatibility also buffers pollen limitation independently of what other traits a plant possesses. For example, among self-compatible species there was no difference using PICs between species that were herbaceous vs. woody, nectariferous vs. nectarless, or temperate vs. tropical. These contrasts were significant, however, when only self-incompatible species were considered. This suggests that these traits have a greater impact on the degree of pollen-limitation in self-incompatible vs. self-compatible species.

Both PICs and TIPs demonstrated less pollen limitation in monocarpic than polycarpic species. The majority of the monocarpic species in the analysis were annuals (68.8%, $N = 16$), so this result lends support to the hypothesis that pollen limitation is less likely in species with short life spans. This could reflect an association between monocarpy and self-compatibility, but this association among the species investigated was not strong ($\chi^2 = 0.95$, $P > 0.30$). This suggests that pollen limitation is decreased in monocarpic species by traits other than self-compatibility.

Herbaceous species demonstrated less pollen limitation than woody species in both PICs and TIPs analyses. However, the shorter life span of herbs is only one explanation for this result. As stated earlier, herbs in the survey tended to be self-compatible, but this confounding influence was removed in the PICs analysis. Reduced pollen limitation in herbs may alternatively reflect differences in display size relative to

woody species. The larger display size of woody species may increase overall visitation rates compared to herbs, but the visitation rate *per flower* may actually be lower (reviewed in Harder and Barrett 1996).

Neither TIPs nor PICs found a difference between pollen limitation in species with specialized versus unspecialized floral morphologies. The objective of this comparison was to determine whether unspecialized flowers are more likely to be visited by pollinators and thus exhibit less pollen limitation than specialized flowers. Unfortunately, knowledge of the pollination systems of most species in the survey was too rudimentary to directly classify the species based on their pollinators. This may account for the non-significant result, because pollinator specialization is not necessarily reflected by morphology (Herrera 1988; McCall and Primack 1992). A comparison between species reliant on a small suite of pollinators with those pollinated by generalists would have been more informative. It would be interesting to compare pollen limitation in oligolectic and more generalized mutualisms to determine which ensures greater visitation (Waser *et al.* 1996). This could be addressed by habitat-specific or sister-species comparisons of taxa differing in pollinator specialization.

TIPs analyses and PICs using self-incompatible species demonstrated that nectariferous species exhibit less pollen limitation than nectarless species. This corroborates the common assertion that rewards have a measurable influence on pollinator attraction (Simpson and Neff 1983). However, in a separate analysis of species in the Orchidaceae, there was no difference between levels of pollen limitation in nectariferous vs. nectarless species ($df = 24$, $t = 0.53$, $P > 0.60$). Comparisons of pollen limitation in nectariferous species and co-occurring "deceitful" sister species could provide a more direct test of the importance of rewards for pollinator visitation (Fritz and Nilsson 1994; Alexandersson and Ågren 1996).

Pollen limitation was marginally reduced in species of open habitats compared to those of forested habitats based on TIPs and PICs. The absence of a strong difference between these classes may reflect the artificial dichotomy between open and forested habitats, because many habitats are intermediate between these extremes. Alternatively,

insect foraging rates may be relatively unaffected by the microclimatic differences between these habitats. This issue could be resolved by comparisons of insect foraging rates and pollen limitation in populations of a species occurring in habitats that are explicitly contrasted.

TIPs analyses and PICs using self-incompatible species demonstrated that temperate species exhibit less pollen limitation than tropical species. The putative mechanism for this difference is the lower densities at which many tropical species are found. An alternative is that tropical species tend to be dependent on more specialized pollinators (Bawa 1990). To determine the mechanistic basis for the observed pattern, pollen limitation in tropical-temperate sister taxa could be compared in relation to their densities and pollinator foraging rates.

TIPs VERSUS PICs

A comparison of the results of TIPs and PICs analyses may provide insight into the evolutionary lability of traits considered (Price 1997). In general, the results of TIPs and PICs analyses in this study were similar. This implies that TIPs analyses were not biased because of historical associations and that selection in current environments influences the degree of pollen limitation within lineages. In other words the influence of phylogeny was not especially strong because closely related species often varied in degree of pollen limitation. Clades such as the Orchidaceae are undoubtedly characterized by pollen limitation ($\overline{X}$ index $\pm$ SE = 0.62 ± 0.06, $N = 26$), but traits influencing pollen limitation generally appear to be evolutionarily labile within most flowering plant families.

By pairing related taxa, PICs may provide control over confounding variables that could influence TIPs analyses. However, PICs provided little control over the effect of self-compatibility in the analyses presented here. In three of the comparisons (nectariferous vs. nectarless, temperate vs. tropical, open vs. forested habitat) TIPs detected differences in pollen limitation but PICs did not unless self-compatible species were excluded. This result supports Price's (1997) conclusion that PICs are unlikely to

control for confounding variables and may also indicate that PICs have less power to detect true differences in the presence of confounding variables.

LIMITATIONS OF THE ANALYSIS

A major limitation of most comparative analyses to date is that interactions between traits cannot be fully investigated. Interactions were included in this analysis to the extent possible. None of the second-order interactions included in the multi-way ANOVA of TIPs were significant. It was not possible, however, to include higher-order interactions. Multiple regression can be used to account for collinearity between continuous variables in PICs analyses (e.g., Rees 1996), but acceptable techniques for considering interactions between categorical traits are still under investigation (see Martins and Hansen 1997). The confounding influence of self-compatibility was removed from some of the PICs analyses, but other correlates may have still influenced the results. The small number of monocarpic species ($N = 16$) in the database precluded this correction in the PICs between monocarpic and polycarpic species.

The results of this analysis in part depend on how well the index of pollen limitation characterizes pollen limitation in the species studied. There are two instances in which the index may not indicate the "true" level of pollen limitation. First, some of the studies in the survey did not conduct pollen supplementations at the whole-plant level, so the indices for these species may be confounded with resource limitation (Zimmerman and Pyke 1988). This may be relatively insignificant, however, because all flowers on plants were supplemented with pollen in 66% of species for which this could be evaluated ($N = 108$, excluding woody species). Second, the index concerns fruit set, which in some cases may not be the most appropriate measure of pollen limitation. Some authors consider seed set per fruit to be a more adequate descriptor of pollen limitation in certain circumstances (Snow 1986; Jennersten and Nilsson 1993). However, for species in which both fruit and seed measures of pollen limitation have been measured, Burd (1994) has shown that fertility is more often determined by maturation of whole fruits than seeds within fruits.

Another deficiency of the index is that it gives no sense of the stochasticity inherent to pollen limitation. Relatively few studies have examined pollen limitation at various times or sites, but these have typically found variation. In Burd's (1994) survey, whether a species was pollen-limited varied for 76.9% of species for which it was measured multiple times within a season, and for 44.7% of those tested in multiple sites or years ($N = 13$ and $N = 38$, respectively). Ideally, the index should incorporate the variability of pollen limitation, which defines the capacity for selective forces to influence traits governing pollen limitation.

Lastly, the validity of PICs is dependent on a number of evolutionary assumptions. In particular, it is based upon one phylogenetic hypothesis for the angiosperms. Sensitivity analyses were not conducted to test the robustness of the results to topological changes (Donoghue and Ackerly 1996), because there is no globally parsimonious tree for the species in the analysis. Although assumptions were also made about branch lengths and the mode of evolutionary change, the general concurrence of TIP and PIC analyses suggests that these did not bias the results (Ricklefs and Starck 1996).

Additional Factors Influencing Pollen Limitation

The relatively low predictive power provided by the traits investigated here indicates that interspecific variation in pollen limitation is caused by a suite of additional factors. Our predictive ability may be increased by incorporating these factors, but will likely be limited by the vagaries of pollen delivery. Additional factors may be roughly categorized as (1) floral traits, (2) pollination system and (3) ecological conditions. The factors discussed below were not included in the current study because data were too few to conduct analyses using PICs. However, preliminary comparative analyses using correlation of TIPs are presented below for some of the factors.

Pollen limitation is influenced by floral traits that determine a plant's attractiveness to pollinators, such as the size and number of flowers displayed. The number of flowers displayed on an inflorescence influences visitation rates and hence

fertility (reviewed in Harder and Barrett 1996). This is supported among species in the sample by the negative relation between daily floral display size and the intensity of pollen limitation ($N = 69$ species, $r^2 = 0.06$, $P < 0.05$, log-transformed indices). Plants could also increase their "attractiveness" by the evolution of longer floral life spans when pollinator visits are infrequent (Primack 1985; Motten 1986; Rathcke 1988; Herrera 1995a). Although PICs were again impractical, there was a positive trend between floral longevity and the intensity of pollen limitation in the species studied ($N = 81$ species, $r^2 = 0.04$, $P < 0.08$, log-transformed indices). This suggests that extended floral longevity rarely compensates for low pollinator visitation rates.

In general, pollen limitation of seed set might be expected to be greater in flowers with more ovules because they require more pollen to be transferred for full seed set. Burd (1995a) has restated this in terms of the adaptiveness of a bet-hedging strategy when pollen delivery is stochastic (see also Motten 1986; Ehrlén 1991). When variance in pollen delivery is large and flower production is sufficiently expensive, his model predicts that plants maximize fitness by oversupplying ovules to take advantage of the infrequent delivery of large pollen loads. The corollary of this view is that plants with many ovules are more likely to be pollen-limited. However, there was little support for this hypothesis among species in the database ($N = 43$ species, $r^2 = 0.02$, $P > 0.35$, log-transformed indices), although ovule number and seed set measures of pollen limitation were available for relatively few species and floral costs could not be incorporated into the analysis. Future testing of these ideas could be enlightening. In particular, Burd's model predicts that ovule numbers should be higher and pollen limitation more intense in stochastic pollination environments.

Information on mating patterns at a finer level than presented here is likely to increase our ability to predict the occurrence of pollen limitation. Self-fertilization in angiosperms can occur via six different modes (Lloyd 1979, 1992; Lloyd and Schoen 1992). Two of these, geitonogamy and facilitated self-fertilization, require insects for pollen transfer, so they do not provide reproductive assurance when pollinators are absent. Conversely, autogamy can provide reproductive assurance in the absence of

pollinators. The three different modes of autogamy, however, differ in the relative timing of self- and cross- pollination. This causes variation in their ability to provide reproductive assurance and in the potential costs of self-fertilization (see Ramsey 1995 for an example). At present, our knowledge of the modes of self-pollination in different species is insufficient to determine their influence on degree of pollen limitation.

The stochasticity of pollen delivery also depends critically upon pollination system, which was not examined here. Noteworthy in this regard are the few studies of pollen limitation in wind-pollinated species (Bertness and Shumway 1992; Holm 1994). Pollen limitation may be severe in some wind-pollinated species because pollen transfer by wind is probably more stochastic than by insects. Until there are more studies of pollen limitation in wind-pollinated species, however, it is difficult to compare them with animal-pollinated taxa. In biotically-pollinated species, the stochasticity of pollen delivery may be linked to type of pollinator. The dependability of pollen transfer should not be determined by pollination syndromes alone, but by detailed observations of which floral visitors actually effect pollen transfer (Schemske and Horvitz 1984; Fishbein and Venable 1996; Waser *et al.* 1996). In general, a diverse pollinator fauna may reduce the likelihood of pollen limitation compared to dependence upon one or a few pollinators (Motten 1986; Rathcke 1988). Pollinators differ in their ability to forage under inclement weather conditions (Herrera 1995b) and in the stability of their populations (Bowers 1985), which will also influence pollen limitation within species.

The degree of pollen limitation in a given species likely depends on a variety of ecological factors other than those considered here. The comparisons of temperate vs. tropical species and species of open vs. forested habitats in this study were relatively crude. It would likely be more informative to compare pollen limitation among specific habitats and geographical regions. For example, pollinator visitation rates are low in certain habitats (McCall and Primack 1992) and this may correspond with intense pollen limitation (Johnson and Bond 1997). The extent to which these among-habitat differences are the result of climatic factors as opposed to historical effects on pollinator faunas is not clear. Another ecological influence on the degree of pollen limitation within

a species is the time at which it flowers. Plants flowering in different seasons likely encounter different weather conditions (Schemske *et al.* 1978; Motten 1986) and different levels of competition for pollinators imposed by the concurrent flowering of other species (Mosquin 1971; Copeland and Whelan 1989). Season may also correlate with other factors that directly reduce pollinator populations (e.g., fire, Johnson and Bond 1997). In addition, the influence of specific weather patterns (e.g., humidity and temperature) on the foraging of pollinators and pollen limitation is seldom studied but has obvious consequences for plant fertility.

Clearly there are numerous factors that influence pollen limitation among flowering plant species. To fully understand these factors experimental studies at the species level are required. These experiments could disentangle the relative roles of intrinsic and extrinsic factors governing pollen limitation and uncover their mechanistic linkage to variation in pollen delivery. Previous intraspecific investigations have considered the effect of plant size (Hainsworth *et al.* 1985; Dudash 1993; Lawrence 1993), population size (Sih and Baltus 1987; Jennersten and Nilsson 1993; Byers 1995; Ågren 1996), insularity (Spears 1987), and seasonal and spatial variation in pollen limitation (reviewed in Burd 1994). Surprisingly, most of these concern ecological factors, rather than variation in plant traits. Future empirical work would be most profitable if sister taxa differing in specific traits are explicitly compared. Comparative investigations at this level are required to increase our understanding of the connection between plant traits and pollen limitation.

CHAPTER THREE

POLLEN LIMITATION IN BUZZ-POLLINATED *RHEXIA VIRGINICA* (MELASTOMATACEAE) IN ONTARIO

"It is evident that [flowers of *Rhexia virginica* are] a very perfect contrivance for securing cross-fertilization, at least to a considerable extent."

W.H. Leggett (1881)

Virginia Meadow-Beauty (*Rhexia virginica*, Melastomataceae) being 'buzzed' by a bumblebee (*Bombus terricola*) at Lake Matchedash, Ontario, during early August 1996. The flowers above the bee are second-day flowers (see text). Photo by Brendon Larson.

ABSTRACT

To determine whether the mechanics of pollen transfer can account for pollen limitation in a flowering plant, the reproductive ecology of *Rhexia virginica* (Melastomataceae) was investigated in the Muskoka region of Ontario. *Rhexia virginica* is pollinated primarily by bumblebees capable of buzz pollination, the sexual function of its flowers lasts one day, and the species occurs at the edge of its familial range in Ontario. Fruit set averaged 52.6% in a total of thirteen populations investigated during 1996 and 1997. Low fruit set was not likely the result of resource limitation, because the fertility of plants on which all flowers were supplementally hand-pollinated was significantly greater than that of open-pollinated plants. The intensity of pollen limitation differed between two populations studied at Lake Matchedash, Ontario, but generally occurred throughout the flowering season in both years. In 1997, the probability of fruit set on seven days was increased an average of 57.6% by supplemental hand-pollination. Fertility was also pollen-limited at three of four other lakes investigated in Muskoka on single days during 1997. Pollen limitation in *R. virginica* results from aspects of its floral biology and the dynamics of local bumblebee populations. Glasshouse experiments demonstrated that *R. virginica* was highly self-compatible but did not self-pollinate autonomously, so Ontario populations are entirely dependent on bumblebees for pollen transfer. However, bumblebee visitation was infrequent and variable at Lake Matchedash. During 15 minute observation periods of 4 m^2 quadrats in 1996 there was a median of one bumblebee visit, but during 30% of the observation periods no visits were observed. Furthermore, field experiments demonstrated that the poricidal anthers of *R. virginica* dispense pollen gradually, with only 10.2% of pollen removed during a single bumblebee visit, and an average of 47.3% of pollen remained in anthers at the end of flowering. There was a negative correlation between the proportion of pollen removed from anthers on a given day and the intensity of pollen limitation, indicating that limited pollen removal is the major cause of pollen limitation in Ontario populations of *R. virginica*.

INTRODUCTION

The fertility of flowering plants is often limited by pollen delivery. Pollen limitation can be demonstrated by an increase in fruit and seed set in flowers that receive supplemental pollination relative to open-pollinated controls. A literature survey of pollen limitation in plants demonstrated that this treatment increased fertility at some times or in some locations in 62% of species that have been studied (Burd 1994). Despite the prevalence of pollen limitation we have little understanding of the proximate ecological factors that influence its occurrence. Pollen limitation is influenced by plant size (Hainsworth *et al.* 1985; Dudash 1993; Lawrence 1993), population size (Sih and Baltus 1987; Jennersten and Nilsson 1993; Byers 1995; Ågren 1996), and insularity (Spears 1987), but few other factors have been studied. Therefore, we are presently unable to predict the general circumstances under which pollen limitation is likely (see Chapter 2). Detailed investigations of the ecology of pollen delivery are required before an understanding of the factors influencing pollen limitation among populations of a particular species is obtained.

Pollen delivery in animal-pollinated plants can be highly stochastic, because it depends upon the vagaries of pollinators that vary both spatially and temporally (Burd 1995). This is reflected by the variable occurrence of pollen limitation. Pollen limitation did not occur consistently in 76.9% of species that have been examined multiple times within a season and in 44.7% of those examined in multiple sites or years ($N = 13$ and $N = 38$ species, respectively; Burd 1994). Variation in the intensity of pollen limitation implies an obvious functional linkage between pollination biology and pollen limitation, but the mechanics of pollen transfer have seldom been shown to influence the magnitude of pollen limitation through field studies. Understanding the interconnections between pollinator behaviour, floral visitation, and plant fertility will likely increase our ability to predict spatial and temporal variation in pollen delivery.

Plant fertility is most susceptible to pollinator stochasticity when floral traits preclude pollination by the majority of floral visitors. Typically, these traits restrict access to floral rewards to all but the visitors that can effect pollination (Proctor *et al.* 1996). An

exemplary case is the syndrome of buzz pollination, where the sole floral reward is pollen hidden within anthers that only open via minute pores (Buchmann 1983). Removal of this pollen requires high frequency vibration of the anthers, which can only be undertaken by certain bees capable of a highly stereotyped behaviour called "buzzing" (Michener 1962; Buchmann 1983). This specialized pollination system may be vulnerable to pollen limitation for two reasons. First, during individual visits by bees the poricidal anthers restrict pollen removal, so that multiple visits are needed for its complete removal (Harder and Thomson 1989; Harder 1990a, b; Harder and Barclay 1994; King and Buchmann 1996). Second, although many species of bees are capable of buzzing (see Buchmann 1983), only a subset are likely efficient at buzz pollination of a particular plant species.

To examine pollen limitation in a buzz-pollinated species, I studied populations of *Rhexia virginica* (Melastomataceae) in southern Ontario, Canada. *Rhexia virginica* is a member of the large tropical family Melastomataceae, which is characterized by buzz pollination (Buchmann 1983; Renner 1989). Pollen limitation has been investigated in three neotropical members of the Melastomataceae (Ramirez and Brito 1990), but details of the ecology of pollen limitation have not been studied in the family. *Rhexia virginica* populations in Ontario are disjunct from their contiguous range on the coastal plain of the United States and represent the northern limit of the Melastomataceae. These populations provide an opportunity to investigate the patterns of pollen limitation in *R. virginica*, with the goal of determining how variation in pollen limitation may be related to pollen transfer. In this study, the following specific questions concerning the relation between pollination biology and pollen limitation in *R. virginica* were addressed: 1. What features of the reproductive biology of *R. virginica* are likely to influence pollen limitation? An understanding of the floral biology and pollinators of *R. virginica* is necessary to interpret patterns of pollen limitation. 2. Are *R. virginica* populations pollen-limited in Ontario? If so, what is the magnitude of pollen limitation at different times during the flowering season? 3. Is there a relation between pollen limitation and patterns of pollen removal and pollen deposition? In particular, might restricted pollen removal associated with buzz pollination in *R. virginica* lead to significant pollen limitation?

MATERIALS AND METHODS

THE STUDY ORGANISM

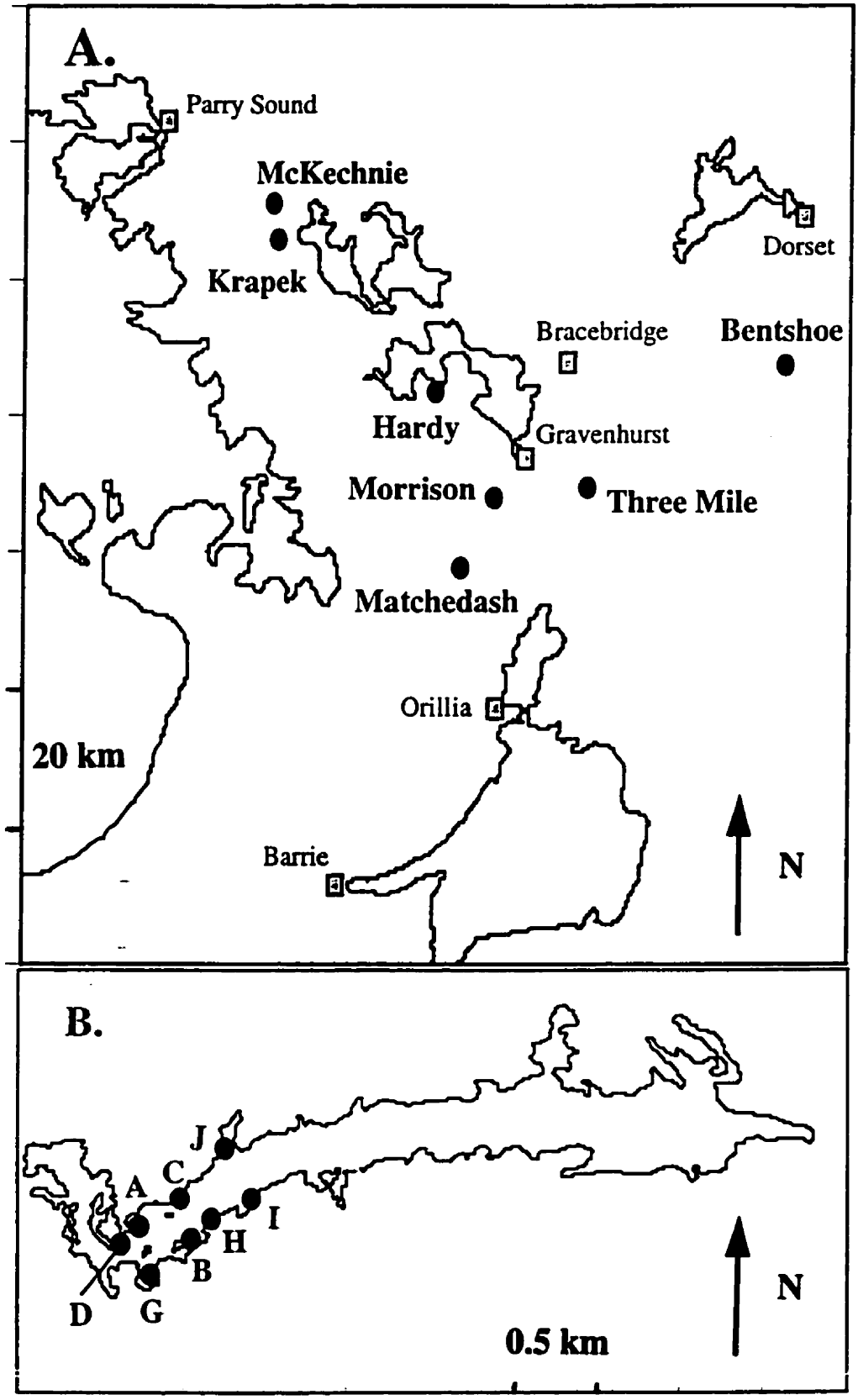
Rhexia virginica L. (Melastomataceae), commonly known as Virginia Meadow-Beauty, is a perennial herb of wetland habitats of the coastal plain of the United States. It also occurs in scattered locations near the Great Lakes, including the Muskoka region of Ontario (Reznicek 1994). Previous research on *R. virginica* has concerned its floral anatomy, vegetative reproduction and some aspects of its pollination biology and population biology (Leggett 1881; Holm 1907; Eyde and Teeri 1967; Sharp 1983; Posluszny *et al.* 1984). These studies are only briefly summarized here. Revisions of the genus *Rhexia* are provided by James (1956), Kral and Bostick (1969), and Snyder (1996).

Flowers of *R. virginica* are borne on cymose inflorescences and consist of four showy pink petals and eight elongate, bright yellow, poricidally-dehiscent anthers borne at the apex of an urceolate hypanthium (see Eyde and Teeri 1967). The style is sigmoidal and directed downwards so the stigma is located below the anthers. *Rhexia virginica* flowers do not produce nectar and possess the syndrome of buzz pollination (*sensu* Buchmann 1983); only bees that "buzz" flowers can release pollen from their poricidal anthers and are thus potential pollinators (Leggett 1881; Eyde and Teeri 1967). *Rhexia virginica* propagates clonally by producing adventitious roots that give rise to new plants. Individual ramets apparently do not overwinter in Ontario populations (Posluszny *et al.* 1984), but may do so in populations located in the southern United States (Holm 1907).

THE STUDY SITE

Unless otherwise stated, all studies were conducted at Lake Matchedash (also called Long Lake), Simcoe County, Ontario, which is located about 20 km north of Orillia (79° 30' 45'' W, 44° 47' 00'' N) (Figure 3.1A, B). Lake Matchedash is 0.3 km wide by 5 km long and has the most significant assemblage of species from the coastal plain flora of the United States located in Ontario (Keddy and Sharp 1989). At least 50 local populations of *R. virginica* are scattered along its shoreline (Keddy and Sharp 1989), which represents one of the largest concentrations of the species in Canada. The populations range in size from

Figure 3.1. Location of populations of *Rhexia virginica* studied in the Muskoka region of Ontario in 1996 and 1997. (A) Seven lakes in the Muskoka region where populations of *Rhexia virginica* were sampled. (B) Close-up map of Lake Matchedash, showing the location of eight populations at the western extremity of the lake in which studies were undertaken.


A.
Parry Sound
McKechnie
Krapek
Dorset
Bracebridge
Bentshoe
Hardy
Gravenhurst
Morrison
Three Mile
Matchedash
Orillia
20 km
Barrie
N
B.
J
C
A
I
H
B
D
G
N
0.5 km

small colonies with dozens of individuals to more extensive shoreline swards consisting of 1000-1500 plants. Eight populations of *R. virginica* were studied at Lake Matchedash in 1996 and 1997 (Figure 3.1B). Intensive research took place in populations B and D, which were located approximately 400 m apart on opposite shores of the lake. Both populations contained 1000-1200 individuals. Population B was relatively dense (140 flowering plants/m^2; $N = 10 \times 1$ m^2 quadrats), about 0.5 m in width and 17 m in length, and located on a sheltered sandy shoreline. Population D was more extensive (20 m by 13 m), but less dense (14 flowering plants/ m^2; $N = 10 \times 1$ m^2 quadrats), and located patchily in an open wet meadow. Plant species that flowered concurrently with *R. virginica* included *Pontederia cordata* L., *Spiraea alba* DuRoi, *S. tomentosa* L., *Cephalanthus occidentalis* L., *Lysimachia terrestris* (L.) BSP., *Utricularia cornuta* Michx. and *Xyris difformis* Chapm. near shorelines and *Melampyrum lineare* Desr. in adjacent woodlands.

The metapopulation structure of *R. virginica* at Lake Matchedash differed greatly between 1996 and 1997. In 1997, water levels until mid-July were approximately 0.5 m higher than in 1996, so populations located closest to water were flooded. Although plants in these populations sprouted when water levels lowered, the size of populations was reduced to below 25% of 1996 levels in half of the populations studied. Population B was relatively unaffected by the water level change, but half of population D was submersed and did not flower in 1997.

FLORAL BIOLOGY OF *RHEXIA VIRGINICA*

Self-Compatibility

To determine whether *R. virginica* plants from Lake Matchedash were self-compatible and whether they depended on insect visits for pollination to occur, an experimental pollination study was performed in a glasshouse at the University of Toronto in July and August 1996. The compatibility status of *R. virginica* is problematic. Kral and Bostick (1969) and Sharp (1983) demonstrated that fruits are set after self-pollination, but based on the prior study Renner (1989) claimed that *R. virginica* is self-incompatible. Neither study compared the seed set resulting from self- and cross-pollination or

investigated the potential for autonomous self-fertilization. Glasshouse experiments in 1996 were used to determine the compatibility status of *R. virginica*, and if flowers were self-compatible, whether they were capable of setting seed autonomously. Treatments were conducted using a minimum of fifteen plants transplanted from populations B, D, G, H, and I at Lake Matchedash (Figure 3.1B) and populations from Krapek Lake and McKecknie Lake. The latter two lakes were located about 55 km northwest of Lake Matchedash, slightly south of Parry Sound (Krapek: 79° 48' W, 45° 13' N; McKechnie: 79° 49' W, 45° 14' N) (Figure 3.1A). Flowers were randomly assigned to one of four treatments: self-pollination, cross-pollination, unpollinated to test for autonomous self-fertilization, or disturbed. Disturbed flowers were watered from above with a watering can for four minutes to simulate rain. Pollen was obtained by slitting anthers and using forceps to remove pollen that was subsequently applied to stigmas. Cross pollinations with Lake Matchedash plants used pollen parents from both the same and different populations from the lake. Treatments and populations were compared using a two-way ANOVA with the square-root of seed set per fruit as the response variable. All statistical analyses reported here were conducted using JMP (Version 3.0.2, SAS Institute, 1994). Standard transformations were performed to stabilize variances and normalize residuals (Sokal and Rohlf 1995). Population was considered a random effect in all analyses because among-population variation was of greater interest than the quantification of differences between populations (Sokal and Rohlf 1995).

Pollen-Ovule Ratio

The pollen-ovule ratio of angiosperm species provides some indication of their mating system (Cruden 1977). Anthers were collected from a total of 154 flowers from populations B and D at Lake Matchedash during the 1996 flowering season and placed in 75% ethanol in separate microcentrifuge tubes. Pollen grains were released from the anthers for counting by rupturing them with a probe sonicator (Vibra-Cell, Sonics and Materials Inc., Danbury, Connecticut, USA). A sample from each microcentrifuge was suspended in a weak aqueous electrolyte (5 g/L NaCl) and the number of grains estimated by averaging four subsample counts obtained using a Particle Data Elzone 282PC particle

counter (Particle Data Inc., Elmhurst, Illinois, USA). To determine whether the number of pollen grains varied among the eight anthers, the grains in individual anthers from ten plants were counted. Ovules were counted in 25 flowers sampled from each of the four terminal nodes on plants in population D at Lake Matchedash on August 10, 1996. The number of ovules in flowers from each node were compared using ANOVA.

Floral Colour Change

Rhexia virginica flowers undergo a colour change after flowering for one day (see Weiss 1995). This phenomenon was investigated at Lake Matchedash in 1996 to assess its relevance to plant fertility. To determine whether colour change was induced by pollinator visits or was environmentally determined (Gori 1983), the colour of thirty flowers located in pollinator exclusion cages was compared qualitatively to flowers outside exclosures. The precise sequence of floral colour change was assessed by monitoring the anthesis of individual flowers. An experiment was also conducted to determine whether flowers having undergone a colour change (second-day flowers) could contribute directly to plant fertility if visited by insects. The receptivity of second-day stigmas and viability of second-day pollen were assessed by comparison with that of first-day flowers using controlled hand-pollinations with outcross pollen. Treatments were applied to plants located within insect exclosure cages in population D during early August 1996.

Flowering Phenology

The number of flowers within plant populations varies through time and pollinator densities can be associated with the distribution of flowering (Thomson 1980). To determine whether this variation affects fertility, the phenology of *R. virginica* at Lake Matchedash was quantified in 1996 and 1997. In 1996, 50 plants were randomly chosen along shoreline transects in populations A, B, C and D prior to flowering. Plants were surveyed every other day until flowering ceased, and the anthesis date of each flower was recorded. In 1997, phenology was assessed at the level of the entire population rather than at the individual plant level. Counts of the total numbers of flowers open in populations B and D were made on nineteen days spanning the entire flowering period.

POLLINATION BIOLOGY OF *RHEXIA VIRGINICA*

Floral Visitation

To identify the pollinators of *R. virginica* flowers, visitors were recorded during observation periods throughout peak flowering in 1996 and 1997 at Lake Matchedash. Bumblebees were identified using the keys in Laverty and Harder (1988), and syrphid flies using the keys in Vockeroth and Thompson (1987) and Vockeroth (1992). Reference insect specimens were placed in the collection of the author. To assess variation in pollinator abundance during the flowering season, rates of floral visitation were also determined. In 1996, the number of visitors entering single 4 m^2 quadrats in populations B and D was recorded every hour from 7 a.m. until 3 p.m. for 15 minute periods on ten days during the flowering period. Since visitation rates proved to be quite low in 1996, this methodology was modified in 1997. During 1997 observation periods, all visits to flowers in population B were recorded. Observation periods were 1.5 to 6 hours in duration and occurred between 6:30 a.m. and noon on fourteen days spanning the flowering period. For each visitor, the number of flowers visited (including those to second-day flowers), time spent within the population, and duration of buzzes on individual flowers were recorded. The behaviour of all visitors was also observed to ascertain whether they were potential pollinators.

Floral Cues to Pollinators

To identify the floral cues that attract bumblebees to *R. virginica* flowers, choice experiments using foraging *Bombus impatiens* were conducted on August 10, 1997 in population B at Lake Matchedash. The experiments were designed to determine the relative roles of the anthers and petals of *R. virginica* flowers in pollinator attraction. Bees were given a choice between flowers that were placed 10 cm apart at the end of a "bee-stick" (Thomson *et al.* 1982) presented to them. Two choice experiments were conducted: (1) intact flowers vs. petal-less ones; (2) intact flowers vs. stamen-less ones. Flowers were replaced and the relative position of the flowers was changed at frequent intervals. Both approaches by bees and visits to flowers were recorded. Results were analyzed using *G*-tests of independence (Sokal and Rohlf 1995).

To investigate whether bumblebees have a preference for unvisited *R. virginica* flowers compared to those that have been previously visited, experimental arrays of sixteen first-day flowers were presented to bees on August 4 and 14, 1996. An important issue addressed in this experiment was whether the bright yellow anthers elicit bees to buzz regardless of whether they contain pollen (Vogel 1978). Flowers were positioned in florist water pics located 5 cm from one another in a square array. Arrays were comprised of equal numbers of flowers of two randomly-arranged treatments: (1) flowers that had been visited during the morning and from which remaining pollen was expelled by repeated tapping using forceps, and (2) unvisited flowers from an exclosure cage. A heterogeneity *G*-test (Sokal and Rohlf 1995) was employed to test for preferences in visitation on the two days as well as for data pooled over both days. Because of low visitation rates, data obtained from individual bees were pooled in statistical analyses.

Pollen Removal During Pollinator Visits

The amount of pollen removed during individual pollinator visits influences the quantity carried by pollinators and levels of deposition on stigmas (Harder and Thomson 1989). To characterize the dynamics of pollen removal in *R. virginica*, an experimental study was conducted to quantify the amount of pollen removed during bumblebee visits. The experiment was conducted in population D at Lake Matchedash on three mornings in early August 1996. Foraging *Bombus bimaculatus* were allowed one, two or three visits to flowers in water pics attached to a stick. The duration of each visit was timed. Following visitation, anthers were placed in 70% ethanol in microcentrifuge tubes and pollen grains were counted as described above. The amount of pollen removed was estimated by comparing the amount remaining after visitation to that in control (unvisited) flowers from the same node ($N = 19$ pairs, $r^2 = 0.78$, $P < 0.0001$). Pollen removal was analyzed by ANCOVA, with number of visits and their cumulative duration treated as main effects.

Observation of bumblebee visits during 1996 suggested that they remove pollen from *R. virginica* flowers simply by contacting the anthers and not only by "buzzing" them. To determine the proportion of pollen that can be removed without "buzzing", an investigation was conducted prior to bumblebee visitation on August 10, 1997 in

population D. Single flowers from ten plants were repeatedly tapped with forceps until no more pollen was expelled. The amount removed was estimated by comparison with the amount in unmanipulated flowers.

VARIATION IN FEMALE FERTILITY OF *RHEXIA VIRGINICA*

Survey of Patterns of Fruit Set

Investigations were conducted in 1996 and 1997 to determine whether fruit set in *R. virginica* was sub-maximal and to what extent it varied in space and time. Fruit set was measured at populations A, B, C, and D at Lake Matchedash in 1996. Fruit set was determined by monitoring the 200 plants marked for the phenology study. In 1997 fruit set was measured at seven populations at Lake Matchedash (Figure 3.1B) and in single populations from Krapek Lake, Three-mile Lake (79° 16' W, 44° 53' N), Bentshoe Lake (78° 55' W, 45° 02' N), Morrison Lake (79° 28' W, 44° 52' N) and Hardy Lake (79° 32' W, 45° 00' N) (Figure 3.1A). In 1997, the number of buds on 25 randomly-chosen plants in each population were counted in early August and the presence of fruits on these plants was recorded in October. *Rhexia virginica* sometimes initiates tiny buds that usually do not flower (B. Larson, pers. obs.), but these were not counted, so estimates of fruit set are conservative. Fruit set of populations was compared using a one-way ANOVA with percent fruit set (arcsine square-root transformed) of individual plants as the response variable. The size of populations visited in 1997 was also estimated, to determine whether there was a relation between female fertility and population size.

Factors Affecting Female Fertility

Variation in female fertility may be affected by any factor that influences pollinator visitation rates. These include the population in which a plant is located, the date of anthesis of a given flower, and the size of the floral display of individual plants. These factors were investigated in *R. virginica* populations A, B, C, and D at Lake Matchedash in 1996. Flowers on plants in the phenology study were individually marked with paint so that fruit set and the number of seeds produced could be related to flowering date and the number of flowers displayed on the date of flowering (daily display size). Flowering date

was treated categorically as early, middle, or late in the season, corresponding to flowering of one-third of all flowers counted on plants in the phenology study. To assess whether second-day flowers increase pollinator visitation rates and hence fertility, they were either included in display size or excluded and the results compared. Fruit set was analyzed using logistic regression (Trexler and Travis 1993), with population, display size and flowering date treated as independent variables. Since the fertility of multiple flowers comprising daily displays were not independent, the proportion of these flowers setting fruit was used in the analysis. All fruit or no fruit were set by the flowers of ninety-four percent of daily displays. In the remaining displays fruit set was considered complete or zero in the logistic regression, depending on whether or not the majority (> 50%) of fruits were set.

To determine the effect of the same variables on the square-root of seed set, a mixed-model ANOVA was conducted. To meet ANOVA assumptions, the mean seed set of flowers in daily displays was used in the analysis and flowers not setting fruit were excluded. Because many plants flowered on more than one day, seed set values were not entirely independent. Repeated measures analyses could be employed to resolve this problem, but numerous plants flowered only once, which introduced imbalance in the dataset. To resolve this problem, error degrees of freedom were adjusted to reflect the number of plants that set fruit rather than total sample size (see Fishbein and Venable 1996). This is a conservative approach because the fertility of displays on a plant on different days are probably largely independent.

POLLEN LIMITATION OF FERTILITY IN *RHEXIA VIRGINICA*

Whole-Plant Pollen Limitation

Pollen limitation may occur at various scales (Burd 1994), so experiments were conducted with *R. virginica* at Lake Matchedash in 1996 and 1997 to investigate its scalar variation. Pollen-limitation is often assessed by comparing the fertility of supplemental hand-pollinated flowers with open-pollinated controls. Although this treatment can demonstrate pollen limitation at the flower level, it cannot alone conclusively demonstrate pollen limitation at the level of the whole plant because resources may simply be re-

allocated to supplemented flowers (see Zimmerman and Pyke 1988). A primary objective of pollen limitation studies at Lake Matchedash was to assay day-to-day variation in pollen limitation, which can only be determined by comparing the fertility of individual flowers that are hand-pollinated with those that are not. To determine whether this objective would be hampered by re-allocation of resources within plants, two experiments were conducted in 1997. First, treatments were conducted at the whole-plant level (Johnston 1991) to assess whether plant fertility could be increased *in toto* by addition of supplemental pollen. Fifteen pairs of plants in population B of similar height and with similar numbers of buds were marked. One of the plants of each pair was randomly chosen and all flowers received supplemental hand cross-pollination. The other plant was left as an unmanipulated control. Plant height, number of flowers, percent fruit set, total number of seeds, seeds per flower and seeds per fruit of pairs were compared using a Wilcoxon's signed-ranks test (Sokal and Rohlf 1995). Since pollen supplementation was expected to increase fertility, one-tailed tests were used to compare the treatments. The second experiment was conducted on two days in populations B and D. The fertility of sixteen open-pollinated flowers on plants on which a flower was supplementally pollinated was compared with that of open-pollinated flowers on sixteen control plants (following Zimmerman and Pyke 1988). Re-allocation of resources within inflorescences would be indicated by reduced fertility of open-pollinated flowers on treated plants relative to those on control plants.

Survey of Pollen Limitation in Muskoka

To assess whether pollen limitation commonly limited female fertility of *R. virginica* plants in the Muskoka region, experiments were conducted at Bentshoe, Hardy, Krapek, and Three-mile Lakes (Figure 3.1A) in 1997. Treatments at Lake Matchedash were more extensive and are described below. All supplementation treatments were undertaken on clear, sunny days unless otherwise stated. Each lake was visited once during August: Three-mile and Bentshoe on August 7, Hardy on August 8, and Krapek on August 14. During visits, the total floral display size of populations was determined, so that its influence on the intensity of pollen limitation could be assessed. On each day, pairs of one-flowered plants of similar size were selected and supplemental pollen was added to the

stigma of one flower. Plants with a floral display size of one were selected because this was the modal daily flower number within populations (see below). The number of pairs chosen varied depending on the size of populations. The fertility of treatments was compared using a two-way ANOVA of square-root of seeds set per flower, with treatment and population as main effects.

Pollen Limitation at Lake Matchedash

An investigation was undertaken at Lake Matchedash in 1996 to determine whether the female fertility of *R. virginica* was pollen-limited. Seasonal variation was assessed by conducting pollination treatments on days throughout the flowering period. Between 15 and 30 plants with two flowers were randomly selected in populations B and D on August 1, 5, 8, and 17. Supplemental pollen from a nearby plant was added to one flower and its fruit set and seeds per fruit were compared to the control flower using *G*-tests of independence and Wilcoxon's signed-ranks tests, respectively.

Data from 1996 indicated that pollen limitation was prevalent at Lake Matchedash, so more detailed studies in 1997 were used to investigate its spatio-temporal variation and ecological correlates. Experiments in 1997 were conducted in populations B and D on seven days spanning the flowering period (see results). On each day, fifteen pairs of one-flowered plants of similar size were selected and supplemental pollen was added to the stigma of one flower. One-flowered plants were chosen for the reason mentioned above. Fruit set of plants was analyzed using logistic regression, with population, pollination treatment and date as categorical variables. A mixed-model ANOVA was used to investigate the significance of these factors on the square-root of seed set per fruit, with date treated as a random effect. Separate analyses were conducted on fruit and seed set because inclusion of zero-fruit set data violated ANOVA assumptions.

Influence of Pollen Removal and Deposition on Pollen Limitation

To investigate the relation between pollen limitation and the removal of pollen grains from anthers and their deposition on stigmas, anthers and stigmas were sampled from populations B and D when pollen limitation treatments were conducted in 1997. The

stigma and anthers from fifteen flowers were collected at the end of each day and placed in 70% ethanol in separate microcentrifuge tubes until pollen grains were counted. The amount of pollen removed during the day was determined as described above. Pollen grains were released from stigmas for counting using acetolysis (Kearns and Inouye 1993). Acetolysis is an acid digestion that degrades all organic matter except the pollen exine and was used to facilitate counting because pollen grains of *R. virginica* are small and obscured by the stigmatic papillae when on the stigmas. Once released, pollen grains were suspended in lactophenol-glycerin with cotton blue stain before four replicate counts were made using a hemacytometer (Lloyd 1965). The mean amount of pollen removed from anthers and deposited on stigmas on each day in each population was calculated. The mean values were used to correlate daily pollen removal and deposition with the intensity of pollen limitation. Pollen limitation on each day in the two populations was summarized as an index of the ratio of seed set in control to supplemental hand-pollinated plants, including those not setting fruit. The intensity of pollen limitation differed in the two populations so they were treated separately.

RESULTS

FLORAL BIOLOGY OF *RHEXIA VIRGINICA*

Self-Compatibility

Experimental hand pollinations conducted under glasshouse conditions demonstrated that *R. virginica* is strongly self-compatible. There was no significant difference between the seed set of flowers after self- or cross-pollination (Figure 3.2). Furthermore, there were no significant differences among cross-pollinations using pollen from the same or different populations at Lake Matchedash (Table 3.1). The self-compatibility of *R. virginica* was confirmed using plants from McKechnie Lake (outcross ($\overline{X} \pm$ SE) = 35.9 ± 7.7, self = 57.4 ± 17.2; t = 1.18, NS, df = 19) and Krapek Lake (outcross = 99.5 ± 29.6, self = 141.9 ± 27.6; t = 1.05, NS, df = 11). *Rhexia virginica* requires insect visits for fruit set to occur. Flowers that were not experimentally hand-pollinated set no

Figure 3.2. Mean seeds per fruit (± SE) for self- and outcross pollinations conducted on flowers of *Rhexia virginica* in a glasshouse at the University of Toronto in July and August 1996. Plants were transplanted to the glasshouse from five populations at Lake Matchedash, Ontario. Unpollinated flowers set no seed autonomously. *N* = number of flowers subjected to each treatment.

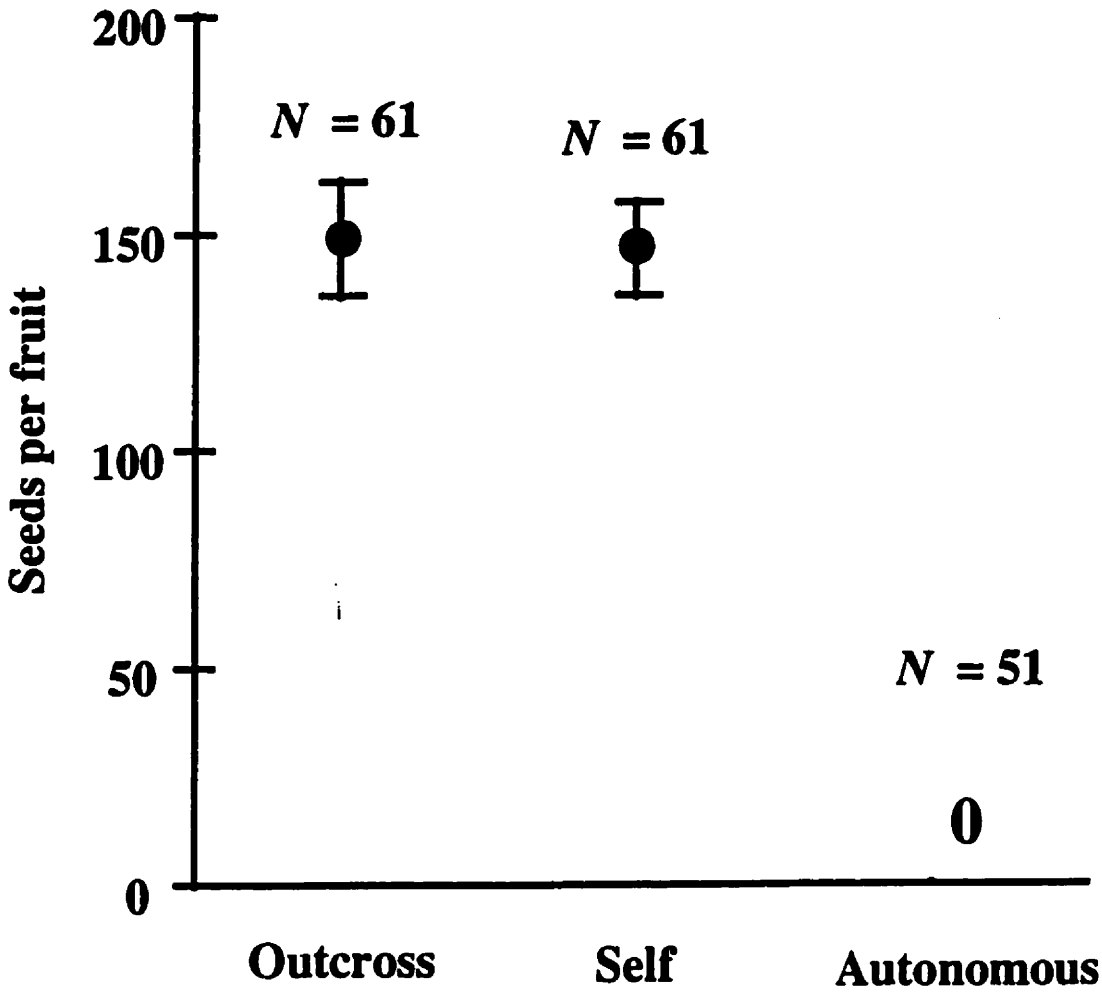

200
150
100
50
0
Seeds per fruit
N = 61
N = 61
N = 51
0
Outcross
Self
Autonomous

Table 3.1. Mixed-model analysis of variance of square-root of seed set per fruit in glasshouse pollinations of *Rhexia virginica* from five populations at Lake Matchedash, Ontario. The three treatments were self-pollination and outcross pollination within and between populations. Population and treatment were random and fixed effects, respectively. Sample sizes were greater than 50 for each of the three treatments. ** $P < 0.01$.

Source of variation	df	SS	*F*
Population	4	735.41	10.77**
Pollination treatment	2	7.85	0.22
Population X Treatment	8	136.40	0.97
Error	198	3492.40	

fruit autonomously ($N = 51$). Furthermore, flowers did not set fruit when subjected to simulated rain ($N = 31$).

Pollen-Ovule Ratio

The pollen-ovule ratio of *R. virginica* flowers at Lake Matchedash was 668, which suggests that it may have a mixed mating system (Cruden 1977). Flowers produced many small pollen grains (number per flower = $3.05 \times 10^5 \pm 5.63 \times 10^3$, $N = 154$; size = 20.47 ± 0.09 µm, $N = 200$) and numerous ovules (number per flower = 456.6 ± 7.5, $N = 87$). Comparison of the quantity of pollen produced by each of the eight anthers in a flower revealed no significant difference among them (number per anther = $3.2 \times 10^4 \pm 8.5 \times 10^3$, $N = 103$, df = 7, $F = 0.65$). Similarly, ovule counts did not differ among flowers from different nodes on the plant ($N = 87$; df = 3, $F = 1.73$).

Floral Colour Change

First and second-day flowers of *R. virginica* were easily differentiated by their colour. Their size was similar, but second-day petals were paler and slightly smaller than first-day petals (first-day petal length = 11.65 ± 0.14 cm, second-day = 11.42 ± 0.15 cm; $N = 48$, $P < 0.06$, Wilcoxon's signed-ranks test). Petals remained attached to the flower for one or two days after anthesis. Colour change was most marked in the filaments, which became reddish and recurved on the second day. Reddening of anthers in second-day flowers was slight, but in their recurved position they were not readily apparent in a frontal view of the flower. Observation of flowers within pollinator exclosures at Lake Matchedash indicated that the sequence of floral colour change was predictable and independent of pollinator visitation. Floral colour change within exclosures differed little from that outside exclosures, indicating that it was not induced by visitation. The rate of visible colour change was affected by temperature; on warm days filaments were dark red and fully recurved by late afternoon, whereas during cool periods they often remained merely pale reddish and only slightly recurved until the next morning.

Controlled hand-pollinations at Lake Matchedash demonstrated that the female and male fertility of second-day flowers were both low. Reduced receptivity of second-day stigmas was indicated by significantly lower fruit set in second-day flowers compared to first-day flowers whether they were pollinated with pollen from first- (fruit set = 11.8%, N = 17) or second-day flowers (fruit set = 6.3%, N = 16) (df = 3, $\chi^2 = 41.84$, $P < 0.0001$). A significant reduction in the viability of pollen from second-day flowers was documented for fruit set but not the number of seeds set per fruit. Fruit set was lower when first-day stigmas were pollinated with second-day pollen than first-day pollen (using first-day pollen, fruit set = 100%, N = 13; using second-day pollen, fruit set = 57.1%, N = 14; df = 1, $\chi^2 = 9.48$, $P < 0.005$), but the number of seeds produced per fruit was similar (first-day pollen = 76.1 ± 16.0; second-day pollen = 53.4 ± 15.9; N = 21, $t = 0.72$, square-root transformed data). These results indicate that second-day flowers are unlikely to contribute directly to plant fitness, but may do so indirectly by increasing floral display size (see below).

Flowering Phenology

Phenological investigations of *R. virginica* at Lake Matchedash indicated that its flowering season lasted from mid-July to early October. However, the vast majority of flowering was concentrated from late July to mid-August (Figures 3.3 and 3.4). Peak flowering was in early August and the distribution of flowering over the season was not markedly skewed (Figure 3.3). These results provide a measure of the time scale over which the floral display of *R. virginica* changes, as viewed by pollinators dependent on its flowers as a resource.

In 1996 the average plant in the phenology study had a total of five flowers, which bloomed on four days over a nine day period (N = 197). When plants flowered they had a median display size of one first-day flower per plant. Of 4884 floral displays on plants observed during phenological studies, 90.3% had one flower in anthesis, 7.8% had two flowers, and the remaining 2.0% had more than two flowers in anthesis. The largest daily floral display size observed consisted of seven first- and four second-day flowers. The preponderance of one-flowered displays on plants flowering at Lake Matchedash indicates

Figure 3.3. Flowering phenology of *Rhexia virginica* at Lake Matchedash, Ontario during late July and August 1996. The data are from 197 plants distributed equally among populations A, B, C, and D. Each data point shows the percentage of all flowers (N = 1041) open on a given day.

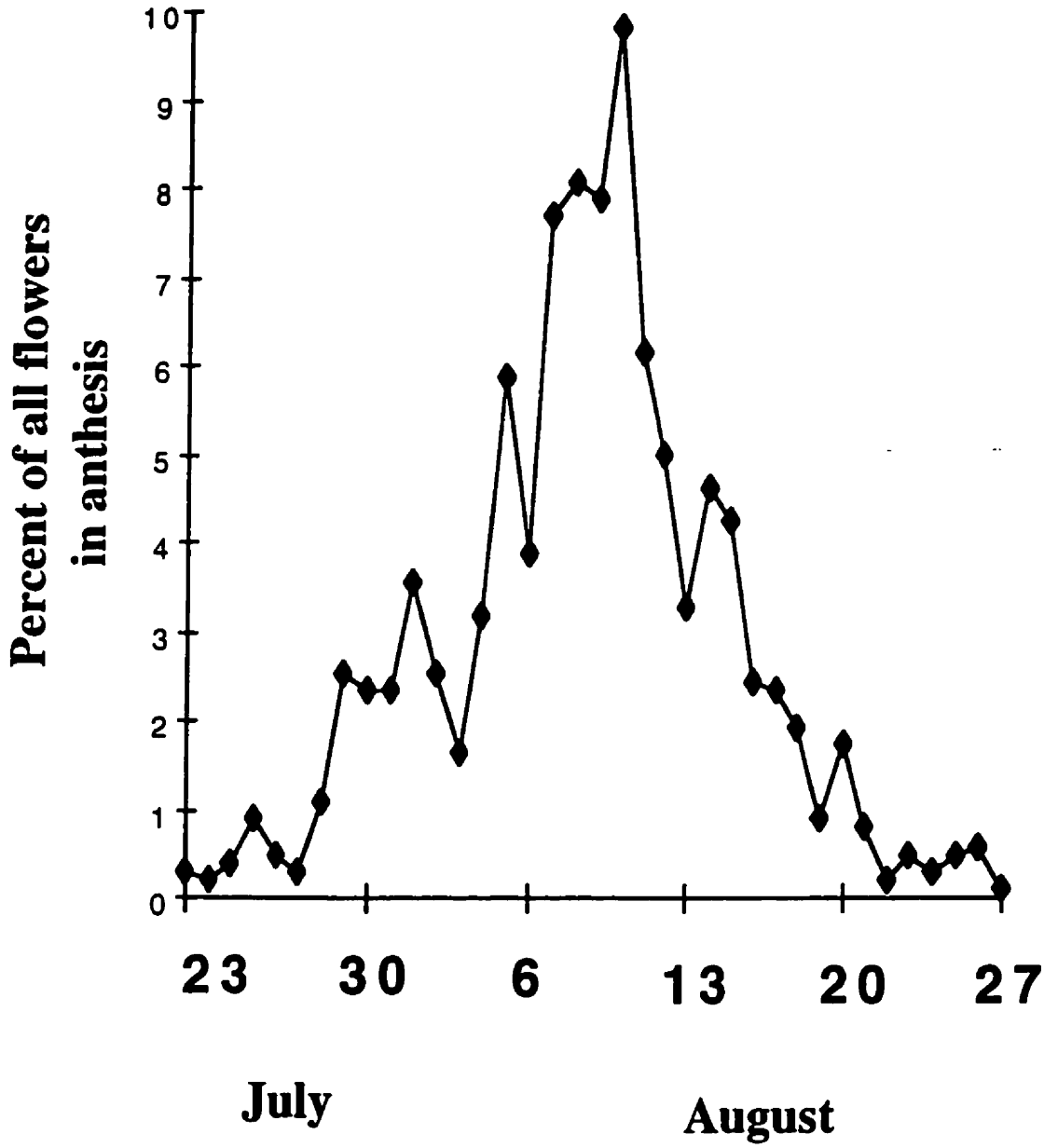
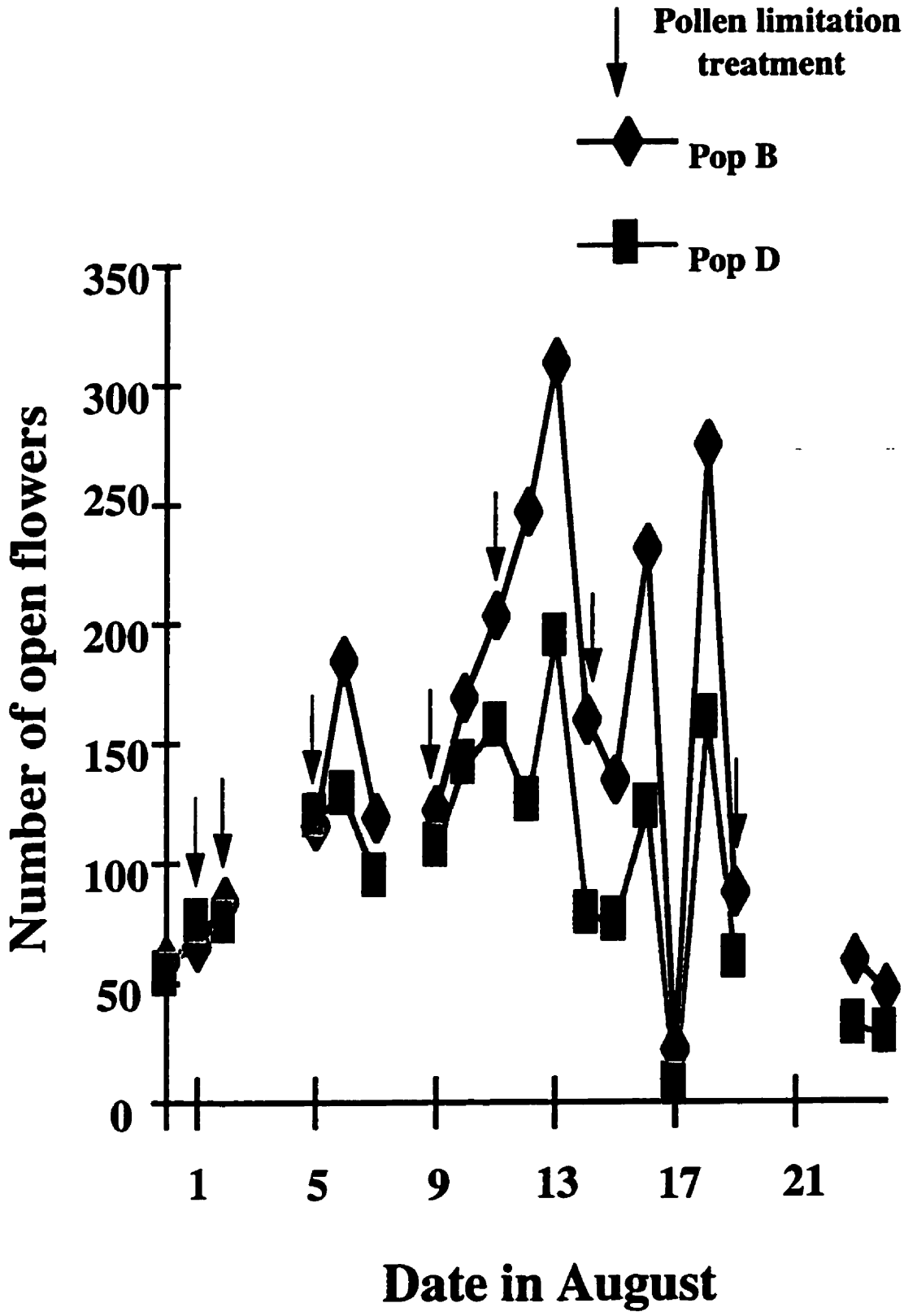

Percent of all flowers in anthesis
10
9
8
7
6
5
4
3
2
1
0
23
30
6
13
20
27
July
August

Figure 3.4. Flowering phenology of *Rhexia virginica* at Lake Matchedash, Ontario in August 1997. The two curves are from populations B and D and present the total number of flowers in each population flowering on each day. Arrows indicate seven days during the flowering period when pollen limitation treatments were conducted in each population (see text for details).


Pollen limitation treatment
Pop B
Pop D
Number of open flowers
350
300
250
200
150
100
50
0
1
5
9
13
17
21
Date in August

that any variation in plant fertility attributable to display size must result from larger displays on a small number of plants. Floral display size could be increased by maintenance of second-day flowers. However, they infrequently augmented the size of displays consisting of first-day flowers. In 1996, 74.6% of the floral displays that contained first-day flowers ($N = 733$) did not contain second-day flowers.

POLLINATION BIOLOGY OF *RHEXIA VIRGINICA*

Floral Visitation

Although 25 species of animal visitors were observed at *R. virginica* flowers at Lake Matchedash (Table 3.2), only bumblebees and small halictid bees were common. Analysis of quadrat data indicated that bumblebees made 82% of visits ($N = 97$ observation periods) to flowers in 1996. The majority (75%) of these visits were by *Bombus bimaculatus*, with declining proportions attributable to *B. terricola*, *B. ternarius*, and *B. affinis*, respectively. Each of these species was infrequent in 1997. Instead, *B. impatiens* was the predominant visitor and made greater than 90% of all visits to flowers. This variation in the pollinator fauna between years illustrates that pollination of *R. virginica* is relatively generalized and probably susceptible to fluctuations in the population size of pollen-foraging bumblebees.

Observation of bumblebee foraging to *R. virginica* populations at Lake Matchedash in 1996 and 1997 indicated visitation was infrequent overall but occasionally reached high levels (Figures 3.5 and 3.6). Visitation generally began soon after sunrise (~ 6:30 a.m. E.S.T.) and typically declined in early afternoon. In 1996, the median number of visits to quadrats during 15-minute quadrat observation periods was one (number of visits = 1.4 ± 0.17, $N = 59$). The majority of the time no visits were recorded, but there were occasionally as many as five visits to quadrats (Figure 3.5). Visitation rates in 1997 were even more sporadic (Figure 3.6). The median number of visits to population B during the fourteen morning observation periods was only 0.65 per hour (number of visits = 1.1 ± 0.34, $N = 14$). On only two of these mornings were there more than ten bumblebee visitation bouts to population B as a whole. However, on these mornings (August 9 and

Table 3.2. Visitors to *Rhexia virginica* flowers at Lake Matchedash, Ontario during 1996 and 1997 (specimens in collection of the author). A total of 25 taxa were recorded.

INSECTS

Hymenoptera

Apidae
Bombus affinis Cresson
Bombus bimaculatus Cresson
Bombus impatiens Cresson
Bombus perplexus Cresson
Bombus ternarius Say
Bombus terricola Kirby
Bombus vagans Smith

Anthophoridae
Ceratina sp.

Halictidae
Augochlorella striata (Provancher)
Lasioglossum (Dialictus) pilosus Smith
Lasioglossum sp.

Megachilidae
Megachile sp.

Vespidae
Dolichovespula arenaria (Fab.)

Diptera

Syrphidae
Eupeodes americanus (Wiedemann)
Helophilus fasciatus Walker
Lejops stipatus Walker
Pipiza sp.
Syritta pipiens (L.)
Syrphus ribesii (L.)
Toxomerus geminatus (Say)
Tropidia quadrata Say

Lepidoptera

Pieridae
Pieris rapae (L.)

Hesperiidae
1 species

Nymphalidae
Speyeria cybele (Fabricius)

BIRDS

Trochilidae
Ruby-throated Hummingbird
(*Archilochus colubris*)

Figure 3.5. The frequency of numbers of bumblebee visits to *Rhexia virginica* during 15 minute observation periods of 4 m^2 quadrats in populations B and D at Lake Matchedash, Ontario in August 1996.

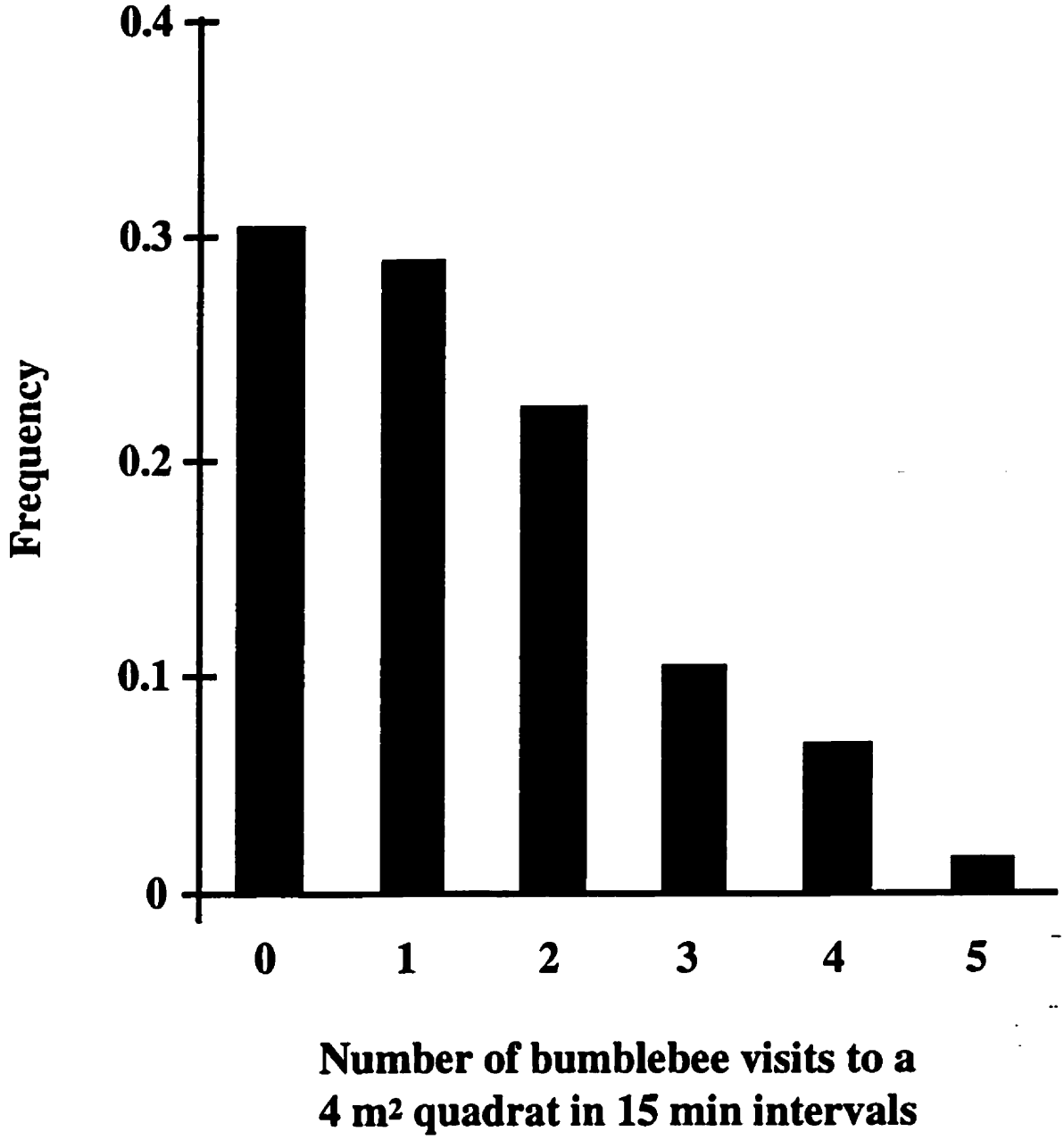
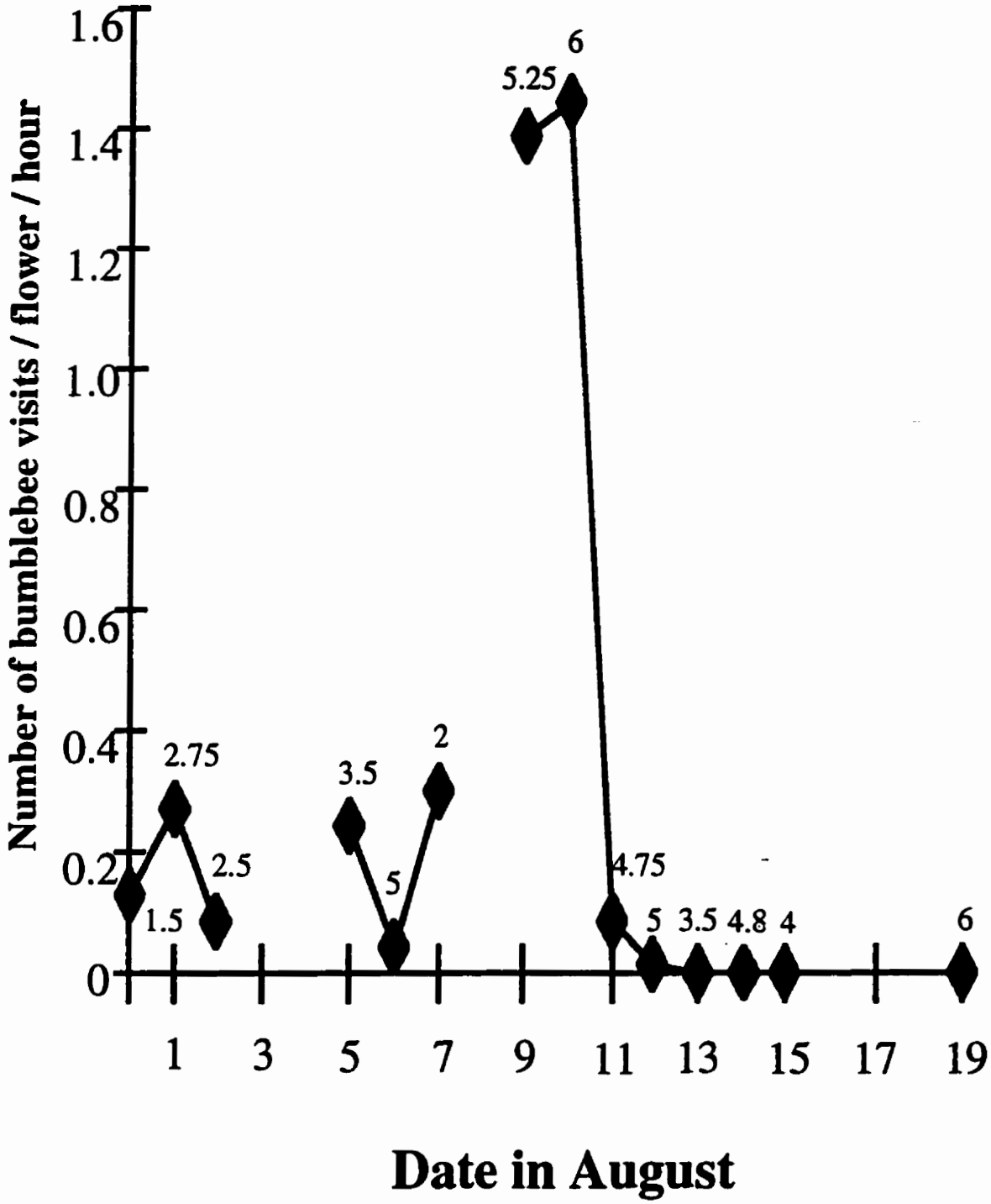

0.4
0.3
0.2
0.1
0
Frequency
0
1
2
3
4
5
Number of bumblebee visits to a
4 m² quadrat in 15 min intervals

Figure 3.6. Daily variation in *Bombus* visitation to population B of *Rhexia virginica* at Lake Matchedash, Ontario during August 1997. Points represent the number of visits recorded during morning observation periods, scaled by the number of flowers in the population (Figure 3.4) and duration of observation (number of hours given above each point).


Number of bumblebee visits / flower / hour
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
1.5
2.75
2.5
3.5
5
2
5.25
6
4.75
5
3.5
4.8
4
6
1
3
5
7
9
11
13
15
17
19
Date in August

10) foraging runs were intensive, with a median bout consisting of 40 flowers (number of flowers visited = 53.7 ± 7.5, range = 1-212, $N = 47$) and lasting four minutes (visit duration in seconds = 298.4 ± 39.2, range = 1-945 seconds). On average, *Bombus impatiens* visited all flowers in population B every 45 minutes on these two mornings (Figure 3.6). On the other mornings, the total number of visits observed was much lower than the number of flowers present in the population. Bees appeared infrequently on these days and only sampled a few flowers before departing the patch. The unusually high rate of foraging on August 9 and 10 was not the result of favourable weather conditions compared to other mornings. Temperatures at 7 a.m. on these two mornings were 16 and 20 °C, respectively, which did not differ markedly from other mornings when observations were conducted (mean temperature at 7 a.m. = 16.4 ± 1.1 °C, $N = 13$, range = 13-23 °C).

Bumblebees were the most significant pollinators of *R. virginica* at Lake Matchedash. They were the most common floral visitors and, moreover, they were the only visitors to effectively buzz flowers. A typical visit by a bee involved grasping the upper filaments with their mandibles and legs and buzzing. While buzzing, their body was arched beneath them and contacted the anthers and often the stigma. In this position, buzzing expelled pollen onto both their thorax and abdominal tergites, imparting their abdomen with a characteristic white tip. Although halictid bees spent lengthy periods on flowers (visit duration in seconds = 16.96 ± 2.92, range = 1-120 seconds, $N = 53$) and buzzed individual anthers, they were relatively uncommon and made little contact with the stigma, so were probably only minor pollinators.

The potential for bumblebees to transfer pollen geitonogamously, within inflorescences, was assessed in 1996 by recording the number of *R. virginica* flowers they visited during foraging on multi-flowered inflorescences. The bumblebees showed a tendency to visit an increasing proportion of flowers on larger inflorescences than smaller ones (39.5, 53.5, and 63.2% of visits were to more than one flower on inflorescences with two, three and four first-day flowers, respectively; $N = 367$, $\chi^2 = 10.52$, $P < 0.01$, G-test of independence). Although this suggests that geitonogamous pollen transfer is likely, the

low frequency of multi-flowered inflorescences within populations at Lake Matchedash minimizes its overall contribution to rates of self-fertilization.

Floral Cues to Pollinators

During observation periods at Lake Matchedash in 1996 and 1997 bumblebees occasionally approached second-day flowers, but rarely visited them. For example, on August 9 and 10, 1997, the frequency of second-day flowers in population B was 46% and 42%, respectively. Despite this, only eight (0.3%) of 2341 bumblebee visits recorded on these two days were to second-day flowers.

Bee-stick experiments at Lake Matchedash in 1996 demonstrated that bumblebees were more likely to approach and visit intact flowers than ones from which either petals or stamens had been removed. Bumblebees were significantly less likely to approach flowers from which petals had been removed (18.2% of approaches to the bee-stick were to petal-less flowers; $N = 55$, $\chi^2 = 22.3$, df = 1, $P < 0.0001$) or to visit them (percent of approaches culminating in a visit, for intact flowers = 87%, for petal-less flowers = 10%; $N = 40$, $\chi^2 = 22.6$, df = 1, $P < 0.0001$). They were also less likely to approach flowers from which stamens had been removed (22.9% of approaches to the bee-stick were to stamen-less flowers; $N = 70$, $\chi^2 = 20.6$, df = 1, $P < 0.0001$) or to visit them (percent of approaches culminating in a visit, for intact flowers = 89%, for stamen-less flowers = 6%; $N = 49$, $\chi^2 = 40.4$, df = 1, $P < 0.0001$). These results indicate that floral visits to *R. virginica* by bumblebees were dependent upon cues provided by both the stamens and petals. In second-day flowers, these cues were modified by the colour change.

Two lines of evidence support the hypothesis that bumblebees made foraging decisions based on an assessment of the amount of pollen within anthers. First, array experiments at Lake Matchedash in 1996 demonstrated that bumblebees were more likely to visit unvisited *R. virginica* flowers than those that had been previously visited. On both days bumblebees made more visits to previously unvisited flowers located in the arrays (visits to unvisited flowers = 78, visits to previously visited flowers = 36; $G_{pooled} = 15.84$, df = 1, $P < 0.0001$), but the strength of this pattern differed between days ($G_{heterogeneity} =$

4.00, df = 1, $P < 0.05$). Second, during periods of frequent visitation (e.g., August 9 and 10, 1997) bumblebees made shorter visits prior to 10 a.m. than afterwards (visit duration in seconds, prior to 10 a.m. = 2.87 ± 0.12, $N = 217$; after 10 a.m. = 3.41 ± 0.17, $N = 193$; Wilcoxon $Z = 2.24$, $P < 0.05$, range = 0.60-13.95 seconds). If one assumes that pollen in anthers was reduced over this period, these results imply that the duration of visits was responsive to the amount of pollen in flowers.

Pollen Removal During Pollinator Visits

Pollen removal experiments conducted at Lake Matchedash in 1996 indicated that a single visit by *Bombus bimaculatus* removed 10.2% of the initial amount of pollen produced by a flower (Figure 3.7). However, pollen removal during bee visits depended less on the number of visits to a flower than on their cumulative duration (number of visits: $N = 29$, df = 2, $F = 0.57$, NS; duration: df = 1, $F = 9.70$, $P < 0.005$). These results suggest that pollen removal during a single visit was somehow metered by the anthers, but that longer pollinator visits overcame this restriction to some extent.

Experiments in 1997 further demonstrated that buzz pollination was not the only mechanism that released pollen grains from *R. virginica* anthers. A significant proportion (49.3%) of pollen could be removed from the anthers by repeated tapping (pollen count prior to manipulation = $2.96 \times 10^5 \pm 1.10 \times 10^4$, $N = 14$; after manipulation = $1.50 \times 10^5 \pm 5.57 \times 10^3$, $N = 10$; $t = 12.02$, $P < 0.0001$, log-transformed data). This result is significant because it implies that bumblebee behaviour at flowers did not have to be highly stereotyped for pollen to be released during visits. Pollen could be expelled from anthers simply by the movement of bumblebees on flowers during visits.

VARIATION IN FEMALE FERTILITY OF *RHEXIA VIRGINICA*

Survey of Patterns of Fruit Set

Population surveys of *R. virginica* in the Muskoka region of Ontario in 1996 and 1997 revealed three clear patterns in female fertility. First, mean fruit set of plants in the populations was consistently low (percent fruit set = 52.6 ± 0.02, $N = 530$; Figure 3.8). In

Figure 3.7. Mean percentage (± SE) of pollen grains in *Rhexia virginica* flowers removed during one, two or three bumblebee visits at Lake Matchedash, Ontario in August 1996. Sample sizes (*N*) are given below the bars. See methods for experimental details.

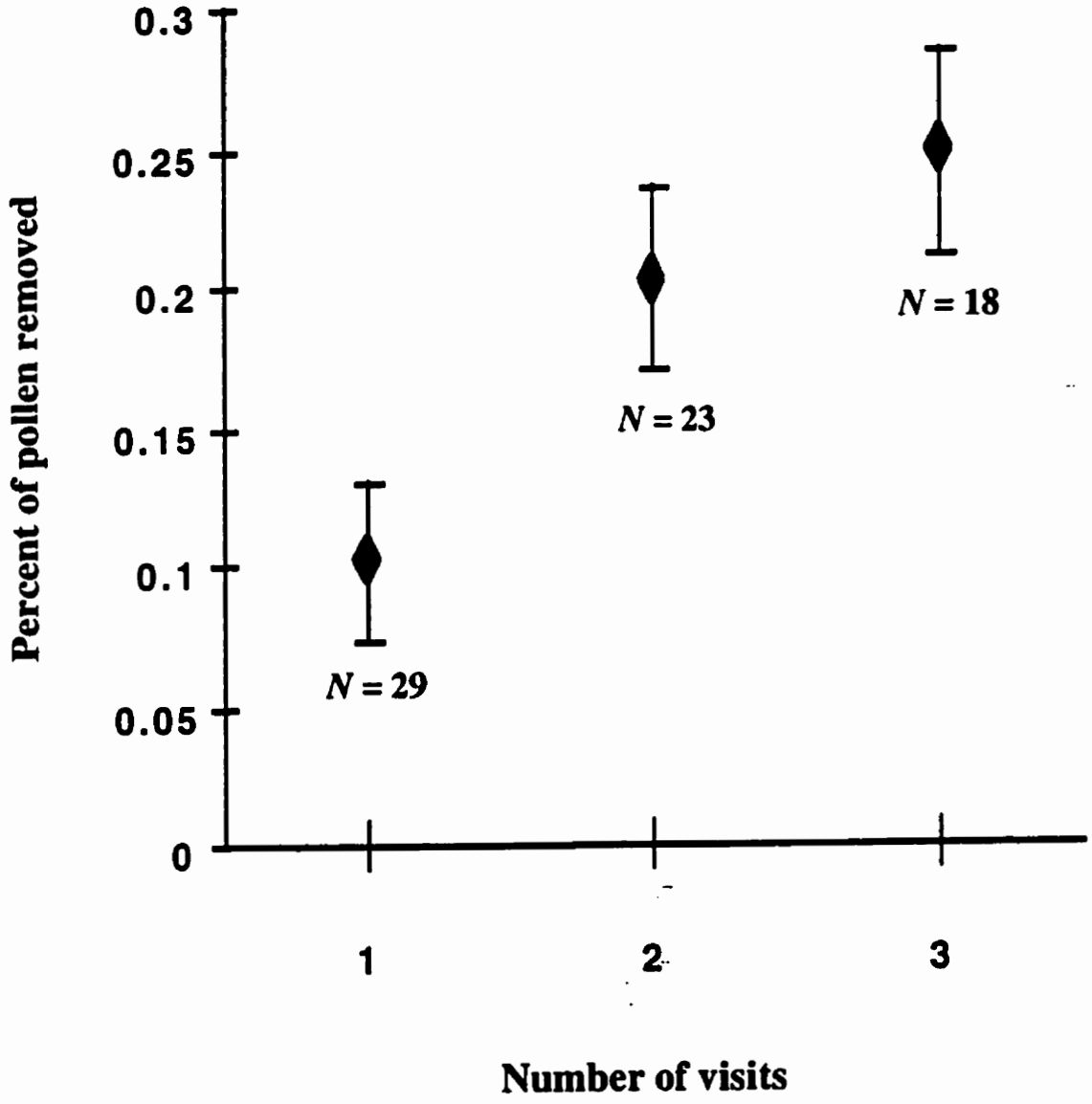
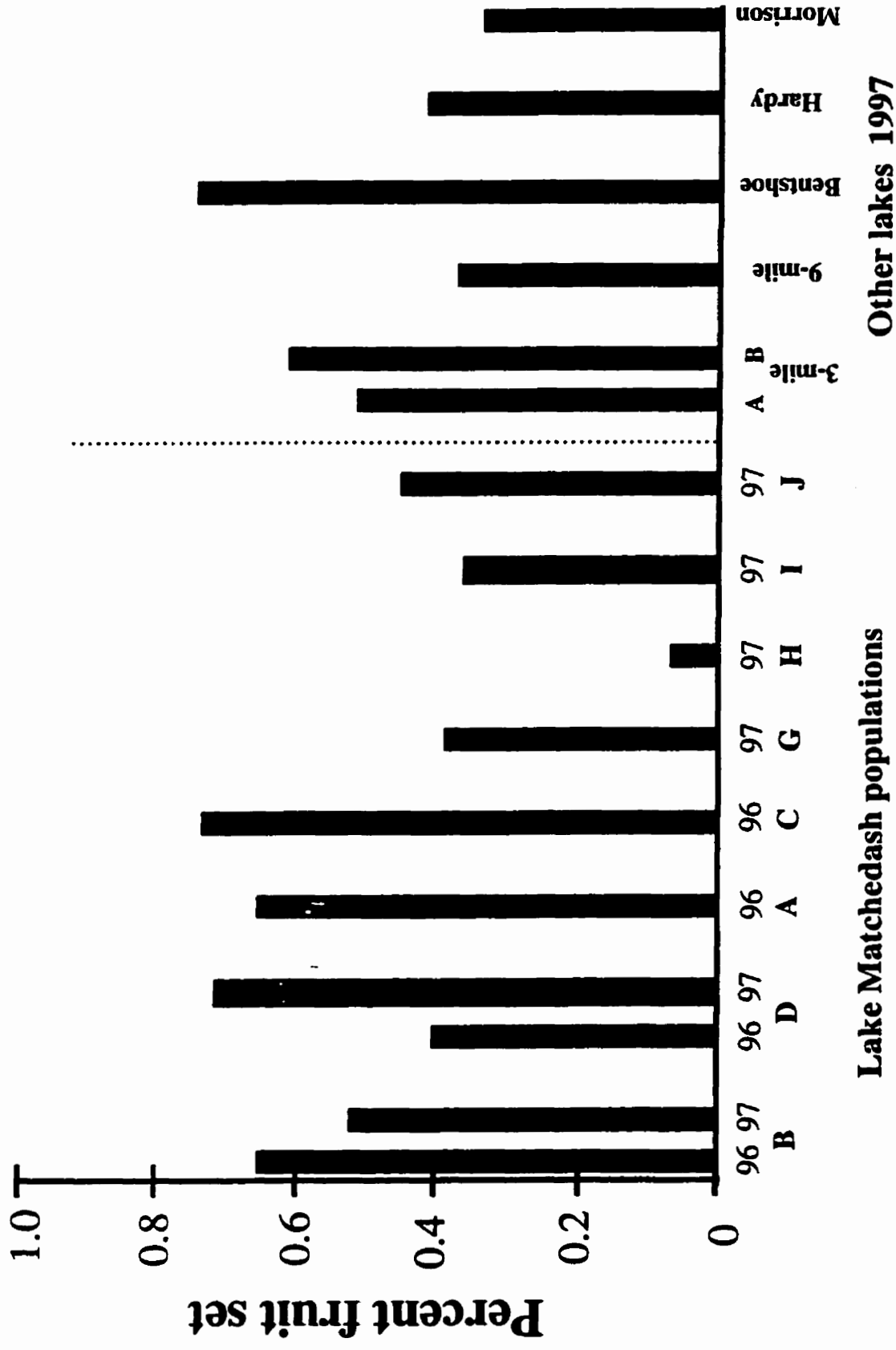

0.3
0.25
0.2
0.15
0.1
0.05
0
Percent of pollen removed
N = 29
N = 23
N = 18
1
2
3
Number of visits

Figure 3.8. Mean percent fruit set of *Rhexia virginica* plants in eight populations at Lake Matchedash, Ontario, and at five other lakes in the Muskoka region. Fruit set was assessed in both 1996 and 1997 at Lake Matchedash, and in 1997 at the other lakes.


Percent fruit set
1.0
0.8
0.6
0.4
0.2
0
96 97 B
96 97 D
96 A
96 C
97 G
97 H
97 I
97 J
A
B
3-mile
9-mile
Bentshoe
Hardy
Morrison
Lake Matchedash populations
Other lakes 1997

1996, fruit set among the four populations studied at Lake Matchedash was 60.6% (N = 195, range = 40.4-72.6%). Amongst the twelve populations surveyed in the Muskoka region in 1997, fruit set was 47.9% (N = 335, range = 5.7-74.2%). Second, in both years levels of fruit set were highly variable among populations (1996: N = 195, df = 3, F = 8.09, $P < 0.0001$; 1997: N = 335, df = 11, F = 10.62, $P < 0.0001$). Lastly, fruit set in the twelve populations surveyed in the Muskoka region in 1997 was significantly related to population size (Figure 3.9). Taken together, these patterns highlight the low and variable fertility of *R. virginica* populations in Muskoka. This was further demonstrated by annual variation in fruit set. Fertility was assessed in both 1996 and 1997 only in populations B and D at Lake Matchedash. An ANOVA indicated that the fertility of these two populations was markedly different in the two years, with only the interaction between year and population significant (N = 203, df = 1, F = 19.92, $P < 0.0001$) (see Figure 3.8).

Factors Affecting Female Fertility

Population, floral display size and flowering time explained a significant proportion of variation in female fertility among *R. virginica* plants at Lake Matchedash in 1996 (Tables 3.3 and 3.4). However, statistical models accounted for a relatively small proportion of this variation (for fruit set, r^2 = 10.4%; for seed set, r^2 = 9.4%), indicating that additional ecological factors must also influence fertility

Floral display size and flowering time explained more of the variation in fertility of *R. virginica* flowers than population membership. Fertility was marginally influenced by the number of first-day flowers displayed on a plant (Tables 3.3 and 3.4), but fruit and seed set did not increase linearly with display size (Figure 3.10C, D). Inclusion of second-day flowers in display size increased its explanatory power in the logistic regression on fruit set (Table 3.3), but not in the ANOVA of seeds per fruit. This indicates a potential adaptive role of second-day flowers through enhancement of total floral display size. The importance of flowering time for fertility was indicated by the significant contribution of the flowering time X population interaction to variation in fruit set (Table 3.3). This indicates that fertility varied through time but that this effect differed between populations,

Figure 3.9. The relation between percent fruit set and log population size in twelve populations of *Rhexia virginica* . Populations were sampled in the Muskoka region of Ontario in 1997 and ranged in size from 75 to 1200 plants. The statistical association (r_s) is Spearman's rank correlation.

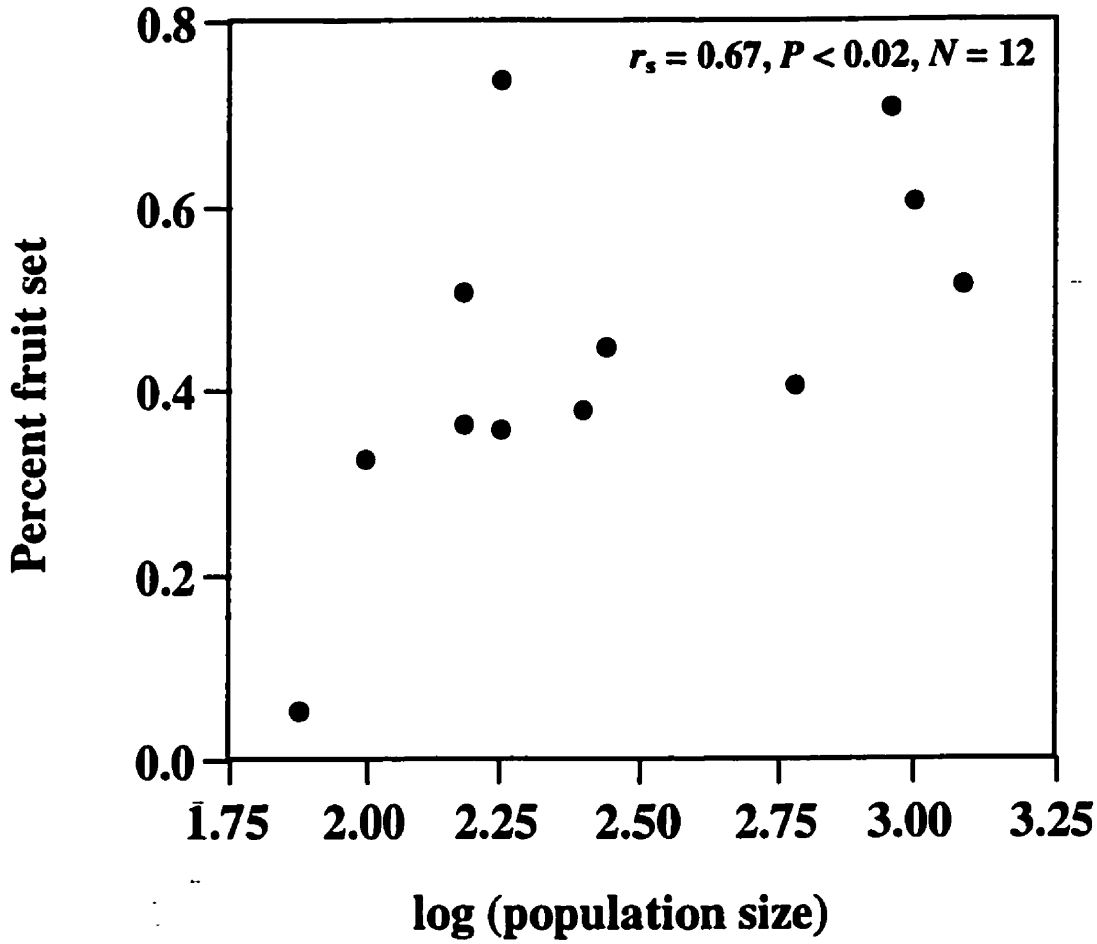

$r_s = 0.67, P < 0.02, N = 12$
Percent fruit set
0.0
0.2
0.4
0.6
0.8
1.75
2.00
2.25
2.50
2.75
3.00
3.25
log (population size)

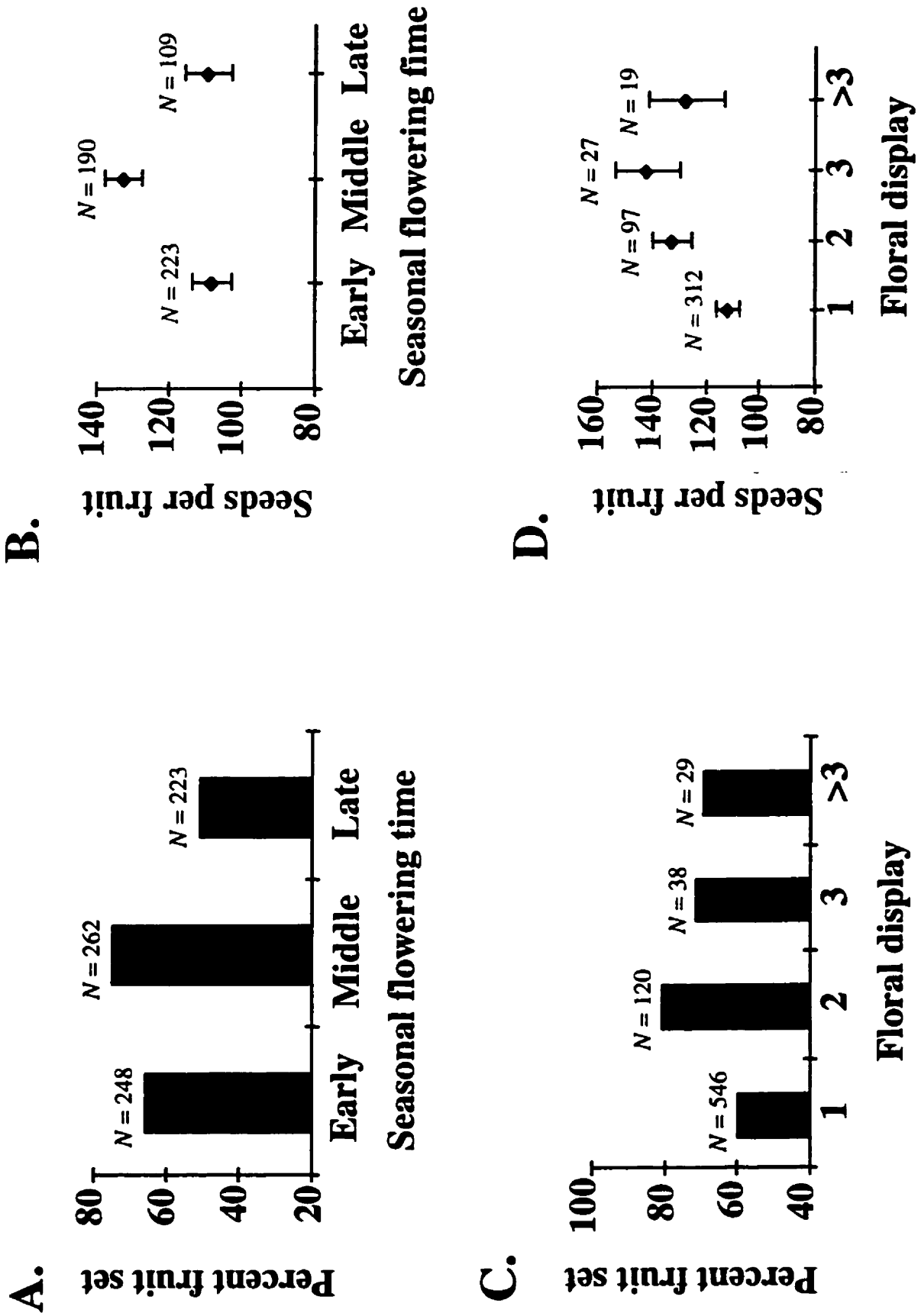
Table 3.3. Logistic regression analysis of the effects of population, floral display size, and flowering time on likelihood of fruit set in *Rhexia virginica* at Lake Matchedash, Ontario in 1996. The results of models where display size consisted of first-day flowers vs. first - and second-day flowers (total display) are contrasted. Insignificant interaction terms ($P > 0.30$) were deleted via backwards stepwise elimination. The percent fruit set of plants with differing display sizes and flowering times is presented in Figure 3.10.

Source of variation	df	1st-day flower display		Total display	
		likelihood ratio χ^2	*P*	likelihood ratio χ^2	*P*
Population	3	8.98	0.03	6.86	0.08
Display size	1	6.34	0.01	10.44	0.0012
Population X Display size	3	5.61	0.13	3.44	0.33
Flowering time	2	5.67	0.06	5.48	0.07
Population X Flowering time	6	28.80	0.0001	28.22	0.0001
Error	717				

Table 3.4. Mixed-model analysis of variance of the effect of population, floral display size, and flowering time on square-root of seed set of *Rhexia virginica* flowers at Lake Matchedash, Ontario in 1996. Only plants that set fruit were included in the analysis. Seed set per fruit of plants with differing displays and flowering times is presented in Figure 3.10. Note that error degrees of freedom reflect the number of plants that set fruit (N = 455), to account for non-independence of effects measured on the same inflorescence on different days (see methods). The whole model $r^2 = 0.094$, * $P < 0.05$.

Source of variation	df	SS	*F*
Population	3	30.34	0.71
Display size	1	64.61	5.56*
Population X Display size	3	30.88	1.15
Flowering time	2	38.83	1.42
Flowering time X Population	6	84.24	1.37
Flowering time X Display	2	8.86	0.49
Flowering time X Population X Display	6	49.63	0.71
Error	162	5003.84	

Figure 3.10. The relation between fertility and seasonal flowering time (A, B) and floral display size (C, D) of *Rhexia virginica* at Lake Matchedash, Ontario in 1996. Data were pooled from populations A, B, C, and D and involved a total of 1041 flowers on 197 plants. Fertility is measured as percent fruit set and mean (± SE) seeds per fruit. The results of a logistic regression on fruit set and an ANOVA on seeds per fruit are presented in Tables 3.3 and 3.4, respectively. N = sample size.


A.
Percent fruit set
80
60
40
20
N = 248
N = 262
N = 223
Early
Middle
Late
Seasonal flowering time
B.
Seeds per fruit
140
120
100
80
N = 190
N = 223
N = 109
Early
Middle
Late
Seasonal flowering time
C.
Percent fruit set
100
80
60
40
N = 546
N = 120
N = 38
N = 29
1
2
3
>3
Floral display
D.
Seeds per fruit
160
140
120
100
80
N = 27
N = 97
N = 312
N = 19
1
2
3
>3
Floral display

possibly reflecting slightly earlier flowering in population B relative to the other three populations. The fertility of flowers in mid-season was higher than flowers early or late in the season (Table 3.3, Figure 3.10A, B).

POLLEN LIMITATION OF FERTILITY OF *RHEXIA VIRGINICA*

Whole-Plant Pollen Limitation

Pollination treatments conducted in population B at Lake Matchedash in 1997 demonstrated that the female fertility of *R. virginica* plants was pollen-limited at the scale of whole plants. Addition of supplemental pollen to all flowers on plants increased percent fruit set and both the total number of seeds they produced and the number of seeds per flower relative to control plants (Table 3.5). The number of seeds per fruit was not increased by supplementation, possibly because fruit initiation requires delivery of a threshold amount of pollen to stigmas. Further support for whole-plant pollen limitation was obtained from the experiment in populations B and D in 1997. Open-pollinated flowers on treatment plants did not have lower fruit set ($N = 82$, $\chi^2 = 0.63$, G-test of independence) or number of seeds per fruit ($N = 46$, $t = 0.139$, square-root transformed data) than open-pollinated flowers on control plants. This indicates that the increased fertility of supplementally hand-pollinated flowers did not occur solely because of resource re-allocation from other flowers on the plant.

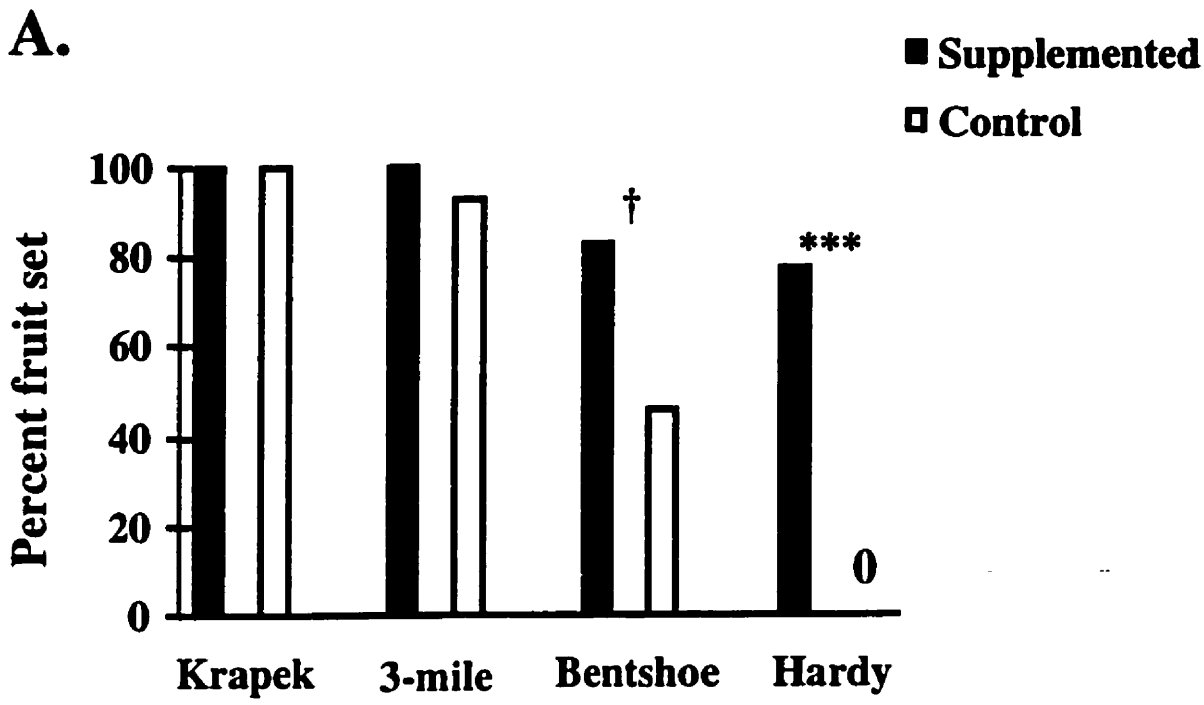
Survey of Pollen Limitation in Muskoka

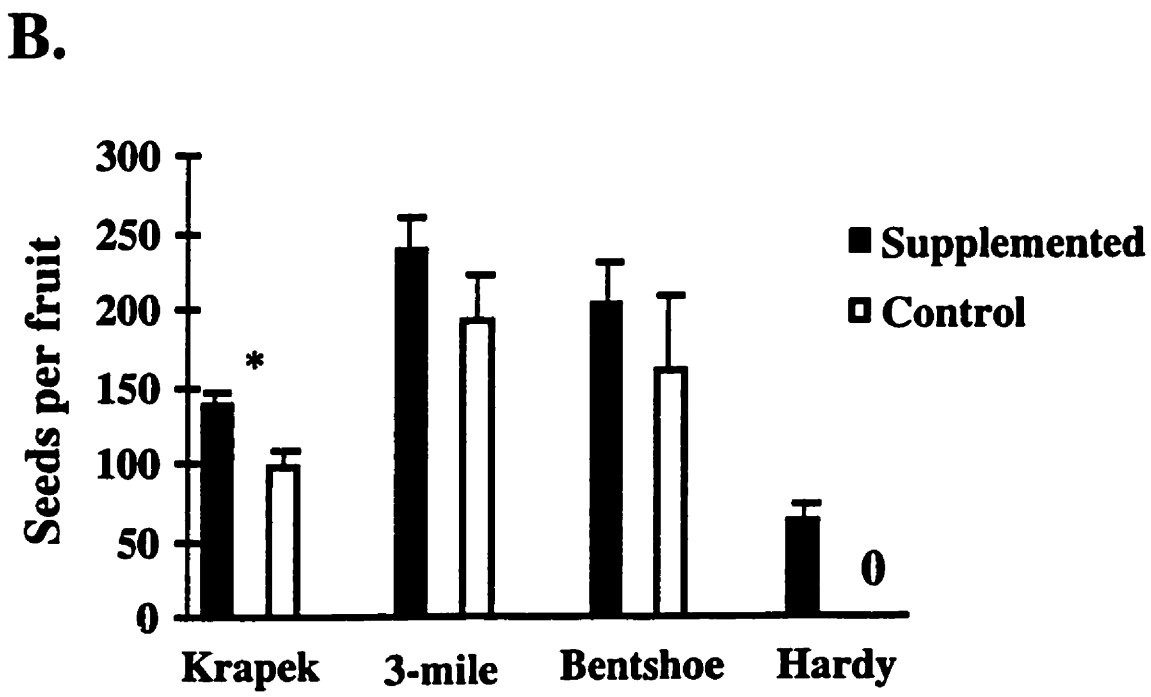
Pollen supplementation increased fruit set or seed set per fruit at three of four populations of *R. virginica* in the Muskoka region (Figure 3.11). This was confirmed by an ANOVA of seeds per flower that indicated a significant treatment effect ($N = 117$, df = 1, $F = 14.31$, $P < 0.05$) and significant variation among lakes (df = 3, $F = 15.01$, $P < 0.05$). Variation among lakes ranged from zero fertility in the open-pollinated treatment at Hardy Lake to an absence of statistically detectable pollen limitation at Three-mile Lake.

Table 3.5. Mean values (± SE) for control and supplemental hand-pollinated *Rhexia virginica* plants in population B at Lake Matchedash, Ontario in 1997. All flowers on supplemented plants received supplemental cross-pollen. Only traits marked by symbols († $P < 0.06$, * $P < 0.05$) differ between treatments, indicating a significant effect of supplemental pollination (one-tailed Wilcoxon's signed-ranks test).

	Control (N = 13)	Supplemented (N = 13)
Number of flowers	3.62 ± 0.51	3.08 ± 0.29
Plant height (cm)	25.8 ± 0.84	24.9 ± 1.04
Proportion fruit set	0.41 ± 0.03	0.59 ± 0.06*
Total seed number	151.8 ± 21.5	194.1 ± 22.3†
Seeds per flower	43.2 ± 4.2	63.2 ± 5.4*
Seeds per fruit	110.3 ± 11.1	113.4 ± 10.3

Figure 3.11. Comparison of fruit and seed set from open- and supplemental cross-pollinations of *Rhexia virginica* at four lakes in the Muskoka region of Ontario in August 1997. (A) Percent fruit set and (B) mean seeds per fruit (± SE) of supplemented and control flowers on separate plants. Sample sizes were 15, 15, 12 and 18 plants per treatment for the four lakes, respectively. Significant increases in fruit and seed set with pollen supplementation are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$, † $P < 0.06$), based on *G*-tests of independence for fruit set and one-tailed Wilcoxon two-sample tests on square-root of seed set.


A.
Supplemented
Control
Percent fruit set
100
80
60
40
20
0
†
***
0
Krapek
3-mile
Bentshoe
Hardy


B.
Seeds per fruit
300
250
200
150
100
50
0
*
0
Supplemented
Control
Krapek
3-mile
Bentshoe
Hardy

Pollen Limitation at Lake Matchedash

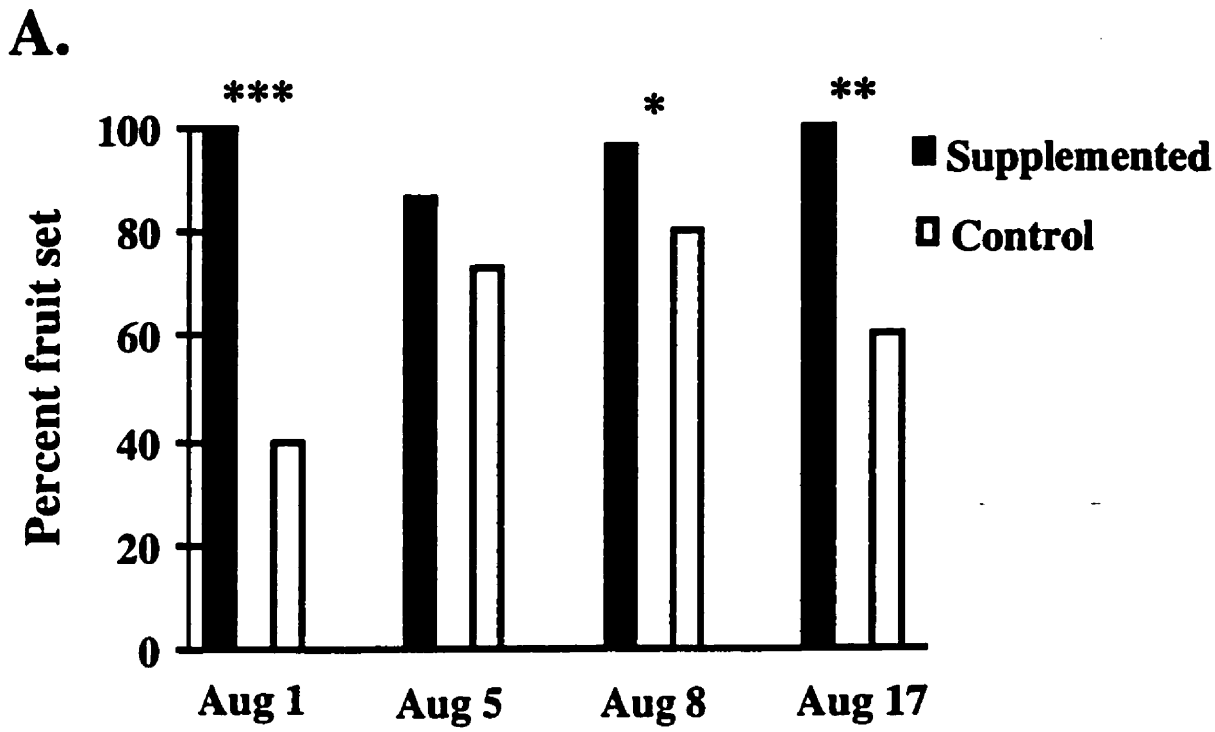
There was pronounced seasonal variation in the degree of pollen limitation at Lake Matchedash in 1996 (Figure 3.12). Over the entire season, fruit set of control flowers was significantly lower than that of supplemented flowers (control = 62.2%, $N = 90$; supplemented = 96.7%, $N = 90$; $\chi^2 = 37.24$, $P < 0.0001$, G-test of independence), as was mean number of seeds per fruit (control = 67.76 ± 7.58, supplemented = 115.75 ± 6.89; $N = 55$ pairs, $T_s = 472.5$, $P_{1\text{-tailed}} < 0.001$, Wilcoxon's signed-ranks test). The fertility of open-pollinated controls was particularly low near the beginning of the flowering season, probably as a result of wet, overcast weather.

Pollen supplementation treatments conducted at Lake Matchedash in 1997 revealed significant variation in pollen limitation that was manifested between populations and sampling dates (Tables 3.6 and 3.7, Figures 3.13 and 3.14). Fruit set was usually increased by pollen supplementation (Figures 3.13A and 3.14A), but the number of seeds set per fruit was rarely increased (Figures 3.13B and 3.14B). In population B, fruit set over the entire season was greatly increased by addition of supplemental pollen (control = 42%, $N = 100$; supplemented = 86.5%, $N = 96$; $\chi^2 = 44.45$, $P < 0.0001$, G-test of independence) whereas in population D it increased only moderately (control = 61.4%, $N = 101$; supplemented = 76.5%, $N = 102$; $\chi^2 = 5.43$, $P < 0.02$, G-test of independence). Overall, the likelihood of fruit set in the two populations was similar (no population effect in Table 3.6), but a significant population-treatment interaction indicates that pollen limitation differed between populations. The probability of fruit set varied among days during the flowering period in both populations (Table 3.6).

Influence of Pollen Removal and Deposition on Pollen Limitation

Analysis of daily patterns of pollen removal and deposition indicated that pollinators transferred only a small amount of the pollen produced by *R. virginica* anthers to stigmas. On average, fifty percent of the pollen produced was removed (52.7% ± 0.01, range = 31.3-67.3), but less than 0.5% was deposited on stigmas (0.46% ± 0.04, range = 0.07-1.04). However, the average number of grains deposited on stigmas in a day

Figure 3.12. Comparison of fruit and seed set from open- and supplemental cross-pollinations of *Rhexia virginica* at Lake Matchedash, Ontario, on four days in August 1996. (A) Percent fruit set and (B) mean seeds per fruit (± SE) of supplemented and control flowers that were paired on individual plants. Sample sizes were 30 pairs on August 1 and 8, and 15 on August 5 and 17. Significant increases in fruit and seed set with pollen supplementation are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$), based on *G*-tests of independence for fruit set and one-tailed Wilcoxon's signed-ranks tests on seed set.


A.
***
*
**
100
80
60
40
20
0
Percent fruit set
Supplemented
Control
Aug 1
Aug 5
Aug 8
Aug 17

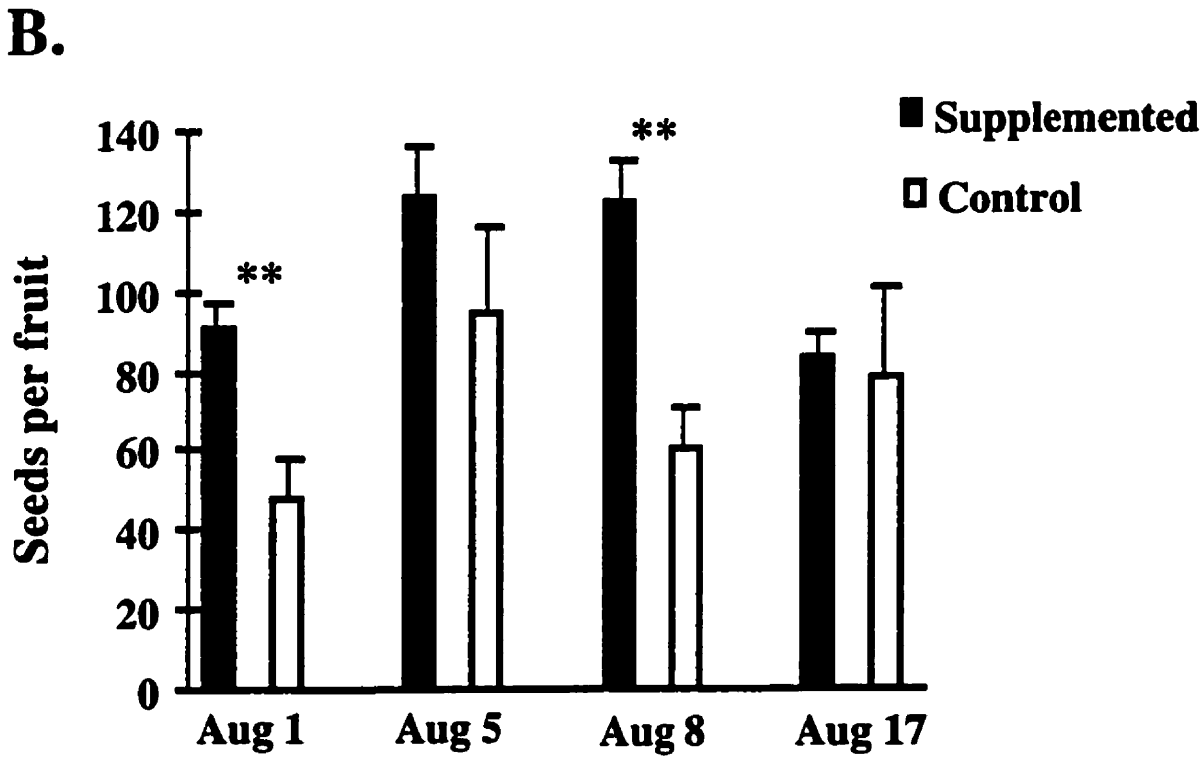

B.
**
**
140
120
100
80
60
40
20
0
Seeds per fruit
Supplemented
Control
Aug 1
Aug 5
Aug 8
Aug 17

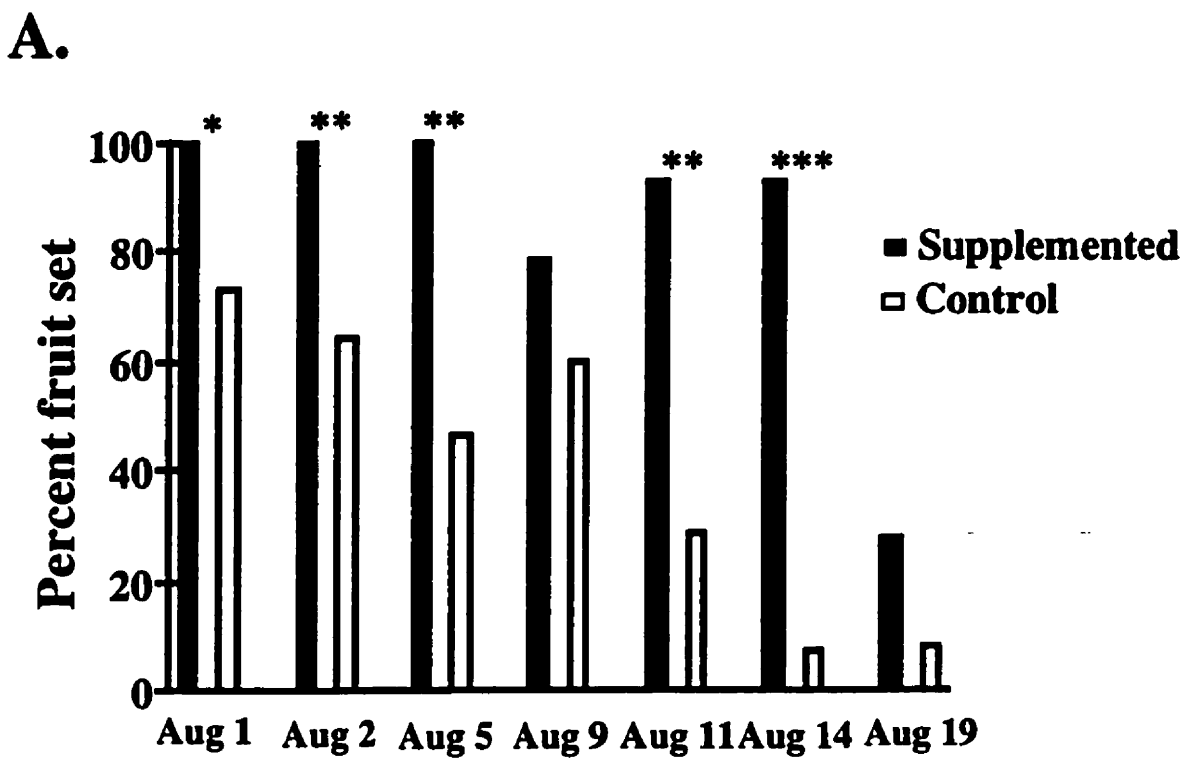
Table 3.6. Logistic regression analysis of the effects of population, pollination treatment (pollen supplementation vs. control) and date on likelihood of fruit set in *Rhexia virginica* at Lake Matchedash, Ontario in 1997. Insignificant interaction terms ($P > 0.30$) were deleted via backwards stepwise elimination. The percent fruit set for the pollen limitation experiment is presented in Figures 3.13 and 3.14.

Source of variation	df	Likelihood ratio χ^2	*P*
Population	1	0.01	0.93
Treatment	1	57.19	< 0.0001
Population X Treatment	1	12.35	0.0004
Date	6	109.13	< 0.0001
Treatment X Date	6	11.00	0.089
Error	383		

Table 3.7. Mixed-model analysis of variance of the effect of population, pollination treatment (pollen supplementation vs. control), and date on square-root of seed set of *Rhexia virginica* flowers at Lake Matchedash, Ontario in 1997. Only plants that set fruit were included in the analysis. Population and date were random effects, treatment was fixed. The whole model $r^2 = 0.235$. Seed set data for the pollen limitation experiment is presented in Figures 3.13 and 3.14.

Source of variation	df	SS	*F*	*P*
Population	1	2.01	1.98	0.69
Treatment	1	66.26	15.13	0.08
Population X Treatment	1	0.33	0.05	0.83
Date	6	245.40	3.02	0.13
Population X Date	6	41.55	1.19	0.42
Treatment X Date	6	74.58	2.14	0.19
Population X Treatment X Date	6	34.84	0.67	0.68
Error	229	1988.06		

Figure 3.13. Comparison of fruit and seed set from open- and supplemental cross-pollinations of *Rhexia virginica* in population B at Lake Matchedash, Ontario, during seven days in August 1997. (A) Percent fruit set and (B) mean seeds per fruit (± SE) of supplemented and control flowers on separate plants. There were fifteen plants in each treatment each day. Significant increases in fruit and seed set with pollen supplementation are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, † $P < 0.06$), based on *G*-tests of independence for fruit set and one-tailed Wilcoxon two-sample tests on square-root of seed set.


A.
Percent fruit set
100
80
60
40
20
0
*
**
**
**
***
Supplemented
Control
Aug 1
Aug 2
Aug 5
Aug 9
Aug 11
Aug 14
Aug 19

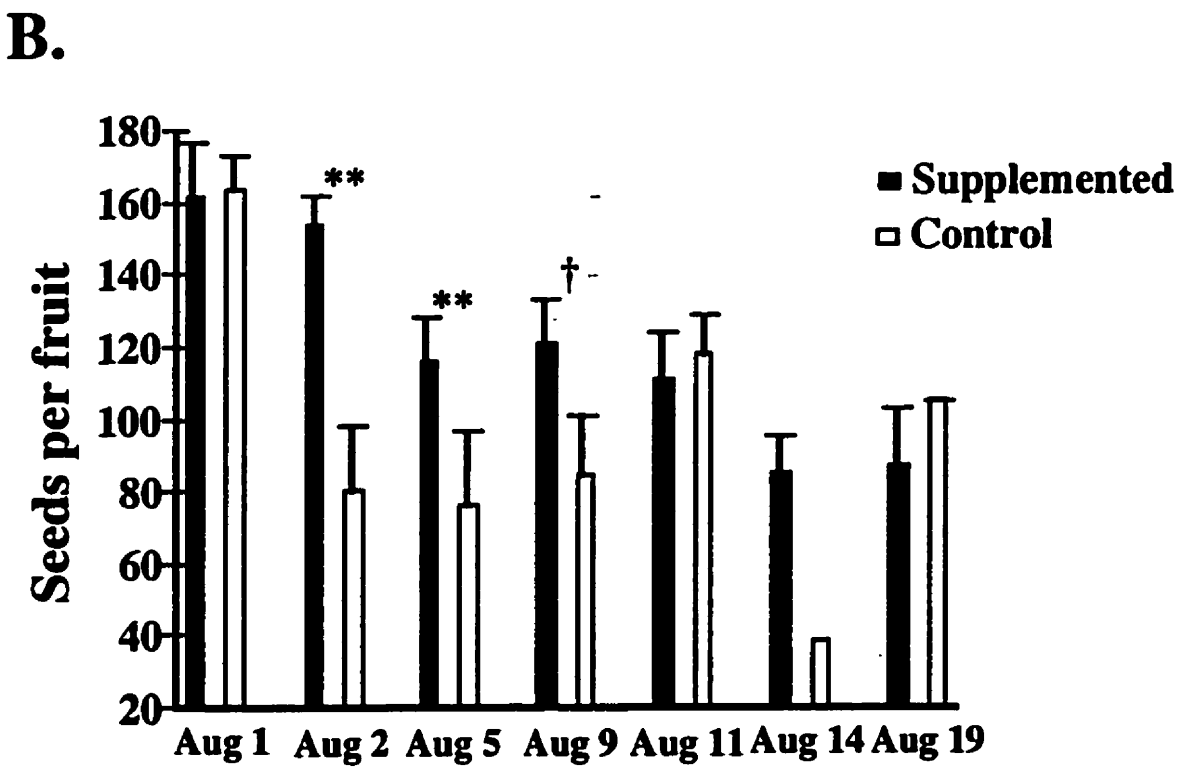
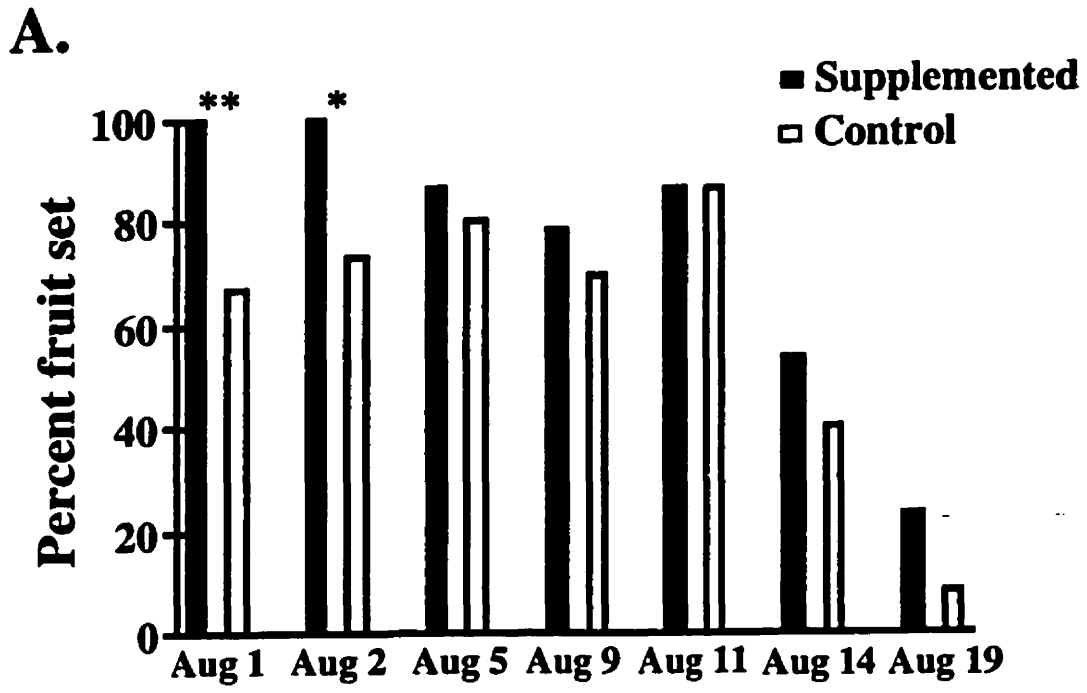
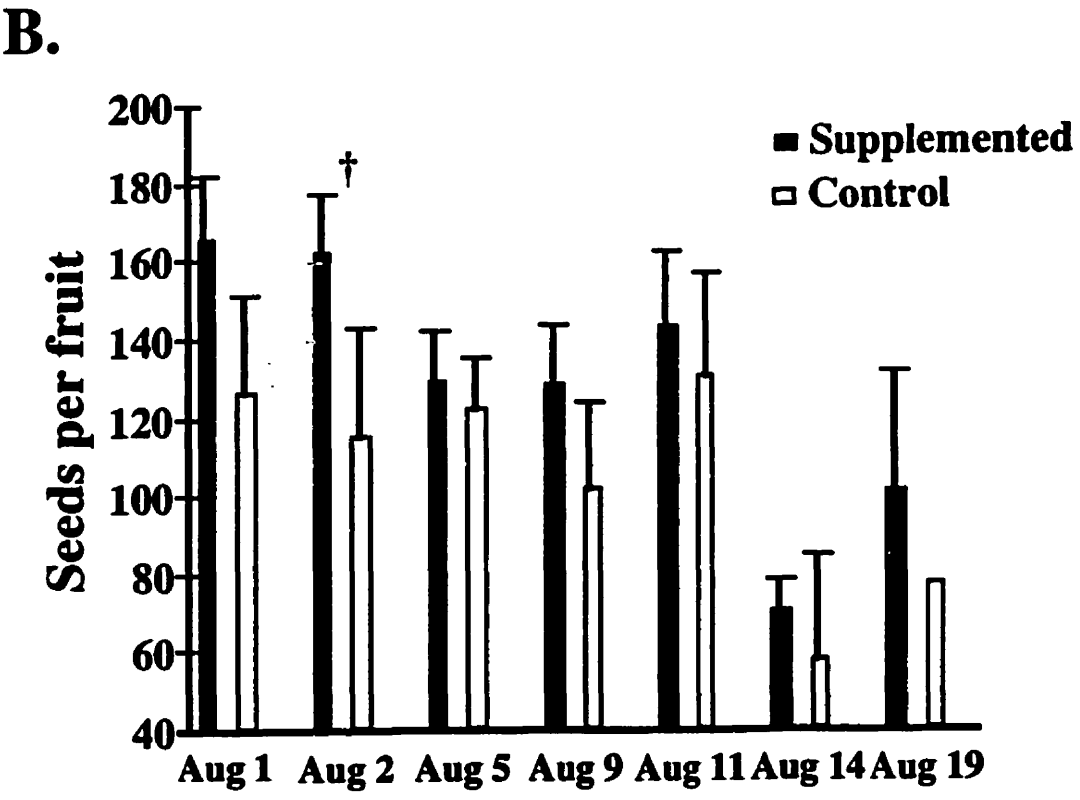

B.
Seeds per fruit
180
160
140
120
100
80
60
40
20
**
**
†
Supplemented
Control
Aug 1
Aug 2
Aug 5
Aug 9
Aug 11
Aug 14
Aug 19

Figure 3.14. Comparison of fruit and seed set from open- and supplemental cross-pollinations of *Rhexia virginica* in population D at Lake Matchedash, Ontario, during seven days in August 1997. (A) Percent fruit set and (B) mean seeds per fruit (± SE) of supplemented and control flowers on separate plants. There were fifteen plants in each treatment each day. Significant increases in fruit and seed set with pollen supplementation are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$, † $P < 0.06$), based on *G*-tests of independence for fruit set and one-tailed Wilcoxon two-sample tests on square-root of seed set.


A.
Supplemented
Control
**
*
Percent fruit set
100
80
60
40
20
0
Aug 1
Aug 2
Aug 5
Aug 9
Aug 11
Aug 14
Aug 19


B.
Supplemented
Control
†
Seeds per fruit
200
180
160
140
120
100
80
60
40
Aug 1
Aug 2
Aug 5
Aug 9
Aug 11
Aug 14
Aug 19

(number of grains = $1.32 \times 10^3 \pm 1.17 \times 10^2$) was larger than the number of ovules in *R. virginica* flowers.

Patterns of pollen removal and deposition reflected the degree of pollen limitation within populations of *R. virginica* at Lake Matchedash. There was a significant decline in pollen limitation for a given day as more pollen was removed from anthers (Figure 3.15A) and as more pollen was deposited on stigmas (Figure 3.15B). The linkage between removal and deposition was demonstrated by a positive correlation between the mean amount of pollen removed from anthers on a given day and mean pollen deposition on stigmas ($N = 14$, $r^2 = 0.49$, $P < 0.006$).

DISCUSSION

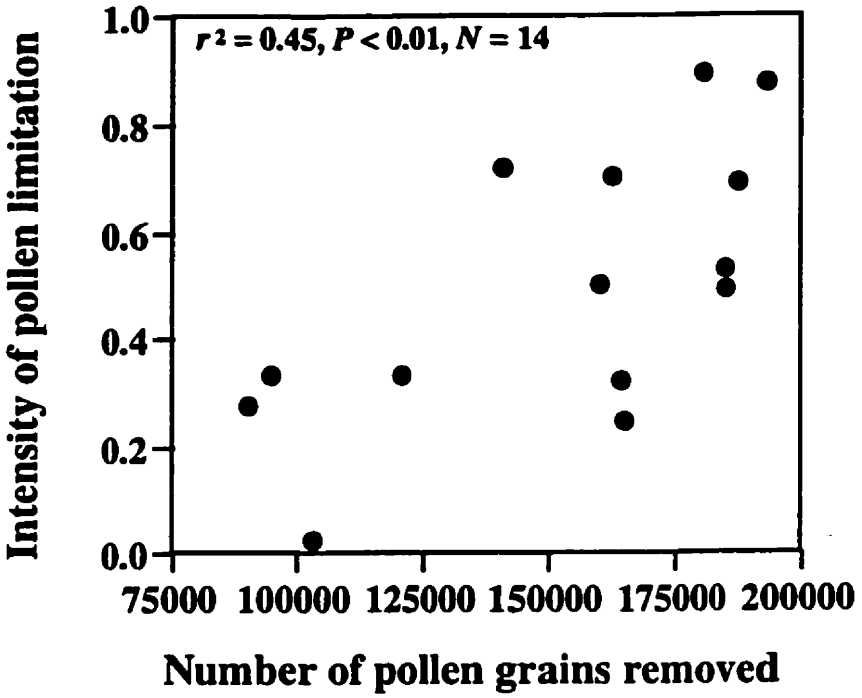
This investigation has demonstrated that the fertility of buzz-pollinated *R. virginica* is frequently limited by pollen delivery in the Muskoka region of Ontario. Although pollen limitation varied in intensity, its prevalence was confirmed at Lake Matchedash in 1996 and 1997 and at three of four other lakes investigated in 1997. Here, I discuss the evidence for pollen limitation in *R. virginica*, then consider the life history traits and ecological factors that contributed to its occurrence. Floral traits of *R. virginica* such as its pollen-dispensing anthers and dependence on pollinators for pollen transfer contributed to its limited fertility. The effect of these floral traits was exacerbated by infrequent pollinator visits in Muskoka, and I consider two potential explanations for low visitation. I conclude by arguing that restricted pollen removal from anthers of *R. virginica*, which is in part the result of low pollinator visitation, is the main proximate mechanism accounting for pollen limitation of this species in Ontario.

POLLEN LIMITATION OF FERTILITY

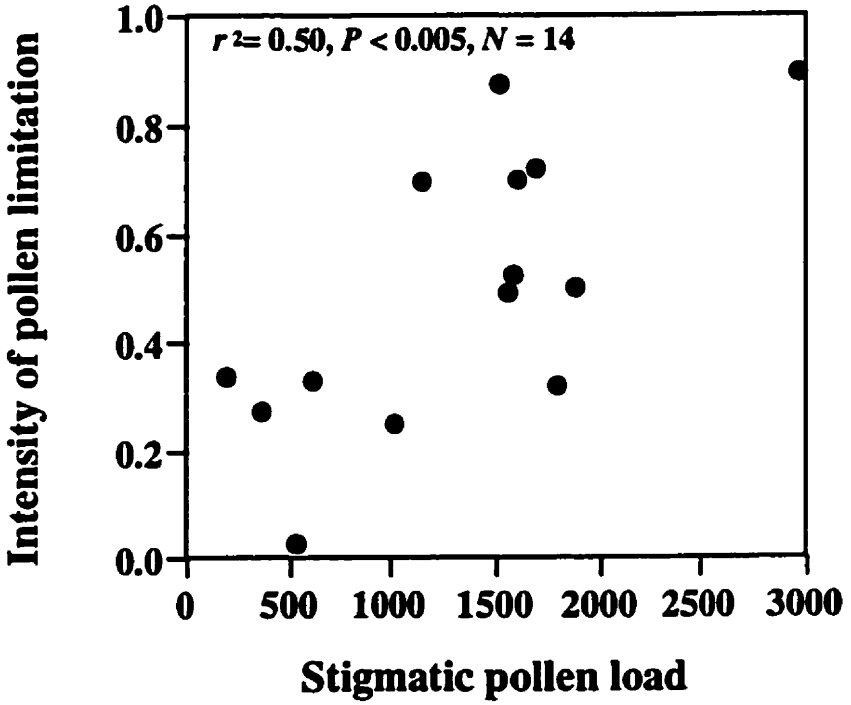
Experiments conducted at Lake Matchedash in 1996 and 1997 demonstrated that the fertility of *R. virginica* plants was often pollen-limited. The addition of pollen to flowers increased fruit set in population B on most days during the flowering period, but

Figure 3.15. The relation between the intensity of pollen limitation and (A) pollen removal and (B) pollen deposition from *Rhexia virginica* at Lake Matchedash, Ontario during August 1997. The ratio of seed set in control to supplemental hand-pollinated flowers, including those not setting fruit, is used as an index of the intensity of pollen limitation. See methods for experimental details.

A.


$r^2 = 0.45, P < 0.01, N = 14$
Intensity of pollen limitation
1.0
0.8
0.6
0.4
0.2
0.0
75000 100000 125000 150000 175000 200000
Number of pollen grains removed

B.


$r^2 = 0.50, P < 0.005, N = 14$
Intensity of pollen limitation
1.0
0.8
0.6
0.4
0.2
0.0
0 500 1000 1500 2000 2500 3000
Stigmatic pollen load

increased fruit set less frequently in population D. When open-pollinated flowers set fruit, they contained similar numbers of seeds as fruits from supplemented flowers, suggesting that fruit were produced only when a threshold number of pollen grains were delivered to stigmas. The increase in fertility resulting from pollen supplementation was unlikely to be the result of resource re-allocation within plants, since whole-plant fertility was increased by the addition of pollen to all flowers. It follows that pollen limitation, rather than resource limitation, was the primary mechanism accounting for levels of fruit set of *R. virginica* in the Muskoka region. The mean fruit set in all populations investigated was 52.6%, which was similar to the 56% recorded at Axe Lake, Ontario in 1982 ($N = 25$ flowers, Sharp 1983). This level of fertility is markedly lower than the average fruit set of 72.5% among 445 self-compatible hermaphroditic species in a survey conducted by Sutherland and Delph (1984). There are several potential adaptive explanations for low fertility in plants (reviewed in Sutherland 1986), but the proximate mechanism in *R. virginica* appears to be insufficient pollen transfer by pollinators.

Studies at five lakes in the Muskoka region demonstrated that pollen limitation is prevalent in this part of the range of *R. virginica*. The degree of pollen limitation varied among lakes, but not in association with population size. For pollinators, the effective size of a population on a given day is of more functional relevance and best expressed in terms of the number of flowers displayed. However, this parameter was not associated with the patterns of pollen limitation observed. For example, the Krapek Lake population (220 flowers) had the largest number of flowers of the populations studied, but seed set was pollen-limited. Fertility was pollen-limited at Bentshoe Lake (60 flowers), but not in the larger population at Three-mile Lake (143 flowers) on August 7, 1997. The weather was less overcast the next day at Hardy Lake (92 flowers), yet no control plants set fruit. In addition, the degree of pollen limitation in two populations at Lake Matchedash on seven days during 1997 was not associated with variation in the number of flowers in anthesis each day. The daily pollen limitation index (fruit set of controls relative to supplemented flowers) was unrelated to the number of flowers displayed in the populations during daily population censuses ($P > 0.70$, $N = 14$). Collectively, these results from five lakes in the

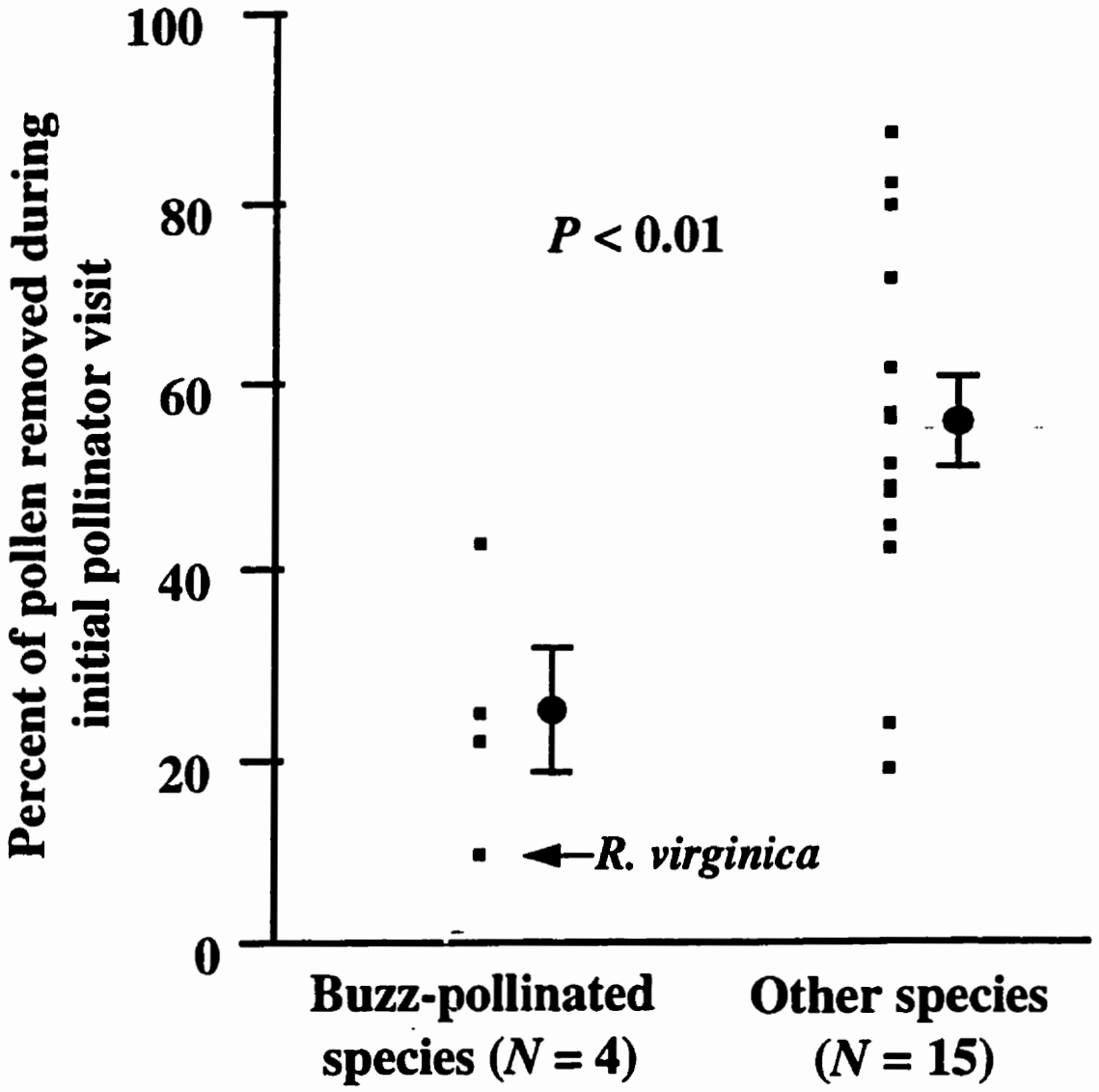
Muskoka region indicate that pollinator activity did not simply vary in response to the quantity of floral resource available. Larger populations may have been more heavily visited by pollinators over the entire season, accounting for the significant positive relation between total population size and the fruit set of individual plants (Figure 3.9), but on a day-to-day basis levels of bumblebee visitation did not vary directly with floral resources.

The investigations reported here do not address whether the lifetime fertility of *R. virginica* plants in Muskoka is pollen- and/or resource-limited. Individual ramets probably do not survive the winter in Ontario (Posluszny *et al.* 1984), so these results imply that ramet fitness is pollen-limited. However, pollen supplementation may have depleted resources in *R. virginica* tubers and reduced clonal propagation. In quadrats at Axe Lake, Ontario in 1982, 60% of *R. virginica* stems produced at least one tuber annually (Sharp 1983), so clonal propagation can contribute significantly to population growth. To determine whether the overall fertility of clones is pollen-limited, demographic studies conducted over multiple years would be required. These could be used to address the issue of whether population growth in *R. virginica* is pollen-limited (see Ehrlén and Eriksson 1995).

CONTRIBUTION OF FLORAL TRAITS TO POLLEN LIMITATION

Pollen limitation in *R. virginica* may, in part, result from the restriction of pollen removal from its poricidal anthers. The amount of pollen removed during a single visit to *R. virginica* is the lowest recorded for an angiosperm species to date (Figure 3.16). The poricidal anthers serve a dispensing function that theoretically counters the decelerating relation between total pollen dispersal to stigmas and the amount of pollen removed during individual visits (Lloyd and Yates 1982; Harder and Thomson 1989; Harder and Wilson 1994). This relation results from the accelerated loss of pollen due to pollen-layering and more intense pollinator grooming as more pollen is removed (Buchmann and Cane 1989; Harder 1990b). However, since pollinator visits to *R. virginica* are quite infrequent, this mechanism appears to overly restrict pollen removal, resulting in a considerable amount of pollen that remains in anthers at the end of anthesis (Figure 3.15A). In other species that dispense pollen, an increasing proportion of remaining pollen is removed during later

Figure 3.16. Percentage of pollen removed from anthers of plant species during an initial visit by a bee, based on a literature survey of buzz-pollinated species ($N = 4$) and those with other pollination systems ($N = 15$). The mean (± SE) for each group of species and significance level based on a two-tailed t -test are also presented. Buzz-pollinated species: *Cassia reticulata* (Snow and Roubik 1987), *Dodecatheon conjugens* (1st-day flowers) (Harder and Barclay 1994), *Pedicularis contorta* (Harder 1990a), and *Rhexia virginica* (this study). Other species: *Aconitum delphinifolium* (Harder 1990a), *A. septentrionale* (Thøstesen and Olesen 1996), *Aralia hispida* (Harder 1990a), *Drosera tracyi* (Wilson 1995), *Echium vulgare* (Strickler 1979; Klinkhamer *et al.* 1991), *Erythronium americanum* (Harder and Thomson 1989), *E. grandiflorum* (Thomson and Thomson 1989), *Impatiens capensis* (Wilson and Thomson 1991), *Lupinus sericeus* (Harder 1990a), *Mertensia paniculata* (Harder 1990a), *Pedicularis bracteosa* (Harder 1990a), *Polemonium viscosum* (Galen and Stanton 1989), *Pontederia cordata* (Wolfe and Barrett 1989), *Raphanus sativus* (Young and Stanton 1990), and *Trifolium pratense* (Dunham 1939).


100
80
60
40
20
0
Percent of pollen removed during initial pollinator visit
P < 0.01
R. virginica
Buzz-pollinated species (N = 4)
Other species (N = 15)

visits, which increases the likelihood that most of the pollen is dispersed by the end of anthesis (Harder and Barclay 1994; Harder and Wilson 1994; Lebuhn and Anderson 1994; King and Buchmann 1996). The extent to which this occurs in *R. virginica* is unknown, but the large amount of pollen remaining in anthers suggests that the adaptiveness of the dispensing mechanism may be compromised in Ontario populations because of low pollinator visitation rates.

Since *R. virginica* flowers do not self-fertilize autonomously, pollinators are required for pollen transfer to occur. However, pollinator visits at Lake Matchedash were relatively infrequent. This caused pollen limitation in *R. virginica* because the quantity of pollen deposited on stigmas did not maximize fruit and seed set (insufficient pollen transfer *sensu* Harder and Barrett 1996). Alternatively, fertility may be limited by the quality of pollen deposited on stigmas (inefficient pollen transfer *sensu* Harder and Barrett 1996). *Rhexia virginica* was self-compatible (*contra* Renner 1989), so pollen quality concerns the relative proportion of self and outcross pollen deposited during pollinator visits. Although the seed set of selfed and outcrossed *R. virginica* flowers was similar, inbreeding depression may depress the fitness of selfed seeds (Charlesworth and Charlesworth 1987; Husband and Schemske 1996). Kral and Bostick (1969) demonstrated reduced germination of *R. virginica* seeds after self-fertilization. If self-pollen is of lower quality than outcross pollen, self-fertilization may also contribute to pollen limitation in Ontario populations. For example, in *Blandfordia grandiflora* (Liliaceae) the main cause of pollen limitation is the abortion of ovules that are self-fertilized during pollinator visits (facilitated self-fertilization *sensu* Lloyd and Schoen 1992), because this process pre-empts ovules that would otherwise have been outcrossed (Ramsey 1995).

Pollinators of *R. virginica* may cause self-fertilization in two ways. They could transfer pollen between flowers on a plant (geitonogamous self-fertilization), but the relative rarity of multiple daily flowers on inflorescences of *R. virginica* at Lake Matchedash suggests that this contributes minimally to rates of selfing. If self-fertilization is occurring at Lake Matchedash, it follows that most would be intrafloral facilitated self-fertilization caused by pollinators during visits. Pollinators of *R. virginica* may cause

intrafloral self-fertilization directly if pollen that lands on their bodies while they buzz is later deposited on the stigma. Alternatively, pollen from the cloud they disperse as they buzz may land on the stigma. Further investigations are required to determine the prevalence of these modes of self-fertilization in *R. virginica*, and the implications of self-fertilization for seed fitness and pollen limitation.

A Comment on the Role of Second-day Flowers

Pollen limitation in *R. virginica* may be reduced by the maintenance of second-day flowers because they increase floral display size. Inflorescences displaying more flowers are generally more likely to be visited by pollinators, but this may be counterbalanced by an increase in the rate of geitonogamous self-fertilization (reviewed in Harder and Barrett 1996). An increase in geitonogamy seems likely in *R. virginica*, because pollinators visited more flowers on larger displays and fertility declined when display sizes were largest (Figure 3.10C, D). Under these circumstances, the maintenance of second-day flowers may represent an evolutionary compromise between the greater fertility that could be obtained by maintaining first-day flowers for longer periods, which would simultaneously increase display size, and the concomitant loss of fertility resulting from increased geitonogamy. Second-day flowers resolve this problem by increasing floral display size while not contributing to geitonogamy. This is supported by the greater ability of floral display size to predict fertility in *R. virginica* when it included second-day flowers. In Ontario populations most *R. virginica* inflorescences had only one flower daily and second-day flowers contributed infrequently to display size, so this mechanism may be relatively unimportant. However, it may be of more significance in the centre of diversity for the genus *Rhexia*, in the southern United States, where *R. virginica* plants are larger and have numerous flowers (James 1956; S. C. H. Barrett pers. obs.). Further investigations would be needed to determine the contribution of second-day flowers to rates of geitonogamy and pollen limitation.

These results concerning second-day flowers of *R. virginica* support an hypothesis for the adaptiveness of floral colour change that is not explicitly considered in the recent literature (reviewed in Gori 1983; Weiss 1991). As demonstrated for *R. virginica*, flowers

that have undergone a colour change are typically infertile and not visited by pollinators (Weiss 1991, 1995). To explain this, recent studies have usually considered independent adaptive explanations for floral maintenance and floral colour change. Floral maintenance has been hypothesized to increase floral display size and thus visitation rates (Cruzan *et al.* 1988; Gori 1989; Weiss 1991; but see Casper and La Pine 1984; Delph and Lively 1989). Conversely, floral colour change has been hypothesized to increase pollinator foraging efficiency and the efficiency of pollen transfer (Gori 1983, 1989). None of these studies have explained why flowers are maintained *and* undergo a colour change. A colour change is not required to increase display size, and petals could simply be dropped to increase foraging efficiency. As hypothesized by Harder and Barrett (1995, 1996), however, the adaptiveness of second-day flowers may be that they increase insect visitation rates by contributing to daily display size, but concurrently undergo a colour change to reduce rates of geitonogamy and pollen discounting. This hypothesis could be tested further by explicit analyses of the relative energetic costs and fitness gains of first- and second-day flowers.

Contribution of Pollinators to Pollen Limitation

Particular floral traits of *R. virginica* discussed above may contribute to the intensity of pollen limitation, but these traits have less relevance if pollinator visitation rates are high. However, pollinator visitation at Lake Matchedash was infrequent in 1996 and 1997. This accords with the few bumblebee visits observed during two seasons of ecological study at Axe Lake, Ontario by Sharp (1983). Although visitation rates to concurrently flowering species were not measured at Lake Matchedash, previous studies on *Pontederia cordata* (Pontederiaceae) at Pothole Lake, Grenville County, Ontario in 1982 and 1983 provide a yardstick for comparison (Wolfe 1985; Wolfe and Barrett 1988). *Pontederia cordata* is similar to *R. virginica* because it is predominately bumblebee-pollinated, has flowers that are only fertile for one day, and has a similar phenology and habitat. In early August of 1982 and 1983, visitation rates to 4 m^2 quadrats of *P. cordata* ranged from 10-80 per hour, compared to four visits to 1 m^2 quadrats of *R. virginica* in 1996, and much lower rates on most days to an entire population in 1997. Visitation rates to *P. cordata* may be higher because it has larger inflorescences and offers nectar as a

reward. It is noteworthy, however, that visitation to *P. cordata* by *Bombus impatiens* and *B. bimaculatus* at Pothole Lake dropped rapidly in early August. This may indicate that the low visitation rates to *R. virginica* in part reflect a general seasonal decline in bumblebee numbers.

Rhexia virginica offers large quantities of pollen for foraging bumblebees at Lake Matchedash, so it is somewhat surprising that visitation was so infrequent. There are two potential explanations for low visitation rates. First, there may be an oversupply of *R. virginica* flowers at Lake Matchedash relative to the pollen required by local bumblebee colonies. It is possible that individuals from few bumblebee colonies visited population B in 1997, because more than two individuals were never observed simultaneously. Furthermore, whether pollen or nectar is a limiting resource for bumblebees may vary through the season. Pollen is most important as a protein source for larval bees, so it is probably only a limiting resource during nest initiation early in the season (cf. Zimmerman and Pleasants 1982; Plowright and Laverty 1984). If pollen demands are lower in early August, the number of bumblebees visiting *R. virginica* at Lake Matchedash may be influenced by how much pollen was available for colony growth earlier in the season.

Despite these considerations, bumblebees likely require some pollen during early August, and *R. virginica* is a readily-available pollen source. However, buzz pollination is a relatively complex behaviour, and bees may take longer to learn it compared to obtaining rewards from flowers with more simple floral morphologies (see Laverty 1980, 1994; Laverty and Plowright 1988). The time needed for bees to learn to buzz-pollinate has not been experimentally investigated (but see King 1993). Regardless, if bees require relatively small amounts of pollen in early August, the amount obtained incidentally while visiting other flowers at Lake Matchedash for nectar may be sufficient. This seems likely since most of the concurrently flowering species at this site had less complex flowers that may require shorter learning times than those of *R. virginica*.

These considerations do not account for the variable patterns of visitation to *R. virginica* at Lake Matchedash. There was a switch in the predominant *R. virginica* pollinator from *Bombus bimaculatus* in 1996 to *B. impatiens* in 1997. This was paralleled

by lower visitation rates in 1997. Although the reason for these changes is unknown, it could be related to weather differences between the years. Weather affects bumblebee colony establishment (Harder 1986), which may influence both species richness and abundance levels.

MECHANICS OF POLLEN LIMITATION

Plant fertility can be considered from both male and female perspectives. Unfortunately, pollen limitation has been viewed almost entirely from the perspective of inadequate pollen deposition and its effect on maternal seed set (Burd 1994; Wilson *et al.* 1994). The possibility that pollen removal from anthers and transport *per se* may limit pollen dispersal to stigmas has not been previously investigated (Harder and Wilson 1997). Although the amount of pollen removed and deposited during single pollinator visits have been compared (Snow and Roubik 1987; Cruzan *et al.* 1988; Harder and Thomson 1989; Thomson and Thomson 1989; Wolfe and Barrett 1989; Harder 1990a; Murcia 1990; Wilson and Thomson 1991), daily pollen removal and deposition have not been explicitly linked to variation in the degree of pollen limitation. Since pollen removal is restricted in many species and most pollen loss occurs during transport (Harder and Thomson 1989), this perspective may inform our understanding of factors limiting fertility in some pollination systems.

In *R. virginica* at Lake Matchedash, there was strong evidence that low rates of pollen removal directly limited plant fertility. Pollen removal from *R. virginica* was restricted by its poricidal anthers and short floral longevity, as well as the infrequency of pollinator visits. For these reasons, much pollen remained in anthers at the end of anthesis, and variation in the degree of pollen limitation was strongly correlated with the amount of pollen removed from anthers on a given day (Figure 3.15A). Although models for the evolution of dispensing mechanisms commonly refer to their potential for limiting fertility when pollinators are infrequent (Harder and Thomson 1989; Harder and Wilson 1994), this is the first empirical demonstration that this can commonly occur in a flowering plant.

In addition to restricted pollen removal, the efficiency of pollen transfer may also limit the fertility of *R. virginica*. Low pollen deposition may result from excessive pollen loss during buzzing or grooming by bees (Wilson and Thomson 1991; Harder and Wilson in press). Neither of these losses were quantified in this study, but bumblebees released a cloud of pollen from anthers during visits that seemed to preclude precise pollen placement on pollinators. This accords with imprecise pollen placement reported for some other buzz-pollinated species (Snow and Roubik 1987; Renner 1989). Conversely, buzz-pollinated species with a solanoid morphology constrain pollinator contact with the flower to increase the precision of pollen deposition (Harder and Barclay 1994; Harder and Wilson 1997). Detailed investigations of the pollination process in *R. virginica* would be required to determine the relative amount of pollen lost during buzzing and grooming by pollinators, and carried in "safe sites" that may subsequently contact stigmas.

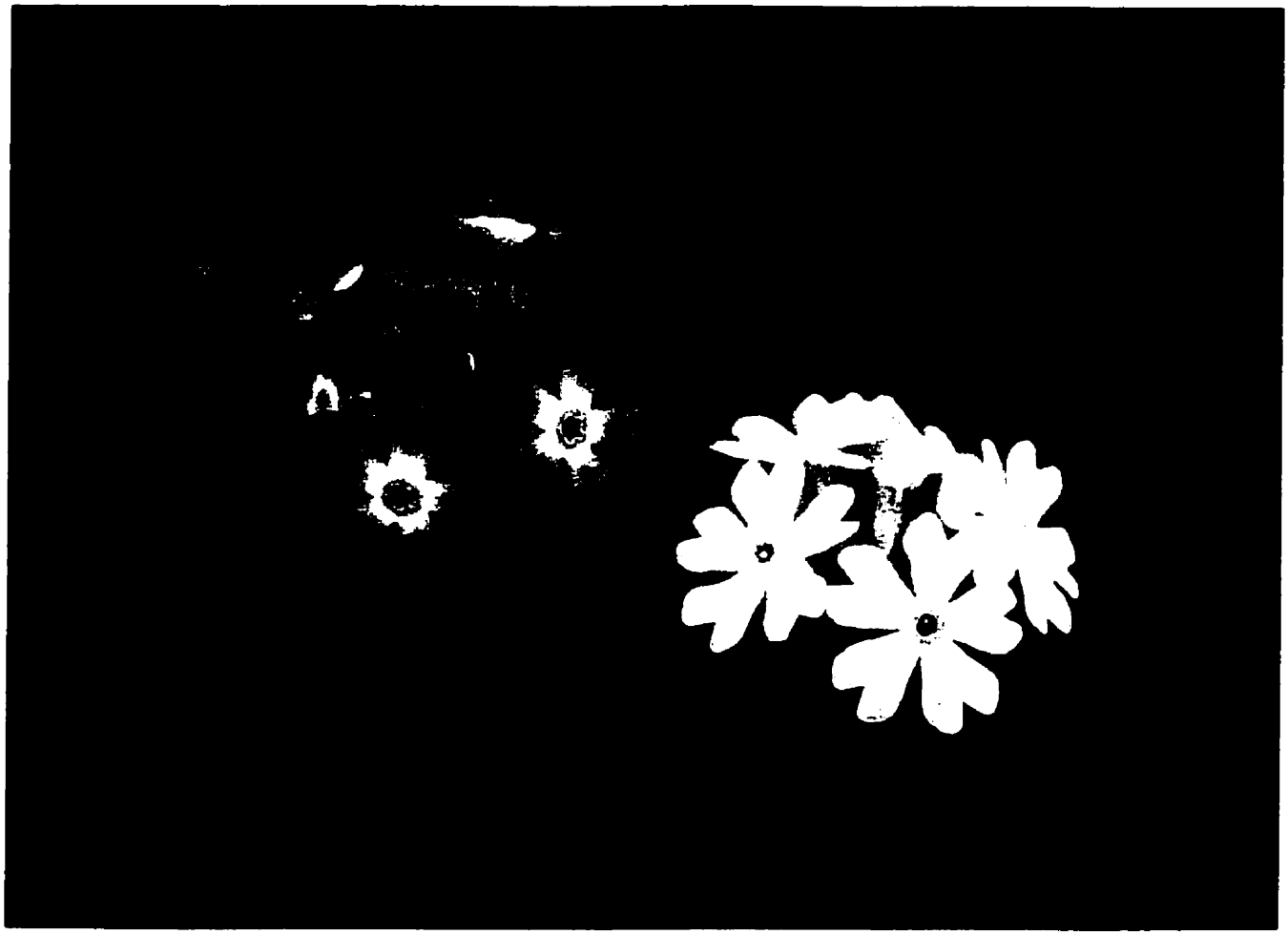
Studies at Lake Matchedash also confirmed that pollen deposition on stigmas influenced the degree of pollen limitation in *R. virginica* (Figure 3.15B). This was a consequence of restricted pollen transport by pollinators as discussed above. On average, only 0.46% of the pollen produced was transferred by pollinators to stigmas, which is comparable to that in other species that have been examined (see Thøstesen and Olesen 1996). Despite the inefficiency of pollen transfer, the average number of grains deposited ($\overline{X}$ = 1320) was three times greater than the number of ovules within flowers ($\overline{X}$ = 456.6). Nevertheless, seed set in *R. virginica* was pollen-limited, which suggests that many of the pollen grains deposited did not germinate or that significant attrition of pollen tubes occurred. This may be particularly likely in *R. virginica* because of the relatively small size of its pollen grains. Studies of the relation between pollination intensity and seed set have consistently demonstrated that the ratio of pollen grains deposited to seed set is greater than three (Snow 1982, 1986; Shore and Barrett 1984; Wolfe 1985; Cruzan 1989; Cruzan and Barrett 1996; Mitchell 1997). If this interpretation is correct, pollen limitation in *R. virginica* would likely be reduced if the amount of pollen circulating in populations was increased. This would be achieved by an increase in levels of pollinator visitation.

CHAPTER FOUR

REPRODUCTIVE BIOLOGY OF ISLAND AND MAINLAND POPULATIONS OF *PRIMULA MISTASSINICA* (PRIMULACEAE) ON LAKE HURON SHORELINES

"In a sense an island is a perturbation relative to a mainland ..."

Y.B. Linhart and P. Feinsinger (1980)



Two inflorescences of the long-styled morph of Bird's-eye Primrose (*Primula mistassinica*, Primulaceae) flowering in a population near Howdenvale, Ontario during May 1997. The inflorescence on the right has flowers that are atypically white. Photo by Spencer Barrett.

ABSTRACT

To investigate the influence of insularity on plant reproductive biology at a local geographic scale, I examined aspects of reproduction in distylous *Primula mistassinica* on Lake Huron shorelines of the Bruce Peninsula and adjacent Tobermory Islands in Ontario, Canada. Controlled pollinations demonstrated that *P. mistassinica* possesses a dimorphic incompatibility system with intermorph crosses setting significantly more seeds than self or intramorph crosses. Floral morphology, population style-morph ratios, and seed fertility were compared in seven mainland and thirteen nearshore island populations to determine whether there was evidence for differences in reproduction between these areas. Style-morph ratios did not differ significantly from equilibrium expectations, and there were no consistent differences between island and mainland populations in floral morphology or fertility. Rather, the generalized pollination system of *P. mistassinica* and extensive historical opportunities for colonization appear to have mitigated insular effects so that proximate ecological factors are likely more relevant to the current reproductive biology of populations.

INTRODUCTION

The reproductive biology and genetics of plant populations on islands may differ from mainland populations in several respects (reviewed in Barrett 1996). First, adaptations for self-fertilization are likely in island populations as they increase the probability of establishment following dispersal. Second, island colonization may lead to a loss of genetic variation because of founder effects and genetic drift in small founding populations. Finally, island pollinator faunas are often depauperate so fertility may decline in island populations unless countered by the evolution of selfing mechanisms. Although recent studies have supported the second prediction by comparing the genetic diversity of island and mainland populations (reviewed in Frankham 1997 and see Affre *et al.* 1997), there have been relatively few island-mainland comparisons of breeding systems and pollination. Existing comparisons typically test Baker's (1955) prediction that self-compatible species are more likely to colonize islands, and have mostly involved interspecific comparisons at large geographic scales (Barrett 1996 and references therein). Intraspecific studies at small scales that would allow explicit comparisons of reproduction in mainland and island populations are generally lacking.

The paucity of intraspecific studies may reflect the assumption that gene flow at small geographic scales will prevent evolutionary divergence (Halkka and Halkka 1974). It seems unlikely, however, given the evidence for local adaptation at more restricted spatial scales in plant populations (Snaydon 1970; Antonovics 1976; Turkington and Aarssen 1984), that colonization of islands within regional archipelagoes will fail to cause significant changes to plant reproductive biology. Recent intraspecific investigations have indeed shown that insular effects can be present at small scales, in terms of lowered reproductive success (Linhart and Feinsinger 1980; Spears 1987) and adaptive reduction of dispersal ability (Cody and Overton 1996). Nonetheless, studies that examine patterns of floral morphology and breeding-system variation between numerous islands and the nearby mainland are rare (but see Ågren and Ericson 1996).

To investigate the possible effects of insularity on island plant populations at a local scale, I compared seven mainland and thirteen nearshore island populations of *Primula mistassinica* (Primulaceae) on Lake Huron shorelines along the Bruce Peninsula and Tobermory Islands (Figure 4.1), paying particular attention to aspects of the species' floral morphology and breeding system. The islands vary in size from about 1 ha (Middle and Harbour Islands) to 875 ha (Cove Island) and are located between 0.5 km (Doctor Island) and 6.3 km (Bear's Rump Island) from the Bruce Peninsula. *Primula mistassinica* is distylous, with populations containing both long-styled (hereafter L-morph) and short-styled (hereafter S-morph) individuals that differ reciprocally in stigma and anther heights. The floral morphs in distylous species are normally self-incompatible, and at equilibrium occur at a ratio of 1 : 1 unless there is frequent self-fertilization (Ganders 1979; Richards 1986; Barrett 1992). Rare, self-compatible homostylous phenotypes occur in most heterostylous taxa, particularly in association with ecologically or geographically marginal environments, where they are best documented in the genus *Primula* (Charlesworth and Charlesworth 1979; Richards 1986, 1993).

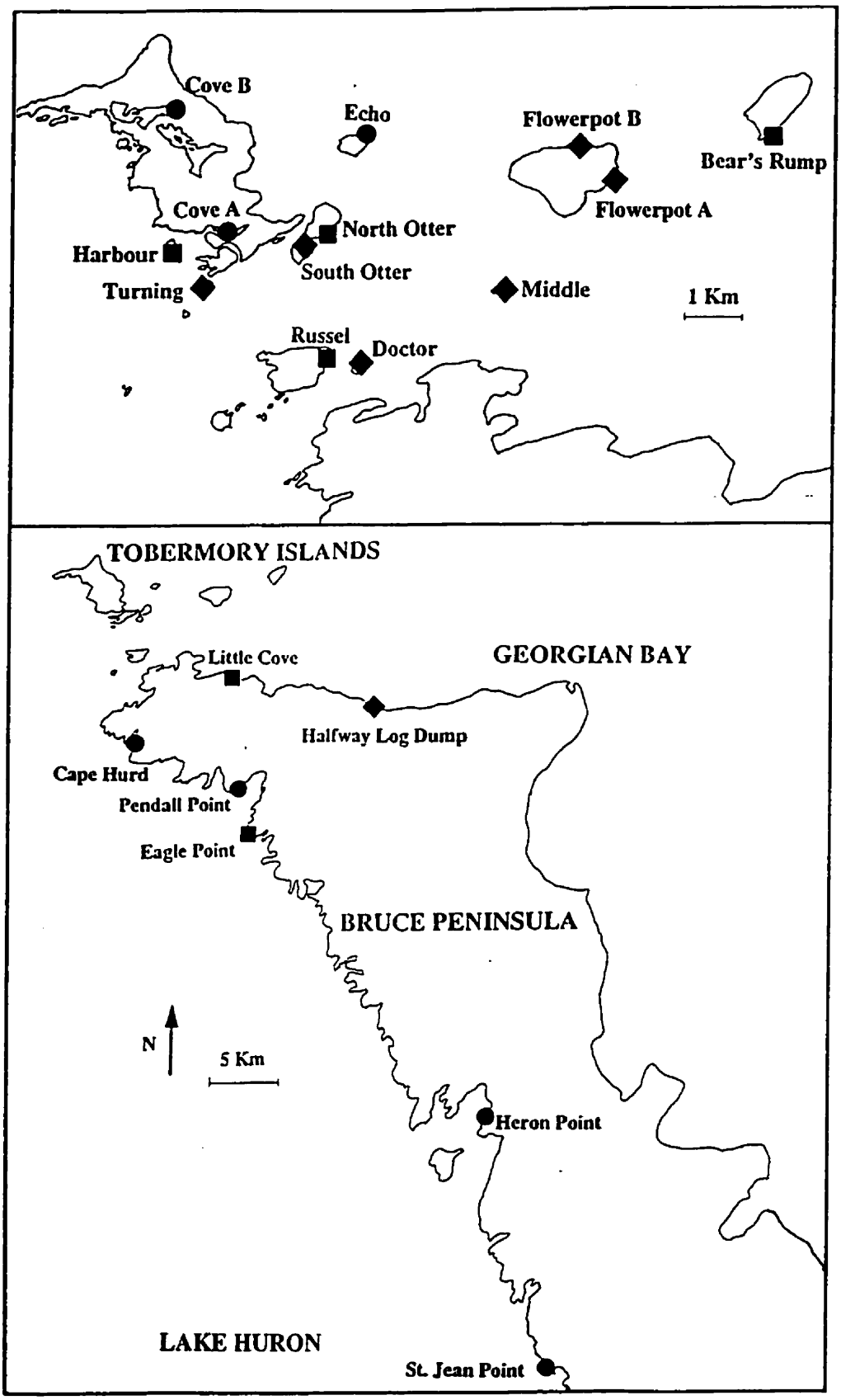
Here I document for the first time the reproductive biology of *P. mistassinica* and pose three questions that explicitly compare mainland and nearshore island populations: 1. Does floral morphology differ between mainland and island populations? In particular, is there any evidence for the occurrence of floral adaptations in island populations that promote self-pollination? 2. Do populations exhibit biased style-morph ratios, and is this correlated with insularity? 3. Is fertility reduced in island populations in comparison with those on the mainland?

MATERIALS AND METHODS

The Study Species

Primula mistassinica Michx., commonly known as Bird's-eye Primrose, is a variable diploid member of *Primula* section *Aleuritia*. It is differentiated from other Nearctic *Primula* by its typically distylous (as opposed to homostylous) condition,

Figure 4.1. Location of seven mainland and thirteen island populations of *Primula mistassinica* sampled on Lake Huron shorelines in Ontario, Canada. The lower panel shows the study area and the location of the mainland Bruce Peninsula populations; the upper panel is an enlargement of the Tobermory Islands showing the location of the island populations. ◆, Small-sized populations (<100 individuals); ■, Medium-sized populations (100 - 1 000 individuals); ●, Large-sized populations (>1 000 individuals).


Cove B
Echo
Flowerpot B
Bear's Rump
Cove A
North Otter
Flowerpot A
Harbour
South Otter
Turning
Middle
1 Km
Russel
Doctor
TOBERMORY ISLANDS
Little Cove
GEORGIAN BAY
Halfway Log Dump
Cape Hurd
Pendall Point
Eagle Point
BRUCE PENINSULA
N
5 Km
Heron Point
LAKE HURON
St. Jean Point

lavender (rarely white) corollas, and heavily yellow farinose or efarinose leaves (Kelso 1991). It is distributed across boreal North America, reaching its southern limit in the Great Lakes region and New England (Soper *et al.* 1965; Kelso 1991). In southern Ontario, peak flowering of the cymose inflorescences occurs during late May; the flowers are scented and last as long as ten days.

Reproductive Biology

To document distyly in *P. mistassinica*, measurements of stigma and anther height, and pollen size and number were made in a population at Cape Hurd on the Bruce Peninsula (see Figure 4.1). Stigma and anther height were measured on single flowers sampled from 25 plants per style morph, using camera lucida and Sigma-Scan (Version 2.6, Jandel Scientific 1986) connected to a digitizing tablet. Ten pollen grains from six plants of each morph were measured using Northern Exposure image analysis software (Release 2.9X, Empix Imaging 1995), and their sizes compared using ANOVA, with morph and plant nested within morph as main effects. To compare pollen production of the floral morphs, anthers from twelve flowers per morph were acetolyzed (Kearns and Inouye 1993) and the pollen grains suspended in lactophenol-glycerin with cotton blue stain before four replicate counts were made using a hemacytometer. Pollen counts for the style morphs were compared using a Student's *t*-test. All statistical analyses were conducted using JMP (Version 3.0.2, SAS Institute 1994).

The compatibility of the floral morphs was investigated by transplanting plants to a glasshouse prior to flowering. Three flowers on ten plants of each style morph were randomly allocated to separate treatments conducted on the same day: self-pollination, intramorph cross-pollination and intermorph cross-pollination. Seed set was quantified when fruits matured two months later, and treatments were compared using a Kruskal-Wallis non-parametric test. Seed set in the two style morphs was compared for each treatment using one-way ANOVA or Kruskal-Wallis tests, depending on whether ANOVA assumptions were met.

To determine the most frequent pollinators visiting flowers of *P. mistassinica*, collections were made during visits to populations at peak flowering. Voucher specimens of taxa collected are deposited in the insect collection of the author. The degree of pollen limitation in the Cape Hurd population was determined by adding supplemental pollen to stigmas of two flowers on twelve plants per morph, marking them, and later comparing their seed set to two unmanipulated flowers on the same plant. Other flowers on each plant were removed. A two-tailed paired *t*-test was used to compare mean seed set of the pollen-supplemented flowers with those of the control flowers in each morph.

ISLAND-MAINLAND COMPARISONS

Floral Morphology

Seven floral characters (listed in Table 4.1) were measured, as described above, on single flowers sampled from 25 plants per style morph (fewer in small populations) in six mainland and eight island populations. Stigma-anther separation was derived from these measurements as the distance between the stigma and the apex or base of the anther in the L- and S-morphs, respectively. Inflorescence samples were also collected from four mainland and eight island populations to determine variation in the total number of flowers per inflorescence. Measurements were analyzed using a mixed-model nested ANOVA, with region (island or mainland) and style morph as fixed effects, and population nested within region as a random effect. Ovary height was log-transformed to meet ANOVA assumptions. A sequential Bonferroni test (Rice 1989) was used to determine table-wide ANOVA probabilities.

Style-Morph Ratios

A random sample of inflorescences from each of the 20 populations was used to determine style-morph ratios. *G*-tests were employed to test for significant deviation from an equilibrium style-morph ratio (1 : 1) within populations and when populations were pooled (Sokal and Rohlf 1995).

Fertility

Mature inflorescences (20 per morph) were collected from six mainland and five island populations in mid-July to assess female fertility. Fruit set was not assessed because the vast majority of flowers set fruit, as in other *Primula* species (e.g., Baker *et al.* 1994). Mean seed set was estimated as the average number of seeds counted in two randomly chosen capsules per plant. Mean seed set was multiplied by the number of flowers on a plant to give an estimate of total fertility. Seed set was analyzed with the same mixed-model nested ANOVA described above for analysis of floral measurements. Total fertility was square root-transformed to meet ANOVA assumptions. A Tukey-Kramer HSD test was used to contrast mean seed set in populations pooled over morphs. To investigate potential predictors of female fertility, mean fertility for each population was regressed on the mean value for several morphological and ecological parameters from the populations, including population size, mean number of flowers per inflorescence, and both island isolation and area.

RESULTS

Reproductive Biology

The pattern of floral variation in the Cape Hurd population demonstrates that *P. mistassinica* possesses a conventional distylous floral syndrome. Plants can be grouped into two distinct morphological groups on the basis of differences in stigma and anther height and pollen size (Figure 4.2). Stamens and pistils were usually entirely within the corolla tube, but the tips of anthers in the S-morph and terminal section of the style in the L-morph were sometimes exserted. Flowers of the L-morph had significantly smaller pollen grains produced in greater numbers than flowers of the S-morph (Table 4.1; pollen size: $F = 1527.2$, df = 2, $P < 0.0001$; pollen number: $t = 3.88$, df = 22, $P < 0.001$).

Controlled self and cross-pollinations of the two morphs demonstrated that *P. mistassinica* possesses a dimorphic self-incompatibility system. Self and intramorph pollination resulted in low levels of seed set, whereas intermorph pollination increased

Figure 4.2. (A) Variation in stigma (☐) and anther (+) height for 25 plants of the L- and S- morphs of *Primula mistassinica* from the Cape Hurd population, ranked by stigma height. (B) Distribution of pollen size for plants of the L- ($N = 60$, white bars) and S-morph ($N = 60$, black bars) of *P. mistassinica* from the Cape Hurd population. Mean sizes of pollen grains in the two morphs are presented in Table 4.1, and are significantly different (see text).

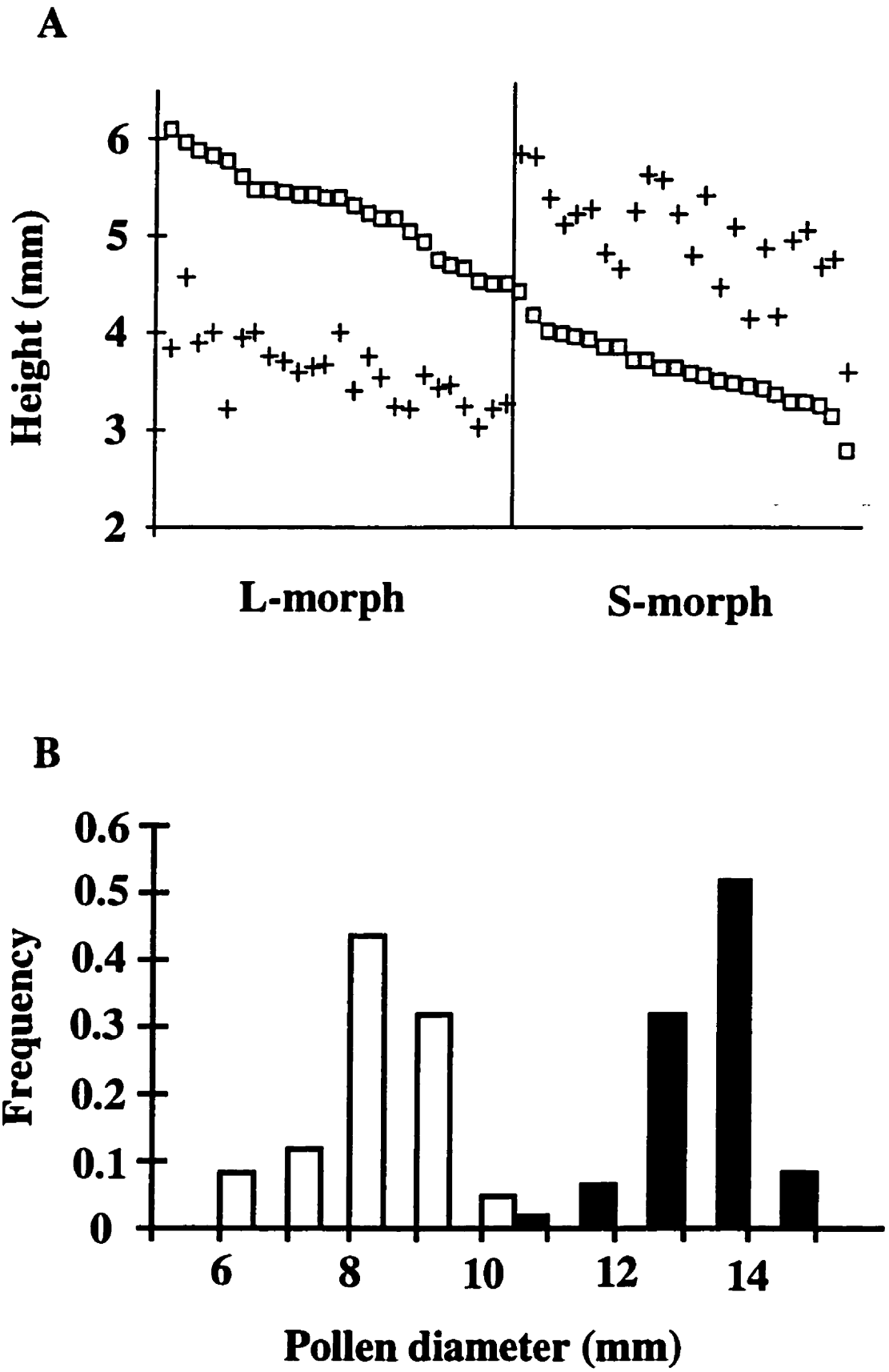

A
Height (mm)
6
5
4
3
2
L-morph
S-morph
B
Frequency
0.6
0.5
0.4
0.3
0.2
0.1
0
6
8
10
12
14
Pollen diameter (mm)

Table 4.1. Mean (mm), standard errors (SE) and sample sizes (*N*) of floral characters measured on an average of 25 plants per morph from six mainland and seven island populations of *Primula mistassinica*. Pollen size (µm) and number measurements are from the Cape Hurd population (see text). An asterisk indicates a significant difference between the morphs (Table 4.2 and see text).

Character	Long-styled Morph			Short-styled Morph		
	Mean	SE	*N*	Mean	SE	*N*
Number of flowers	3.89	0.085	475	3.69	0.086	465
Calyx length*	4.48	0.029	297	4.37	0.030	295
Corolla tube length*	5.39	0.032	297	5.71	0.038	293
Petal length*	5.51	0.040	297	5.29	0.037	295
Ovary height	1.83	0.014	297	1.80	0.014	295
Style length*	3.72	0.022	297	1.99	0.015	295
Filament length*	1.34	0.014	297	2.64	0.023	295
Anther length*	1.17	0.009	297	1.31	0.011	295
Stigma-anther separation*	1.20	0.019	297	0.65	0.022	295
Pollen number*	30 315	2 382	12	16 667	2 586	12
Pollen size*	8.69	0.12	60	13.12	0.09	60

seed set significantly (Figure 4.3; Kruskal-Wallis test: $H = 14.05$, 14.84 for L- and S-morphs, respectively, df = 2, $P < 0.001$). The strength of self-incompatibility was stronger in the S-morph than the L-morph (Figure 4.3).

Remarkably few insects were seen visiting the flowers of *P. mistassinica*. Flower flies (Syrphidae) were the most prevalent visitors and particularly noticeable on warm, sunny days. Species of *Eristalis* (*E. arbustorum* (L.), *E. bardus* (Say), *E. dimidiatus* Wiedemann, *E. tenax* (L.), and *E. transversus* Wiedemann) and *Platycheirus* (2 species) were most common, but *Eupeodes americanus* (Wiedemann), *Helophilus fasciatus* Walker, *Lejops stipatus* Walker, *Orthonevra* sp., and *Parhelophilus rex* Curran and Fluke were also collected. Other visitors to flowers included six additional families of Diptera (mosquitoes (Culicidae), fungus gnats (Sciaridae), soldier flies (Stratiomyiidae), blow flies (Calliphoridae), muscid flies (Muscidae), and dung flies (Scathophagidae)), locally common thrips (Thysanoptera) and sap beetles (Nitidulidae: *Carpophilus* sp.), and rarely, the halictid bee *Augochlorella striata* (Provancher).

In the Cape Hurd population, there was no significant difference between seed set in pollen-supplemented flowers and control flowers in either the L-morph (supplemented = 93.5 ± 6.6; control = 84.6 ± 9.0; $t = 1.34$, df = 9) or the S-morph (supplemented = 95.7 ± 6.4; control = 101.8 ± 9.2; $t = 0.51$, df = 10). Preliminary studies with smaller samples at St. Jean Point and Little Cove on the mainland and in the Flowerpot B island population corroborated these results.

Island-Mainland Comparisons

Does Floral Morphology Differ?

Comparison of floral traits failed to reveal any significant differences between mainland and island populations. This was indicated by the absence of a significant region effect in the ANOVA (Table 4.2). Morph and population main effects accounted for most variation in floral characters. Plants of the L-morph had longer calyces and petals, but shorter corolla tubes and anthers, than the S-morph (Table 4.1). Differences

Figure 4.3. Mean seed set per fruit (± SE) in *Primula mistassinica* flowers of the long-styled ($N = 10$, white bars) and short-styled ($N = 6$, black bars) morphs after self-pollination, intramorph cross-pollination and intermorph cross-pollination treatments in the glasshouse. Probabilities after non-parametric Kruskal-Wallis test are indicated by asterisks (* $P < 0.01$; $\chi^2 = 8.54, 9.36$ for self and intramorph pollination, respectively, df = 1). NS = not significant after one-way ANOVA ($F = 2.00$, df = 14).

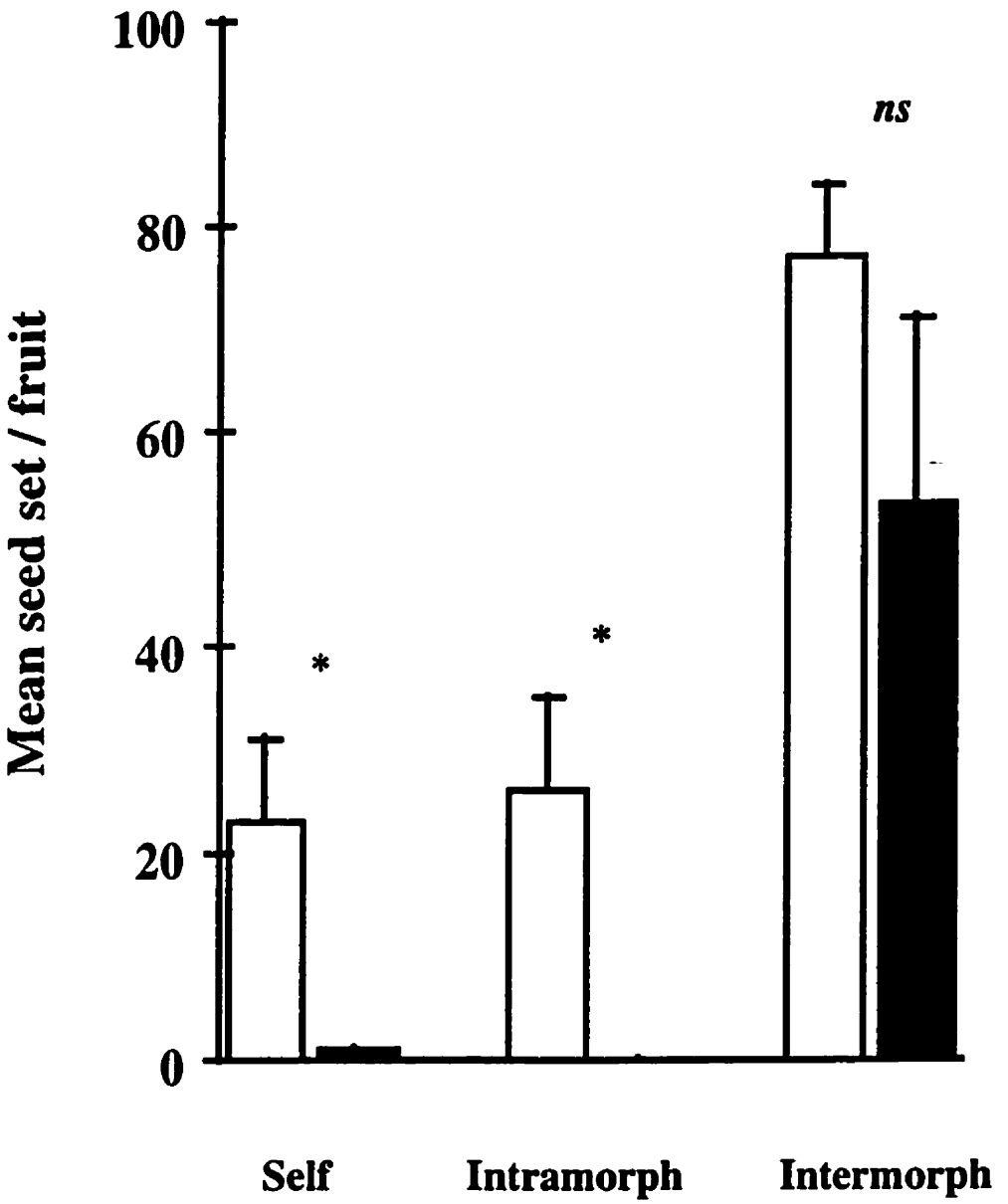

100
80
60
40
20
0
Mean seed set / fruit
*
*
ns
Self
Intramorph
Intermorph

Table 4.2. *F*-values from mixed-model ANOVA of the effect of region (island or mainland), morph and population nested within region on floral characters measured on an average of 25 plants per morph from six mainland and seven island populations of *Primula mistassinica*. Mean values are presented in Table 4.1. Table-wide probabilities after a sequential Bonferroni test are indicated by asterisks (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$). There is one fewer degree of freedom for the effect of population (region) on flower number.

Source of variation	df	number of flowers	calyx length	corolla tube length	petal length	log ovary height	style length	filament length	anther length	stigma-anther separation
Region	1	0.22	0.001	0.54	0.97	1.78	0.49	0.45	1.72	0.99
Morph	1	1.55	13.19 **	79.17 ***	10.32 *	1.75	1916.3 ***	950.1 ***	270.2 ***	77.07 ***
Region X Morph	1	0.04	0.07	0.004	0.90	2.00	0.079	0.69	2.50	0.12
Population (Region)	11	15.15 ***	22.50 ***	26.08 ***	11.29 **	8.29 **	4.40 *	4.66 *	40.98 ***	1.29
Morph X Population (Region)	11	0.90	0.45	0.71	1.40	1.36	2.43 *	3.06 **	0.38	5.70 ***

between the two morphs in style and filament length were those expected for a distylous species. Despite thorough searches at all sites, no homostylous plants were encountered in any of the populations.

Are Population Style-Morph Ratios Biased?

A total of 1243 plants from seven mainland populations and 869 plants from thirteen island populations were sampled to determine style-morph ratios. When populations were pooled, there was no significant departure from equilibrium expectations on the mainland (L-morph 0.511: S-morph 0.489; $G_{pooled} = 0.59$, df = 1) or the islands (L-morph 0.487: S-morph 0.513; $G_{pooled} = 0.61$, df = 1). In only one mainland population (Heron Point) was a significant deviation from an equilibrium style-morph ratio detected (L-morph 0.61: S-morph 0.39; $N = 100$, $G = 4.88$, df = 1, $P < 0.05$).

How Does Fertility Vary?

Comparisons of female fertility in mainland and island populations failed to detect any significant difference between the two regions (Table 4.3). Fruits of the S-morph had significantly greater seed set (78.3 ± 2.0; $N = 195$) than those of the L-morph (70.6 ± 2.2; $N = 187$), but total fertility was not associated with style morph. The effect of population accounted for most of the variation in seed set and total fertility (Table 4.3). Within regions, seed set varied greatly among populations, but was not correlated with simple predictors of fertility such as population size (Figure 4.4). Female fertility within island populations was unrelated to either island isolation or area. The only morphological trait associated with seed set per fruit was inflorescence size. Among nine populations, there was a significant positive relation between mean number of flowers per inflorescence and mean seed set per fruit (Figure 4.5). This association was unrelated to region.

Table 4.3. *F*-values from mixed-model ANOVA of the effect of region (island or mainland), morph and population nested within region on female fertility in six mainland and five island populations of *Primula mistassinica*. Probabilities are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.001$, * $P < 0.01$).

Source of variation	df	Mean seeds	Square root of total fertility
Region	1	0.58	0.50
Morph	1	10.06 *	1.14
Region X Morph	1	0.29	0.20
Population (Region)	9	46.22 ***	15.66 **
Morph X Population (Region)	9	0.61	1.50

Figure 4.4. Mean seed set per fruit (± SE) in six mainland and five island populations of *Primula mistassinica*. Within these two regions, population size generally decreases from left to right. ◆, Long-styled morph; ■, Short-styled morph. Mean seed set between populations sharing the same letter is not significantly different ($P < 0.05$) after Tukey-Kramer HSD test pooled over morphs. Mainland: 1 Heron Point, 2 Cape Hurd, 3 St. Jean Point, 4 Eagle Point, 5 Little Cove, and 6 Halfway Log Dump. Island: 1 Echo, 2 Bear's Rump, 3 Flowerpot B, 4 South Otter, and 5 Turning.

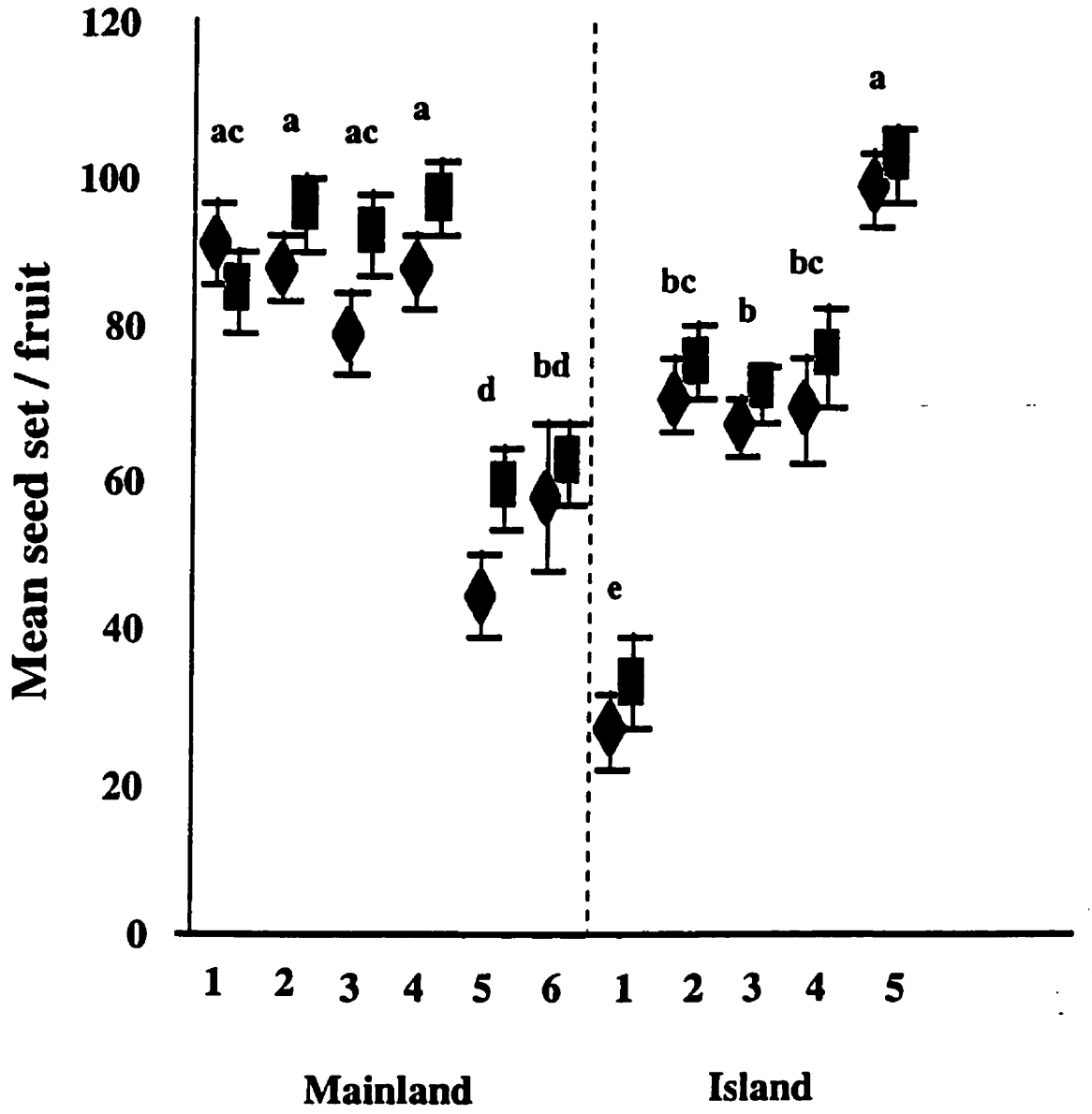
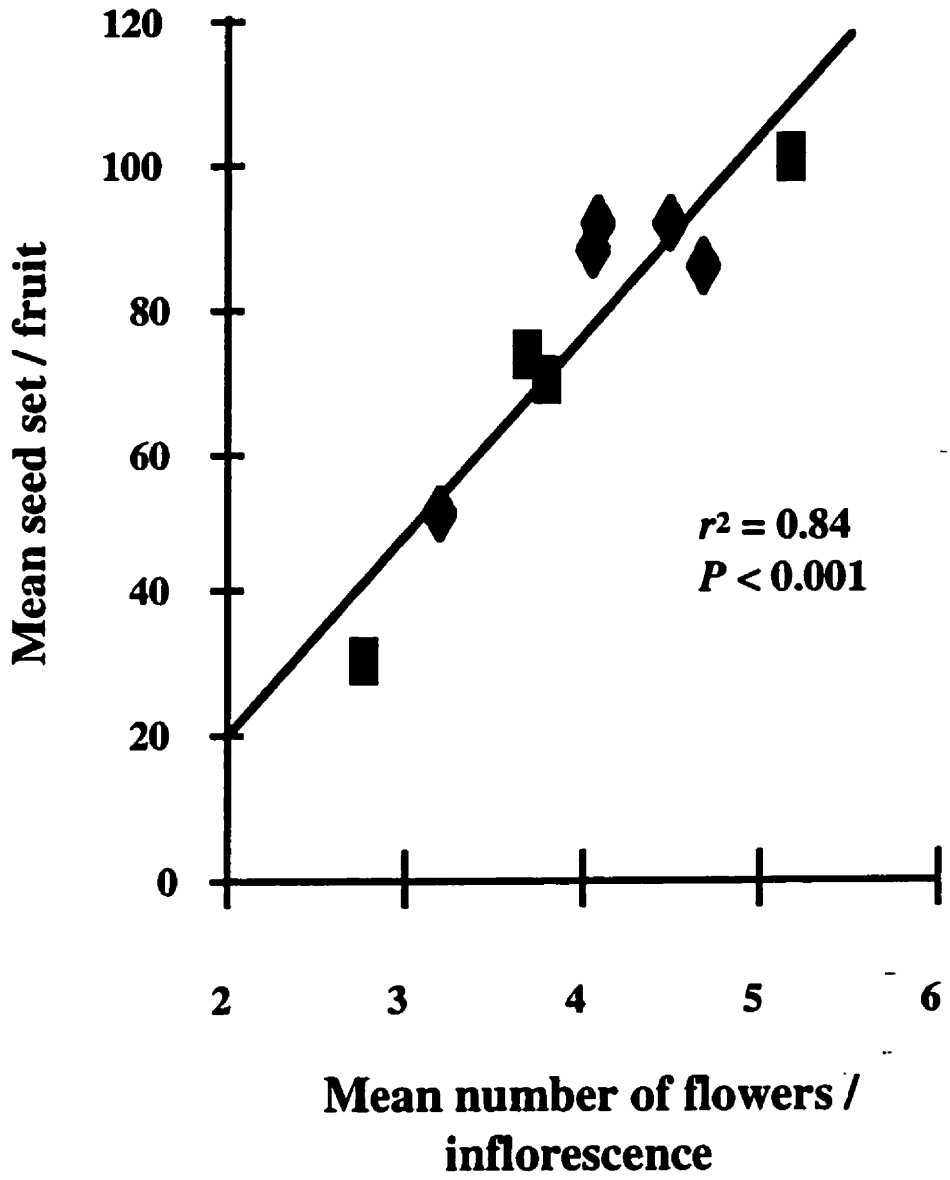

120
100
80
60
40
20
0
Mean seed set / fruit
ac
a
ac
a
d
bd
e
bc
b
bc
a
1
2
3
4
5
6
1
2
3
4
5
Mainland
Island

Figure 4.5. Relation between mean seed set per fruit and mean number of flowers per inflorescence in five mainland (●) and four island (■) populations of *Primula mistassinica*. Seed set and flower number were counted on separate random samples from the populations.


120
100
80
60
40
20
0
Mean seed set / fruit
$r^2 = 0.84$
$P < 0.001$
2
3
4
5
6
Mean number of flowers / inflorescence

DISCUSSION

Comparison of the reproductive biology of distylous *P. mistassinica* in mainland and nearshore island populations on Lake Huron shorelines in Ontario found no evidence for consistent differences in their floral morphology, style-morph ratios or fertility. While somewhat unexpected, this finding probably results from a synergism between aspects of the life-history and floral biology of the particular species investigated and the geographic scale of the study. Below I discuss in more detail the reproductive ecology and biogeography of *P. mistassinica* to account for the results.

The differences in floral morphology detected in *P. mistassinica* were those befitting the two morphs of a distylous species. Other characters may have been expected to vary between populations from mainland and island areas, but only population-level differences were found. For example, if pollinators were less abundant or reliable on the islands, flower size and stigma-anther separation may have decreased, as these transitions are correlated with increasing self-fertilization and have been documented in other mainland-island comparisons (Barrett 1985; Inoue *et al.* 1996). The results indicate that these changes are more responsive to local conditions found within populations than to larger scale ecological factors related to insularity.

Failure to detect self-pollinating homostyles within populations of *P. mistassinica* is not entirely unexpected, because homostyles are generated rather infrequently (on the order of 10^{-3}) through crossovers within the supergene controlling heterostyly (Charlesworth and Charlesworth 1979). Furthermore, in *Primula* the evolution of homostyly tends to be associated with polyploidy, so homostyly may be less likely in a diploid species such as *P. mistassinica* (Kelso 1991, 1992; Richards 1993). Even if rare homostyles were present in the populations I surveyed, it does not appear that the reproductive assurance provided by self-fertilization has been a strong enough selective pressure to permit their spread (Charlesworth and Charlesworth 1979), whether or not this is in association with island colonization.

The observed style-morph ratios in populations of *P. mistassinica* suggest that self-fertilization occurs rarely, regardless of insularity. Significant selfing rates are undoubtedly prevented by the dimorphic incompatibility system of this species. The leaky self-incompatibility in the L-morph, which is not unusual (see Barrett and Cruzan 1994), could provide some capacity for self-fertilization. However, the equilibrium style-morph ratios consistently found in populations of *P. mistassinica* cast doubt on whether this occurs. In addition, offspring resulting from self-fertilization would likely have limited survivorship because of inbreeding depression in this predominately outcrossing species (Charlesworth and Charlesworth 1987). It is important to note that in the absence of self-fertilization, founder effects (e.g., over-representation of one style-morph among founders) alone cannot bias population style-morph ratios in a distylous species. After a single generation of disassortative mating within a founding population a 1 : 1 style-morph ratio would be restored, because intermorph cross-pollinations produce seeds of the two morphs at equal frequencies (Barrett 1992).

The lack of any consistent differences between the fertility of mainland and island populations of *P. mistassinica* indicates that intermorph pollen transfer by pollinators must be effective in both areas. Anecdotal reports of butterfly pollination of *P. mistassinica* occur in the literature (Soper *et al.* 1965), but syrphid flies were the predominant visitors to flowers in the populations I investigated. Syrphid flies are generalist pollinators that visit flowers to consume pollen grains (Proctor *et al.* 1996) and their hairy bodies give them some capacity for intermorph cross-pollination. Small bees were observed infrequently, but this may simply reflect their more restricted foraging during the inclement weather conditions that often occur on the Bruce Peninsula in May. Bees may be significant pollinators during the instances within the lengthy flowering period of *P. mistassinica* when weather is more amenable to their activity. Syrphid flies (*Helophilus groenlandicus* O. Fabricius) were reported as the most important pollinators of homostylous *P. laurentiana* Fern. in Maine, even though it was visited frequently by bumble bees (Campbell *et al.* 1986). Inter-morph pollen transfer by the beetles and thrips observed in *P. mistassinica* flowers is probably relatively insignificant, but little is known regarding their patterns of inter-plant movement and potential for pollen transfer.

The generalized pollinators of *P. mistassinica* probably account for the successful cross-pollination of the species even on small, isolated islands. In instances where insular effects on the pollination of island plants have been detected (Spears 1987; Inoue *et al.* 1996) the species involved were dependent on specialized bee pollinators that have more restricted larval substrates than flies and hence are less able to inhabit small islands. The larvae of *Eristalis* species, in particular, breed in substrates ranging from shallow water and muck to decaying vegetable matter (Gilbert 1986), habitats that are available on both the mainland and islands. Bumblebees and moths are often reported as pollinators of larger-flowered *Primula* species (e.g., Schou 1983 for *P. elatior* (L.) Hill; Boyd *et al.* 1990 for *P. vulgaris* Huds.; Antrobus and Lack 1993 for *P. veris* L.; Miller *et al.* 1994 for *P. angustifolia* Torrey and *P. parryi* Gray; Washitani *et al.* 1995 for *P. sieboldii* E. Morren), which might make these taxa more susceptible to pollinator declines, as has been documented for the rare *P. sieboldii* in isolated Japanese populations (Washitani *et al.* 1994).

Island size and isolation and population size were not good predictors of fertility within individual populations, indicating that local ecological factors were more important in governing reproductive success. The strongest association detected in the data was between mean seed set per fruit and the number of flowers per inflorescence (Figure 4.5). If inflorescence size is influenced by resource status, then this correlation would suggest that fertility may be resource-limited. This hypothesis was invoked to explain the increase in seed set per capsule with capsule number per inflorescence in *P. farinosa* L., but the alternative hypothesis of greater pollinator visitation to larger inflorescences could not be excluded (Baker *et al.* 1994). Low seed set in some of the populations most exposed to offshore winds (Little Cove, Halfway Log Dump and Echo Island) suggests that exposure may be a significant factor limiting fertility in *P. mistassinica* populations. To the extent that weather conditions are less severe as the flowering season progresses, the flowering time of populations may also influence the probability of pollinator visitation. Studies of pollinator visitation and the intensity of pollen limitation in populations exposed to varying microclimatic conditions would be necessary to evaluate these ideas.

Even if ecological factors associated with insularity were capable of causing character divergence between mainland and island populations, historical and biogeographical factors may have mitigated their effects. The northern section of the Bruce Peninsula and all of the Tobermory Islands, except the higher-elevation portions of Flowerpot and Bear's Rump, were submerged at least until Nipissing phase lake levels began to fall about 4 000 years ago (Morton and Venn 1987). When lake levels receded, colonies founded on both the mainland and the islands would have been equally insular, and there may not yet have been enough time for subsequent evolutionary adjustment to conditions in contemporary island and mainland environments. Furthermore, the high floatation capacity of *P. mistassinica* seeds (Category A of Morton and Hogg 1989: more than 50% of seeds remain afloat after one week with periodic mechanical shaking; B. Larson, unpubl. data) may allow frequent gene flow between populations and constrain evolutionary divergence. Fluctuating lake levels provide an opportunity for seeds to be washed from shoreline environments and to be dispersed among the network of small islands that surround the tip of the Bruce Peninsula. Recurrent migration through seed dispersal between the populations studied is aided by the relative proximity of *P. mistassinica* populations on the Lake Huron shoreline. Frequent gene flow was similarly invoked by Halkka and Halkka (1974) to explain the consistent equilibrium style-morph ratios found in island populations of tristylous *Lythrum salicaria* L. in southern Finland.

These results do not undermine the potential ecological and evolutionary consequences of insularity to plant populations. Rather, they highlight the dependence of these effects on the particular species and spatial scale investigated. It appears that the reproductive versatility of *P. mistassinica* and limited isolation of the Tobermory Islands buffer island populations from insular selective forces on their reproductive biology. Despite the results, further examination of microevolutionary patterns and processes in small insular environments are worth conducting and will likely reveal significant evolutionary changes to plant reproductive biology accompanying island colonization. Island plant populations represent a naturally fragmented system, so the results also illustrate the reduced susceptibility of species with effective dispersal and generalized pollination systems to pollinator declines in fragmented habitats.

CHAPTER FIVE

GENERAL CONCLUSIONS

"We know so little about this strange planet we live on, this haunted world where all answers lead only to more mystery."

Edward Abbey, *The Journey Home*, 1977

The major objective of this thesis was to explore factors limiting pollen delivery and hence fertility in flowering plants. There have been numerous investigations of pollen limitation within species (reviewed in Burd 1994), but patterns in its occurrence have seldom been assessed. Comparative analyses were conducted to determine whether pollen limitation among species (Chapter 2) or fertility among populations (Chapter 4) was associated with simple predictor variables. Broad-scale comparative studies may uncover patterns in the occurrence of pollen limitation, but are often uninformative with regard to potential mechanisms. In Chapter 2, predictions concerning the association of particular plant traits and ecological conditions with the occurrence of pollen limitation were generally supported, but the mechanisms responsible were often open to speculation. Similarly, the specific ecological factors that caused variable fertility among populations of *Primula mistassinica* on Lake Huron shorelines were unknown (Chapter 4). To examine a potential mechanism accounting for pollen limitation within flowering plants, the mechanics of pollen transfer within populations of *Rhexia virginica* at Lake Matchedash, Ontario were investigated and related to the intensity of pollen limitation (Chapter 3). By documenting interspecific patterns in the occurrence of pollen limitation and by using experimental approaches to evaluate potential mechanisms accounting for it, this thesis has served to increase our understanding of the ecological factors associated with pollen limitation.

In Chapter 2, comparative methods were used to discern patterns in pollen limitation among species, based on floral, life history and ecological traits. The analysis used an index of pollen limitation calculated for 224 species to determine whether these traits were associated with the intensity of pollen limitation. The study represents one of the first comparative analyses of plant reproductive traits that has accounted for the phylogenetic relationships among taxa using independent contrasts (see also Barrett *et al.* 1996). Although there was generally little difference between analyses that considered these relationships (PICs) versus those that did not (TIPs), this concordance strengthens the main results. Comparative analyses are nevertheless correlative, so conclusions must be tempered with caution. In particular, interactions between traits considered in the analysis were not fully

explored, and the PICs analysis rests on a single phylogenetic hypothesis for the species considered.

Despite these caveats, the main conclusion from this study was that the features considered did influence the intensity of pollen limitation to some extent. Pollen limitation was lower in species that have less dependence on pollinators because of their capacity to self-fertilize, whether this occurs autonomously or not. Pollen limitation was also lower in species with shorter lifespans. This may result from stronger selection on traits that increase the likelihood of pollinator visitation because these species have fewer opportunities within their lifetime to reproduce. Pollen limitation was lower in nectariferous than nectarless species, probably reflecting their greater rewards to foraging pollinators. Differences between species with specialized vs. unspecialized floral morphology were minimal, probably because morphology does not adequately depict the degree of specialization of floral visitors. Pollen limitation also tended to be lower in species of temperate regions and open habitats, but the reasons for these patterns were not entirely clear. Many of these comparisons require clarification using empirical studies that combine experimental manipulations in field populations with phylogenetic insights. For example, pollinator visitation rates and pollen limitation could be compared among sister species occurring in contrasting environments or having pollinators that differ in degree of specialization.

Because of variable pollen delivery, floral traits of a given species cannot necessarily ensure maximal fertility on a daily or seasonal basis, even if they reflect adaptation to average historical levels of pollen delivery. It may be for this reason that the predictive ability of the traits considered in the comparative analysis was limited. Variation in pollen limitation in ecological time may be relatively insignificant to floral evolution if lifetime fitness is resource-limited. Pollen limitation has not yet been shown to limit the lifetime fitness of a perennial plant (Ehrlén and Eriksson 1995), so it may be that pollen limitation is best interpreted in terms of adaptive explanations for the production of more flowers and ovules than are likely to be matured into fruits and seeds. Future analyses may benefit from incorporation of the stochasticity of pollen limitation, which could not be included here because it has been adequately characterized in so few species.

The results of the comparative analysis have implications for assessing the likelihood that plant species will be affected by anthropogenic activities such as habitat fragmentation (Kearns and Inouye 1997). Sexual reproduction in species that have traits associated with a greater likelihood of pollen limitation is more likely to be impacted by pollinator declines. This predictive ability may increase our ability to develop suitable management plans for rare species located in fragments before their populations decline because of inadequate reproduction (Caughley 1994; Schemske *et al.* 1994). Some species may have compensatory reproductive modes such as vegetative reproduction that are not influenced by pollinator declines (Bond 1994), but failed sexual reproduction greatly limits long-term survival prospects. Although the predictive ability provided by the single traits considered here was relatively low, future multivariate studies may detect suites of traits associated with intensity of pollen limitation.

In Chapter 3, experimental techniques were used to investigate a mechanism accounting for pollen limitation in buzz-pollinated *Rhexia virginica* in Ontario populations. This study required background research to document the floral ecology and pollination biology of *R. virginica*. This is the first detailed study of the reproductive ecology of a member of the Melastomataceae and one of the first for buzz-pollinated species in general. Limited pollen removal from anthers was associated with pollen limitation, indicating that the pollen-dispensing anthers of *R. virginica* could be viewed as 'inefficient' in the context of pollinators at Lake Matchedash. This result provides the first empirical evidence that pollen removal can limit fertility in a natural plant population. However, there were several limitations of the study that prevented full comprehension of the linkage between pollinator behaviour, pollen transfer and pollen limitation. In particular, pollinator visitation was far too low and erratic for visitation patterns to be fully described in relation to weather and time during the season. Hence, the link between pollinators, pollen removal patterns and pollen limitation could only be inferred and could not be related quantitatively to the causes of variable bumblebee visitation.

The results of the *R. virginica* study suggest a number of interesting research avenues for future consideration. In particular, this system is especially suited to further

examination of the floral and ecological factors that limit plant fertility. First, how prevalent is pollen limitation in other parts of the range of *R. virginica* and in other species of *Rhexia*? It would be interesting to determine whether the dispensing function of the anthers is more effective in the context of the pollinator fauna of the southeastern United States. One might predict that pollen limitation would be less severe on the coastal plain because the flowering season is longer, plants are larger and pollinators may be more abundant. If not, what are potential explanations for the large amount of pollen left in anthers at the end of anthesis? Second, the anthers of *R. virginica* do not dispense pollen in accordance with theoretical models for maximizing pollen dispersal (Harder and Thomson 1989), because more pollen was removed during longer visits by pollinators. Further investigations would be required to determine whether this was offset by diminishing returns once visits reached a certain duration. Third, why were pollinator visitation rates at Lake Matchedash so low? This question is testimony to our lack of understanding of mutualisms at a landscape scale (Bronstein 1995). Pollen limitation in *R. virginica* at Lake Matchedash may vary over space and time in association with the dynamics of local bumblebee populations. Although there is some understanding of the foraging patterns of individual bumblebees (Heinrich 1976, 1979a), foraging patterns have rarely been linked to the dependence of colonies on floral resources through the season. Studies of the association between bumblebee colony dynamics and pollen limitation of *R. virginica* populations could help us to understand the factors that limit the function of mutualisms over time. Eyde and Teeri (1967) stated that "... the floral biology of *Rhexia* might well be worth the attention of an enterprising graduate student ..." My research has to some extent confirmed this supposition and has shown that the reproductive biology of *R. virginica* may be of general interest to plant evolutionary ecologists.

In Chapter 4, a comparison of mainland and nearshore island populations of *Primula mistassinica* along Lake Huron shorelines in Ontario detected no consistent differences in their floral morphology and fertility. The 'naive prediction' was initially that insularity would cause differences in aspects of reproduction that influence fertility. Retrospectively, the results may not seem surprising given the proximity of the islands to the mainland. However, the wide variation in floral morphology and fertility between populations suggests

that ecological factors affected fertility, but simply were not consistently different between island and mainland environments. Logistical difficulties prevented full investigation of pollen limitation within the populations studied, particularly those on the islands, but it seems likely that pollen limitation did to some extent account for levels of fertility observed. Testing of this hypothesis would require empirical exploration of the prevalence of pollen and resource limitation in *P. mistassinica* populations on the rocky shorelines of Lake Huron.

It is partly a mystery why fertility levels in populations of *P. mistassinica* were as high as they were. Extensive observations suggested that pollinator visits were very infrequent. Fertility could be high despite low visitation rates because of the extended longevity (about ten days) of flowers of *P. mistassinica*. It is also possible that visitation rates were sporadically high. A full understanding of the ecological factors affecting the fertility of *P. mistassinica* would require intensive observation of pollinator visitation in relation to microclimatic conditions in different populations. These studies could also explicate whether syrphid flies are the major pollinators of *P. mistassinica* or whether infrequent visits by bees account for most pollen transfer. These potential pollinators are likely to be affected differently by local environmental conditions, which could, in part, account for fertility differences among populations.

Pollen limitation has traditionally been studied from the plant perspective. Given a plant's dependence on pollinators, however, the renaissance of pollen limitation research should in the future involve more explicit consideration of the ecological factors that influence pollinator visitation rates. How do different types of pollinators respond to variable weather patterns and how does their foraging ability vary between habitats? This type of knowledge would inform the results of the studies of *R. virginica* and *P. mistassinica* conducted in this thesis. In association with the experimental assessment of pollen limitation and knowledge of pollinator population dynamics, these investigations could serve to increase our understanding of pollination as a mutualism, rather than simply as a plant-specific phenomenon (Bronstein 1994). The interaction between floral traits, pollinating insects and ecological conditions provides a rich milieu for elucidating general principles of evolutionary ecology.

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Species included in the comparative analysis of pollen limitation in angiosperms (Chapter 2) that were not in Burd's (1994) compilation, listed alphabetically by family (* = new family): Amaryllidaceae: two species of *Cyrtanthus*, *Haemanthus rotundifolius*, *Nerine sarniensis* (Johnson and Bond 1997) and *Narcissus longispathus* (Herrera 1995a); Arecaceae*: *Neodypsis decaryi* (Ratsirarson and Silander 1996) and *Calyptrogyne ghiesbreghtiana* (Cunningham 1996); Caryophyllaceae: *Silene virginica* (Dudash and Fenster 1997); Ericaceae: *Andromeda polifolia* and four species of *Vaccinium* (Fröborg 1995); Gentianaceae: *Gentiana cruciata* (Petanidou *et al.* 1995); Iridaceae: *Iris lacustris* (G. Hannan, unpubl. data) and *I. versicolor* (Kron *et al.* 1993); Lamiaceae: hermaphroditic individuals of *Glechoma hederacea* (Widén 1992); Lentibulariaceae*: *Utricularia vulgaris* (P. Kron and B. Husband, unpubl. data); Lythraceae*: *Decodon verticillatus* (Eckert and Barrett 1995); Melastomataceae: *Rhexia virginica* (see Chapter 3); Orchidaceae: *Calypso bulbosa* (Alexandersson and Ågren 1996), six species of *Disa* (Johnson and Bond 1997) and *D. draconis* (Johnson and Steiner 1997); Primulaceae: legitimate pollination of *Primula sieboldii* lumped over morphs (Washitani *et al.* 1994) and *P. veris* (Lehtilä and Syrjänen 1995); Proteaceae*: *Grevillea barklyana* (Vaughton 1996) and *Persoonia mollis* (Krauss 1994); Rosaceae: *Sorbus aucuparia* (Sperens 1996); and Trilliaceae: *Trillium grandiflorum* (D. Smith, unpubl. data).

Dataset used for comparative analyses of pollen limitation in 224 species of flowering plants (Chapter 2), listed alphabetically by species. PLI = Pollen limitation index. A coding of '1' corresponds to the character state listed at the top of each column (auto = autogamous; forest = forested habitats; herb = herbaceous; mono = monocarpic; nectar = nectariferous; sc = self-compatible; spec = specialized floral morphology; temp = temperate). A coding of '2' corresponds to the alternative character state for each trait (see Table 2.1). Blanks indicate missing data.

GENUS	SPECIES	PLI	1=sc	1=auto	1=mono	1=herb	1=spec	1=nectar	1=temp	1=forest
Adesmia	*montana*	0.36	1	2	2	1	1		1	1
Adesmia	*mucronata*	0.37	1	1	1	1	1		1	1
Agalinis	*strictifolia*	0.07	2		1	1	1	2	1	
Agave	*mckelveyana*	0.11	2		2		1		2	
Allium	*tricoccum*	0.00	1		2	1	2		1	1
Alstroemeria	*ligtu*	0.48	1	1	2	1	2		1	1
Alstroemeria	*pallida*	0.29	1	1	2	1	2		1	1
Amelanchier	*arborea*	0.17	2		2	2	2	1	1	1
Amianthum	*muscaetoxicum*	0.44	1	2	2	1	2	1	1	1
Anchusa	*officinalis*	0.24	2		2	1	1	1	1	2
Andira	*inermis*	0.98	2		2	2	1	1	2	2
Andromeda	*polifolia*	0.00	1	1	2	2	1	1	1	2
Angelonia	*bisaccata*	0.28	2		2	2	1	1	2	
Angelonia	*hirta*	0.35	2		2	1	1	1	2	
Angelonia	*hookeriana*	0.30	2		2	2	1	1	2	
Angelonia	*pubescens*	0.00	1	1	1	1	1	1	2	
Aplectrum	*hyemale*	0.06	1	1	2	1	1	2	1	1
Aquilegia	*caerulea*	0.26	1		2	1	1		1	
Aralia	*nudicaulis*	0.22	2		2	1	2	2	1	1
Ardisia	*revoluta*	0.11	1		2	2	2		2	
Argyroxiphium	*sandwicense*	0.56	1	2	2		2		2	2
Asimina	*obovata*	0.50	1	2	2	2	2	2	2	1
Asimina	*parviflora*	0.82	1	2	2	2	2	2	2	1
Asimina	*pygmaea*	0.87	2		2	2	2	2	2	1
Asimina	*triloba*	0.96		2	2	2	2	2	1	1
Aspasia	*principissa*	0.84	1	2	2	1	1	2	2	1
Bauhinia	*pauletia*	0.68	1	2	2	2	1	1	2	1
Bauhinia	*ungulata*	0.51	1	2	2	2	2		2	1
Begonia	*involucrata*	0.03		2	2	1	2	2	2	2
Berberis	*sp*	0.93	2		2	2	2		1	1
Bourreria	*quirosii*	0.66	1		2	2	2		2	
Brassavola	*nodosa*	0.82	1	2	2	1	1	1	2	2
Byrsonima	*crassifolia*	0.65	1		2	2	2		2	
Caesalpinia	*eriostachys*	0.94	2		2	2	2		2	1
Calandrina	*grandiflora*	0.06	1	1	2	1	2		1	1
Calandrina	*prostrata*	0.00	1	1	1	1	2		1	1

GENUS	SPECIES	PLI	1=sc	1=auto	1=mono	1=herb	1=spec	1=nectar	1=temp	1=forest
Calathea	*ovandensis*	0.09		2	2	1	1	1	2	1
Calceolaria	*purpurea*	0.64	1	2	2	1	1		1	1
Calceolaria	*sp*	0.55	1	2	2	1	1		1	1
Calopogon	*tuberosus*	0.92	1	2	2	1	1	2	1	2
Calypso	*bulbosa*	0.29	1	2	2	2	1	2	1	1
Calyptrogyne	*ghiesbreghtiana*	0.65	2		2		2		2	1
Campsis	*radicans*	0.80	1	2	2	2	1	1	1	2
Cardamine	*angustata*	0.07	1	2	2	1	2	1	1	1
Cassia	*nictitans*	0.00	1	1	1	1	2	2	1	2
Centrosema	*virginianum*	0.30	1		2	1	1		2	
Chimaphila	*umbellata*	0.23	1	2	2	1	2	1	1	1
Claytonia	*virginica*	0.02	1	2	2	1	2	1	1	1
Clidemia	*capitellata*	0.20	1	1	2	2	2		2	1
Clintonia	*borealis*	0.29	1	1	2	1	1	1	1	1
Comparettia	*falcata*	0.79	1	2	2	1	1	1	2	1
Cordia	*alliodora*	0.64	1	2	2	2	2	1	2	1
Cordia	*dentata*	0.60	2		2	2	2	1	2	1
Cornus	*canadensis*	0.50	2		2	1	2	2	1	1
Crepis	*tectorum*	0.60	1	1	1	1	2		1	2
Crinum	*erubescens*	0.19	1		2	1	2	1	2	2
Cristaria	*dissecta*	0.12	1	1	1	1	2		1	1
Curatella	*americana*	0.00	1		2	2	2		2	
Cyclopogon	*cranichoides*	0.69		2	2	1	1	2	2	1
Cypripedium	*acaule*	0.99	1	2	2	1	1	2	1	1
Cyrtanthus	*guthrieae*	0.87		2	2	1	2	1	1	2
Cyrtanthus	*ventricosus*	0.69		2	2	1	2	1	1	2
Dalbergia	*retusa*	0.88	2		2	2	1		2	1
Decodon	*verticillatus*	0.06	1	2	2	1	2	1	1	2
Dieffenbachia	*longispatha*	0.38	1	2	2	1	2	2	2	1
Diervilla	*lonicera*	0.17	2		2	1	1	1	1	2
Disa	*bivalvata*	0.41		2	2	1	1	2	1	2
Disa	*draconis*	0.84	1	2	2	1	1	2	1	2
Disa	*fasciata*	0.52		2	2	1	1	2	1	2
Disa	*racemosa*	0.63		2	2	1	1	2	1	2
Disa	*tenella*	0.24		2	2	1	1	2	1	2
Disa	*tenuifolia*	0.39		2	2	1	1	2	1	2
Disa	*uniflora*	0.50		2	2	1	1	1	1	2
Dryas	*integrifolia*	0.16		1	2	1	2	1	1	2
Eccremocarpus	*scaber*	0.53	1	2	2	2	2		1	1
Eichhornia	*crassipes*	0.67	1	2	2	1	1	1	2	2
Encyclia	*cordigera*	0.90	1	2	2	1	1	1	2	
Encyclia	*krugii*	0.92	2		2	1	1	2	2	1
Enterolobium	*cyclocarpum*	1.00	2		2	2	2		2	
Epidendrum	*ciliare*	0.84	1	2	2	1	1	2	2	2
Eriotheca	*gracilipes*	0.50	2		2	2	2	1	2	1
Eriotheca	*pubescens*	0.40	1	2	2	2	2	1	2	1
Erythronium	*albidum*	0.00	1	2	2	1	2	1	1	1
Erythronium	*umbilicatum*	0.00	1	2	2	1	2	1	1	1
Espeletia	*batata*	0.67	2		2	2	2		2	2
Espeletia	*floccosa*	0.00	1		1	2	2		2	2
Espeletia	*lindenii*	0.15	2		1	2	2		2	2
Espeletia	*moritziana*	0.47	1		2	2	2		2	2

GENUS	SPECIES	PLI	1=sc	1=auto	1=mono	1=herb	1=spec	1=nectar	1=temp	1=forest
Espeletia	*neriifolia*	0.00	2		2	2	2		2	2
Espeletia	*schultzi*	0.42	2		2	2	2		2	2
Espeletia	*semiglobulata*	0.77	2		2	2	2		2	2
Espeletia	*spicata*	0.17	1		2	2	2		2	2
Espeletia	*timotensis*	0.07	1		2	2	2		2	2
Euthamia	*graminifolia*	0.10	1	2	2	1	2	1	1	2
Frasera	*caroliniensis*	0.00	1	2	1	1	2	1	1	1
Gauzuma	*tomentosa*	0.99	2		2	2	2		2	
Gaylussacia	*frondosa*	0.26	1	2	2	2	1	1	1	2
Gentiana	*cruciata*	0.00	1	2	2	1	1	1	1	2
Geranium	*berterianum*	0.01	1	1	2	1	2		1	1
Geranium	*maculatum*	0.00	1	2	2	1	2		1	1
Glechoma	*hederacea*	0.36	1		2	1	1		1	2
Grevillea	*barklyana*	0.00	1		2	2	2		1	
Haemanthus	*rotundifolius*	0.81		2	2	1	2	1	1	2
Hebe	*stricta*	0.19	1		2	2	2	1	1	2
Hepatica	*americana*	0.02	1	1	2	1	2	2	1	1
Heterotropa	*tamaensis*	0.32	1		2		2		1	
Hippeastrum	*bicolor*	0.63	2		2	1	2		1	1
Hirtella	*racemosa*	0.94	2		2	2	2		2	
Houstonia	*caerulea*	0.18	2		2	1	1	1	1	2
Hymenaea	*courbaril*	0.95	2		2	2	1		2	
Hyptis	*suaveolens*	0.00	1	1	2	1	1	1	2	2
Ilex	*opaca*	0.06	2		2	2	2	1	1	2
Impatiens	*capensis*	0.27	1	2	1	1	1	1	1	2
Impatiens	*pallida*	0.19	1	2	1	1	1	1	1	2
Inga	*brenesii*	0.91	2		2	2	2	1	2	1
Inga	*densiflora*	0.80	2		2	2	2	1	2	1
Inga	*mortoniana*	0.96	1	2	2	2	2	1	2	1
Inga	*oerstediana*	0.58	1	2	2	2	2	1	2	1
Inga	*punctata*	0.90	1	2	2	2	2	1	2	1
Inga	*quaternata*	0.00	2		2	2	2	1	2	1
Ionopsis	*utricularioides*	0.68	1		2	1	1	2	2	2
Ipomopsis	*aggregata*	0.52	2		1	1	1	1	1	2
Iris	*cristata*	0.39	1	2	2	1	1	1	1	1
Iris	*lacustris*	0.51	1	2	2	1	1	1	1	2
Iris	*versicolor*	0.00	1	1	2	1	1	1	1	2
Kalmia	*angustifolia*	0.16	1	1	2	2	1	1	1	2
Kalmia	*latifolia*	0.34	1	2	2	2	1	1	1	2
Lathyrus	*vernus*	0.55	1	2	2	1	1		1	1
Leptospermum	*scoparium*	0.15	1		2	2	2	1	1	2
Leucocoryne	*ixioides*	0.39	1	2	2	1	2		1	1
Linnaea	*borealis*	0.92	1	1	2	1	1	1	1	1
Lobelia	*cardinalis*	0.37	1	2	2	1	1	1	1	2
Lobelia	*siphilitica*	0.41	1	2	2	1	1	1	1	2
Lonchocarpus	*costaricensis*	0.97	2		2	2	1		2	
Lonchocarpus	*eriocarinalis*	0.89	2		2	2	1		2	
Luehea	*candida*	0.79	2		2	2	2	1	2	1
Luehea	*seemannii*	0.16	1	2	2	2	2	1	2	1
Luehea	*speciosa*	0.80	2		2	2	2	1	2	
Lysimachia	*quadrifolia*	0.44	1	2	2	1	2	2	1	1
Magnolia	*hypoleuca*	0.60			2	2	2	2	1	1

GENUS	SPECIES	PLI	1=sc	1=auto	1=mono	1=herb	1=spec	1=nectar	1=temp	1=forest
Maianthemum	*canadense*	0.13	2		2	1	2		1	1
Malpighia	*glabra*	0.70	1		2	2	2		2	
Mazus	*miquelii*	0.00	1		2	1	1		1	
Medeola	*virginiana*	0.73	1	2	2	1	2	1	1	1
Melampyrum	*pratense*	0.23		2	1	1	1	1	1	2
Miconia	*stephananthera*	0.32	1	1	2	2	2		2	1
Mitchella	*repens*	0.14	1	2	2	1	1	1	1	1
Monnina	*angustifolia*	0.36	2		2	1	1		1	1
Myrosmodes	*cochleare*	0.28	1	2	2	1	1	1	2	2
Myrospermum	*fructescens*	0.42	1		2	2	1		2	1
Narcissus	*longispathus*	0.09	1	2	2	1	1	2	1	2
Neodypsis	*decaryi*	0.20	2		2		2		2	
Nepeta	*cataria*	0.11	1	1	2	1	1		1	2
Nerine	*sarniensis*	0.19		2	2	1	2	1	1	2
Nerium	*oleander*	0.96	1		2	2	1	2	1	2
Ochroma	*pyramidale*	0.00	1		2	2	2		2	
Oncidium	*variegatum*	0.98	2		2	1	1	1	2	
Oxalis	*montana*	0.65	1	1	2	1	2		1	1
Passiflora	*vitifolia*	0.63	2		2	2	2	1	2	1
Persoonia	*mollis*	0.16	1	2	2	2	2		1	
Phellodendron	*amurense*	0.70	2		2	2	2		1	
Physalis	*longifolia*	0.36	2		2	1	2		1	2
Piscidia	*carthagenensis*	0.62	2		2	2	1		2	
Pithecelobium	*saman*	0.82	2		2	2	2		2	
Platanthera	*blephariglottis*	0.37	1	2	2	1	1	1	1	2
Platanthera	*ciliaris*	0.09	1	2	2	1	1	1	1	2
Platanthera	*stricta*	0.42	1	2	2	1	1	1	1	2
Podophyllum	*peltatum*	0.76	2		2	1	2	2	1	1
Primula	*sieboldii*	0.80	2		2	1	1		1	2
Primula	*veris*	0.31	2		2	1	1		1	1
Pseudowintera	*colorata*	0.70	2		2	2	2	1	1	2
Pterocarpus	*rohrii*	0.83	2		2	2	1		2	1
Pterolepis	*glomerata*	0.06	1	1	2	1	2		2	1
Pyrola	*secunda*	0.47	1	1	2	1	2	1	1	1
Raphanus	*sativus*	0.00	1	2	1	1	2	1	1	2
Relbunium	*hypocarpium*	0.00	1	1	2	1	1		1	1
Rhexia	*virginica*	0.36	1	2	2	1	2	2	1	2
Rubus	*chamaemorus*	0.04		2	2	1	2		1	2
Sabatia	*angularis*	0.11		2	1	1	2	2	1	2
Salpiglossis	*sinuata*	0.58	1	2	2	1	1		1	1
Sanguinaria	*canadensis*	0.00	1	1	2	1	2	2	1	1
Sapranthus	*palanga*	0.99	2		2	2	2		2	
Saxifraga	*oppositifolia*	0.00		2	2	1	2	1	1	2
Schultesia	*brachyptera*	0.00	1	1	2	1	2		2	1
Silene	*alba*	0.55	2		2	1	1	1	1	2
Silene	*virginica*	0.16			2	1	1	1	1	2
Siparuna	*neglecta*	0.94	2		2	2	2	2	2	1
Sisyrinchium	*arenarium*	0.53	1	2	2	1	2		1	1
Sisyrinchium	*philippii*	0.43	1	2	2	1	2		1	1
Solanum	*ligustrinum*	0.46	2		2	2	1		1	1
Solanum	*marginatum*	0.00	1	2	2	2	1	2	2	
Solidago	*canadensis*	0.22	1	2	2	1	2	1	1	2

GENUS	SPECIES	PLI	1=sc	1=auto	1=mono	1=herb	1=spec	1=nectar	1=temp	1=forest
Solidago	*juncea*	0.00		2	2	1	2	1	1	2
Sorbus	*aucuparia*	0.21	1	2	2	2	2		1	2
Spondias	*mombin*	0.99	2		2	2	2		2	
Stachys	*albicaulis*	0.18	1	1	2	1	1		1	1
Stellaria	*pubera*	0.00	1	2	2	1	2	1	1	1
Syzygium	*cormiflorum*	0.01	1	2	2	2	2	1	2	1
Tabebouia	*neochrysantha*	0.96	2		2	2	2		2	1
Tabebouia	*palmeri*	0.90	2		2	2	2		2	1
Tabebouia	*rosea*	0.98	2		2	2	2		2	1
Talquena	*quinquenervia*	0.41	1	2	2	2	2		1	1
Thalictrum	*thalictroides*	0.02	1	1	2	1	2	2	1	1
Tiarella	*cordifolia*	0.12	1	2	2	1	2	2	1	1
Tipularia	*discolor*	0.64	1	2	2	1	1	1	1	1
Tolumnia	*variegata*	1.00	2		2	1	1	2	2	1
Tricyrtis	*affinis*	0.03	1		2	1	2		1	1
Tricyrtis	*flava*	0.00	1	2	2	1	2	1	1	1
Tricyrtis	*latifolia*	0.00	1	2	2	1	2	1	1	1
Tricyrtis	*nana*	0.07	1	1	2	1	2	1	1	1
Trientalis	*borealis*	0.47	2		2	1	2	2	1	1
Trientalis	*europaea*	0.77	1	2	2	1	2	-	1	1
Trillium	*catesbaei*	0.10	1	1	2	1	2	2	1	1
Trillium	*grandiflorum*	0.09	1	2	2	1	2	1	1	1
Trillium	*undulatum*	0.00	1	1	2	1	2	2	1	1
Tropaeolum	*tricolor*	0.00		1	2	1	2		1	1
Utricularia	*vulgaris*	0.20	1	2	2	1	1	1	1	2
Uvularia	*sessilifolia*	0.05	2		2	1	1	1	1	1
Vaccinium	*myrtillus*	0.00	1	2	2	2	1		1	2
Vaccinium	*oxycoccos*	0.13	1	2	2	2	1		1	2
Vaccinium	*uliginosum*	0.00	2		2	2	1		1	2
Vaccinium	*vitis-idaea*	0.00	1	2	2	2	1		1	2
Viscaria	*vulgaris*	0.00	1	1	2	1	1	1	1	2
Viviana	*marifolia*	0.46	2		2	2	2		1	1