2005

Interspecific Competition for Pollination Lowers Seed Production and Outcrossing in Mimulus Ringens

John M. Bell
University of Wisconsin - Milwaukee

Jeffrey D. Karron
University of Wisconsin - Milwaukee

Randall J. Mitchell
University of Akron Main Campus, rjm2@uakron.edu

Ecology, 86(3), 2005, pp. 762–771

INTERSPECIFIC COMPETITION FOR POLLINATION LOWERS SEED PRODUCTION AND OUTCROSSING IN *MIMULUS RINGENS*

JOHN M. BELL,[1,3] JEFFREY D. KARRON,[1,4] AND RANDALL J. MITCHELL[2]

[1]*Department of Biological Sciences, P.O. Box 413, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201 USA*
[2]*Department of Biology, University of Akron, Akron, Ohio 44325-3908 USA*

Abstract. Sympatric plant species with similar flowering phenologies and floral morphologies may compete for pollination, and as a consequence potentially influence each other's reproductive success and mating system. Two likely competitors are *Mimulus ringens* and *Lobelia siphilitica*, which co-occur in wet meadows of central and eastern North America, produce blue zygomorphic flowers, and share several species of bumble bee pollinators. To test for effects of competition for pollination, we planted experimental arrays of *Mimulus ringens*, each consisting of genets with unique combinations of homozygous marker genotypes. In two arrays we planted mixtures of *Mimulus* and *Lobelia*, and in two additional arrays we planted *Mimulus* without a competitor for pollination. Bumble bee pollinators frequently moved between *Mimulus* and *Lobelia* flowers in the mixed-species arrays, with 42% of plant-to-plant movements being interspecific transitions. Pollinator movements between species were associated with a reduction in the amount of conspecific pollen arriving on *Mimulus* stigmas. The presence of *Lobelia* led to a significant 37% reduction in the mean number of *Mimulus* seeds per fruit. In addition, *Mimulus* had a significantly lower rate of outcrossing in the mixed-species arrays (0.43) than in the ''pure'' arrays (0.63). This is the first study to demonstrate that competition for pollination directly influences outcrossing rates. Our work suggests that in self-compatible populations with genetic load, competition for pollination may not only reduce seed quantity, but may also lower seed quality.

Key words: Bombus fervidus; competition for pollination; field experiment; improper pollen transfer; Lobelia siphilitica; *mating system;* Mimulus ringens; *outcrossing rate; seeds per fruit; seed set; pollen loss; visitation rate.*

INTRODUCTION

When two or more sympatric plant species have overlapping flowering phenologies, they may compete for pollination (Levin and Anderson 1970, Schemske et al. 1978, Waser 1978, Pleasants 1980, 1983, Campbell 1985*a*, Feinsinger et al. 1986, Caruso 1999, 2000). This competition may lower the quantity and quality of pollen deposited on conspecific stigmas (Harder and Barrett 1996, Caruso 1999, Brown et al. 2002), and may reduce reproductive success and outcrossing rates (Campbell 1985*a*, *b*).

Two potential mechanisms of competition for pollination are pollinator preference and improper pollen transfer. Competition through pollinator preference occurs when plant species B attracts pollinators away from species A, reducing the reproductive success of species A (Levin and Anderson 1970, Waser 1978, 1983, Campbell 1985*a*, Campbell and Motten 1985, Sih and Baltus 1987, Brown et al. 2002). Competition through improper pollen transfer occurs when heterospecific pollen is deposited on stigmas of one or both competitors (Rathcke 1983). Accumulation of foreign pollen on a recipient's stigma may interfere with fertilization of ovules by conspecific pollen (Waser 1978, Rathcke 1983, Brown and Mitchell 2001). Heterospecific pollen deposition may also lead to pollen loss (pollen wastage), a reduction in the amount of pollen deposited on conspecific stigmas (Waser 1983). Both of these mechanisms of competition may affect plant reproductive success and outcrossing rates by altering the amount and type of pollen arriving on stigmas.

The extent of competition for pollination largely depends upon patterns of pollinator foraging within a population (Levin 1978, Campbell 1985*a*, Campbell and Motten 1985, Feinsinger et al. 1986, Brown and Mitchell 2001, Brown et al. 2002). Improper pollen transfer is much more likely when pollinators move frequently between co-occurring species on a single foraging bout (Grant 1950, Waser 1978, 1986). Campbell (1985*a*, *b*) demonstrated that frequent pollinator movements between co-occurring species may lead to substantial pollen loss. She hypothesized that in self-compatible species, this pollen loss would reduce the amount of pollen deposited on stigmas of other conspecific individuals, leading to a lower rate of outcrossing (Campbell 1985*b*). However, the effect of

Manuscript received 22 April 2004; revised 23 August 2004; accepted 24 August 2004. Corresponding Editor: L. M. Wolfe.
[3] Present address: Applied Ecological Services, Inc., 17921 Smith Road, Brodhead, Wisconsin 53520 USA.
[4] Corresponding author. E-mail: karron@uwm.edu

competition for pollination on outcrossing rates has not yet been demonstrated empirically.

We tested Campbell's outcrossing-rate hypothesis by quantifying the effects of *Lobelia siphilitica*, a competitor for pollination, on outcrossing rates and seed production in *Mimulus ringens*. These two blue-flowered perennials broadly overlap in flowering phenology, and bumble bee pollinators move freely between them (Bell 2003), so there is strong potential for competition. A rigorous test of Campbell's hypothesis requires accounting for many other variables that can potentially influence plant mating systems, including floral morphology (Karron et al. 1997), population density (Karron et al. 1995*a*), and floral display size (Karron et al. 2004). To control these variables, we planted experimental arrays of *Mimulus ringens* with constant spacing and floral display size. In two arrays we planted *Mimulus ringens* without a competitor for pollination. In two other arrays we added a competitor for pollination by planting mixtures of *Mimulus* and *Lobelia* in close sympatry. Our study addressed the following questions: (1) How does the presence of a competitor for pollination influence patterns of pollinator movement? (2) How does competition for pollination influence patterns of pollen receipt and numbers of seeds per fruit? (3) How does competition for pollination influence outcrossing rates and pollen-mediated gene dispersal?

Methods

Study species

Mimulus ringens L. (Scrophulariaceae) is an herbaceous perennial native to wet meadows of central and eastern North America. Populations tend to be small (<50 genets), with distances between conspecifics ranging from 0.5 m to 3 m (J. M. Bell, J. D. Karron, and R. J. Mitchell, *personal observations*). Plants produce showy blue zygomorphic flowers with corolla tube length ~19 mm and corolla tube width ~5 mm. Flowering occurs from mid-July through early September in southeastern Wisconsin, USA. Flowers last for half a day, and are visited primarily by bumble bees foraging for nectar and pollen (Mitchell et al. 2004).

Mimulus ringens flowers are self-compatible and have a mixed mating system (Karron et al. 1995*a*). Nearly all flowers develop into fruits, even in the absence of pollinator visitation, due to delayed self-fertilization at the time of corolla abscission (Dole 1990, Karron et al. 2004). Outcrossing rates vary widely among fruits, both within and among genets (Karron et al. 2004). This variation may reflect the composition of the pollen load deposited on each stigma (Karron et al. 2004, Mitchell et al. 2005).

Lobelia siphilitica L. (Campanulaceae) is an herbaceous perennial that grows in moist soils along the edges of lakes, streams, mesic woodlands, and wet meadows in central and eastern North America. The azure-blue zygomorphic flowers are similar in size to *Mimulus* flowers, with typical *Lobelia* corolla tube length ~17 mm and corolla tube width ~6 mm (Caruso et al. 2003*b*). Plants flower from early August to early October in southeastern Wisconsin. Flowers last for 5–6 days, and are bumble bee pollinated (Johnston 1991).

Lobelia siphilitica is gynodioecious and the frequency of females varies considerably among populations (see references cited in Caruso et al. [2003*a*]). Some *L. siphilitica* populations lack females (Caruso et al. 2003*a*), and in our experimental arrays only hermaphroditic plants were present. The hermaphroditic flowers are strongly protandrous. On each day of our experimental study, every *Lobelia* plant had both male phase and female phase flowers present.

To assess whether these species co-occur and share pollinators in the wild, in the summer of 2002 we surveyed 23 natural populations of *Mimulus ringens* in southeastern Wisconsin. Five of these populations (21.7%) co-occurred with *Lobelia siphilitica*, and bumble bees were observed moving between the two species (Bell 2003). The *Mimulus* populations, ranging in size from 12 to more than 50 individuals, were found along the edges of wet meadows and deciduous mesic forests.

When worker bumble bees probe *Mimulus ringens* flowers, pollen is transported on the face and proboscis of the bee (Mitchell et al. 2004: cover photo). *Lobelia siphilitica* pollen is also transported on the face and proboscis, and pollen of the two species mixes at these locations on the bee (J. M. Bell, *personal observation*).

Propagation of genets with unique marker genotypes

To facilitate mating-system analysis, we utilized 16 *Mimulus ringens* genets with unique multilocus combinations of homozygous genotypes at four unlinked allozyme loci. This design enabled us to unambiguously assign paternity to all sampled seeds, and to quantify the outcrossing rate of individual plants. We used this same approach in our earlier work on selfing rates and gene dispersal in *Mimulus ringens* (Karron et al. 1995*a*, *b*, 1997, 2004).

Mimulus ringens occasionally produces vegetative offshoots in natural populations and is readily cloned in the greenhouse. In October 2000 we divided ramets from each of the 16 genets with unique combinations of genetic markers and transplanted them into 10-cm (four-inch) pots. We then stored these ramets in the dark at 4°C for seven months. In early May 2001 we moved the ramets to a cool greenhouse, where they were hardened off prior to planting in the field.

Hand-outcrossed *Lobelia siphilitica* seeds collected from 40 plants in a natural population in Cook County, Illinois, USA, were stratified and germinated in January 2001. In May 2001 we transplanted *Lobelia* seedlings to 10-cm (four-inch) pots and hardened them off in a cool greenhouse prior to planting in the field.

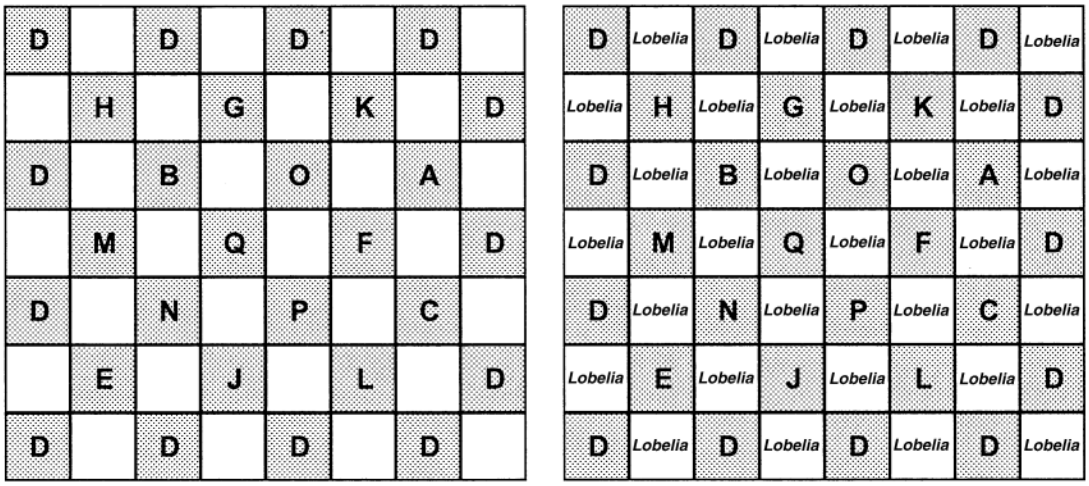

FIG. 1. Arrangement of *Mimulus ringens* genets in two of four experimental arrays: one with no competitor (left), and one with a mixture of *Mimulus* and *Lobelia siphilitica* (right). Each *Mimulus* genet is identified with a capital letter on a shaded background. Single ramets of 15 genets are surrounded by 13 ramets of border genet ''D.'' *Lobelia* plants are shown on a white background and identified with the word *Lobelia*. In both gardens there is 1.0-m spacing between *Mimulus* plants along the diagonal.

Experimental arrays

On 6 June, 2001 we planted four experimental arrays at the University of Wisconsin-Milwaukee Field Station (Saukville, Wisconsin, USA; 43°23′29.4″ N, 88°01′25.0″ W). To minimize pollen dispersal between experimental populations, the arrays were planted in isolated gardens separated by at least 75 m of vegetation containing several bumble bee-pollinated plant species. We fenced the gardens to exclude mammalian herbivores, then tilled and mulched with hay before planting to control weeds and maintain similar moisture levels. In two of the four experimental arrays (arrays 1 and 3) we planted *Mimulus* without a competitor (Fig. 1). Neighboring *Mimulus* plants were separated by 1.0 m along the diagonal. In the remaining two arrays (arrays 2 and 4) we planted a mixture of *Mimulus* and *Lobelia* in a checkerboard pattern (Fig. 1). The density of *Mimulus* in the mixed-species arrays was the same as the density in the ''pure'' arrays. We watered the well-drained gardens 2–3 times each week to ensure that these wetland plants did not experience drought stress. Because plants were watered well and post-experiment excavations revealed that root systems of neighboring *Mimulus* and *Lobelia* plants were separated by at least 25 cm, we concluded that any difference among arrays in reproductive success of *Mimulus* individuals was unlikely to be due to belowground competition.

To facilitate mating-system analysis, we planted one ramet of each of 15 *Mimulus* genets with unique multilocus genotypes in the center of each array. To minimize edge effects on pollinator visitation to these 15 ''central genets,'' we surrounded them with a buffer row consisting of 13 ramets of genet D (Fig. 1). We randomly planted the central genets in ''pure'' array 1 and used a different random order in ''pure'' array 3. We used the same spatial arrangement of *Mimulus* genets in array 1 to plant array 2 in competition with *Lobelia*. Similarly, we used the same spatial arrangement of *Mimulus* genets in array 3 to plant *Mimulus* genets in competition with *Lobelia* in array 4.

Manipulation of floral display

Because natural variation in *Mimulus* floral display strongly affects outcrossing rate (Karron et al. 2004), we trimmed all *Mimulus* in each array to eight flowers per plant during our experimental observations. We trimmed these flowers in the pre-dawn morning, before pollinators were foraging in our populations. Since *Mimulus* plants in our gardens often grew to be slightly taller than in the wild, *Mimulus* floral displays were trimmed so that displays of the two species were similar in height, as they are in natural populations.

Pollinator observations

On three consecutive fair-weather days, 12–14 August 2001, a single two-person team observed and recorded a total of 12 hours of pollinator visitation patterns in the four arrays. We initiated 20-min observation periods at sunrise, immediately following completion of floral-display manipulations. Observations continued until 11:00 hours, when pollinator visitation noticeably declined and *Mimulus* stigmas had begun to close. We rotated observation periods randomly among arrays. Each array was observed three times each day (60 min total), which was ~20% of the total time between anthesis and stigma closure.

In each observation period we followed the first bumble bee to arrive in the array for as long as possible, recording the full floral-visitation sequence. We then

quantified the sequence of floral probes for each subsequent bumble bee visitor until the end of the 20-min observation period. When two bumble bees were foraging simultaneously in an array, we did not begin following the second visitor until the first bee had departed. For each floral-visitation sequence we recorded the pollinator species and noted the spatial position and species of each plant visited. Pollinator movements between flowers were classified as follows: (a) transitions within displays of individual *Mimulus* plants; (b) transitions between *Mimulus* plants; (c) transitions from *Mimulus* to *Lobelia*; (d) transitions within displays of individual *Lobelia* plants; (e) transitions between *Lobelia* plants; (f) transitions from *Lobelia* to *Mimulus*. From these data we calculated visitation rate per flower per hour of observation by tallying the total number of floral probes to each *Mimulus* plant during each observation period (transitions (a) + (b) + (f) from the list above), and dividing by the number of open flowers per *Mimulus* plant (eight), and by the amount of time observed (0.333 h).

Analysis of pollen loads on stigmas

To quantify patterns of improper pollen transfer, we measured pollen deposition on *Mimulus* and *Lobelia* stigmas. Pollen grains of the two species can readily be distinguished with a dissecting microscope. *Mimulus ringens* pollen is small (12 μ diameter), granular, and white, whereas *Lobelia siphilitica* pollen is larger (26 μ diameter), yellow-orange, and usually found in clumps. *Mimulus ringens* stigmas are papillose and *Mimulus* pollen is often deposited in several layers, each with thousands of pollen grains (J. M. Bell, *personal observation*). Therefore, we could not accurately count numbers of conspecific and heterospecific pollen grains on stigmas. Instead, we noted the presence or absence of conspecific and heterospecific pollen, and visually estimated the proportion of stigmatic area occluded by conspecific or heterospecific pollen.

Sampling of stigmas to characterize pollen loads is destructive. Therefore, we did not sample stigmas on 12–14 August because these flowers were needed for quantifying seed number and outcrossing rate of each fruit. Instead, we sampled stigmas on 15 August, the first day following the three consecutive days of pollinator observation. Like the preceding three days, 15 August was a fair-weather day and we manipulated floral displays in the early morning. We collected eight stigmas from each of the 15 central *Mimulus* genets in both ''pure'' array 3 and mixed-species array 4. We also sampled eight stigmas from each of the 15 central *Lobelia* plants in array 4. Sampling of stigmas was performed at 11:00 hours, when most stigma lobes were still open and therefore could readily be spread apart on a wet-mount microscope slide. Although this sampling period facilitated analysis of pollen loads, it may have led to a slight underestimate of the proportion of stigmas receiving *Mimulus* pollen, since stigmas were harvested prior to delayed selfing, which typically occurs after 14:00 hours.

Seeds per fruit

To quantify seed production in the two competition treatments, we tied labeled plastic tags to pedicels of open flowers on each *Mimulus* central genet in each array. Pedicels were tagged immediately following pollinator observations on 12–14 August. We then air dried fruits for 14 d and stored them in a low-humidity chamber at 4°C. We used a dissecting microscope to count seeds in each of two randomly selected fruits from each ramet for each of the three days of pollinator observation ($N = 355$ fruits).

Genotyping progeny

To quantify selfing rates we germinated seeds from each of four fruits on each central genet ($N = 235$ fruits). Two fruits from each plant were derived from flowers open on 12 August, and two fruits were derived from flowers open on 14 August. We germinated progeny arrays in separate pots, and transplanted two-week-old seedlings into individual cells in plastic flats. After three additional weeks of growth, the seedlings were large enough for genotyping. We genotyped 10 randomly selected seedlings from each progeny array using the tissue extraction and electrophoretic methods of Karron et al. (2004). Seed germination rates were high (>85%) and seedling mortality was near zero. Also an earlier study (J. D. Karron, R. J. Mitchell, K. G. Holmquist, and J. M. Bell, *unpublished manuscript*) found no evidence for inbreeding depression at early stages of the life cycle. Therefore it is unlikely that our estimates of outcrossing rate are biased due to early mortality of inbred zygotes (Farris and Mitton 1984).

Data analysis

All analyses were conducted using SAS 8.02 (SAS Institute 2000) statistical software. We compared proportion data on pollinator species composition in the ''pure'' and mixed arrays using log-likelihood ratio χ^2 analyses.

In order to understand how patterns of pollinator movement within and among *Mimulus* genets might influence outcrossing rates in our ''pure'' and mixed-species arrays, we determined the ratio $[b/(a + b)]$, where a = transitions within displays of individual *Mimulus* genets and b = transitions between *Mimulus* genets. These proportions were calculated for the ''pure'' and mixed arrays and tested with a log-likelihood ratio χ^2 analysis.

To quantify the effects of competition treatment on pollinator visitation rate or behavior, we used a split-plot analysis (Steel and Torrie 1980), with competition treatment and day of measurement as whole-plot factors, and observation period as a sub-plot factor. Because the unit of sampling for whole-plot factors is the individual array, we tested for their significance using

a denominator mean square of array within treatment using the ''test'' statement in SAS Procedure GLM (SAS Institute 2000).

To determine whether the proportion of *Mimulus* flowers receiving conspecific pollen was influenced by the presence of *Lobelia*, we used a *t* test to compare means for the two competition treatments. It was necessary to perform a *t* test rather than a split-plot analysis because quantification of pollen loads on stigmas was very labor intensive, and we were only able to sample from two arrays on a single day. To determine whether competition treatment influenced the amount of *Mimulus* pollen received per flower we used one-way ANOVA. We arcsine transformed pollen variables to approximate a normal distribution of residuals (Zar 1999). Following arcsine transformation, these variables satisfied the assumptions of normality and homogeneity of variance.

To test the hypothesis that competition treatment affected number of seeds per fruit and outcrossing rate, we used a split-plot analysis, with competition treatment and day of measurement as whole-plot factors, and genet as a sub-plot factor. Because the unit of sampling for whole-plot factors is the individual array, we tested for their significance using a denominator mean square of array within treatment. For sub-plot factors, we took the conservative approach of testing for significance using a denominator MS of Genet × Day × Array(Treatment), which reflects variation among ramet–day combinations.

To analyze patterns of pollen-mediated gene dispersal, we excluded seeds sired by self-pollen and calculated the distance between the pollen donor and the maternal parent for each outcrossed seedling in our paternity data set. We used χ^2 analysis to test for the effect of competition treatment on pollen dispersal distance, assigning each outcrossed seedling to 2-m distance categories based on distance to the paternal plant. We combined the two longest distance categories to ensure that expected values in each cell were >5 progeny.

RESULTS

Pollinator observations

During 12 h of observation we recorded 2160 floral probes, 626 plant visits, and 67 separate bumble bee floral-visitation sequences. The predominant pollinator visiting *Mimulus* flowers in both ''pure'' and mixed-species arrays was *Bombus fervidus* Fabricius, which was responsible for 86.3% of visits to *Mimulus* plants in pure arrays, and 86.8% of visits to *Mimulus* plants in mixed-species arrays (Appendix A). The next most common visitor to *Mimulus* plants in the two types of arrays was *Bombus impatiens* Cresson, which contributed 13.3% of visits to *Mimulus* plants in pure arrays, and 10.4% of visits to *Mimulus* plants in mixed-species arrays. The species composition of pollinators visiting *Mimulus* did not differ significantly between pure and mixed-species arrays ($P > 0.7$; likelihood ratio $\chi^2_1 = 0.08$; all non-*B. fervidus* visitors were pooled to avoid expected cells <5).

Bombus fervidus also was the predominant pollinator to *Lobelia* in the mixed-species arrays, accounting for 75.5% of pollinator visits to *Lobelia* plants. *Lobelia* received a slightly higher proportion of visits by *B. impatiens* (16.6%) and *B. vagans* Smith (8.0%) than did *Mimulus* in the mixed-species arrays (*B. impatiens* = 10.4%; *B. vagans* = 2.8%). These differences in composition of bee species visiting *Mimulus* and *Lobelia* in the mixed-species arrays were significant ($P < 0.003$, likelihood ratio $\chi^2_1 = 9.0$, again pooling non-*B. fervidus* visitors) (Appendix A).

Bee activity in the pure arrays was about half that in the mixed-species arrays. In the pure arrays we observed 25 bumble bee floral-visitation sequences with 783 floral probes, while in the mixed-species arrays we observed a total of 42 visitation sequences with 1377 floral probes to the two species. The higher total visits in the mixed-species arrays was largely due to the presence of *Lobelia*, which received 814 of the 1377 floral probes. The combined number of *Mimulus* and *Lobelia* flowers probed by a bee during a floral-visitation sequence was similar in the two competition treatments (31.3 ± 6.4 flowers [mean ± 1 SE] in the pure arrays, 32.8 ± 5.2 flowers in the mixed-species arrays).

The visitation rate to *Mimulus* flowers tended to be higher in the pure arrays (0.65 ± 0.04 probes per flower per hour) than in the mixed-species arrays (0.47 ± 0.04 probes per flower per hour). However, because of substantial variation among arrays, statistical power for among-plot factors was low (power = 0.05), and the split-plot ANOVA (Appendix B) showed no significance for this 28% difference between competition treatments ($P > 0.3$). Probes to *Mimulus* flowers that were immediately preceded by probes to *Lobelia* flowers may be ineffective in transferring *Mimulus* pollen. Therefore, we also calculated a rate of ''effective'' visitation to *Mimulus* flowers in the mixed-species arrays, which excludes *Mimulus* probes that were immediately preceded by a probe to a *Lobelia* flower. The rate of effective visitation to *Mimulus* flowers in the mixed-species arrays was 0.40 ± 0.04 probes per flower per hour. This rate is slightly lower than the overall visitation rate to *Mimulus* in the mixed-species arrays (0.47 ± 0.04 probes, reported above), but patterns of significance were unchanged (unpublished analysis).

In the mixed-species arrays, bees frequently moved between species during individual visitation sequences. Nearly half (42%) of the 321 plant-to-plant movements were interspecific transitions (either *Mimulus* to *Lobelia* or *Lobelia* to *Mimulus*), while 10.1% of 1335 movements between flowers were interspecific.

The ratio of transitions between *Mimulus* genets divided by all intraspecific *Mimulus* transitions was higher in the pure arrays (0.314) than in the mixed-species

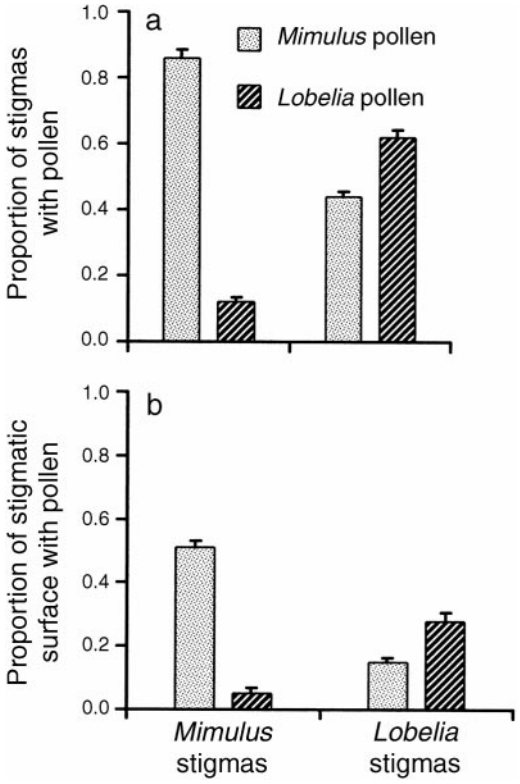

FIG. 2. Conspecific and heterospecific pollen loads on *Mimulus ringens* and *Lobelia siphilitica* stigmas in the mixed-species arrays: (a) the proportion of *Mimulus* and *Lobelia* stigmas with conspecific or heterospecific pollen present; (b) the proportion of stigmatic surface occluded by conspecific and heterospecific pollen. Data are means and 1 SE; $N = 120$–127 stigmas for each bar.

arrays (0.227) (likelihood ratio $\chi^2_2 = 10.7$, $P = 0.005$). Such among-plant movements may promote outcross pollen transfer. Bees did not change the number of flowers they probed consecutively on *Mimulus* plants in response to competition treatment (in the pure arrays, 2.80 ± 0.19 flowers [mean ± 1 SE], in the mixed-species arrays, 2.68 ± 0.16 flowers; $P > 0.6$ [from split-plot ANOVA]; Appendix C). This suggests that geitonogamy on *Mimulus* plants is similar in the two competition treatments.

Pollen load analysis

The proportion of *Mimulus* flowers receiving conspecific pollen prior to 11:00 hours was significantly lower in the mixed-species arrays (85.8%) than in the pure arrays (95.9%) ($t = 2.75$, $P = 0.007$). In addition, the proportion of *Mimulus* stigmatic area occluded with conspecific pollen was significantly lower in the mixed-species arrays (0.51 ± 0.01) than in the pure arrays (0.62 ± 0.02) ($F_{1,1} = 6.43$, $P = 0.012$).

The presence of *Lobelia* did not lead to much heterospecific pollen deposition on *Mimulus* stigmas. Only 12% of *Mimulus* stigmas in the mixed-species arrays had detectable *Lobelia* pollen present (Fig. 2a). By contrast, 44% of *Lobelia* stigmas received *Mimulus* pollen (Fig. 2a). In addition, the proportion of *Mimulus* stigmatic area occluded with *Lobelia* pollen was lower (0.05 ± 0.01) than the proportion of *Lobelia* stigmatic area (0.15 ± 0.019) occluded with *Mimulus* pollen (Fig. 2b).

Seeds per fruit

The presence of *Lobelia* led to a significant 37% reduction in the mean number of *Mimulus* seeds per fruit (ANOVA, $F_{1,2} = 114.76$, $P < 0.009$, Table 1, Fig. 3). Genets also differed significantly in seeds per fruit, and these differences were reasonably consistent across

TABLE 1. Results of split-plot ANOVA of the effects of competition treatment (abbreviated "Trt"), day, and genet on the number of seeds per fruit.

Source of variation	df	MS	*F*	*P*
Whole-plot				
Treatment	1	4.61×10^7	**114.76**	**0.009**
Day	2	3.48×10^6	8.68	0.10
Trt × Day	2	5.95×10^5	1.48	0.4
Array(Trt)	2	4.02×10^5		
Sub-plot				
Genet	14	1.43×10^6	**2.78**	**0.004**
Trt × Genet	14	8.10×10^5	1.57	0.12
Day × Array(Trt)	4	1.95×10^6	**3.78**	**0.009**
Genet × Array(Trt)	28	5.07×10^5	0.98	0.5
Genet × Day	28	3.28×10^5	0.64	0.9
Trt × Genet × Day	28	4.07×10^5	0.79	0.7
Genet × Day × Array(Trt)	54	5.16×10^5	**1.64**	**0.008**
Residual error	177	3.14×10^5		

Notes: Competition treatment and day were applied to whole plots (arrays), so their effects were tested over Array(Trt), which is among-array error. The subplot effect was Genet, which was conservatively tested over Genet × Day × Array(Trt). The variation among ramet–day combinations [Genet × Day × Array(Trt)] was tested over the variation among individual fruits. $N = 355$ fruits. Model $R^2 = 0.739$. Significant values are in boldface type.

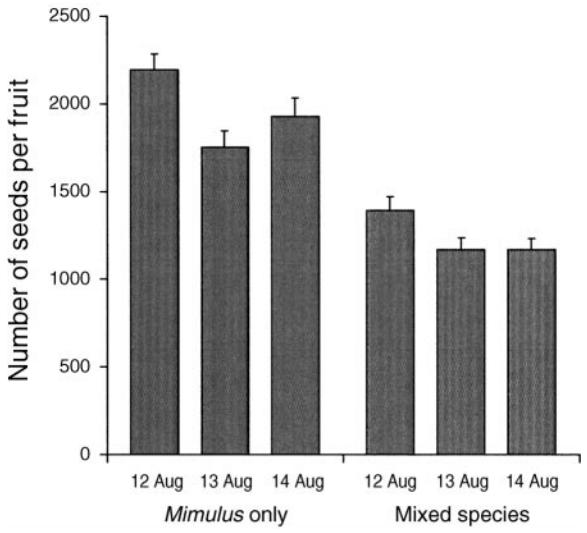

FIG. 3. *Mimulus ringens* seeds per fruit in the presence (mixed species) and absence (*Mimulus* only) of *Lobelia siphilitica*, a competitor for pollination. Seed number was determined by counting seeds from two randomly selected fruits from each of the 15 central genets in each array (Fig. 1). Separate means are reported for each day of pollinator observation. Data are means and 1 SE; $N = 56$–71 fruits for each bar.

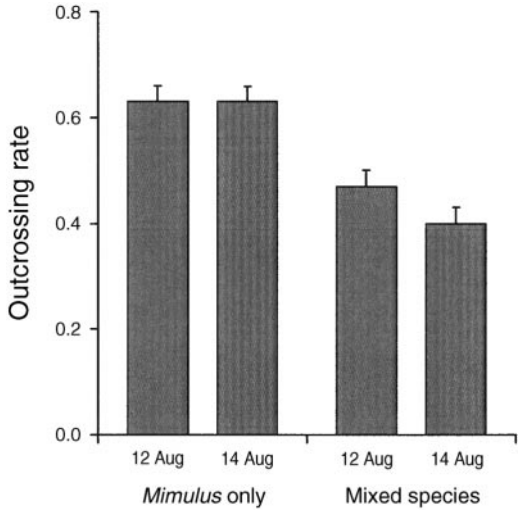

FIG. 4. *Mimulus ringens* outcrossing rate (proportion of seeds that are outcrossed) in the presence and absence of *Lobelia siphilitica*, a competitor for pollination. Outcrossing rates for *Mimulus* were determined by genotyping 10 offspring from each of two randomly selected fruits from each of the 15 central genets in each array. Data are means and 1 SE; $N = 58$–59 fruits for each bar. The total number of seedlings genotyped was 2344 seedlings from 238 fruits.

competition treatments (interaction $P > 0.12$). The other significant interactions concern sub-plot factors, and do not alter conclusions about the whole-plot effects.

Outcrossing rates

The rate of outcrossing (proportion of seeds that are outcrossed) in *Mimulus ringens* was significantly lower in the mixed-species arrays (0.43 ± 0.02) than in the pure arrays (0.63 ± 0.02) (mean $\pm$ 1SE) (Table 2, Fig. 4). This effect was consistent across days and across genets. No other main effects were significant, and most interactions were weak. The only significant interaction indicates that individual ramets responded differently to competition treatment on different days. Outcrossing rates were not correlated with seeds per fruit in either treatment ($P > 0.8$ in both cases, $N > 116$). Additionally, patterns of pollen-mediated gene dispersal did not vary significantly across competition treatments ($\chi^2_4 = 6.9$, $P = 0.14$); both had similar, strongly leptokurtic distributions.

DISCUSSION

Competition for pollination with *Lobelia siphilitica* dramatically lowered both the number of seeds per fruit and the rate of outcrossing in *Mimulus ringens*. To our

TABLE 2. Results of split-plot ANOVA of the effects of competition treatment, day, and genet on outcrossing rate.

Source	df	MS	*F*	*P*
Whole-plot				
Treatment	1	2.1432	**148.36**	**0.0067**
Day	2	0.0772	5.35	0.15
Trt × Day	2	0.1228	8.50	0.10
Array(Trt)	2	0.0144		
Sub-plot				
Genet	14	0.0636	0.64	0.8
Trt × Genet	14	0.0314	0.32	0.9
Day × Array(Trt)	4	0.0619	0.63	0.5
Genet × Array(Trt)	28	0.0783	0.79	0.7
Genet × Day	28	0.0458	0.46	0.9
Trt × Genet × Day	28	0.0901	0.91	0.6
Genet × Day × Array(Trt)	54	0.0988	**2.40**	**0.0008**
Residual error	177	0.0412		

Notes: Denominator MS are as in Table 1. $N = 2344$ seeds, 235 fruits. Model $R^2 = 0.690$. Significant values are in boldface type.

knowledge, this is the first study to empirically demonstrate that pollinator sharing directly influences outcrossing rates, as predicted by Campbell (1985*b*). This result adds competition for pollination to a growing list of ecological and demographic variables known to influence outcrossing rates, including population density, population size, floral-display size, weather conditions, and availability of pollinators (Barrett and Eckert 1990, de Jong et al. 1992, Jarne and Charlesworth 1993, Williams et al. 2001, Karron et al. 2004). In our study, the effect of competition for pollination was striking. The 20-percentage-point reduction in outcrossing rate is comparable to the effects of other ecological factors known to influence outcrossing in *Mimulus*. For example, Karron et al. (1995*a*) noted that a 16-fold decrease in population density led to a 16-percentage-point reduction in outcrossing rate. Also, Karron et al. (2004) found that outcrossing rates of plants with large daily floral displays (16 open flowers) were 14 percentage points lower than outcrossing rates of plants with small daily floral displays (two open flowers).

Our findings have important implications for fitness in mixed-species populations because reproductive success depends upon both the quality and quantity of offspring produced. The *Mimulus* genets used in this study are derived from a population that exhibits a moderate level of inbreeding depression (mean fitness of self progeny is 21% lower than mean fitness of outcross progeny (J. D. Karron, *unpublished data*)). To quantify the overall effects of competition for pollination on fitness through seed function, we used these data on inbreeding depression to weight the relative fitness of self and outcross progeny in our two competition treatments. These calculations indicate that, on average, competition with *Lobelia siphilitica* resulted in a 39.4% reduction in maternal fitness for *Mimulus ringens*. The effects of competition on both seed set and outcrossing rate may be different in natural communities, where competitors may be clumped or may occur at a range of densities (Caruso 1999, Fishman and Wyatt 1999). Separate clusters or patches of conspecifics may reduce the proportion of interspecific transitions by pollinators, and may lower the amount of improper pollen transfer (Campbell 1985*b*, Campbell and Motten 1985). Alternatively, the presence of several co-occurring species competing for pollination could lead to a greater reduction in fitness than we observed in this study. Additional research is needed to quantify the effects of competition for pollination on offspring quality in natural populations.

Competition mechanisms influencing seed set

Our study was designed primarily to examine the consequences of competition for pollination on seed set and outcrossing rates in *Mimulus ringens*. However, our results also provide some indications of the mechanisms responsible for the significant responses we found. Lower seed set in mixed-species arrays is sometimes attributed to a reduced rate of pollinator visitation (Waser 1978, 1983, Brown and Mitchell 2002). In our study, the frequency of ''effective'' pollinator visitation to *Mimulus* flowers was 38% lower in the mixed- species arrays than in the ''pure'' arrays. This result was not significant, but we had low statistical power for detecting differences in visitation rate. If such differences in visitation indeed exist, they may partially explain the significant reduction in amount of conspecific pollen deposited on *Mimulus* stigmas in the mixed-species arrays.

Reduced seed set in species mixtures may also result from improper pollen transfer and pollen loss (Waser 1978, Campbell and Motten 1985, Galen and Gregory 1989). Frequent pollinator moves from *Mimulus* to *Lobelia* led to considerable deposition of *Mimulus* pollen on *Lobelia* stigmas, and probably also to loss on other floral surfaces. These factors may have contributed to the smaller loads of conspecific pollen on *Mimulus* stigmas in the mixed-species arrays.

Pollinator moves from *Lobelia* to *Mimulus* may also cause improper pollen transfer through deposition of *Lobelia* pollen on *Mimulus* stigmas. However, only 12% of *Mimulus* stigmas received any *Lobelia* pollen (Fig. 2b). Therefore, heterospecific pollen deposition on *Mimulus* stigmas was probably not a major factor influencing the number of ovules fertilized per fruit. Multiple factors may have limited the amount of *Lobelia* pollen deposited on *Mimulus* stigmas. For example, *Lobelia* anthers usually do not dehisce until late morning, after many *Mimulus* stigmas have already closed. In addition, *Lobelia* pollen grains are much larger than *Mimulus* grains, and do not adhere well to the small papillae on *Mimulus* stigmas.

Effect of pollen loss on outcrossing

The lower rate of outcrossing in the mixed-species arrays (Fig. 4, Table 2) is most likely caused by the loss of *Mimulus* pollen to *Lobelia*. Such heterospecific pollen loss reduces the amount of bee-transported *Mimulus* pollen that could potentially be deposited on flowers of other conspecifics (outcrossing). The presence of *Lobelia* is unlikely to have had much influence on the extent of *Mimulus* self-pollination. In particular, geitonogamous self-pollination is likely to have been similar in the two competition treatments, since there was no significant difference in the distribution of numbers of consecutively probed flowers on *Mimulus* plants in the ''pure'' and mixed-species arrays. Also, the presence of a competitor for pollination may have little effect on autogamous (within-flower) self-pollination. Three of the modes of autogamy (prior, competing, and delayed self-pollination) occur without involvement of a pollinator (Lloyd and Schoen 1992). The fourth mode, facilitated selfing, may be modest in *Mimulus* due to the position of the stigma above the anthers (LeClerc-Potvin and Ritland 1994). If the number of outcross pollen grains on *Mimulus* stigmas declines,

but the number of self pollen grains on stigmas remains unchanged, the outcrossing rate would be lower in the mixed-species arrays. Note that there is some similarity between the effects of heterospecific pollen loss on outcrossing rates and the effects of pollen discounting on outcrossing rates (Holsinger 1996). In both processes a reduced amount of pollen transfer to conspecifics leads to a lower rate of outcrossing.

Pollen-mediated gene dispersal

Campbell (1985*b*) suggested that competition for pollination would not only influence outcrossing rates, but might also influence the distance pollen disperses between conspecific plants. She empirically confirmed this prediction by finding that the presence of *Claytonia virginica* decreased the distance of dye dispersal in *Stellaria pubera* (Campbell 1985*b*). We found no evidence for an effect of competition treatment on realized pollen-mediated gene dispersal. It is possible that a different result would be obtained in larger arrays or large natural populations, which would be less subject to edge effects, and might permit detection of longer distance gene dispersal.

Conclusion

When *Mimulus ringens* and *Lobelia siphilitica* grow in close sympatry, they share bumble bee pollinators and strongly compete for pollination. The outcrossing rate of *Mimulus* in mixed-species arrays was 20 percentage points lower than the outcrossing rate in pure *Mimulus*. arrays. Also, seed set in *Mimulus* fruits was 37% lower in the presence of *Lobelia* than in *Mimulus*-only arrays. Therefore, competition influences both seed quality and seed quantity, resulting in a dramatic reduction in reproductive success. In the present study the effect of competition for pollination on seed quantity was stronger than the effect on seed quality, largely because the experimental population had a fairly low level of genetic load. However, our results suggest that studies of competition for pollination in self-compatible species should consider effects on seed quality as well as seed quantity. It is possible that in a species with substantial delayed self-fertilization and fairly high levels of genetic load, competition for pollination might have little effect on seed quantity, but might dramatically affect seed quality. Further research is needed to determine whether competition for pollination influences outcrossing rates of other flowering plant species.

Acknowledgments

We thank T. Schuck for assistance in the greenhouse and K. Havens for providing seeds of *Lobelia siphilitica*. We are grateful to T. Bell, B. Funk, R. Claire, J. Reinartz, G. Meyer, and L. Nelson for help and encouragement in the field. We thank N. Waser, S. Schnitzer, J. Reinartz, G. Meyer, P. Dunn, and T. Ehlinger for helpful comments on an earlier version of the manuscript. A Botanical Society of America Karling Research Award to J. M. Bell and National Science Foundation grants DEB 9816712 to J. D. Karron and DEB 9903308 to R. J. Mitchell supported this research. This paper is derived from a Ph.D. dissertation submitted by J. M. Bell to University of Wisconsin—Milwaukee.

Literature Cited

Barrett, S. C. H., and C. G. Eckert. 1990. Variation and evolution of mating systems in seed plants. Pages 229–554 *in* S. Kawano, editor. Biological approaches and evolutionary trends in plants. Academic Press, London, UK.

Bell, J. M. 2003. The effect of interspecific competition for pollinator service on seed production and outcrossing rates in *Mimulus ringens* (Scrophulariaceae). Dissertation. University of Wisconsin-Milwaukee, Milwaukee, Wisconsin, USA.

Brown, B. J., and R. J. Mitchell. 2001. Competition for pollination: effects of pollen of an invasive plant on seed set of a native congener. Oecologia **129**:43–49.

Brown, B. J., R. J. Mitchell, and S. A. Graham. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. Ecology **83**:2328–2336.

Campbell, D. R. 1985*a*. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. Ecology **66**: 544–553.

Campbell, D. R. 1985*b*. Pollen and gene dispersal: the influences of competition for pollination. Evolution **39**:418–431.

Campbell, D. R., and A. F. Motten. 1985. The mechanism of competition for pollination between two forest herbs. Ecology **66**:554–563.

Caruso, C. M. 1999. Pollination of *Ipomopsis aggregata* (Polemoniaceae): effects of intra- vs. interspecific competition. American Journal of Botany **86**:663–668.

Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. Evolution **54**:1546–1557.

Caruso, C. M., H. Maherali, and R. B. Jackson. 2003*a*. Gender-specific floral and physiological traits: implications for the maintenance of females in gynodioecious *Lobelia siphilitica*. Oecologia **135**:524–531.

Caruso, C. M., S. B. Peterson, and C. E. Ridley. 2003*b*. Natural selection on floral traits of *Lobelia* (Lobeliaceae). American Journal of Botany **90**:1333–1340.

deJong, T., N. M. Waser, M. V. Price, and R. M. Ring. 1992. Plant size, geitonogamy, and seed set in *Ipomopsis aggregata*. Oecologia **89**:310–315.

Dole, J. A. 1990. Role of corolla abscission in delayed self-pollination of *Mimulus guttatus* (Scrophulariaceae). American Journal of Botany **77**:1505–1507.

Farris, M. A., and J. B. Mitton. 1984. Population density, outcrossing rate, and heterozygote superiority in ponderosa pine. Evolution **38**:1151–1154.

Feinsinger, P., K. G. Murray, S. Kinsman, and W. H. Busby. 1986. Floral neighborhood and pollination success in four hummingbird-pollinated cloud forest plant species. Ecology **67**:449–464.

Fishman, L., and R. Wyatt. 1999. Pollinator-mediated competition, reproductive character displacement, and the evolution of selfing in *Arenaria uniflora* (Caryophyllaceae). Evolution **53**:1723–1733.

Galen, C., and T. Gregory. 1989. Interspecific pollen transfer as a mechanism of competition: consequences of foreign pollen contamination for seed set in the alpine Wildflower, *Polemonium viscosum*. Oecologia **81**:120–123.

Grant, V. 1950. The flower constancy of bees. Botanical Review **16**:379–398.

Harder, L. D., and S. C. H. Barrett. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. Pages 140–190 *in* D. G. Lloyd and S. C. H. Barrett, editors. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York, New York, USA.

Holsinger, K. E. 1996. Pollination biology and the evolution of mating systems in flowering plants. Evolutionary Biology **29**:107–149.

Jarne, P., and D. Charlesworth. 1993. The evolution of the selfing rate in functionally hermaphrodite plants and animals. Annual Review of Ecology and Systematics **24**:441–466.

Johnston, M. O. 1991. Natural selection on floral traits in two species of *Lobelia* with different pollinators. Evolution **45**:1468–1479.

Karron, J. D., R. T. Jackson, N. N. Thumser, and S. L. Schlicht. 1997. Outcrossing rates of individual *Mimulus ringens* genets are correlated with anther–stigma separation. Heredity **79**:365–370.

Karron, J. D., R. J. Mitchell, K. G. Holmquist, J. M. Bell, and B. Funk. 2004. The influence of floral display size on selfing rates in *Mimulus ringens*. Heredity **92**:242–248.

Karron, J. D., N. N. Thumser, R. Tucker, and A. J. Hessenauer. 1995*a*. The influence of population density on outcrossing rates in *Mimulus ringens*. Heredity **75**:175–180.

Karron, J. D., R. Tucker, N. N. Thumser, and J. A. Reinartz. 1995*b*. Comparison of pollinator flight movements and gene dispersal patterns in *Mimulus ringens*. Heredity **75**: 612–617.

Leclerc-Potvin, C., and K. Ritland. 1994. Modes of self-fertilization in *Mimulus guttatus* (Scrophulariaceae): a field experiment. American Journal of Botany **81**:199–205.

Levin, D. A. 1978. Pollinator behaviour and the breeding structure of plant populations. Pages 133–150 *in* A. J. Richards, editor. The pollination of flowers by insects. Academic Press, London, UK.

Levin, D. A., and W. W. Anderson. 1970. Competition for pollinators between simultaneously flowering species. American Naturalist **104**:455–467.

Lloyd, D. G., and D. J. Schoen. 1992. Self- and cross-fertilization in plants I. Functional dimensions. International Journal of Plant Science **153**:358–369.

Mitchell, R. J., J. D. Karron, K. G. Holmquist, and J. M. Bell. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. Functional Ecology **18**:116–124.

Mitchell, R. J., J. D. Karron, K. G. Holmquist, and J. M. Bell. 2005. Patterns of multiple paternity in fruits of *Mimulus ringens* (Phrymaceae). American Journal of Botany **92**(5), *in press*.

Pleasants, J. M. 1980. Competition for bumblebee pollinators in Rocky Mountain plant communities. Ecology **61**:1446–1459.

Pleasants, J. M. 1983. Structure of plant and pollinator communities. Pages 375–393 *in* C. E. Jones and R. J. Little, editors. Handbook of experimental pollination ecology. Van Nostrand Reinhold, New York, New York, USA.

Rathcke, B. 1983. Competition and facilitation among plants for pollination. Pages 305–329 *in* L. Real, editor. Pollination biology. Academic Press, Orlando, Florida, USA.

SAS Institute. 2000. SAS/STAT User's Guide, Version 8. SAS Institute, Cary, North Carolina, USA.

Schemske, D. W., M. F. Willson, M. N. Melampy, L. J. Miller, L. Verner, K. M. Schemske, and L. B. Best. 1978. Flowering ecology of some spring woodland herbs. Ecology **59**: 351–366.

Sih, A., and M. Baltus. 1987. Patch size, pollinator behavior, and pollinator limitation in catnip. Ecology **68**:1679–1690.

Steel, R. G. D., and J. H. Torrie. 1980. Principles and procedures of statistics: a biometrical approach. Second edition. McGraw-Hill Book Company, New York, New York, USA.

Waser, N. M. 1978. Competition for hummingbird pollination and sequential flowering in two Colorado wildflowers. Ecology **59**:934–944.

Waser, N. M. 1983. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. Pages 277–293 *in* C. E. Jones and R. J. Little, editors. Handbook of experimental pollination biology. Van Nostrand Reinhold New York, New York, USA.

Waser, N. M. 1986. Flower constancy: definition, cause, and measurement. American Naturalist **127**:593–603.

Williams, C. F., J. Ruvinsky, P. E. Scott, and D. K. Hews. 2001. Pollination, breeding system, and genetic structure in two sympatric *Delphinium* (Ranunculaceae) species. American Journal of Botany **88**:1623–1633.

Zar, J. H. 1999. Biostatistical analysis. Fourth edition. Prentice-Hall, Upper Saddle River, New Jersey, USA.

APPENDIX A

A figure showing species composition of bumble bee visitors to *Mimulus ringens* and *Lobelia siphilitica* in the experimental arrays is available in ESA's Electronic Data Archive: *Ecological Archives* E086-039-A1.

APPENDIX B

A table showing results of a split-plot ANOVA for pollinator visitation as a function of Treatment, Day, and Array(Trt) is available in ESA's Electronic Data Archive: *Ecological Archives* E086-039-A2.

APPENDIX C

A figure showing the number of consecutively probed flowers on individual *Mimulus* plants in the pure-species and mixed-species arrays is available in ESA's Electronic Data Archive: *Ecological Archives* E086-039-A3.