

THE POLLINATION ECOLOGY OF BUZZ-POLLINATED *RHEXIA VIRGINICA* (MELASTOMATACEAE)¹

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We examined the function of floral traits associated with buzz pollination through studies of *Rhexia virginica* (Melastomataceae) in the Muskoka region of Ontario, Canada. Controlled pollinations demonstrated that the species is self-compatible, but dependent on insects for pollen transfer. Bumble bees made 82 and 90% of observed insect visits to *R. virginica* in 1996 and 1997, respectively, and effectively buzzed flowers. Buzz pollination did not appear to be highly “specialized” since various species of bumble bee were capable of pollination, and pollen transfer efficiency appeared to be relatively low. Experimental manipulations provided little support for the hypothesis that the yellow color of melastome anthers mimics abundant pollen, thereby deceiving pollinators to visit regardless of whether most pollen has been removed. Fruit set averaged 52.6% among populations, owing largely to infrequent pollinator visits and pollen limitation. Flowers of *R. virginica* were infertile after a single day of anthesis, but petals were subsequently maintained for 1–2 d and stamens underwent a color change from bright yellow to red. Second-day flowers may function to increase floral display size and hence fertility, without a concomitant increase in pollen discounting. Studies of bumble bee foraging behavior and correlates of seed set provided indirect support for this hypothesis.

Key words: bumble bee behavior; floral color change; Melastomataceae; phenology; pollen limitation; *Rhexia virginica*; specialization.

The anthers of 6–8% of flowering plant species dehisce via pores that restrict pollen removal by insects (Buchmann, 1983). Pollen removal requires bees that land on the flowers and vibrate their indirect flight muscles at a high frequency, which causes pollen to stream out of the anthers. In reference to the sound made by the bee vibrations, this pollination system is called buzz pollination. Recent studies consider the mechanics of pollen removal from poricidal anthers (King and Lengoc, 1993; King and Ferguson, 1994) and its implications for pollen dispersal (Harder and Barclay, 1994; King and Buchmann, 1996). Nonetheless, field investigations of the reproductive ecology of buzz-pollinated species are few (Macior, 1964; Buchmann, Jones, and Colin, 1977; Cane and Payne, 1988; Knudsen and Olesen, 1993), and several questions concerning the response of pollinators to floral traits associated with buzz pollination remain unanswered.

The Melastomataceae, a large predominately tropical family characterized by buzz pollination, exemplify two aspects of the buzz pollination syndrome that have received little empirical consideration (Buchmann, 1983; Renner, 1989). First, anthers in the family are typically bright yellow in color, which mimics abundant pollen

even when they are empty. This has been interpreted as a deceptive adaptation because insects must visit the flowers to determine whether they are rewarding (Vogel, 1978; Buchmann, 1983). Second, melastome flowers commonly undergo a color change after anthesis (Renner, 1989; Weiss, 1995). Floral color change in flowering plants has been hypothesized to increase the efficiency of pollinator foraging and pollen transfer (Gori, 1983, 1989), and the maintenance of flowers that have undergone a color change is thought to increase floral display size and thus visitation rates (Cruzan, Neal, and Willson, 1988; Gori, 1989; Weiss, 1991; but see Casper and La Pine, 1984; Delph and Lively, 1989). Here, we investigate the function of these two aspects of the buzz pollination syndrome in the melastome *Rhexia virginica* L. (Virginia meadow-beauty), using experimental manipulations and observations of bee foraging behavior.

Rhexia virginica is a perennial wetland herb found predominately on the coastal plain of the United States, but following glacial retreat it migrated inland to the vicinity of the Great Lakes (Reznicek, 1994; Fig. 1A). Populations in the Muskoka region of Ontario, Canada, are significantly disjunct in the distribution of the species and are at the edge of the familial range of the Melastomataceae. In this region, the floral syndrome of *R. virginica* is unique because the few co-occurring buzz-pollinated taxa have either a solanoid or ericoid floral morphology. In contrast, the nectarless flowers of *R. virginica* consist of four large, showy pink petals and eight elongate, bright yellow, poricidally dehiscent anthers that spread laterally from the center of the flower. The style is sigmoidal and directed downwards so that the stigma is below the anthers. Given the distinctiveness of *R. virginica* in Ontario, an investigation of its pollination syndrome provided an opportunity to assess its function in a geographic context that is marginal with respect to the family's distribution.

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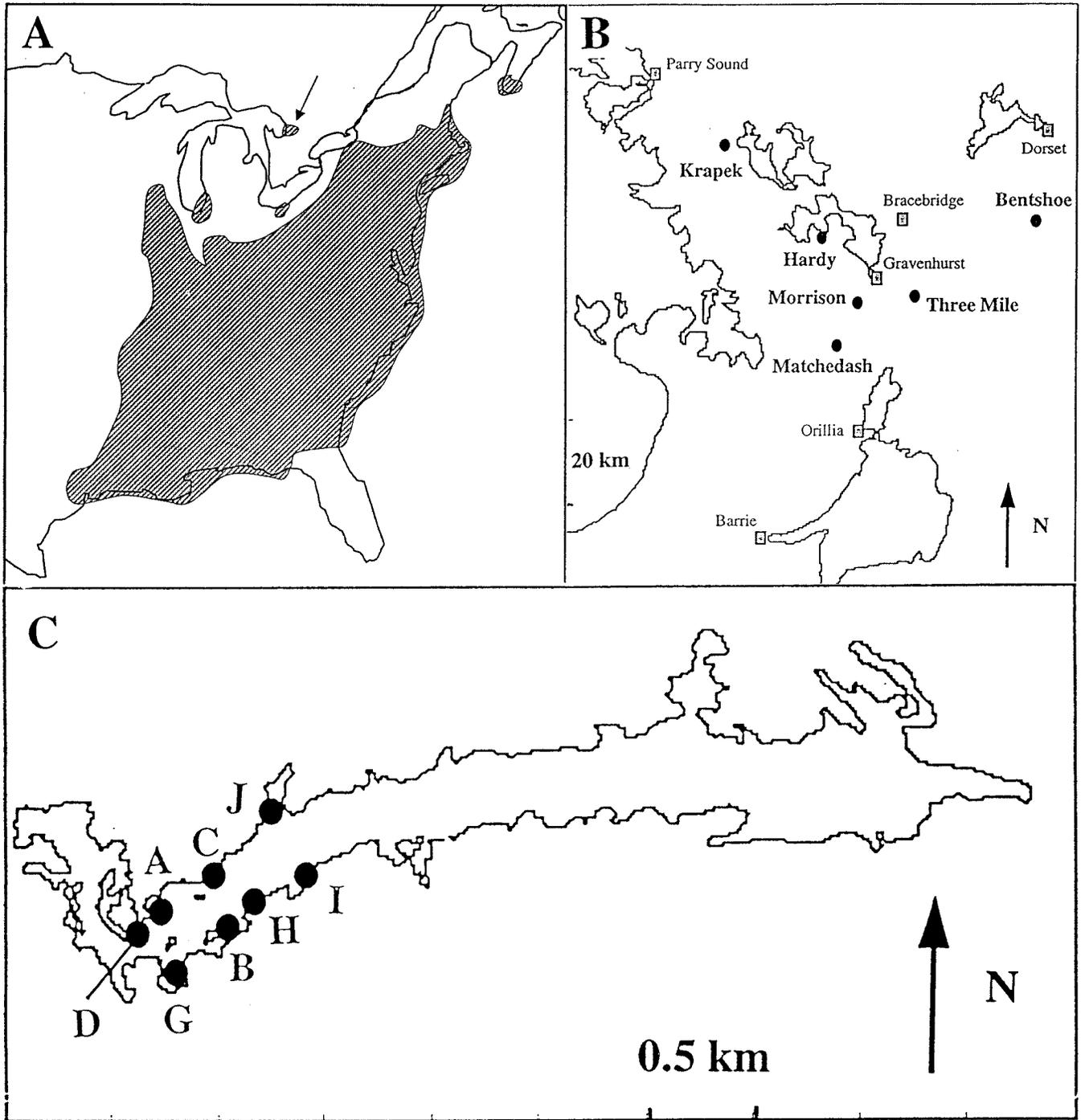


Fig. 1. Location of populations of *Rhexia virginica* studied in the Muskoka region of Ontario in 1996 and 1997. (A) The range of *Rhexia virginica* in eastern North America (modified from Sharp and Keddy, 1983), with the Muskoka region indicated by an arrow. (B) Six lakes in the Muskoka region where populations were sampled. (C) Close-up map of Lake Matchedash, showing the location of eight populations at the western extremity of the lake where studies were undertaken.

In this study we document the floral biology of *R. virginica* in Ontario by characterizing its compatibility system, dependence on pollinators for pollen transfer, and flowering phenology. With this background, we then address the following specific questions concerning its pollination ecology: (1) What are the pollinators of *R. virginica* and how do they respond to cues provided by

flowers? In particular, do they visit flowers even if the anthers have been emptied of their pollen? (2) What is the function of floral color change and the maintenance of second-day flowers in *R. virginica*? (3) What levels of female fertility characterize populations of *R. virginica* in Ontario, and is the fruit and seed set of populations pollen limited? Following presentation of our results, we

assess the extent to which buzz pollination of *R. virginica* may be considered a "specialized" pollination syndrome.

MATERIALS AND METHODS

The study site—Unless otherwise stated, we conducted studies at Lake Matchedash (also called Long Lake), Simcoe County, Ontario (79°30'45" W, 44°47'00" N) (Fig. 1). 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. We conducted research in a total of eight populations of *R. virginica* at Lake Matchedash in 1996 and 1997 (Fig. 1). We undertook detailed investigations of the ecology of pollen limitation in populations B and D in 1997 (Larson and Barrett, in press).

Floral biology of *Rhexia virginica*—Mating system—The compatibility status of *R. virginica* is uncertain. Kral and Bostick (1969) and Sharp (1983) demonstrated that fruits are set after self-pollination. Based on the former study, however, Renner (1989) claimed that *R. virginica* is self-incompatible because seeds from selfed flowers did not germinate. To determine whether *R. virginica* flowers from Lake Matchedash set seed after self-pollination and whether they depend on insect visits for pollination to occur, we performed an experimental pollination study in a glasshouse at the University of Toronto in July and August 1996. Each treatment included a minimum of 15 plants transplanted from populations B, D, G, H, and I at Lake Matchedash. We assigned flowers randomly to one of four treatments: self-pollination, cross-pollination, unpollinated to test for autonomous self-fertilization, or "disturbed." We obtained pollen for pollination treatments by slitting anthers and using forceps to remove pollen that was subsequently applied to stigmas. We watered "disturbed" flowers from above with a watering can for 4 min to simulate whether rain could potentially cause self-pollination (see Renner, 1989).

The pollen:ovule ratio of angiosperm species provides some indication of their mating system (Cruden, 1977). To calculate the pollen:ovule ratio of *R. virginica*, we counted ovules in 25 flowers sampled from each of the four terminal nodes on plants in population D at Lake Matchedash on 10 August 1996. We also collected anthers from a total of 154 flowers during 1996 and placed them in 75% ethanol in separate microfuge tubes. We released pollen grains from the anthers using a probe sonicator and counted them using a particle counter (see Harder, 1990, for details).

Floral color change—After flowering for 1 d *R. virginica* flowers undergo a color change (Weiss, 1995). To determine whether pollinator visits or environmental cues induce this color change (Gori, 1983), we qualitatively compared the color of 30 flowers located in pollinator enclosure cages at Lake Matchedash to flowers outside enclosures. To assess whether flowers having undergone a color change (second-day flowers) could contribute directly to plant fertility if visited by insects, we compared the receptivity of second-day stigmas and viability of second-day pollen to that of first-day flowers using controlled hand-pollinations with outcross pollen. Treatments were applied to plants located within enclosure cages in population D during early August 1996.

Flowering phenology—The number of flowers within plant populations varies through time, and pollinator densities may be associated with the distribution of flowering (Thomson, 1980). We characterized the phenology of *R. virginica* at Lake Matchedash in 1996 by randomly choosing 50 plants along shoreline transects within populations A, B, C, and D prior to flowering. We surveyed plants every other day until flowering ceased and recorded the anthesis date of each flower. We also

recorded second-day flowers to quantify their contribution to floral display size.

Pollination biology of *Rhexia virginica*—Floral visitation—To identify the pollinators of *R. virginica* flowers, we watched visitors during observation periods throughout peak flowering in 1996 and 1997 at Lake Matchedash. We quantified rates of floral visitation in 1996 by recording the number of visitors entering single 4-m² quadrats in populations B and D every hour from 0700 until 1500 for 15-min periods on 10 d during the flowering period.

Bumble bee behavior—We undertook four investigations of bumble bee foraging behavior on *R. virginica* flowers at Lake Matchedash in 1996 and 1997. (1) We recorded bumble bee visits to second-day flowers to determine whether they were visited in proportion to their abundance or whether the bees preferentially visited first-day flowers. (2) To determine whether bumble bees had a preference for unvisited *R. virginica* flowers compared to previously visited flowers, we presented bees with experimental arrays of 16 first-day flowers on 4 and 14 August 1996. We positioned flowers in florist water pics located 5 cm from one another in a square array comprising equal numbers of flowers of two randomly arranged treatments: (a) flowers that had been visited during the morning and from which we expelled remaining pollen by repeated tapping using forceps and (b) unvisited flowers from an enclosure cage. (3) To quantify the proportion of pollen that can be removed from *R. virginica* flowers without "buzzing," we conducted an investigation prior to bumble bee visitation on 10 August 1997 in population D. We tapped single flowers from ten plants repeatedly with forceps until we could remove no more pollen and then estimated the amount removed by comparison with the amount in unmanipulated flowers. (4) Bumble bees could cause self-pollination in *R. virginica* by transferring pollen (i) within flowers during visits (facilitated self-pollination sensu Lloyd and Schoen, 1992) or (ii) between flowers on a plant (geitonogamous self-pollination). We assessed the potential for bumble bees to transfer pollen geitonogamously, within inflorescences, by recording the number of *R. virginica* flowers they visited during foraging on multi-flowered inflorescences in 1996.

Female fertility of *Rhexia virginica*—Survey of patterns of fruit set—We conducted investigations in 1996 and 1997 to assay fruit set in *R. virginica* and to determine the extent to which it varied in space and time. We measured fruit set in populations A, B, C, and D at Lake Matchedash in 1996 by monitoring the 200 plants marked for the phenology study. We measured fruit set in 1997 at seven populations at Lake Matchedash and in single populations from Krapek Lake (79°48' W, 45°13' N), 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) (Fig. 1). We counted the number of buds on 25 randomly chosen plants in each population in early August and recorded the presence of fruits on these plants in October. We also estimated the size of populations visited in 1997 to determine whether there was a relation between female fertility and population size.

Factors affecting female fertility—We investigated the effect of various factors on the female fertility of *R. virginica* in populations A, B, C, and D at Lake Matchedash in 1996. We marked flowers on plants in the phenology study individually with paint so that fruit set and the number of seeds produced could be related to the population in which a plant was located, flowering date, and the number of flowers displayed on the date of flowering (daily display size). We treated flowering date 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 increased pollinator visitation rates and hence fertility, we either included or excluded them in display size and compared the results. We analyzed fruit set using logistic regression, with population, display size, and flowering date treat-

ed as independent variables. Since fertility levels in the multiple flowers comprising some daily displays were not independent, we used the proportion of these flowers setting fruit in the analysis. In 94% of daily displays, plants set either all fruit or no fruit. In the remaining displays we considered fruit set complete or zero in the logistic regression, depending on whether or not the majority (>50%) of fruits set. To determine the effect of the same variables on seed set, we conducted a mixed-model ANOVA. All statistical analyses were conducted using JMP (Version 3.0.2, SAS, 1994).

Pollen limitation of fertility—We undertook an investigation at Lake Matchedash in 1996 to determine whether the female fertility of *R. virginica* was pollen limited. We randomly selected between 15 and 30 plants with two flowers in populations B and D on 1, 5, 8, and 17 August. The two populations were of similar size and contained ~1000 plants. We added supplemental pollen from a nearby plant to one flower and compared its fruit set and seeds per fruit to the control flower. We conducted all pollination treatments on clear, sunny days unless otherwise noted. Preliminary investigations indicated that pollen limitation occurred at the whole-plant level (*sensu* Johnston, 1991) in *R. virginica* at Lake Matchedash, so confounding of treatment effects with resource re-allocation within inflorescences was unlikely (B. M. H. Larson and S. C. H. Barrett, unpublished data).

To assess whether pollen limitation limited female fertility of *R. virginica* flowers elsewhere in the Muskoka region, we conducted surveys at Bentshoe, Hardy, Krapek, and Three-mile Lakes (Fig. 1) in 1997. We visited each lake once during the period 7–14 August, selected pairs of one-flowered plants of similar size, and added supplemental pollen to the stigma of one flower per pair. We selected plants with a floral display size of one because this was the modal daily flower number (see Results).

RESULTS

Floral biology of *Rhexia virginica*—Mating system—Experimental hand pollinations conducted under glasshouse conditions demonstrated that *R. virginica* at Lake Matchedash was strongly self-compatible. All flowers that were either self- or cross-pollinated set fruit, and there was no significant difference between the seed set of flowers in these two treatments (outcross, $\bar{X} \pm 1$ SE = 140.4 ± 7.9 seeds; self = 142.4 ± 11.1 ; $t_{166} = 0.15$, NS). Neither flowers that were not experimentally hand-pollinated ($N = 51$) nor those subjected to simulated rain ($N = 31$) set fruit.

The pollen:ovule ratio of *R. virginica* flowers at Lake Matchedash was 668. Flowers produced many small pollen grains ($\bar{X} \pm$ SE = $3.05 \times 10^5 \pm 5.63 \times 10^3$ grains, $N = 154$; $\bar{X} \pm$ SE = 20.47 ± 0.09 μ m, $N = 200$) and numerous ovules ($\bar{X} \pm$ SE = 456.6 ± 7.5 ovules, $N = 87$). Comparison of the quantity of pollen produced by each of the eight anthers in a flower revealed no significant difference among them ($\bar{X} \pm$ SE = $3.2 \times 10^4 \pm 8.5 \times 10^3$ grains, $F_{7,95} = 0.65$, NS). Similarly, ovule counts did not differ among flowers from different nodes on the plant ($F_{3,83} = 1.73$, NS).

Floral color change—Floral color change within enclosures at Lake Matchedash differed little from that outside enclosures, indicating that it was not induced by visitation. Color change was most marked in the filaments, which became red and recurved on the second day. Reddening of anthers in second-day flowers was slight, but their yellow color was not apparent in a frontal view of the flower because they were hidden by the curved fila-

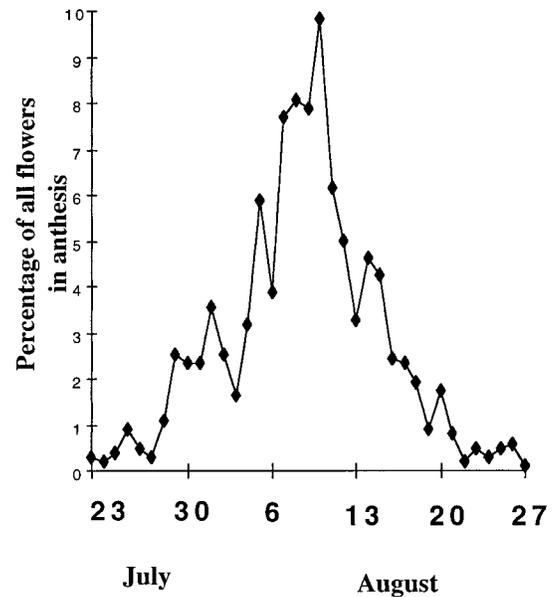


Fig. 2. Flowering phenology of *Rhexia virginica* at Lake Matchedash, Ontario, during late July and August 1996. The data are from 197 plants distributed equally among populations A, B, C, and D. Each data point shows the percentage of all flowers ($N = 1041$) open on a given day.

ments. Temperature affected the rate of visible color change; on warm days filaments were dark red and fully recurved by late afternoon, whereas during cool periods they often remained pale reddish and only slightly recurved until the next morning. The size of first- and second-day flowers was similar, but second-day petals were paler and slightly smaller than first-day petals (first-day petal length, $\bar{X} \pm$ SE = 11.65 ± 0.14 cm; second-day = 11.42 ± 0.15 cm; $N = 48$ pairs, $T_s = 182.5$, $P < 0.06$, Wilcoxon's signed-ranks test). Petals occasionally remained attached to the flower for 2 d after anthesis.

Controlled hand-pollinations at Lake Matchedash demonstrated that the female and male fertilities of second-day flowers were both low. Second-day flowers had significantly lower fruit set compared to first-day flowers whether we pollinated them with pollen from first-day (fruit set = 11.8%, $N = 17$) or second-day flowers (fruit set = 6.3%, $N = 16$) ($df = 3$, $\chi^2 = 41.84$, $P < 0.0001$, G test of independence). Fruit set was lower when we pollinated first-day stigmas 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, $\bar{X} \pm$ SE = 76.1 ± 16.0 seeds; second-day pollen = 53.4 ± 15.9 ; $t_{19} = 0.72$, NS, square-root transformed data).

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 occurred from late July to mid-August. Peak flowering was in early August, and the distribution of flowering over the season was not markedly skewed (Fig. 2).

The flowering phenology of individual plants of *R. vir-*

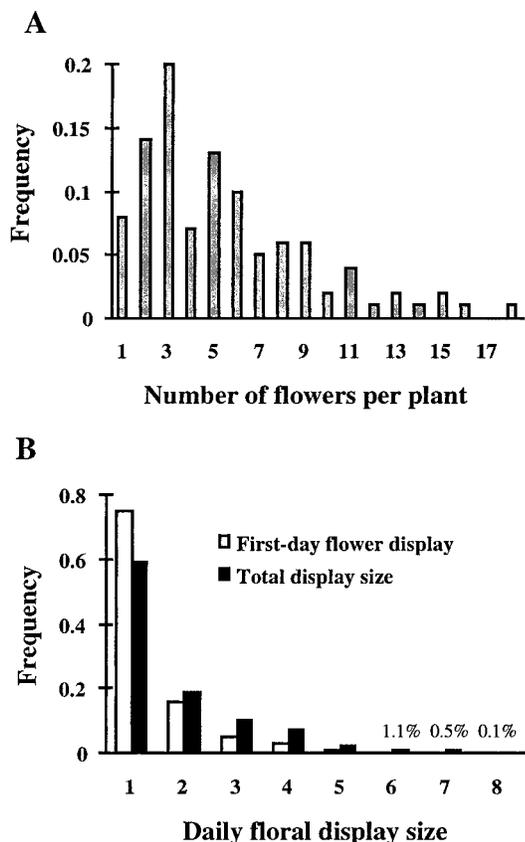


Fig. 3. Frequency distributions of the number of flowers on 197 *Rhexia virginica* plants at Lake Matchedash, Ontario in late July and August 1996. (A) Total number of flowers per plant measured over the entire flowering season (range = 1–18). (B) Size of floral display on individual plants on the days they flowered ($N = 735$), contrasting total display size, which includes second-day flowers, with the size of displays when only first-day flowers were considered.

ginica may be considered from three perspectives (Fig. 3). Over the entire flowering season an average plant had a total of five flowers (median = 5 flowers, $\bar{X} \pm \text{SE} = 5.3 \pm 0.3$, range = 1–18, $N = 197$; Fig. 3A), which bloomed over a 9-d period. On days that plants flowered they displayed a median of one first-day flower ($\bar{X} \pm \text{SE} = 1.4 \pm 0.03$ flowers, range = 1–5, $N = 735$; Fig. 3B). Second-day flowers infrequently augmented the size of displays consisting of first-day flowers: 74.6% of the daily floral displays that contained first-day flowers did not contain second-day flowers. Therefore, the median total daily display size was only slightly greater than display sizes that included first-day flowers (median = 1 flower, $\bar{X} \pm \text{SE} = 1.8 \pm 0.05$, range = 1–8; Fig. 3B). The largest daily floral display size we observed consisted of seven first- and four second-day flowers.

Pollination biology of *Rhexia virginica*—Floral visitation—Although we observed 25 species of animal visitors to *R. virginica* flowers at Lake Matchedash (Appendix), only bumble bees and small halictid bees were common. Visitation generally began soon after sunrise (~ 0630 EST) and typically declined in early afternoon. Analysis of quadrat data indicated that bumble bees

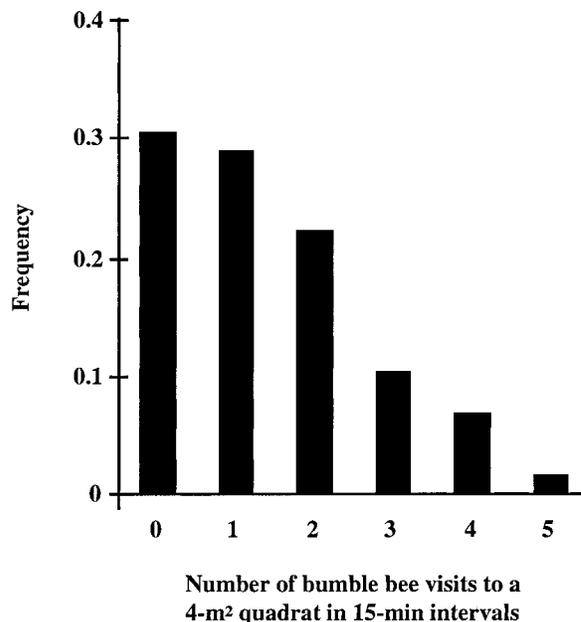


Fig. 4. The frequency of numbers of bumble bee visits to *Rhexia virginica* during 15-min observation periods of 4-m² quadrats in populations B and D at Lake Matchedash, Ontario, in August 1996.

made 82% of visits ($N = 97$) 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 >90% of recorded floral visits ($N = 65$). Visitation rates during 1996 were quite variable. The median number of visits to quadrats during 15-min observation periods was one ($\bar{X} \pm \text{SE} = 1.4 \pm 0.17$ visits, $N = 59$). The majority of the time we recorded no visits, but there were occasionally as many as five visits to quadrats (Fig. 4).

Bumble bees 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 buzz flowers effectively. A typical visit by a bee involved grasping the upper filaments with their mandibles and legs and buzzing for ~3 s ($\bar{X} \pm \text{SE} = 3.13 \pm 0.10$ s, range = 0.6–13.95 s, $N = 410$). While buzzing, they arched their body 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. Halictid bees spent lengthy periods on flowers ($\bar{X} \pm \text{SE} = 16.96 \pm 2.92$ s, range = 1–120 s, $N = 53$) and buzzed individual anthers, but they were relatively uncommon and made little contact with the stigma, so they were probably minor pollinators.

Bumble bee behavior—During observation periods at Lake Matchedash in 1996 and 1997 bumble bees occasionally approached second-day flowers, but rarely visited them. For example, on 9 and 10 August 1997, the frequency of second-day flowers in population B was 46 and 42%, respectively. Despite this, only eight (0.3%) of

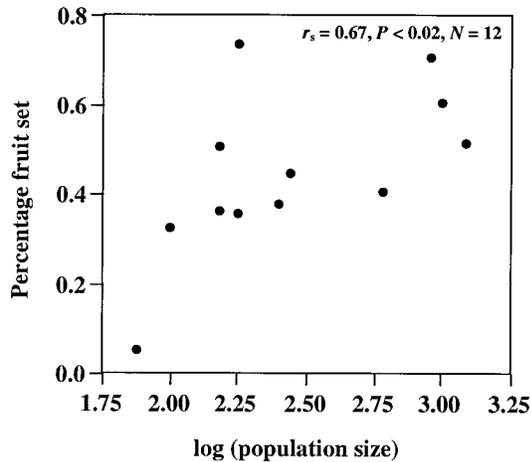


Fig. 5. The relation between percentage fruit set and log population size in 12 populations of *Rhexia virginica*. Populations were sampled in the Muskoka region of Ontario in 1997 and ranged in size from 75 to 1200 plants. The statistical association (r_s) is Spearman's rank correlation.

2341 bumble bee visits recorded on these two days were to second-day flowers.

Array experiments at Lake Matchedash in 1996 demonstrated that bumble bees were more likely to visit unvisited *R. virginica* flowers than those that had been previously visited. On both days bumble bees 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 ($G_{heterogeneity} = 4.00$, $df = 1$, $P < 0.05$).

Experiments in 1997 demonstrated that buzz pollination was not the only mechanism that released pollen grains from *R. virginica* anthers. We removed a significant proportion (49.3%) of pollen by repeated tapping of the anthers (pollen count prior to manipulation, $\bar{X} \pm SE = 2.96 \times 10^5 \pm 1.10 \times 10^4$ grains; after manipulation = $1.50 \times 10^5 \pm 5.57 \times 10^3$, $t_{22} = 12.02$, $P < 0.0001$, log-transformed data).

Observation of bumble bee foraging indicated that their behavior could result in geitonogamous pollen transfer. Bumble bees 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; $N = 367$, $\chi^2 = 10.52$, $df = 2$, $P < 0.01$, G test of independence).

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 below maximum (percentage fruit set, $\bar{X} \pm SE = 52.6 \pm 0.02\%$, $N = 530$). In 1996, fruit set among the four populations studied at Lake Matchedash was 60.6% ($N = 195$, population range = 40.4–72.6%), and among the 12 populations surveyed in the Muskoka region in 1997 it was 47.9% ($N = 335$, population range = 5.7–74.2%). Second, in both years levels of fruit set were highly variable among populations (1996: $F_{3,191} = 8.09$, $P < 0.0001$; 1997: $F_{11,323} = 10.62$, $P < 0.0001$, arcsine square-root transformed data). Lastly, in the 12 Muskoka populations in 1997, we found a significant relation between fruit set and size (Fig. 5). 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. We assessed fertility in 1996 and 1997 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 ($F_{1,199} = 19.92$, $P < 0.0001$).

Factors affecting female fertility—Logistic regression analysis indicated that population, floral display size, and flowering time explained a significant proportion of variation in fruit set among *R. virginica* plants at Lake Matchedash in 1996 (Table 1). The number of first-day flowers displayed on a plant marginally influenced fruit set, but inclusion of second-day flowers increased the explanatory power of display size. The effect of flowering time on fruit set was mainly mediated through its interaction with the population effect, which indicates that flowering time differed between populations. The fertility of flowers in midseason was higher than flowers early or late in the season ($N = 733$, $\chi^2 = 30.86$, $df = 2$, $P < 0.001$, G test of independence). The logistic model accounted for a relatively small proportion of variation in fruit set ($r^2 = 10.4\%$), indicating that additional ecological factors must also be important. The only factor that contributed significantly to the three-way ANOVA of seed set in flowers setting fruit was display size (first-day

TABLE 1. 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.

Source of variation	df	First-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 \times display size	3	5.61	0.13	3.44	0.33
Flowering time	2	5.67	0.06	5.48	0.07
Population \times flowering time	6	28.80	0.0001	28.22	0.0001
Error	717				

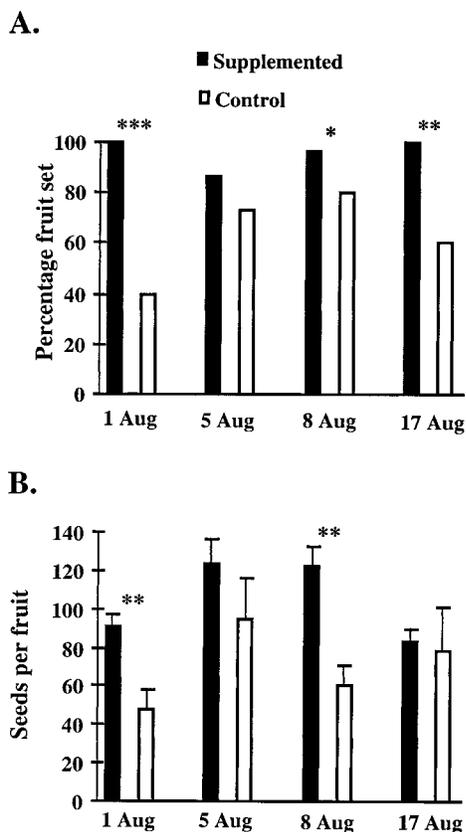


Fig. 6. Comparison of fruit and seed set from open- and supplemental cross-pollinations of *Rhexia virginica* at Lake Matchedash, Ontario, on 4 d in August 1996. (A) Percentage fruit set and (B) mean seeds per fruit (\pm SE) of supplemented and control flowers that were paired on individual plants. Sample sizes were 30 pairs on 1 and 8 August and 15 pairs on 5 and 17 August. 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.

flowers: $F_{1,431} = 5.56$, $P < 0.05$; total display: $F_{1,431} = 4.10$, $P < 0.05$, square-root transformed data).

Pollen limitation of fertility—Pollen limitation was prevalent at Lake Matchedash in 1996 (Fig. 6). 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%, $N = 90$; $\chi^2 = 37.24$, $df = 1$, $P < 0.0001$, G test of independence), as was mean number of seeds per fruit (control, $\bar{X} \pm SE = 67.76 \pm 7.58$ seeds; supplemented = 115.75 ± 6.89 seeds; $N = 55$ pairs, $T_s = 472.5$, $P_{\text{one-tailed}} < 0.001$, 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 increased fruit set at two of four populations of *R. virginica* in the Muskoka region in 1997 (Fig. 7). Overall, pollen supplementation also increased seed set per fruit ($F_{1,109} = 14.31$, $P < 0.05$, square-root transformed data), but its effect varied among lakes ($F_{3,109} = 15.01$, $P < 0.05$). Seed set per fruit did

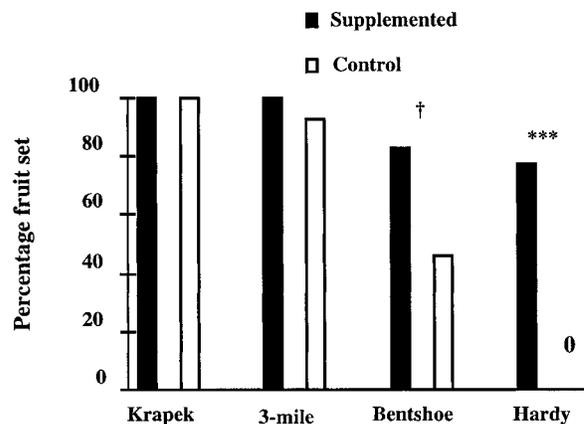


Fig. 7. Comparison of fruit set from open- and supplemental cross-pollinations of *Rhexia virginica* at four lakes in the Muskoka region of Ontario in August 1997. The percentage fruit set of supplemented and control flowers on separate plants is presented. For the four lakes, respectively, sample sizes were 15, 15, 12, and 18 plants per treatment, and there were 220, 143, 60, and 92 flowers displayed, respectively. Significant increases in fruit set with pollen supplementation are indicated by asterisks (***) $P < 0.0001$, † $P < 0.06$) and are based on G tests of independence.

not differ significantly between the two treatments at Three-mile and Bentshoe Lakes, but was significantly greater when pollen delivery was supplemented at Krapek Lake (control, $\bar{X} \pm SE = 97.87 \pm 11.31$ seeds, $N = 15$; supplemented = 138.88 ± 8.28 , $N = 15$; Wilcoxon $Z = 2.57$, $P < 0.01$). This variation in pollen limitation was not directly associated with the number of flowers displayed within populations (Fig. 7).

DISCUSSION

Our investigation of the floral and pollination biology of *R. virginica* in Ontario helps to elucidate the function of floral traits in buzz-pollinated species. We first discuss our results concerning the mating system and flowering phenology of *R. virginica*. We then consider the function of floral color change and evaluate evidence from our study concerning hypotheses for its adaptiveness. We conclude by arguing that the variety of bumble bees that visit *R. virginica* and apparently low pollen transfer efficiency suggest that buzz pollination of this species in the Muskoka region of Ontario is relatively unspecialized.

Floral biology of *Rhexia virginica*—Flowers of *R. virginica* depend on insects for pollen transfer because fruits were not set autonomously or when we subjected flowers to simulated rain. Contrary to the statement by Renner (1989), flowers were self-compatible, and the occurrence of self-pollination is suggested by the pollen: ovule ratio of *R. virginica* and observations of pollinator behavior. Bumble bees were the predominant pollinators of *R. virginica* at Lake Matchedash, and they may cause self-fertilization in two ways. They could transfer pollen between flowers on a plant (geitonogamous self-fertilization), but the relative paucity of multiple daily flowers on inflorescences at Lake Matchedash (Fig. 3B) implies that this contributes minimally to rates of selfing. A great-

er source of self-fertilization at Lake Matchedash is likely to be intrafloral facilitated self-fertilization caused during bumble bee visits. This may occur 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. Selfed seeds from populations of *R. virginica* in North Carolina did not germinate (Kral and Bostick, 1969), but this has not been examined for populations elsewhere in the range.

This study highlights the various perspectives from which floral phenology can be viewed. At a population level the flowering distribution of *R. virginica* was not markedly skewed (Fig. 2). Positively skewed flowering distributions are common and may be favored when a rapid initiation of flowering counteracts the initial hesitancy of pollinators to visit novel floral resources (Thomson, 1980). If the floral syndrome of a species is similar to species previously flowering in the community, less skewed distributions may result. This is unlikely to account for the nonskewed phenology of *R. virginica*, because its flowers were quite dissimilar to all other species found at Lake Matchedash. It is possible that its flowering distribution reflects the lag time required for bumble bees to sample it as a "minor" species and to learn to buzz pollinate, before adopting it as a "major" species (Heinrich, 1976a). This hypothesis could be explored by determining the "innateness" of buzzing by bumble bees and the amount of experience required before they efficiently acquire pollen from *R. virginica* flowers (Laverty, 1994).

The anthesis of flowers on plants of *R. virginica* was quite dispersed, with typically only one flower in anthesis during a single day (Fig. 3B). This results in an extended flowering period, with few individual flowers in anthesis simultaneously, as has been noted for buzz-pollinated species in general (Buchmann, 1983) and for *R. virginica* in particular (Leggett, 1881). The apportioning of pollen into numerous flowers functions as a pollen-packaging mechanism that may reduce the diminishing returns associated with the removal of a large proportion of a plant's pollen during single pollinator visits (Harder and Thomson, 1989). A lengthy flowering period also increases the probability that some flowers set seed, even if occasional poor weather precludes some flowers from fruiting.

Function of floral color change—As reported for other flowers that undergo color change, second-day flowers of *R. virginica* were infertile and not visited by pollinators (Weiss, 1991, 1995). One potential function of these flowers is to increase floral display size, which attracts greater numbers of pollinators to the inflorescence (reviewed in Klinkhamer and de Jong, 1990) and, hence, increases the probability of fruit set. The maintenance of second-day flowers generally increased the size of floral displays of *R. virginica* at Lake Matchedash (Fig. 3B), and their contribution to fertility was demonstrated by the greater ability of floral display size to predict fruit set in *R. virginica* when it included second-day flowers (Table

1). Nonetheless, most inflorescences at Lake Matchedash had only one daily flower and second-day flowers contributed infrequently to display size. Second-day flowers may enhance floral display size more markedly in the center of diversity for the genus *Rhexia*, in the southeastern United States, where *R. virginica* plants are larger and have more flowers (James, 1956; S. C. H. Barrett, personal observations). Adaptive explanations for floral color change and the maintenance of second-day flowers may apply more to these populations, rather than those at the edge of the range where a shortened growing season appears to limit plant size and hence flower production.

The maintenance of sterile flowers contributes to floral display size, but what is the function of concurrent color change? Without color change, visitors would likely visit both first- and second-day flowers on inflorescences. Previous studies have recognized that this may result in pollen wastage if pollen from one plant is transferred to sterile flowers on another (Gori, 1983; Cruzan, Neal, and Willson, 1988). However, these studies have not explicitly considered the wastage of pollen resulting from transfer between first- and second-day flowers *within* an inflorescence. Pollen transferred in this way is unavailable for deposition on stigmas of other plants (pollen discounting sensu Harder and Barrett, 1995). Increased visitation associated with larger displays would therefore be counterbalanced by reductions in outcrossed male fertility. More pollen discounting with larger displays seems likely in *R. virginica* because pollinators visited a larger proportion of flowers as more were displayed. With color change, however, pollinators avoid sterile flowers. Collectively, our results are consistent with the hypothesis proposed by Harder and Barrett (1996) that the adaptiveness of floral color change is that it increases insect visitation rates by contributing to daily display size, without the associated mating costs resulting from pollen discounting. This hypothesis could be tested by analyses of the costs and benefits of variation in floral display size in different parts of the range of *R. virginica*.

Floral color change modifies the cues provided by petals and anthers to foraging pollinators. Petals generally act as long-distance attractants to foraging bumble bees, but it may be the contrast between anthers and petals that determines whether a visit occurs (Lunau, 1990, 1996). Preliminary choice experiments with *R. virginica* demonstrated reduced visitation to flowers from which either petals or anthers had been removed, suggesting that visitation depends upon cues provided by both of these structures (B. M. H. Larson and S. C. H. Barrett, unpublished data). In *R. virginica*, floral color change was localized to the androecium. This is relatively uncommon among flowering plants, occurring in only 14% ($N = 77$) of families for which floral color change has been documented (Weiss, 1995). Nonetheless, in species where the sole reward is pollen, the status of anthers often provides a short-distance cue of whether flowers are rewarding (Harder and Barclay, 1994; Weiss, 1995). The yellow anthers of *R. virginica* did not appear to be deceptive to visiting bumble bees, because they were less likely to approach flowers that had previously been visited than virgin flowers (see also Zimmerman, 1982; Cresswell and Robertson, 1994). It is uncertain whether the signal to

bumble bees was the lower pollen content of the anthers, small red necrotic marks ("bee kisses") left where bees held the anthers while buzzing (e.g., Renner, 1989) or scent markings (e.g., Goulson, Hawson, and Stout, 1998).

*Is buzz pollination in *Rhexia virginica* specialized?*—Buzz pollination may be considered specialized if only one or a few bees act as pollinators. However, unlike flowers offering nectar rewards, which partition the bumble bee fauna according to proboscis length (Heinrich, 1976b; Harder, 1985), buzz-pollinated flowers offering pollen rewards could potentially be rewarding to all bumble bee species in a given region. In the case of *R. virginica* at Lake Matchedash, a variety of bumble bee species were pollinators, and there was a switch from *Bombus bimaculatus* as the predominant pollinator in 1996 to *B. impatiens* in 1997. The varied pollinators of *R. virginica* imply that there is little partitioning of the local bumble bee fauna. Generalized visitation by multiple bumble bee species has also been reported in other buzz-pollinated taxa (e.g., Knudsen and Olesen, 1993; Harder and Barclay, 1994).

Pollination systems dependent upon bumble bees are relatively specialized, because bumble bee learning capacity gives them a high capacity for floral constancy (Heinrich, 1976a). In Ontario populations, however, three observations imply that bumble bee foraging on *R. virginica* may not be particularly specialized in terms of the efficiency of pollen transfer. First, a large amount of pollen could be removed without buzzing, which contrasts with the statement by Buchmann (1983) that pollen is very difficult to remove from poricidal anthers without high-frequency vibration. This result indicates that the dispensing function of poricidal anthers (sensu Harder and Thomson, 1989) may be compromised in *R. virginica* because pollen can be removed simply by bee movement on the flowers. Second, pollen dispersed as a cloud when bumble bees buzzed, which suggests that pollen deposition on their bodies was not particularly localized. This observation supports Renner's (1989) hypothesis that deposition is generalized in most melastomes, but quantification of the distribution of pollen on bumble bee bodies would be required to explicitly test this hypothesis. In particular, it is quite unusual for pollen to accumulate on the dorsal surface of the abdomen (Buchmann, 1983; Renner, 1989), and the amount present here could be compared to that on the ventral section of the thorax, which is probably exposed to pollinator grooming, as well as to that elsewhere on the body. Lastly, Buchmann (1983) suggested that little pollen is wasted while bees buzz, but the previous observation suggests otherwise. The "efficiency" of pollen deposition could be assessed by comparisons with taxa related to *R. virginica*.

The consistent submaximal fertility of *R. virginica* in Muskoka suggests that bumble bees were unreliable pollinators. 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 adaptive explanations for low fertility in plants (reviewed in Suth-

erland, 1986), but the proximate mechanism in Ontario populations of *R. virginica* appears to be insufficient pollen transfer by pollinators (Figs. 6, 7; Larson and Barrett, in press). Pollen limitation may have been less in larger populations, accounting for the significant positive relation between total population size and the fruit set of individual plants (Fig. 5). Prevalent pollen limitation of *R. virginica* in Ontario suggests that the geographic marginality of populations may have compromised the functioning of its pollination syndrome. However, whether consistent pollen limitation has any demographic and fitness consequences for Ontario populations is not clear. Comparative investigations of the pollination ecology and demography of populations in the center of the range in the southeastern United States with those we studied in Ontario would be required to determine whether geographic marginality influences the functioning of the buzz pollination syndrome of *R. virginica*.

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APPENDIX. Visitors to *Rhexia virginica* flowers at Lake Matchedash, Ontario during 1996 and 1997. Reference specimens are located in the collection of the senior author.

INSECTS	Diptera
Hymenoptera	Syrphidae
Apidae	<i>Eupeodes americanus</i> (Wiedemann)
<i>Bombus affinis</i> Cresson	<i>Helophilus fasciatus</i> Walker
<i>Bombus bimaculatus</i> Cresson	<i>Lejops stipatus</i> Walker
<i>Bombus impatiens</i> Cresson	<i>Pipiza</i> sp.
<i>Bombus perplexus</i> Cresson	<i>Syritta pipiens</i> (L.)
<i>Bombus ternarius</i> Say	<i>Syrphus ribesii</i> (L.)
<i>Bombus terricola</i> Kirby	<i>Toxomerus geminatus</i> (Say)
<i>Bombus vagans</i> Smith	<i>Tropidia quadrata</i> Say
Anthophoridae	Lepidoptera
<i>Ceratina</i> sp.	Pieridae
Halictidae	<i>Pieris rapae</i> (L.)
<i>Augochlorella striata</i> (Provancher)	Hesperiidae
<i>Lasioglossum (Dialictus) pilosus</i>	1 species
Smith	Nymphalidae
<i>Lasioglossum</i> sp.	<i>Speyeria cybele</i> (Fabricius)
Megachilidae	BIRDS
<i>Megachile</i> sp.	Trochilidae
Vespidae	Ruby-throated hummingbird
<i>Dolichovespula arenaria</i> (Fab.)	(<i>Archilochus colubris</i>)