

The ecology of pollen limitation in buzz-pollinated *Rhexia virginica* (Melastomataceae)

BRENDON M.H. LARSON and SPENCER C.H. BARRETT

Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, Ontario, Canada M5S 3B2

Summary

1 Pollen limitation of female fertility has often been documented in animal-pollinated plants, but seldom have the ecological mechanisms responsible been investigated. We examined factors influencing pollen limitation in *Rhexia virginica* (Melastomataceae), a species in which pollen transfer depends on bumblebees capable of buzz pollination. Experimental studies were conducted in two populations at Lake Matchedash, southern Ontario, Canada, where the species occurs at the edge of its familial range.

2 Supplemental hand pollination of flowers increased their probability of fruit set compared with open-pollinated control flowers by an average of 57.6%. Pollen limitation was assessed on a per flower basis because the median floral display size at Lake Matchedash was one flower. Pollen limitation was prevalent throughout the 3–4-week blooming period in one population, whereas in the other it was only evident at the beginning of flowering, despite their close proximity.

3 Bumblebee visits (primarily *Bombus impatiens*) to *R. virginica* flowers were infrequent and variable in their occurrence. Visitation was recorded on 14 days during flowering. The median number of visits was 0.65 bees per hour; on 6 days there was virtually no bee activity, but on 2 days visitation rates were high. Variation in pollinator activity was apparently unrelated to local weather conditions.

4 Field experiments demonstrated that the poricidal anthers of *R. virginica* dispense pollen gradually, with only 10.2% of pollen removed from flowers during a single bumblebee visit. This level of pollen removal is lower than reported in other flowering plants. On average, 47.3% of pollen remained in anthers at the end of anthesis.

5 Investigation of the relation between pollen dispersal and pollen limitation demonstrated a significant negative correlation between the proportion of pollen removed from anthers on a given day and the intensity of pollen limitation. It appears that the pollen-dispensing mechanism of *R. virginica* and infrequent visitation by bumblebees compromise pollen dispersal, causing pollen limitation in Ontario populations.

Keywords: bumblebee, insufficient pollination, pollen removal, poricidal anthers, reproductive ecology

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Introduction

The fertility of flowering plants is often limited by pollen delivery. In animal-pollinated species, pollen dispersal can be highly stochastic because it depends upon the vagaries of pollinators that vary in their spatial and temporal abundance (Burd 1995). Unreliable pollinator service is likely to result in the variable occurrence of pollen limitation of fruit and seed set. Pollen limitation can be demonstrated exper-

imentally by an increase in the fertility of flowers that receive supplemental pollination relative to open-pollinated controls (Bierzuchudek 1981; Rathcke 1983). A literature survey of pollen limitation among flowering plants demonstrated that this treatment increased fertility at some times or in some locations in 62% of species studied (Burd 1994). Despite the prevalence of pollen limitation and its ecological and evolutionary significance (Haig & Westoby 1988; Ehrlén & Eriksson 1995; Morgan & Schoen 1997), there has been little experimental work on the proximate ecological factors involved. Remarkably few studies demonstrating pollen limitation have inves-

tigated which events are critical in the pollination process and may thus lead to insufficient pollination. In particular, examination of the relations between pollinator visitation, pollen dispersal and plant fertility are required to enable predictions concerning the magnitude of pollen limitation.

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 only those visitors that can effect pollination (Proctor *et al.* 1996). An exemplary case is the syndrome of buzz pollination, where the sole 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 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 & Thomson 1989; Harder & Barclay 1994; King & Buchmann 1996). Secondly, although many species of bees are capable of buzzing (Buchmann 1983) only a subset is likely to be efficient at buzz pollination of a particular plant species.

To examine pollen limitation in a buzz-pollinated species, we studied populations of *Rhexia virginica* L. (Melastomataceae) in southern Ontario, Canada, where the species occurs at the edge of its familial range. Melastomataceae is a large tropical family characterized by many buzz-pollinated species (Buchmann 1983; Renner 1989), and although pollen limitation has been investigated in three neotropical members of the family (Ramirez & Brito 1990) details of the ecology of pollen limitation were not considered. A previous survey of *R. virginica* populations in Ontario demonstrated that their fertility was quite low (mean fruit set = 52.6%, $n = 13$ populations) and that plants were frequently pollen-limited (Larson & Barrett 1999). Populations of *R. virginica* are particularly abundant at Lake Matchedash, Ontario, where they occur in discrete colonies around the shoreline of the lake. Preliminary studies at this location conducted in 1996 revealed that populations were strongly pollen-limited. In this study, we investigated the ecological causes of pollen limitation at Lake Matchedash by addressing the following specific questions. (i) What is the magnitude of pollen limitation and does it vary among populations and at different times during the flowering period? (ii) Are rates of pollinator visitation associated with variation in pollen limitation? (iii) Is there a relation between pollen limitation and patterns of pollen removal and pollen deposition? (iv) In particular, might restricted pollen removal associated with buzz pollination in *R. virginica* lead to significant pollen limitation? Fol-

lowing presentation of our results, we discuss the extent to which the functioning of the buzz-pollination syndrome of *R. virginica* may be compromised in Ontario populations, owing to low pollinator visitation rates.

Methods

THE STUDY ORGANISM AND STUDY SITE

Rhexia virginica (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). The flowers of *R. virginica* are orientated vertically and consist of four large, showy pink petals and eight elongate, bright yellow poricidal anthers that spread laterally from the centre of the flower. The style is sigmoidal and directed downwards so that the stigma is below the anthers. The flowers are nectarless, with pollen as the sole floral reward. Pollen removal from the poricidal anthers is accomplished by bumblebees capable of buzz pollination. In Ontario populations, *R. virginica* is self-compatible but does not self-pollinate autonomously, so it is entirely dependent on bumblebees for pollen transfer. Furthermore, its flowers are functional for 1 day, so their fertility depends on bumblebee activity during the single day of anthesis (Larson & Barrett 1999).

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). Two populations, B and D, which were located 400 m apart on opposite shores of the lake, were investigated in August 1997. Both populations contained between 1000 and 1200 flowering plants. Population B was relatively dense (140 flowering plants m⁻²; $n = 10 \times 1\text{-m}^2$ 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 × 13 m) but less dense (14 flowering plants m⁻²; $n = 10 \times 1\text{-m}^2$ quadrats) and located patchily in an open wet meadow. A variety of plants around the shores of Lake Matchedash bloomed concurrently with *R. virginica* and were also visited by bumblebees. These plants included *Cephalanthus occidentalis* L., *Lysimachia terrestris* (L.) BSP., *Pontederia cordata* L., *Spiraea alba* DuRoi and *S. tomentosa* L.

MEASUREMENT OF POLLEN LIMITATION IN *RHEXIA VIRGINICA*

Spatial and temporal components of pollen limitation in *R. virginica* at Lake Matchedash were investigated in populations B and D on 7 days spanning the 1997

flowering period (Fig. 1). On each day, 30 plants with one flower in anthesis were selected. Supplemental out-cross pollen from a single donor was added to stigmas of flowers on 15 plants, and flowers on the other plants were left as open-pollinated controls. Plants with one flower were chosen because this was the median daily display size in both populations (mean = 1.08 ± 0.01 , $n = 4176$, range = 1–5). Although this treatment can demonstrate pollen limitation at the flower level, it cannot alone conclusively demonstrate pollen limitation at the whole-plant level because resources may be re-allocated to flowers receiving supplemental pollen (Zimmerman & Pyke 1988; see the Discussion). Nevertheless, our primary objective here 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.

Fruit set of plants was analysed 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 population and date treated as random effects. Separate analyses were conducted on fruit and seed set because inclusion of zero fruit set data violated assumptions of the ANOVA of seed set. All statistical analyses here were conducted using JMP (Version 3.0.2; SAS Institute 1994).

RATE OF FLORAL VISITATION BY POLLINATORS

To determine the frequency of pollinator visits to *R. virginica*, bumblebees were observed throughout the flowering period in population B during 1997. We present visitation data for bumblebees only, as they were the predominant visitors to *R. virginica* at this

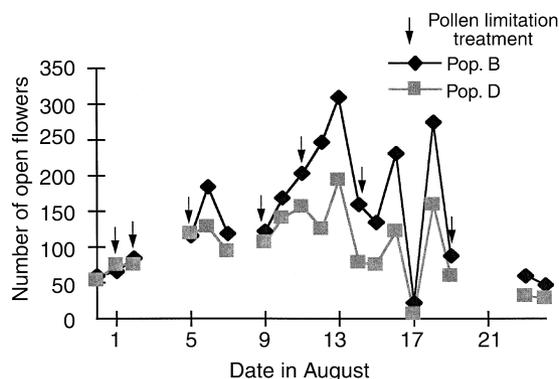


Fig. 1 Dates on which pollen limitation treatments were conducted on *Rhexia virginica* at Lake Matchedash, Ontario, Canada in August 1997, in relation to flowering phenology. The two curves are from populations B and D and present the total number of flowers in each population in anthesis on each day. Arrows indicate 7 days during the flowering period when pollen limitation treatments were conducted in each population.

site and the only insects that effectively ‘buzzed’ flowers. Because of the localized distribution and high density of population B, a single observer could monitor the arrival and departure of bumblebees to the entire population without difficulty. Visitor observations totalling 56.5 h were made on 14 days spanning the flowering season, and ranged in duration from 1.5 to 6 h per day. Initially, observations were conducted for 15 min per hour each morning, but low visitation rates necessitated a change to continual observation throughout the morning. Observations were not conducted from mid-afternoon onwards owing to a general cessation of bumblebee activity. The duration of each foraging bout, the number of flowers visited, daily temperatures and weather conditions were recorded. All days were sunny with no rainfall.

POLLEN REMOVAL DURING POLLINATOR VISITS

To determine whether restricted pollen removal from poricidal anthers could, in part, account for pollen limitation 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 early in the flowering season. Foraging bumblebees were allowed one, two or three visits to flowers in water pics attached to a stick (the method is described in Thomson *et al.* 1982). The duration of each visit was timed. Following visitation, anthers were placed in 70% ethanol in separate microcentrifuge tubes. The anthers were ruptured with a probe sonicator (Vibra-Cell, Sonics and Materials Inc., Danbury, CT) to release pollen grains for counting. A sample from each microcentrifuge tube was suspended in a weak aqueous electrolyte (5 g l^{-1} 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, IL). 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, $r^2 = 0.78$, $P < 0.0001$).

RELATIONS BETWEEN POLLEN REMOVAL, POLLEN DEPOSITION AND 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 on days when the pollen limitation treatments discussed above were conducted. The stigma and anthers from 15 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 from anthers during the day was determined as described above. Pollen grains were released from stigmas for counting using acetolysis (Kearns & 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 haemocytometer (Lloyd 1965). The mean amount of pollen removed from anthers and the amount deposited on stigmas on each day in each population were calculated. These values were used to correlate daily pollen removal and deposition with the intensity of pollen limitation. The intensity of pollen limitation on each day was summarized by an index, $P = 1 - (F_0/F_s)$, where F_0 is the fertility (seed set) of open-pollinated control flowers, and F_s is the fertility of flowers that received supplemental cross-pollen. A value of $P = 0$ indicates that no pollen limitation was detected.

Results

MEASUREMENT OF POLLEN LIMITATION IN *RHEXIA VIRGINICA*

Pollen supplementation treatments revealed significant variation in pollen limitation that was manifested between populations and sampling dates (Table 1 and Fig. 2). Fruit set was usually increased by pollen supplementation (Fig. 2) but the number of seeds set per fruit was relatively unaffected (no significant effects in three-way ANOVA). In population B, fruit set over the entire season was usually higher when supplemental pollen was added to flowers (control = 42%, $n = 100$ flowers; supplemented = 86.5%, $n = 96$ flowers; $\chi^2 = 44.45$, $P < 0.0001$, G -test of independence), whereas in population D increases were observed less frequently (control = 61.4%, $n = 101$ flowers;

supplemented = 76.5%, $n = 102$ flowers; $\chi^2 = 5.43$, $P < 0.02$, G -test of independence). Overall, the likelihood of fruit set was similar in the two populations (no significant population effect in Table 1) but a significant population-treatment interaction indicated that pollen limitation differed between them. The probability of fruit set varied among days during the flowering period in both populations (Table 1). Overall levels of fruit set in both control and supplementally pollinated flowers declined at the end of the flowering season (Fig. 2), probably as a result of resource limitation.

RATE OF FLORAL VISITATION BY POLLINATORS

Observation of bumblebee foraging to *R. virginica* populations at Lake Matchedash in 1997 indicated that visitation was infrequent overall but reached high levels on 9 and 10 August (Fig. 3). The median number of visitation bouts to population B during the 14 morning observation periods was 0.65 per hour (mean number of bouts = 1.1 ± 0.34) but was significantly greater on 9 and 10 August than on the other days (9 and 10 August = 3.36 ± 0.31 ; other days = 0.76 ± 0.25 ; Wilcoxon $Z = 2.11$, $P < 0.05$). The number of flowers visited per hour and per bout was also greater on these two mornings (visits per hour: 9 and 10 August = 205.6 ± 13.1 ; other days = 10.5 ± 5.3 ; Wilcoxon $Z = 2.11$, $P < 0.05$; visits per bout: 9 and 10 August = 60.8 ± 6.1 ; other days = 15.2 ± 2.9 ; Wilcoxon $Z = 2.00$, $P < 0.05$). On 9 and 10 August, *Bombus impatiens* visited all flowers in population B every 45 min. On the remaining 12 mornings, the total number of visits to flowers was dramatically lower than the total number of flowers present in the population. The unusually high rate of foraging on 9 and 10 August was not the result of favourable weather conditions compared with 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).

Table 1 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, Canada, in 1997. Insignificant interaction terms ($P > 0.30$) were deleted via backwards stepwise elimination

| Source of variation | d.f. | Likelihood ratio | |
|------------------------|------|------------------|----------|
| | | χ^2 | P |
| Population | 1 | 0.01 | 0.93 |
| Treatment | 1 | 57.19 | < 0.0001 |
| Population × treatment | 1 | 12.35 | 0.0004 |
| Date | 6 | 109.13 | < 0.0001 |
| Treatment × date | 6 | 11.00 | 0.089 |
| Error | 383 | | |

POLLEN REMOVAL DURING POLLINATOR VISITS

Pollen removal experiments conducted at Lake Matchedash indicated that a single bumblebee visit to previously unvisited flowers of *R. virginica* removed an average of 10.2% (SE = 1.8, $n = 29$) of the pollen produced by a flower. There was a gradual increase in the amount of pollen removed from flowers receiving two and three visits (Fig. 4). The amount of pollen removed during bee visits depended less on their number than on their cumulative duration (ANCOVA number of visits: $F_{2,25} = 0.57$, NS; duration: $F_{1,25} = 9.70$, $P < 0.005$). These results suggest that pollen removal

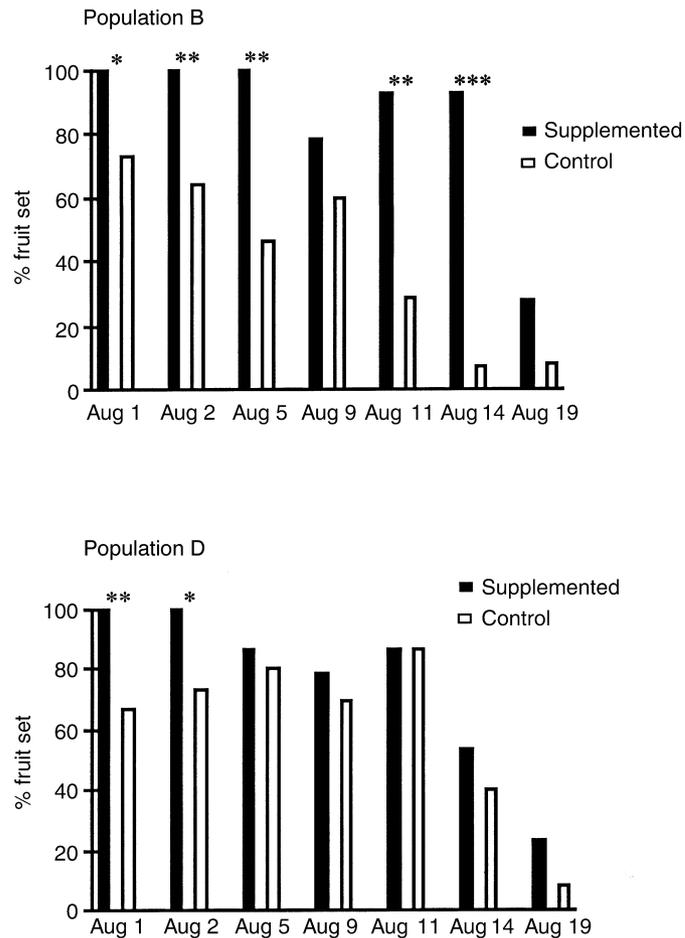


Fig. 2 Comparison of fruit set from open- and supplemental cross-pollinations of *Rhexia virginica* flowers in populations B and D at Lake Matchedash, Ontario, Canada, during 7 days in August 1997. The percentage fruit set of supplemented and control flowers on separate plants is presented. There were 15 plants in each treatment each day. Significant increases in fruit set with pollen supplementation are indicated by asterisks (*** $P < 0.0001$, ** $P < 0.01$, * $P < 0.05$) and are based on G -tests of independence.

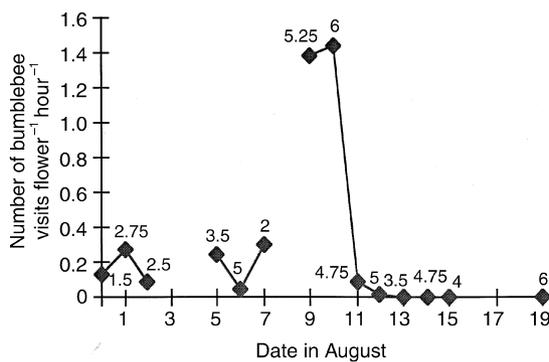


Fig. 3 Day-to-day variation in *Bombus* visitation to population B of *Rhexia virginica* at Lake Matchedash, Ontario, Canada, during August 1997. Points represent the number of visits recorded during morning observation periods, scaled by the number of flowers in the population (Fig. 1) and duration of observation (number of hours given above each point).

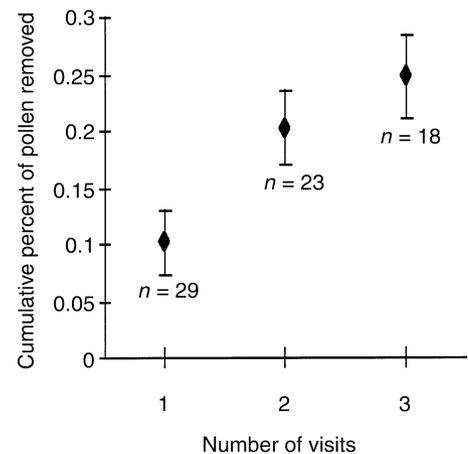


Fig. 4 Mean cumulative percentage (\pm SE) of pollen grains in *Rhexia virginica* flowers removed after one, two or three bumblebee visits at Lake Matchedash, Ontario, Canada. Sample sizes (n) are given below the bars.

during a single visit was restricted by the poricidal anthers of *R. virginica*, but that longer pollinator visits overcame this to some extent.

RELATIONS BETWEEN POLLEN REMOVAL, POLLEN DEPOSITION AND 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 50% of the pollen produced was removed ($52.7 \pm 0.01\%$, range = 31.3–67.3) but less than 0.5% was deposited on stigmas ($0.46 \pm 0.04\%$, range = 0.07–1.04). The average number of grains on stigmas at the end of a given day (number of grains = $1.32 \times 10^3 \pm 1.17 \times 10^2$) was about three times larger than the mean number of ovules in *R. virginica* flowers (number of ovules = 456.6 ± 7.5 , $n = 87$).

Patterns of pollen removal and deposition reflected the degree of pollen limitation of *R. virginica* at Lake Matchedash. When data for the two populations investigated were combined, there was a significant decline in pollen limitation for a given day both as more pollen was removed from anthers (Fig. 5a) and as more pollen was deposited on stigmas (Fig. 5b). 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 an association between pollen removal and pollen limitation in buzz-pollinated *R. virginica*. Although many studies have reported pollen limitation, our results represent one of the first attempts to link the mechanics of the pollination process to the intensity of pollen limitation. While pollen limitation at its most elementary level most often arises from inadequate pollination, low fertility can result from a variety of potential mechanisms operating at fruit and seed set. Here, we examine the likely causes of pollen limitation in *R. virginica* by considering interactions between the species' reproductive traits and the prevailing pollinator environment at Lake Matchedash. The pollen-dispensing anthers of *R. virginica* and its dependence on pollinators for pollen transfer were shown to be critical factors influencing fertility. The effect of these floral traits on pollen limitation was exacerbated by infrequent pollinator service, and we consider two potential explanations for low visitation rates. We 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

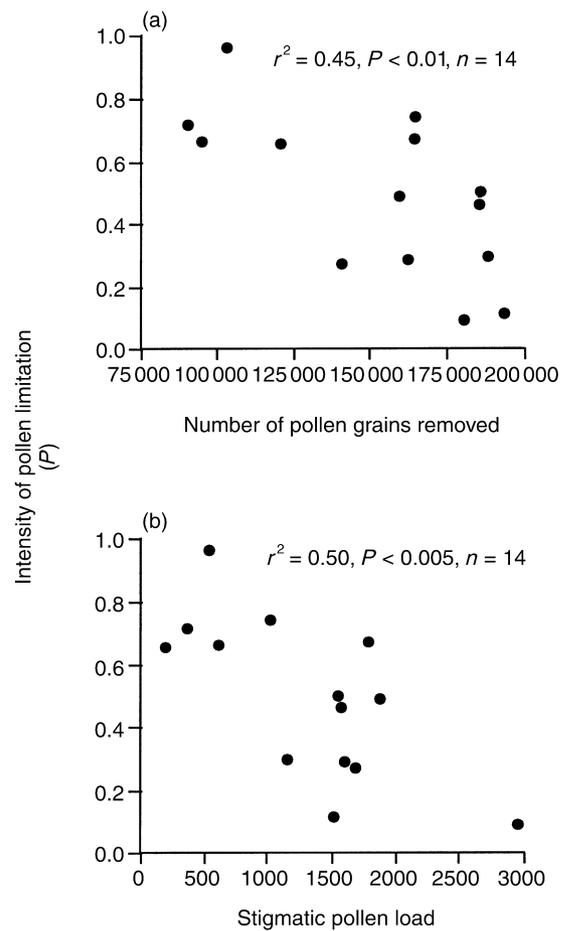


Fig. 5 The relation between the intensity of pollen limitation, P , and (a) pollen removal from anthers and (b) pollen deposition on stigmas of *Rhexia virginica* flowers at Lake Matchedash, Ontario, Canada, during August 1997. Mean daily pollen removal from anthers, deposition on stigmas, and pollen limitation were measured on 7 days during the flowering period and data from the two populations were combined (see the Methods).

mechanism accounting for pollen limitation of this species at Lake Matchedash.

POLLEN LIMITATION OF FERTILITY

Relatively few studies have examined the magnitude of pollen limitation in different populations and at different times during the flowering period of a given species. For example, in Burd's survey (Burd 1994), discussed above, about 81% of the species ($n = 258$) were investigated in a single population at one time in the flowering season. Our results caution against generalizations about the occurrence of pollen limitation in a species based on such limited sampling. We found significant spatial and temporal variation in pollen limitation at the two populations we investigated at Lake Matchedash. Particularly striking was the difference in magnitude of pollen limitation between them on several of the sampling dates. They were only 400 m apart, but their independent

responses to the pollen supplementation treatment suggest that their pollination environments were quite different. It is possible that the open water separating the two populations restricted interpopulation movement by foraging bumblebees. The inability to predict the extent of pollen limitation for populations located in close proximity and flowering at the same time serves to emphasize the stochastic nature of the pollination environment.

For species with multi-ovulate flowers, pollen limitation can be assayed at either fruit or seed set. Pollen limitation of fruit set is caused by either of two potential mechanisms. Perhaps most commonly, it results from the absence of a pollinator visit; alternatively, it may result from deposition of an insufficient number of pollen grains on the stigma to prevent fruit abortion (reviewed in Stephenson 1981). Variation in seed set among fruits most often results from different amounts of pollen deposition (see below). Pollen limitation of *R. virginica* at Lake Matchedash was largely manifested as reduced fruit set. When open-pollinated flowers set fruit they contained similar numbers of seeds as those from flowers receiving supplemental pollen. This suggests that fruits were produced only when a sufficient number of pollen grains were delivered to stigmas. Given the low pollinator visitation rates recorded in this study and the number of flowers available in populations, it seems likely that low fruit set results from the presence of surplus flowers relative to the number of bumblebees available for pollination. While recent studies of plant fertility have emphasized the increased likelihood of pollen limitation in small populations (Sih & Baltus 1987; Jennersten 1988; Ågren 1996), our study raises the possibility of the reverse pattern occurring, where undervisitation by pollinators occurs in large populations containing more floral resources than are required (Fritz & Nilsson 1994).

These investigations of *R. virginica* do not address the relative importance of pollen and resource limitation to fertility or the demographic and evolutionary consequences of pollen limitation (Haig & Westoby 1988; Ehrlén & Eriksson 1995). These issues are best evaluated using information on the lifetime fertility of plants and were outside the scope of this particular study, which focused instead at the flower level. This is the appropriate ecological unit for determining the reproductive mechanisms accounting for pollen limitation in this species, because the median daily display size was one flower and individual ramets are annual in Ontario (Posluszny *et al.* 1984). Preliminary evidence indicated that resources were unlikely to have played an important role in governing the percentage of fruit set that we observed, except towards the very end of the blooming period. Pollen supplementation of all flowers on plants at Lake Matchedash increased the proportion that set fruit and the total number of seeds plants produced relative to open-pollinated control plants. This method is

sufficient to demonstrate that pollen limitation also occurs at the scale of entire plants, at least during a single year (Johnston 1991). Nonetheless, it would be of interest to investigate whether population growth via clonal propagation is influenced by the degree of pollen limitation in *R. virginica*. Because this species forms tubers, heavy and repeated fruiting may divert resources away from these structures, and thereby limit population growth.

CONTRIBUTION OF FLORAL TRAITS TO POLLEN LIMITATION

Pollen limitation in *R. virginica* was associated with restricted 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 (Fig. 6). 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 & Yates 1982; Harder & Thomson 1989; Harder & Wilson 1994). This relation

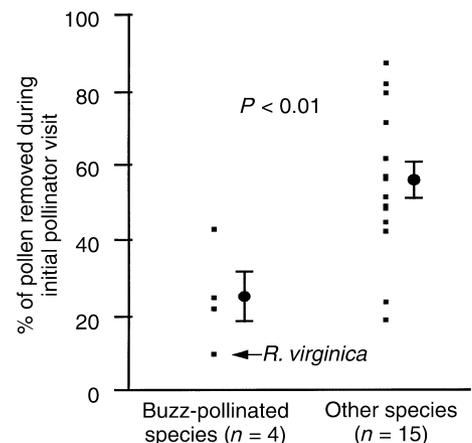


Fig. 6 Average percentage of pollen removed from anthers of plant species during an initial visit by a bee, based on a literature survey of buzz-pollinated species ($n = 4$) and those with other pollination systems ($n = 15$). The mean (\pm SE) for each group of species and significance level based on a two-tailed t -test (d.f. = 17, $t = 2.94$) are also presented. Buzz-pollinated species: *Cassia reticulata* (Snow & Roubik 1987), *Dodecatheon conjugens* (1st-day flowers) (Harder & Barclay 1994), *Pedicularis contorta* (Harder 1990a) and *Rhexia virginica* (this study). Other species: *Aconitum delphinifolium* (Harder 1990a), *A. septentrionale* (Thøstenes & Olesen 1996), *Aralia hispida* (Harder 1990a), *Drosera tracyi* (Wilson 1995), *Echium vulgare* (Strickler 1979; Klinkhamer *et al.* 1991), *Erythronium americanum* (Harder & Thomson 1989), *E. grandiflorum* (Thomson & Thomson 1989), *Impatiens capensis* (Wilson & Thomson 1991), *Lupinus sericeus* (Harder 1990a), *Mertensia paniculata* (Harder 1990a), *Pedicularis bracteosa* (Harder 1990a), *Polemonium viscosum* (Galen & Stanton 1989), *Pontederia cordata* (Wolfe & Barrett 1989), *Raphanus sativus* (Young & Stanton 1990) and *Trifolium pratense* (Dunham 1939).

probably results from the accelerated loss of pollen due to pollen-layering and more intense pollinator grooming as more pollen is removed (Buchmann & Cane 1989; Harder 1990b). However, as pollinator visits to *R. virginica* were quite infrequent, this mechanism appeared to overly restrict pollen removal, resulting in a considerable amount of pollen remaining in anthers at the end of anthesis. In other species that dispense pollen, a greater proportion of the pollen remaining is removed during later visits, which increases the likelihood that most of the pollen is dispersed (Harder & Barclay 1994; Harder & Wilson 1994; Lebuhn & Anderson 1994; King & 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, perhaps associated with the species' geographical marginality (Larson & Barrett 1999). This hypothesis could be evaluated by studies of populations of *R. virginica* from the central portion of its range in the southern United States, where pollinator visitation may be more frequent.

Because *R. virginica* flowers do not self-fertilize autonomously, pollinators are required for pollen transfer to occur. Low visitation may have caused pollen limitation in *R. virginica* at Lake Matchedash because the quantity of pollen deposited on stigmas did not maximize fruit and seed set (insufficient pollen transfer; *sensu* Harder & Barrett 1996). Fertility may also be limited by the quality of pollen deposited on stigmas (inefficient pollen transfer; *sensu* Harder & Barrett 1996). *Rhexia virginica* is self-compatible (*contra* Renner 1989; Larson & Barrett 1999) so pollen quality largely concerns the relative proportion of self- and out-cross pollen deposited during pollinator visits. If self-pollen is of lower quality than out-cross pollen, self-fertilization may also contribute to pollen limitation in Ontario populations. Evidence for pollen limitation arising from low pollen quality was found in *Blandfordia grandiflora* R. Br. (Liliaceae), where pollinator-mediated selfing pre-empted ovules that would otherwise have been out-crossed (Ramsey 1995). The extent to which self-pollen deposition causes pollen limitation is poorly appreciated and may be more significant than is generally thought.

CONTRIBUTION OF POLLINATORS TO POLLEN LIMITATION

The floral traits of *R. virginica* discussed above contribute to the intensity of pollen limitation, but would probably be less relevant if pollinator visitation rates were high. It is somewhat surprising that visitation was so infrequent at Lake Matchedash, because *R. virginica* offers large quantities of pollen for foraging bumblebees. There are two potential explanations for

low visitation rates. First, as mentioned earlier, there may be an oversupply of *R. virginica* flowers at Lake Matchedash relative to the pollen required by local bumblebee colonies. Secondly, 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 (Plowright & Lavery 1984). The number of bumblebees visiting *R. virginica* at Lake Matchedash may be determined by the availability of pollen for colony growth earlier in the season, rather than simply the number of flowers available within populations during August.

Despite these considerations, bumblebees require some pollen towards the end of the summer, and *R. virginica* is a readily available source. However, buzz pollination is a relatively complex behaviour, and bees may take longer to learn it compared with obtaining rewards from flowers with less specialized floral morphologies (Lavery 1994). The time needed for bees to learn to buzz pollinate has not been investigated experimentally (but see Buchmann 1983; King 1993). Regardless, if bees require relatively small amounts of pollen in August, the amount obtained incidentally while visiting other flowers at Lake Matchedash for nectar may be sufficient. This seems possible because most of the concurrently flowering species visited by bumblebees at this site (listed in the Methods) had less complex flowers that probably require shorter learning times than those of *R. virginica*. Furthermore, *R. virginica* flowers do not produce nectar, which may make them relatively unrewarding in comparison with other flowering species in the community.

While it is clear that pollen limitation in *R. virginica* is associated with low pollinator service, our attempts to establish a functional link between rates of pollinator visitation and the intensity of pollen limitation were unsuccessful. These variables were measured on 7 days during the blooming period in population B. However, because visitation rates were so variable among days (Fig. 1), the statistical power for testing this association was weak. There was a negative relation between pollinator visitation rate and pollen limitation, and positive relations between visitation and both pollen removal and deposition, but none of these relations was statistically significant ($n = 7$ days; removal: $P = 0.25$; deposition: $P = 0.17$; pollen limitation of fruit set: $P = 0.13$; pollen limitation of seed set: $P = 0.34$). These results highlight the difficulty in determining the quantitative relation between the number of pollinator visits to a population and pollen limitation when visitation rates are both low and highly stochastic. Long-term studies would be valuable to determine the causes behind the dramatic increases in pollinator visitation we observed in population B on 9 and 10 August, and whether they occur with any regularity.

Plant fertility can be considered from both male and female perspectives. 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 investigated previously (Harder & Wilson 1997). Although the amounts of pollen removed and deposited during single pollinator visits have been compared (Snow & Roubik 1987; Cruzan *et al.* 1988; Harder & Thomson 1989; Thomson & Thomson 1989; Wolfe & Barrett 1989; Harder 1990a; Murcia 1990; Wilson & Thomson 1991), pollen removal and deposition have not been explicitly linked to variation in the degree of pollen limitation. As mechanisms that restrict pollen removal are widespread in flowering plants and considerable pollen is lost during transport (Harder & Thomson 1989), a perspective that includes male function is likely to inform our understanding of factors limiting female fertility in other 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 (Fig. 5a). Although models for the evolution of pollen-dispensing mechanisms commonly refer to their potential for limiting fertility when pollinators are infrequent (Harder & Thomson 1989; Harder & Wilson 1994), this is the first empirical demonstration that this occurs 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 & Thomson 1991; Harder & Wilson 1998). Neither of these losses was quantified in this study, but bumblebees released a cloud of pollen from anthers during visits that seemed to preclude precise pollen placement. This fits with descriptions of imprecise pollen placement reported for several other buzz-pollinated species (Snow & Roubik 1987; Renner 1989). Conversely, buzz-pollinated species with a solanoid morphology constrain pollinator contact with the flower, thereby increasing the precision of pollen deposition (Harder & Barclay 1994; Harder & 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 revealed that pollen deposition on stigmas influenced the degree of pollen limitation in *R. virginica* (Fig. 5b). This was a consequence of restricted pollen transport by pollinators as discussed above. On average, only 0.46% of the pollen produced by flowers was transferred to stigmas, which is comparable to that in several other species that have been examined (Thøstesen & Olesen 1996). Despite this level of pollen transfer, the number of grains deposited (mean = 1320) was three times greater than the number of ovules within *R. virginica* flowers (mean = 457). Nevertheless, seed set was pollen-limited, which suggests that many of the pollen grains deposited either did not germinate or that significant attrition of pollen tubes occurred in the style, perhaps due to low pollen quality (Cruzan 1989). Studies on the relation between pollination intensity and seed set have consistently demonstrated that the ratio of pollen grains deposited on stigmas to seed set is greater than three (Shore & Barrett 1984; Snow 1986; Cruzan & Barrett 1996; Mitchell 1997). Pollen limitation in *R. virginica* would probably be reduced if greater amounts of cross-pollen were deposited on stigmas by bumblebees. This would occur through an increase in pollen circulation within populations caused by higher rates of bumblebee visitation to *R. virginica* flowers.

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