



Effects of multiple competitors for pollination on bumblebee foraging patterns and *Mimulus ringens* reproductive success

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When co-occurring plant species overlap in flowering phenology they may compete for the service of shared pollinators. Competition for pollination may lower plant reproductive success by reducing the number of pollinator probes or by decreasing the quality of pollen transport to or from a focal species. Pair-wise interactions between plants sharing pollinators have been well documented. However, relatively few studies have examined interactions for pollination among three or more plant species, and little is known about how the outcomes and mechanisms of competition for pollination may vary with competitor species composition. To better understand how the dynamics of competition for pollination may be influenced by changes in the number of competitors, we manipulated the presence of two competitors, *Lythrum salicaria* and *Lobelia siphilitica*, and quantified reproductive success for a third species, *Mimulus ringens*. Patterns of pollinator preference and interspecific transitions in mixed-species arrays were significantly influenced by the species composition of competitor plants present. Both pair-wise and three-species competition treatments led to a similar ~ 40% reduction in *Mimulus ringens* seed set. However, the patterns of pollinator foraging we observed suggest that the relative importance of different mechanisms of competition for pollination may vary with the identity and number of competitors present. This variation in mechanisms of competition for pollination may be especially important in diverse plant communities where many species interact through shared pollinators.

In many flowering plant communities multiple species bloom simultaneously and compete for the service of shared pollinators (Mitchell et al. 2009). The consequences of competition for pollination have been well documented for species pairs (Waser 1978, Caruso 2000, Brown et al. 2002, Montgomery 2009, Takakura et al. 2009), but have received much less attention in more diverse plant communities (but see Ghazoul 2006, Lopezaria-Mikel et al. 2007, Lazaro et al. 2009). Several studies of interspecific competition for abiotic resources have demonstrated that competitive outcomes may not be a simple additive function of pair-wise interactions (Miller 1994, Dormann and Roxburgh 2005, Weigelt et al. 2007, Perkins et al. 2007). Differences between pair-wise and multispecies interactions may also occur in pollination systems, but this topic has received little experimental study (Mitchell et al. 2009).

Competitors for pollination may lower the reproductive success of a focal species via two nonexclusive mechanisms. First, if pollinators are drawn to more attractive competitors, the number of pollinator probes to a less attractive focal species may be reduced (Levin and Anderson 1970, Campbell 1985, Rathcke 1988, Bell et al. 2005). Second, if pollinators move between species, heterospecific pollen may be deposited onto a focal species' stigma or pollen of a focal species may be lost during probes to competitors (Waser 1983a, 1983b, Campbell 1985, Waser and Fugate 1986,

Murcia and Feinsinger 1996, Brown and Mitchell 2001, Mitchell et al. 2009). Competition for pollination may be more severe when there is a greater diversity of competitors due to an increase in the number of competitive mechanisms acting on a focal species (Vamosi et al. 2005, Mitchell et al. 2009). For example, a very attractive competitor species may draw pollinators away from a focal species, while a second competitor simultaneously competes with that focal species through interspecific pollen transfer. The combined influence of reduced visitation and increased interspecific pollen transfer may lead to strong reductions in plant reproductive success. Alternatively, an increased number of co-flowering species may instead facilitate the pollination of a focal species, offsetting any competitive effects. A more diverse plant community may attract more pollinators and/or a greater diversity of pollinators, to the benefit of the entire community (Feinsinger 1978, Rathcke 1983, Feldman et al. 2004, Ghazoul 2006, Lazaro et al. 2009).

Interactions for pollination may also be driven by a single, highly attractive "cornucopian species" (Mosquin 1971) that flowers abundantly and offers such a large supply of floral rewards relative to co-occurring plants that it can reduce the pollination success of other species regardless of the community context. Highly attractive invasive plants may be particularly likely to fall into this category (Mitchell et al. 2009). For example, showy invasive plants may strongly

influence plant–pollinator networks in multi-species plant communities (Morales and Aizen 2006, Lopezaria-Mikel et al. 2007, Bartomeus et al. 2008) and may compete strongly for pollination with native species (Brown and Mitchell 2001, Bjercknes et al. 2007, Kandori et al. 2009).

The extent of competition for pollination among multiple species may also depend upon two aspects of pollinator foraging behavior: pollinator preference and flower constancy (Waser 1986, Husband and Barrett 1992, Gegeer and Thomson 2004). Pollinator preference is the over-visitation of a plant species relative to its availability (Waser 1986, Aldridge and Campbell 2007), leading to reduced visitation to a less preferred competitor. Flower constancy refers to the tendency of pollinators to probe the same flower type or species consecutively and by-pass other equally rewarding types (Waser 1986, Chittka et al. 1999, Gegeer and Laverty 2005). Both of these behaviors may influence the amount and quality of pollen deposited onto conspecific stigmas (Waser 1986, Husband and Barrett 1992), and therefore may influence a focal species' reproductive success.

To understand how the dynamics of competition for pollination may be influenced by changes in the number of competitors, we manipulated the presence of two competitors, *Lythrum salicaria* and *Lobelia siphilitica*, and quantified reproductive success for a third species, *Mimulus ringens*. We first explored these competitive interactions in a pair-wise fashion. We then examined whether competitive interactions and outcomes were modified when all three species were present in mixed species arrays. We address the following questions: (1) are patterns of pollinator preference and constancy influenced by changes in the identity and number of competitors? (2) Is the number of seeds per *M. ringens* fruit influenced by changes in the identity and number of competitors?

Material and methods

Study species

Mimulus ringens (Phrymaceae) is a perennial herb native to central and eastern North America (Karron et al. 1995a). Individual plants produce displays with 1–22 showy, blue, zygomorphic flowers that open at 5 a.m. and last for a half day (Karron et al. 1995a, 2009, Mitchell et al. 2004). At our field site in southeastern Wisconsin, USA, plants flower from mid July through mid September. *Mimulus ringens* flowers are pollinated by bumblebees as they contact the stigma and anthers with their face and proboscis (Karron et al. 1995a, 1995b, Mitchell et al. 2004). The flowers are self-compatible and have a bilobed, papillose stigma that generally closes < 90 min following pollination (Mitchell et al. 2005). Nearly all flowers produce a capsule, regardless of pollination history (Karron et al. 2004, Mitchell et al. 2005). Each flower has approximately 6000 ovules. Following a bumblebee probe, fruits typically produce 1600–2300 seeds (Karron et al. 2006).

Lythrum salicaria (Lythraceae) is a perennial herb native to Eurasia that has become invasive in North America (Hager and McCoy 1998, Farnsworth and Ellis 2001). In its invasive range, this species can become very large and showy,

with up to 50 stems bearing terminal spikes. In southeastern Wisconsin plants typically bloom in July and August. Each spike is densely packed with numerous showy, actinomorphic magenta flowers. *Lythrum salicaria* is tristylous, producing three floral morphs that differ in relative style and filament lengths: short-style morphs bear long and mid-length filaments, mid-style morphs bear long and short filaments, and long-style morphs bear mid-length and short filaments (Mal and Hermann 2000). Anthers of each filament length have different-sized pollen grains (small, medium, and large) that correspond to the three filament lengths (Mal and Hermann 2000). The small and mid-sized grains fit in among the papillae of *M. ringens* stigmas, and therefore have the potential to interfere with conspecific pollen germination (Flanagan et al. 2009). *Lythrum salicaria* occasionally co-occurs with *M. ringens* in the wild. In regions of sympatry, bumblebees move freely between the two species.

Lobelia siphilitica (Campanulaceae) is an herbaceous perennial native to central and eastern North America (Caruso 2004). This species has blue zygomorphic flowers similar in size to *M. ringens* flowers (Bell et al. 2005). In southeastern Wisconsin, *L. siphilitica* flowers from early August to early October and therefore overlaps substantially in flowering phenology with *M. ringens* and *L. salicaria*. In regions where *M. ringens* and *L. siphilitica* co-occur, bumblebees move between them, carrying both species' pollen on the face and proboscis (Bell et al. 2005).

Experimental arrays

Since a major goal of our study was to explore the effects of competitors for pollination on the reproductive success of a focal species, our experimental design utilized an “addition experiment” (Mitchell et al. 2009). This approach keeps the abundance and density of the focal species constant so that the effects of competitors are not confounded with changes in the density of the focal species. Plants were grown in 35.6 cm diameter pots at the Univ. of Wisconsin-Milwaukee Field Station (Saukville, WI, USA). We established control and competition treatments by placing pots of each species into a single square grid. This design enabled us to sequentially expose all competition treatments to the same local pollinator assemblage. Plants not used in an array were stored in a pollinator-free screenhouse.

In each array we positioned 15 *M. ringens* plants in a checkerboard arrangement (Fig. 1). In the control treatment no competitor plants were present (Fig. 1A). To quantify competition for pollination between *Mimulus* and *Lobelia*, we added 15 *Lobelia* plants to the checkerboard design (Fig. 1B). A similar two-species array was established with 15 *M. ringens* and 15 *L. salicaria* plants (Fig. 1C). To quantify the effects of two competitors on reproductive success of *M. ringens*, we established arrays that maintained the same number and density of competitors as in the other competition treatments (Fig. 1D). Therefore, there were 15 total competitor plants, which were arranged in an alternating pattern. Because it was not possible to have an equal number of *L. salicaria* and *L. siphilitica* plants in this treatment, we consistently used eight *L. siphilitica* and seven *L. salicaria* plants to minimize variation among arrays. All three *L. salicaria* style morphs were represented in each competitive array.

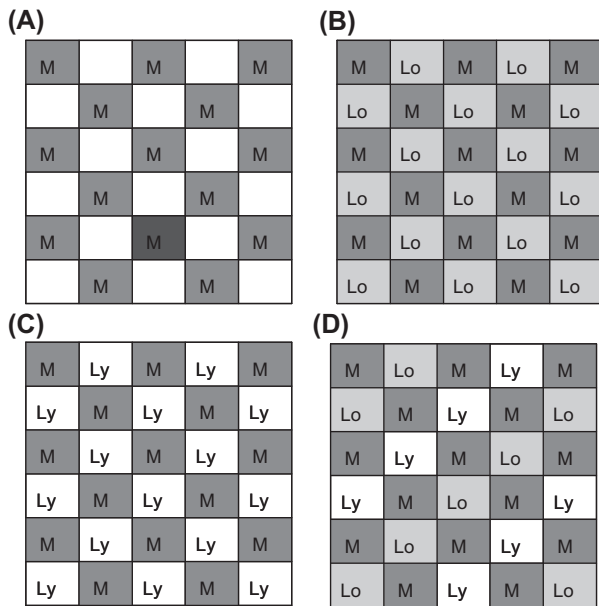


Figure 1. Experimental design for quantifying the effects of competition for pollination. *Mimulus ringens* plants are grown either in the absence of competitors (A), in the presence of a single competitor (B and C), or in the presence of two competitors (D). In each array there was 2m spacing between *Mimulus* plants. M = *Mimulus ringens*, Lo = *Lobelia siphilitica*, Ly = *Lythrum salicaria*.

We established each competition treatment on a separate day. For each competition treatment, we recorded pollinator foraging patterns and collected the resulting *M. ringens* fruits. A set of three different competition treatments and one control comprised a statistical block. Therefore, a block included four total days of pollinator observation (one day for the control and one day for each of the three competition treatments). We replicated each of these four-day blocks twice in August 2006. Because we established all four array types in the same physical location, we allowed one day between observations so that pollinators could acclimate to each newly-established array. We used a fresh set of *M. ringens* plants for each four-day block. At the end of each block, we returned *M. ringens* plants to a screenhouse to protect them from further pollinator visitation and pests while fruits ripened. In addition to changing competitor plants after each block, when necessary we replaced competitor plants with fresh plants from the screenhouse to ensure that all competitor plants had an ample number of freshly-opened, unvisited flowers. On the day following the acclimation day we quantified pollinator visitation and tagged flowers to measure seed number per fruit. Each block lasted 8-10 days, depending on weather conditions. We measured pollinator foraging patterns and the resulting seeds per *M. ringens* fruit for two blocks during August 2006 (n = 8 total observation days).

We trimmed each vigorously growing *M. ringens* plant to eight flowers to reflect the size of a typical *M. ringens* floral display in nature (Karron et al. 2009). To control for possible variation due to the position of flowers on the plant, we made sure the locations of the eight flowers were spread evenly over the entire plant. We trimmed the *M. ringens* displays at 5 a.m., before pollinators became active. We did not manipulate *L. salicaria* displays, which already reflected

display sizes typical of nearby populations (Flanagan et al. 2009). We trimmed *L. siphilitica* plants to 2-3 stems, each with 4-10 flowers per stem to make them more representative of plants in the wild.

Pollinator observations were recorded by a team of two observers. Observations began at 5 a.m., prior to arrival of the first pollinator, and continued until late afternoon, when *Mimulus* stigmas had closed and the corollas had dropped off. We collected two types of observational data for each treatment: visitation rate and pollinator foraging sequences. We alternated between the collection of these two types of data continually for the entire observation day. To quantify the effects of competition treatment on *Mimulus ringens* reproductive success, we tied labeled plastic tags to the pedicels of two randomly selected flowers on all 15 *M. ringens* plants in each array. We tagged a total of 240 flowers (2 flowers × 15 plants × 4 treatments × 2 blocks). We harvested the fruits upon ripening in late September and counted seeds in each fruit using a dissecting microscope.

Pollinator visitation rates

On each observation day we recorded pollinator visitation rates during 10 min intervals scheduled every 30 min between 5 a.m. (prior to the arrival of the first bee) and 1 p.m. (after all stigmas had closed). Because multiple bees frequently foraged simultaneously in the plot, we divided the 30 positions in each array (e.g. 15 *M. ringens* plants and, if present, 15 competitor plants) into five sections, each with three *M. ringens* and three (or zero) competitor plants. During each 10 min window we recorded the number of probes to *M. ringens* flowers on plants in one section, with each section observed at least once per day. We were able to observe and record all bee probes to the three *Mimulus* plants during the observation period, even when multiple bees were present. We randomly rotated among the five sections throughout the day. We used these data to estimate the number of probes per *M. ringens* flower per hour over the eight hour window of observation.

For all analyses we used JMP ver. 5.1.2 (SAS Inst. 2004). To test the effects of the number and identity of competitors on visitation rate to *M. ringens* we used a one-way ANOVA. We used a single measurement of probes per flower per hour for each 8 h observation day as the unit of replication (n = 8).

Pollinator foraging patterns

Immediately following each visitation rate census we recorded the complete floral visitation sequence for each pollinator foraging in an array during a 20-min period (n = 101 bees total). We noted the species and location of each plant visited and the number of consecutive floral probes on that plant. We used these data to quantify pollinator preference for *Mimulus ringens* or a competitor in each mixed species treatment and to determine if patterns of pollinator movement between species might promote interspecific pollen transfer.

To test for pollinator preference, defined as the over-visit of a species relative to its abundance (Aldridge and Campbell 2007), we compared the frequency of visits to each species in the array to expected values based on the

relative abundance of each species. For example, if a particular species receives a high number of visits relative to the number of individuals of that species present, it would indicate pollinator preference for that species. We tested for significant departure from expected values with a replicated G test (Husband and Barrett 1992, Sokal and Rohlf 1995). In this analysis we used blocks as a replicated measure. Therefore we present a G statistic for the pooled frequencies across days (G_p) and for heterogeneity between days (G_H).

We also used replicated G tests to test for flower constancy (whether the species visited by a pollinator was independent of the previous species visited). These analyses were based on pollinator transitions among plants, rather than transitions among individual flowers. Because we were interested in how foragers that moved between species would influence interspecific pollen transfer with respect to *Mimulus*, we pooled *L. salicaria* and *L. siphilitica* transitions in the three species arrays. We used blocks as a replicated measure, and expected values were calculated based on the frequency of probes made to each species by the subset of pollinators analyzed. For example, if a particular species received a high number of conspecific transitions relative to the overall number of times that particular species was visited, it would indicate constancy for that species. Therefore, deviation from expected values should be independent from patterns of pollinator preference (Husband and Barrett 1992). To ensure that foraging bouts were long enough for pollinators to have opportunity to transfer pollen, our analyses were based on all bouts with four or more plants.

We also evaluated the degree of constancy of individual foragers to *Mimulus ringens*. The constancy index, adapted from Jacob's preference index (Jacobs 1974) by Gegeer and Lavery (2005), has been used to measure the constancy of bees for particular colors while taking into account preference for that color. Here we use the index to measure pollinator constancy for a particular species (i.e. *M. ringens*) while taking into account the level of preference for *M. ringens* exhibited by pollinators. The constancy index can be calculated as: $CI = (c - e) / (c + e - 2ce)$, where c = the proportion of conspecific *M. ringens*-to-*M. ringens* transitions and e = the proportion of conspecific *M. ringens* to *M. ringens* transitions expected based on the overall frequency with which bees in a given treatment probed *M. ringens*. CI values range from +1 (complete constancy: all probes to *M. ringens* consisting of *M. ringens* to *M. ringens* transitions) to 0 (random foraging) to -1 (complete inconstancy: all probes to *M. ringens* preceded and followed by transition to a competitor). To determine whether the number and identity of competitors had an influence on the degree of pollinator preference for *M. ringens*, we used one-way ANOVA to compare mean constancy index values for foragers visiting each mixed species treatment. There was no significant heterogeneity between treatment days in patterns of pollinator preference (Results), so we pooled data across days in this analysis to increase statistical power. Because there were so few foraging sequences by the minority bee species (*Bombus vagans*) we were unable to do separate analyses for each pollinator species. Therefore, in our preference and constancy analyses we pooled data across pollinator species. If we exclude the minority bee, *B. vagans*, the outcomes of the analyses of preference and constancy are not affected.

Seed production in *Mimulus ringens*

We used a one-way ANOVA to test the effects of competition treatment on the number of seeds per *M. ringens* fruit. In this analysis a single measurement of mean seeds per fruit for each treatment day was the unit of replication ($n = 8$).

Results

Pollinator visitation rates

During 430 minutes of observation, *Mimulus ringens*, *Lobelia siphilitica*, and *Lythrum salicaria* were pollinated by two bumblebee species: *Bombus impatiens* and *B. vagans*. *Bombus impatiens* was the predominant pollinator of *M. ringens* in all four control and mixed-species treatments, accounting for 93.4% of all floral probes (242 of 259 probes). *Bombus vagans* also made occasional visits to *M. ringens* flowers, accounting for the remaining 6.6% of floral probes. *Bombus impatiens* was a less prevalent pollinator to both competitor species. *Bombus impatiens* accounted for 44% of all floral probes to *L. siphilitica* (93 of 210 total probes), with the remaining 56% of probes by *B. vagans*. Similarly, *B. impatiens* accounted for 40% of all visits to *L. salicaria* (739 of 1841 total probes) with the remaining 60% of floral probes by *B. vagans*.

The rate of pollinator visitation to *M. ringens* flowers did not vary significantly as a function of competition treatment (ANOVA, $F_{3,4} = 1.56$; $p = 0.33$). When no competitor was present, *M. ringens* received 1.92 ± 0.78 probes per flower per hour. In arrays with a mixture of *M. ringens* and *L. siphilitica*, *M. ringens* received 2.75 ± 0.78 probes per flower per hour. In arrays with a mixture of *M. ringens* and *L. salicaria*, *M. ringens* received 0.64 ± 0.78 probes per flower per hour. In three species arrays, the visitation rate to *M. ringens* was 0.87 ± 0.78 probes per flower per hour.

Pollinator foraging patterns

Bumblebees often probed two or more species during individual foraging bouts (Fig. 2). When pollinators visited mixed species arrays of *M. ringens* and *L. siphilitica* ($n = 32$ foraging sequences), 16% of these visitors foraged only on *M. ringens*, 31% foraged only on *L. siphilitica*, and 53% foraged on both species. When bees visited mixed species arrays of *M. ringens* and *L. salicaria* ($n = 33$ foraging sequences), none of the visitors foraged only on *M. ringens*, 64% foraged only on *L. salicaria*, and 36% foraged on both species. When pollinators visited arrays with all three species ($n = 36$ foraging sequences), 2% of visitors foraged only on *M. ringens*, 2% of visitors foraged only on *L. siphilitica*, 39% of visitors foraged only on *L. salicaria*, 2% of visitors foraged on both *M. ringens* and *L. siphilitica*, 21% of visitors foraged on both *M. ringens* and *L. salicaria*, 17% of visitors foraged on both *L. siphilitica* and *L. salicaria*, and 17% of visitors foraged on all three species.

In arrays with both *M. ringens* and *L. siphilitica*, pollinator probes to *M. ringens* plants did not deviate significantly from expected values. However, in mixtures of *M. ringens* and *L. salicaria* or in arrays with all three species, pollinators

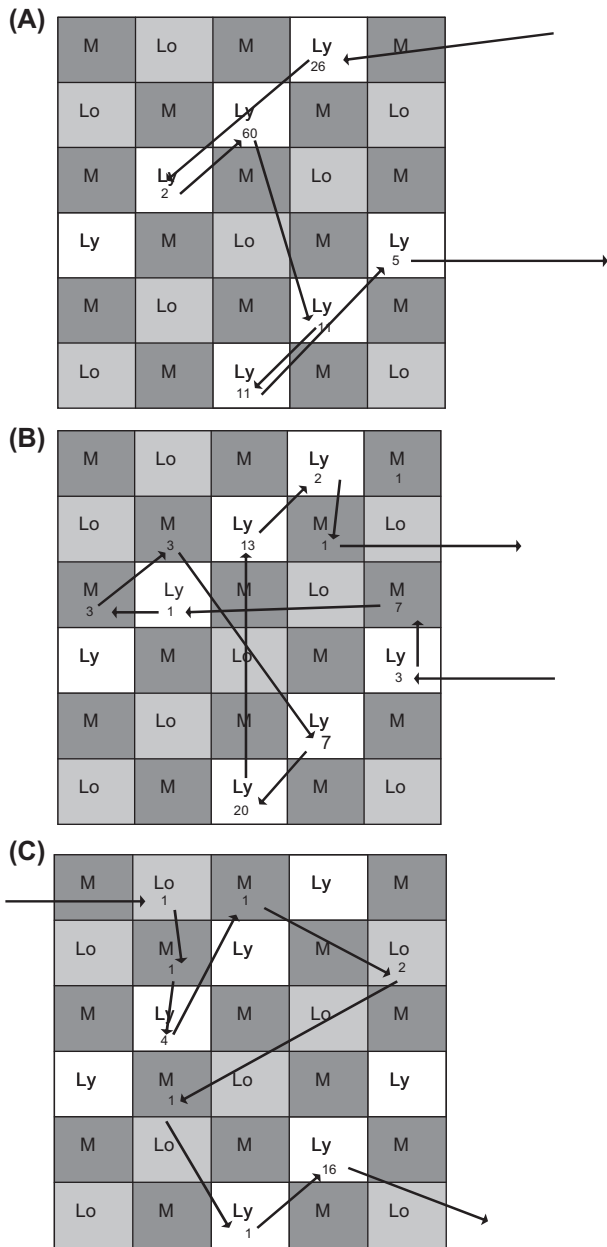


Figure 2. The three most common foraging sequences observed when pollinators visited multi-species arrays. Diagrams show the foraging sequence of three different *Bombus impatiens* workers visiting (A) only *L. salicaria*, (B) *M. ringens* and *L. salicaria*, or (C) all three species. Arrows indicate pollinator transitions and numbers indicate consecutive floral probes per plant.

Table 1. The observed and [expected] number of pollinator probes to *M. ringens*, *L. siphilitica* and *L. salicaria* in experimental arrays. G-statistics are presented for data pooled across treatment days (G_p) and for heterogeneity among days within each treatment (G_H). For all pair-wise comparisons DF = 1. In three-species arrays DF = 2.

Treatment	n foragers	Number of probes observed [expected]			G_p	G_H
		<i>M. ringens</i>	<i>L. siphilitica</i>	<i>L. salicaria</i>		
<i>M. ringens</i> only	27	133	–	–	–	–
<i>M</i> + <i>L. siphilitica</i>	32	157 [147]	137 [147]	–	1.36	1.13
<i>M</i> + <i>L. salicaria</i>	33	55 [133.5]	–	212 [133.5]	98.55*	0.009
Three species	36	50 [98]	27 [52]	118 [45]	126.60*	2.94

* $p < 0.05$.

M = *M. ringens*.

significantly over-visited *L. salicaria* and under-visited *M. ringens* (Table 1).

When pollinators foraged in mixtures of *M. ringens* and *L. siphilitica*, 72% of all interplant transitions were between species and were more common than expected based on patterns of preference of these foragers. When pollinators foraged in mixtures of *M. ringens* and *L. salicaria*, heterospecific transitions were also common (53% of all interplant transitions), but did not differ significantly from expected values. In arrays with all three species, 62% of all transitions involving *M. ringens* were to or from a competitor and were more common than expected, with the remaining 38% being conspecific *Mimulus*-to-*Mimulus* transitions (Table 2). Flower constancy to *M. ringens* was low in all mixed species treatments (mean constancy index values for all mixed species treatments were well below zero), and did not vary significantly among competition treatments (Table 2; ANOVA, $F_{2,98} = 2.11$; $p = 0.13$).

Mimulus ringens seeds per fruit

Seed number per *Mimulus ringens* fruit varied significantly among competition treatments (Fig. 3; ANOVA, $F_{3,4} = 7.67$; $p < 0.05$). When either *Lobelia siphilitica* or *Lythrum salicaria* was present, the number of seeds produced per *M. ringens* fruit was ~ 40% lower than the control treatment. When both competitors were present, seeds per *M. ringens* fruit was reduced by an additional 11% (Fig. 3). In a posteriori test, *M. ringens* seed number was significantly lower when a competitor was present but did not differ significantly among the mixed species treatments (Tukey–Kramer HSD).

Discussion

Pair-wise competition with either *Lobelia siphilitica* or *Lythrum salicaria* dramatically reduced *Mimulus ringens* seed production. In both cases, the number of seeds per *M. ringens* fruit was approximately 40% lower than in the absence of a competitor. When both competitors were present, *M. ringens* seed production was 51% lower, but this additional reduction was not significant. However, patterns of pollinator preference and interspecies movement varied significantly with competitor species composition in mixed species arrays. This suggests that similar competitive effects (reduction in seed production) may have resulted from different mechanisms of competition for pollination.

Table 2. The observed and [expected] number of inter-plant pollinator transitions in experimental arrays. Table also includes mean constancy index scores (CI) \pm 1 SE for each competition treatment. Data shown are restricted to foragers that moved between *M. ringens* and a competitor within a single foraging bout. G statistics are presented for data pooled across treatment days (G_p) and for heterogeneity among days within each treatment (G_H). DF = 3 for all comparisons.

Treatment	n foragers	Transition type observed [expected]				G_p	G_H	CI
		M to C	C to M	M to M	C to C			
M+ <i>L. siphilitica</i>	17	65 [45]	65 [45]	24 [50]	25 [39]	37.24*	1.12	-0.46 \pm 0.13
M+ <i>L. salicaria</i>	12	26 [23.5]	24 [23.5]	19 [21]	25 [26]	0.52	1.62	-0.66 \pm 0.12
Three species	15	27 [18.5]	25 [18.5]	8 [9]	24 [38]	11.23*	0.34	-0.74 \pm 0.13

* $p < 0.05$.

M = *M. ringens*, C = competitor.

Pollinator preference

When *M. ringens* was in pair-wise competition with *L. siphilitica*, pollinators did not show preference for either species; each species received an approximately equal proportion of pollinator probes. By contrast, when *M. ringens* was in pair-wise competition with *L. salicaria*, pollinators strongly preferred *L. salicaria*, which received the vast majority of all pollinator probes. When both competitors were present, *L. salicaria* was still preferred, with *M. ringens* again receiving a low proportion of pollinator probes. Although not significant, likely due to low statistical power ($n = 8$ replicates), visitation rate data also supports the general trend of *Lythrum* preference, with *Mimulus* receiving a lower number of visits when *Lythrum* was present. The preference pollinators exhibit for *L. salicaria* suggests that this plant is likely to draw pollinators away from *M. ringens* whenever it is present. Highly attractive invasive plants have been shown to strongly influence plant-pollinator networks in multi-species plant communities (Larson et al. 2006, Morales and Aizen 2006, Lopezaria-Mikel et al. 2007, Aizen et al. 2008, Bartomeus et al. 2008) and compete strongly for pollination with native species (Brown and Mitchell 2001, Chittka and Schurkens 2001, Bjerknes et al. 2007, Kandori et al. 2009). *Lythrum salicaria* is an attractive and rewarding competitor species, especially in its invasive range, and it can readily attract pollinators from other species (Brown et al. 2002, Flanagan et al. 2010). Therefore, this species may dominate interactions

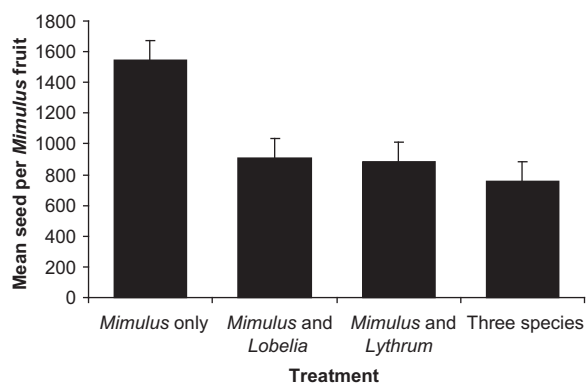


Figure 3. Effect of presence and abundance of competitors for pollination on mean number of seed produced per *M. ringens* fruit. Bars are mean seed per *M. ringens* fruit ($+1$ SE) when no competitor was present, when either *L. salicaria* or *L. siphilitica* was present, and when both competitors were present. See Results for data analyses.

with pollinators in a variety of community contexts (Morales and Traveset 2009).

Patterns of pollinator movement

In the two-species arrays, individual bee workers employed different foraging strategies. Some workers foraged solely on *M. ringens*, some solely on a competitor, and some on both *M. ringens* and a competitor. In the three-species arrays, all possible combinations of visitation patterns were observed (Fig. 2). Although interspecific transitions were common in all mixed-species arrays, there were differences in the extent of interspecific transitions depending on the number and identity of competitors. For example, pollinators exhibited inconstancy to *M. ringens* in mixtures of *M. ringens* and *L. siphilitica* compared to other array types, making competition through interspecific pollen transfer a likely mechanism lowering *Mimulus* seed production when *Lobelia* was present.

These results suggest that there is ample opportunity for interspecific pollen transfer to lower *M. ringens* reproductive success in all competition treatments. However, in pair-wise competition with *L. salicaria*, interspecific transitions were not as prevalent as in other mixed species arrays, even when taking pollinator preference for *L. salicaria* into account. The increased tendency of pollinators to move between *M. ringens* and *L. siphilitica* versus *M. ringens* and *L. salicaria* may reflect the greater morphological similarity of *M. ringens* and *L. siphilitica* flowers. Constancy is often lower when pollinators forage among species with similar floral morphology (Waser 1986, Chittka et al. 1999, Gegear and Laverty 2005), and therefore interspecific pollen transfer may be a particularly important mechanism of competition between these two species (Waser 1983a, 1983b, Campbell 1985, Waser and Fugate 1986, Murcia and Feinsinger 1996, Brown and Mitchell 2001, Mitchell et al. 2009). Although the present study did not quantify *Mimulus* pollen loss to *Lobelia* and *Lythrum* stigmas, our previous work has shown that large quantities of *M. ringens* pollen may be deposited on *L. siphilitica* stigmas (Bell et al. 2005). By contrast, Flanagan et al. (2009) found that very little *M. ringens* pollen was lost to *L. salicaria* stigmas or other floral structures. However, substantial *Mimulus* pollen is lost when pollinators groom while foraging on *L. salicaria* (Flanagan et al. 2009, Mitchell et al. 2009).

Although patterns of pollinator movement between species suggest that the severity of competition should vary with competitor species composition, the effects of competition treatment on *Mimulus* seed production was fairly

consistent across competition treatments, with similar ~40% reductions. A possible factor influencing this result could be differences among conspecific worker bees in patterns of flower constancy and floral preference (Fig. 2, Thompson and Chittka 2001). In our study conspecific individuals had very different foraging responses to the same arrays. Variation in floral preference and floral constancy may have important consequences for the outcome of competition for pollination and may play an important role in the dynamics of competition in multi-species arrays (Mitchell et al. 2004). For example, just a few pollinators with strong preference for *Mimulus* could enhance seed production in competitive arrays, even if overall behavior patterns suggest strong competition should occur (Thompson and Chittka 2001).

Interspecific differences in pollinator behavior and morphology may also be important to outcomes of competition for pollination. For example, *Mimulus* was visited more frequently by *B. impatiens* than by *B. vagans*. Although our design had the advantage that each treatment was subjected to the same local assemblage of pollinators, any factor that affects pollinator species composition, such as season or habitat quality, could influence the outcomes of competition for pollination. Our experimental approach maximized our ability to observe pollinators for the entire day (Methods). However, a limitation of this approach is that it was logistically impossible to manipulate all combinations and densities of the competitors and focal species during the window in which the three species were in flower. Consequently, we could not completely disentangle the effects of competitor species diversity and total floral density on *Mimulus* reproductive success. For example, the higher attractiveness of mixed-species arrays could have compensated for affects of competition among plant species due to increased pollinator recruitment (Feinsinger 1978, Rathcke 1983, Johnson et al. 2003, Ghazoul 2006, Feldman 2004, Lazaro et al. 2009). It would be valuable to understand how a range of densities of both the focal species and the competitors may influence pollinator behavior and reproductive success (Flanagan et al. 2010). However, we feel that our design, a standard addition experiment, was the most appropriate strategy for detecting the effects of competition for pollination on *Mimulus* reproductive success (Snaydon 1991) since it controlled the abundance and density of the focal species.

Another logistical limitation of our experimental design was the slightly unbalanced representation of *L. salicaria* morphs in the three species arrays. However, we found in an earlier study (Flanagan et al. 2009) that very little *Lythrum* pollen is transferred to *Mimulus* stigmas, regardless of morph type. Therefore, the representation of morphs in our arrays is unlikely to have influenced our results.

Conclusion

When two or more competitors for pollination interact with a focal species, multiple competitive mechanisms may operate simultaneously, potentially leading to strong reductions in the reproductive success of a focal species. Differences in the number and identity of competitors present may influence pollinator preference and constancy, leading to differences in the mechanism and outcome of

competition for pollination. The patterns of pollinator foraging we observed in three-species arrays provide partial support for this hypothesis: pollinators under-visited *M. ringens* when *L. salicaria* was present and moved frequently between species when *L. siphilitica* was present. These results demonstrate that the mechanisms of competition for pollination may vary with the community context experienced by a focal species, even if the magnitude of competitive effects is not affected. This work also emphasizes the need for additional studies exploring competition for pollination between three or more species.

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References

- Aizen, M. A. et al. 2008. Invasive mutualists erode native pollination webs. – *PLOS Biol.* 6: 396–403.
- Aldridge, G. and Campbell, D. R. 2007. Variation in pollinator preference between two *Ipomopsis* contact sites that differ in hybridization rate. – *Evolution* 61: 99–110.
- Bartomeus, I. et al. 2008. Contrasting effects of invasive plants in plant–pollinator networks. – *Oecologia* 155: 761–770.
- Bell, J. M. et al. 2005. Interspecific competition for pollination lowers seed production and outcrossing rate in *Mimulus ringens*. – *Ecology* 86: 762–771.
- Bjerknes, A. L. et al. 2007. Do alien plant invasions really affect pollination success in native plant species? – *Biol. Conserv.* 138: 1–12.
- Brown, B. J. and Mitchell, R. J. 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. et al. 2002. Competition for pollination between an invasive species (purple loosestrife) and a native congener. – *Ecology* 83: 2328–2336.
- Campbell, D. R. 1985. Pollinator sharing and seed set of *Stellaria pubera*: competition for pollination. – *Ecology* 66: 544–563.
- Caruso, C. M. 2000. Competition for pollination influences selection on floral traits of *Ipomopsis aggregata*. – *Evolution* 54: 1546–1557.
- Caruso, C. M. 2004. The quantitative genetics of floral trait variation in *Lobelia*: potential constraints on adaptive evolution. – *Evolution* 58: 732–740.
- Chittka, L. and Schurkens, S. 2001. Successful invasion of a floral market. – *Nature* 411: 653.
- Chittka, L. et al. 1999. Flower constancy, insect psychology and plant evolution. – *Naturwissenschaften* 86: 361–377.
- Dormann, C. F. and Roxburgh, S. H. 2005. Experimental evidence rejects pairwise modelling approach to coexistence in plant communities. – *Proc. R. Soc. Lond. B* 272: 1279–1285.
- Farnsworth, E. J. and Ellis, D. R. 2001. Is purple loosestrife (*Lythrum salicaria*) an invasive threat to freshwater wetlands? Conflicting evidence. – *Wetlands* 21: 199–209.
- Feinsinger, P. 1978. Ecological interactions between plants and hummingbirds in a successional tropical community. – *Ecol. Monogr.* 48: 269–287.
- Feldman, T. S. et al. 2004. When can two plants facilitate each other's pollination? – *Oikos* 105: 197–207.

- Flanagan, R. J. et al. 2009. Interspecific pollinator movements reduce pollen deposition and seed production in *Mimulus ringens* (Phrymaceae). – *Am. J. Bot.* 96: 809–815.
- Flanagan, R. J. et al. 2010. Increased relative abundance of an invasive competitor for pollination, *Lythrum salicaria*, reduces seed number in *Mimulus ringens* – *Oecologia* doi 10.1007/s00442-010-1693-2
- Gegear, R. and Thomson, J. D. 2004. Does the flower constancy of bumble bees reflect foraging economics? – *Ethology* 110: 793–805.
- Gegear, R. and Laverty, T. M. 2005. Flower constancy in bumblebees: a test of the trait variability hypothesis. – *Anim. Behav.* 69: 939–949.
- Ghazoul, J. 2006. Floral diversity and the facilitation of pollination. – *J. Ecol.* 94: 295–304.
- Hager, H. and McCoy, K. D. 1998. The implications of accepting untested hypotheses: A review of the effects of purple loosestrife (*Lythrum salicaria*) in North America. – *Biodiv. Conserv.* 7: 1069–1079.
- Husband, B. C. and Barrett, S. C. H. 1992. Pollinator visitation in populations of tristylous *Eichhornia paniculata* in northeastern Brazil. – *Oecologia* 89: 365–371.
- Jacobs, J. 1974. Quantitative measurement of food selection. – *Oecologia* 14: 413–417.
- Johnson, S. D. et al. 2003. Pollination success in a deceptive orchid is enhanced by co-occurring rewarding magnet plants. – *Ecology* 84: 2919–2927.
- Kandori, I. et al. 2009. An invasive dandelion unilaterally reduces the reproduction of a native congener through competition for pollination. – *Oecologia* 159: 559–569.
- Karron, J. D. et al. 1995a. The influence of population density on outcrossing rates in *Mimulus ringens*. – *Heredity* 75: 175–180.
- Karron, J. D. et al. 1995b. Comparison of pollinator flight movements and gene dispersal patterns in *Mimulus ringens*. – *Heredity* 75: 612–617.
- Karron, J. D. et al. 2004. The influence of floral display size on selfing rates in *Mimulus ringens*. – *Heredity* 92: 242–248.
- Karron, J. D. et al. 2006. Multiple pollinator visits to *Mimulus ringens* (Phrymaceae) flowers increase mate number and seed set within fruits. – *Am. J. Bot.* 93: 1306–1312.
- Karron, J. D. et al. 2009. Pollinator visitation patterns strongly influence among-flower variation in selfing rate. – *Ann. Bot.* 103: 1379–1383.
- Larson, D. L. et al. 2006. Insect visitation and pollen deposition in an invaded prairie plant community. – *Biol. Conserv.* 130: 148–159.
- Lazaro, A. et al. 2009. Co-flowering neighbors influence the diversity and identity of pollinator groups visiting plant species. – *Oikos* 118: 691–702.
- Levin, D. A. and Anderson, W. W. 1970. Competition for pollinators between simultaneously flowering species. – *Am. Nat.* 104: 455–467.
- Lopezaraiza-Mikel, M. E. et al. 2007. The impact of an alien plant on a native plant-pollinator network: an experimental approach. – *Ecol. Lett.* 10: 539–550.
- Mal, T. K. and Hermann, B. P. 2000. Quantitative evaluation of pollen polymorphism in a tristylous weed, *Lythrum salicaria* (Lythraceae). – *Can. J. Bot.* 78: 1086–1094.
- Miller, T. E. 1994. Direct and indirect species interactions on an early old-field plant community – *Am. Nat.* 143: 1007–1025.
- Mitchell, R. J. et al. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. – *Funct. Ecol.* 18: 116–124.
- Mitchell, R. J. et al. 2005. Patterns of multiple paternity in fruits of *Mimulus ringens* (Phrymaceae). – *Am. J. Bot.* 92: 885–890.
- Mitchell, R. J. et al. 2009. New frontiers in competition for pollination. – *Ann. Bot.* 103: 1403–1413.
- Montgomery, B. R. 2009. Effect of introduced *Euphorbia esula* on the pollination of *Viola pedatifida*. – *Botany* 87: 283–292.
- Morales, C. L. and Aizen, M. A. 2006. Invasive mutualisms and the structure of plant–pollinator interactions in the temperate forests of northwest Patagonia, Argentina. – *J. Ecol.* 94: 171–180.
- Morales, C. L. and Traveset, A. 2009. A meta-analysis of impacts of alien vs. native plants on pollinator visitation and reproductive success of co-flowering native plants. – *Ecol. Lett.* 12: 716–728.
- Mosquin, T. 1971. Competition for pollinators as a stimulus for the evolution of flowering time. – *Oikos* 22: 398–402.
- Murcia, C. and Feinsinger, P. 1996. Interspecific pollen loss by hummingbirds visiting flower mixtures: effects of floral architecture. – *Ecology* 77: 550–560.
- Perkins, T. A. et al. 2007. Multi-species interactions in competitive hierarchies: new methods and empirical test. – *J. Veg. Sci.* 18: 685–692.
- Rathcke, B. 1983. Competition and facilitation among plants for pollination. – In: Real, L. (ed), *Pollination biology*. Academic Press, pp. 305–309.
- Rathcke, B. 1988. Interactions for pollination among co-flowering shrubs. – *Ecology* 69: 446–457.
- Sokal, R. R. and Rohlf, F. J. 1995. *Biometry: the principles and practice of statistics in biological research* (3rd ed.). – W.H. Freeman.
- Snaydon, R. W. 1991. Replacement or additive designs for competition studies? – *J. Appl. Ecol.* 28: 930–946.
- Takakura, K. et al. 2009. Alien dandelion reduces the seedset of a native congener through frequency-dependent and one-sided effects. – *Biol. Invas.* 11: 973–981.
- Thomson, J. D. and Chittka, L. 2001. Pollinator individuality: when does it matter? – In: Chittka, L. and Thomson, J. D. (eds), *Cognitive ecology of pollination: animal behavior and floral evolution*. Cambridge Univ. Press, pp. 191–213.
- Vamosi, J. C. et al. 2005. Pollination decays in biodiversity hotspots. – *Proc. Natl Acad. Sci. USA* 103: 956–961.
- Waser, N. M. 1978. Competition for pollination and sequential flowering in two Colorado wildflowers. – *Ecology* 59: 934–944.
- Waser, N. M. 1983a. Competition for pollination and floral character differences among sympatric plant species: a review of evidence. – In: Jones, C. E. and Little, R. J. (eds), *Handbook of experimental pollination biology*. Van Nostrand Reinhold, pp. 277–293.
- Waser, N. M. 1983b. The adaptive nature of floral traits: ideas and evidence. – In: Real, L. (ed), *Pollination biology*. Academic Press, pp. 241–285.
- Waser, N. M. 1986. Flower constancy: definition, cause and measurement. – *Am. Nat.* 127: 593–603.
- Waser, N. M. and Fugate, M. L. 1986. Pollen precedence and stigma closure: a mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata*. – *Oecologia* 70: 573–577.
- Weigelt, A. et al. 2007. Identifying mechanisms of competition in multi-species communities – *J. Ecol.* 95: 53–64.