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INTERSPECIFIC POLLINATOR MOVEMENTS REDUCE POLLEN DEPOSITION AND SEED PRODUCTION IN *MIMULUS RINGENS* (PHRYMACEAE)[1]

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Movement of pollinators between coflowering plant species may influence conspecific pollen deposition and seed set. Interspecific pollinator movements between native and showy invasive plants may be particularly detrimental to the pollination and reproductive success of native species. We explored the effects of invasive *Lythrum salicaria* on the reproductive success of *Mimulus ringens*, a wetland plant native to eastern North America. Pollinator flights between these species significantly reduced the amount of conspecific pollen deposited on *Mimulus* stigmas and the number of seeds in *Mimulus* fruits, suggesting that pollen loss is an important mechanism of competition for pollination. Although pollen loss is often attributed to pollen wastage on heterospecific floral structures, our novel findings suggest that grooming by bees as they forage on a competitor may also significantly reduce outcross pollen export and seed set in *Mimulus ringens*.

Key words: *Bombus vagans*; bumble bee; competition for pollination; interspecific pollen transfer; *Mimulus ringens*; monkey flower; Phrymaceae; pollen loss; pollination; seed set.

In many plant communities, two or more species overlap in flowering phenology and share pollinators (Levin and Anderson, 1970; Waser, 1978, 1983a, b; Campbell, 1985; Stone et al., 1998; Mitchell et al., 2009). Pollinator movement between species may lead to deposition of a focal species' pollen onto a competitor's floral surfaces (Waser, 1983a; Kohn and Waser, 1985; Feinsinger et al., 1988; Murcia and Feinsinger, 1996). Such wastage of the focal species' pollen on heterospecific flowers may lower the outcross siring success of donor flowers as well as the seed set of the next flower visited (Brown et al., 2002; Harder and Routley, 2006). Inconstant pollinators may also deposit heterospecific pollen onto stigmas of the focal species. Such deposition may block or clog the stigma so that subsequently deposited conspecific pollen cannot fertilize ovules (Shore and Barrett, 1984; Campbell and Motten, 1985; Waser and Fugate, 1986; Galen and Gregory, 1989; Brown and Mitchell, 2001; Morales and Traveset, 2008).

Two behaviors, preference and constancy, may determine the extent to which pollinators move between plant species. Pollinator preference results in the overvisitation of a preferred species relative to its availability (Waser, 1986; Husband and Barrett, 1992; Aldridge and Campbell, 2007). Floral constancy is the tendency of pollinators to sequentially visit the same species or floral morph, bypassing other equally rewarding species. This behavior results in a series of conspecific pollinator transitions and thus may lower interspecific pollen transfer (Waser, 1986; Leebens-Mack and Milligan, 1998; Gegear and Laverty, 2005).

When pollinators probe flowers, contact with anthers determines initial placement of pollen on a pollinator's body (Muchhala and Potts, 2007). However, many pollinators scrape this pollen off themselves while foraging, displacing it from its original location to sites that are less likely to contact stigmas (Harder and Wilson, 1998). This grooming behavior, characteristic of bees and flies, may reduce the pollen available for cross-fertilization (Johnson et al., 2005; Harder and Routley, 2006). When an inconstant forager departs focal species A and then probes several flowers of species B, frequent grooming during visits to B may reduce the amount of species A pollen available for deposition during subsequent visits to flowers of A. Such losses due to grooming are likely to lower both male and female reproductive success of the focal species, but this mechanism of competition for pollination has received little empirical study (Mitchell et al., 2009).

We investigated the effects of invasive *Lythrum salicaria* on conspecific pollen deposition and seed set in *Mimulus ringens,* a wetland plant native to southeastern Wisconsin. These species occasionally co-occur in nature, with flowering phenologies that broadly overlap. In areas of sympatry, bumble bees often move between these species during a single foraging bout. Preliminary experimental research suggested that *Mimulus* seed set was dramatically lowered when *Lythrum* was present (R. Flanagan, unpublished manuscript). In this study, we explore the mechanisms responsible for this reduction in seed set and address the following questions: (1) Does inconstant pollinator foraging decrease conspecific pollen deposition and resulting seed set in *Mimulus*? (2) Does inconstant pollinator foraging increase heterospecific pollen deposition on both *Mimulus* and *Lythrum*? (3) Does pollinator grooming decrease conspecific pollen deposition on *Mimulus*?

MATERIALS AND METHODS

Study species—*Mimulus ringens* L. (Phrymaceae) is a wetland, perennial herb native to central and eastern North America (Grant, 1924). Most *M. ringens*

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populations are small, typically with fewer than 50 individuals. In southeastern Wisconsin, *M. ringens* flowers from mid-July through mid-September, producing large blue zygomorphic flowers that last for a single day (Karron et al., 1995a, b). Daily floral displays bear 1–23 flowers, which are generally scattered across separate stems (Karron et al., 2009). Anthers dehisce before dawn, and pollen grain size is ~12 μm (Mitchell et al., 2004; Bell et al., 2005). This self-compatible species has a bilobed, papillose stigma that generally closes <90 min following pollination (Karron et al., 2004; Fig. 1A). Each *Mimulus ringens* flower produces a capsule containing up to 6000 seeds (Mitchell et al., 2005; Karron et al., 2006).

Lythrum salicaria (Lythraceae) is native to Eurasia. It was introduced to North America in the early 1800s and subsequently became invasive (Hager and McCoy, 1998; Blossey et al., 2001). This perennial often grows to a large size in its invasive range, with up to 50 stems bearing spikes of densely packed, showy magenta flowers that are very attractive to pollinators (Mal et al., 1992). *Lythrum* plants near our study site tend to be smaller, with a mean (±1 SE) of 8.48 ± 0.91 spikes per plant with 32.03 ± 2.636 flowers per spike (N = 45 plants). This species has a heteromorphic incompatibility system, producing three floral morphs that differ in the relative length of stigma and anthers (Darwin, 1877; Agren, 1996). Short-style morphs have long and mid filaments, mid-style morphs have long and short filaments, and long-style morphs have mid and short filaments (Darwin, 1877; Mal et al., 1992). In addition, anthers of each filament length have different sizes of pollen grains (Mal and Hermann, 2000). Small and midsized *Lythrum* pollen grains overlap in size with *Mimulus* pollen grains and appear to fit within *Mimulus* papillae (Fig. 1B).

Pollen transfer experiments—To quantify interspecific pollen transfer between *Mimulus* and *Lythrum*, we placed potted plants of each species in 44 linear arrays, each with two *Mimulus* plants on either side of a single *Lythrum* plant. The four *Mimulus* plants were unrelated genets from a single population (Karron et al., 1995a). To minimize variation among arrays, we only used long-style *Lythrum* morphs in the arrays. Before presenting an array to pollinators, plants were kept in a large screenhouse to ensure that all open flowers were unvisited prior to observations. We trimmed each *Mimulus* plant to a four-flower display, and each *Lythrum* plant to 8–10 spikes of newly opened flowers. The goal of these floral manipulations was to encourage pollinators to move between plants in our array, rather than to become satiated and leave prior to visiting other plants in the array. We conducted pollinator observations on eight fair weather days during August 2007. We removed the 44 arrays one at a time from a pollinator-free screenhouse and placed them outside, allowing the first free-flying bumblebee worker that arrived to forage solely on the array. Only one five-plant array was exposed to visitors at a time, and each of these arrays was only visited once. We recorded the species and sequence of flowers that each pollinator visited. During some of the visitation sequences, bees foraged only on *Mimulus* flowers, whereas during other sequences bees probed both *Mimulus* and *Lythrum* flowers. When a bee visited only *Mimulus*, we allowed it to forage on at least two different genets and then tagged the first flower probed on the third genet. When a bee foraged on both *Mimulus* and *Lythrum* during a single visitation sequence, we only tagged a *Mimulus* flower if the bee foraged on at least two *Mimulus* genets then visited at least four flowers on *Lythrum* before visiting another *Mimulus* genet. The tag was then placed on the first *Mimulus* flower probed following visits to *Lythrum* flowers. We then returned the entire array to the screenhouse to protect plants from further visitation.

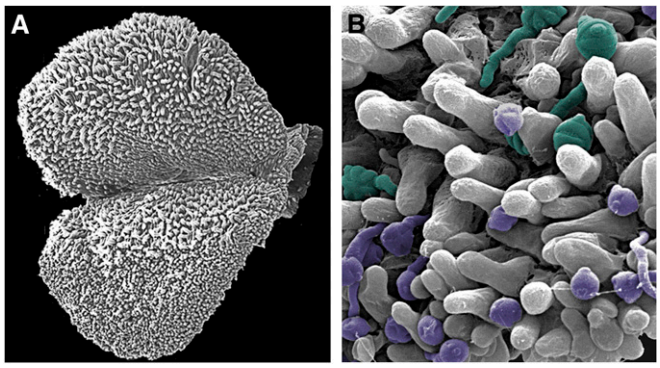

Fig. 1. Scanning electron micrographs of stigma of *Mimulus ringens*. (A) Pollen grains deposited during a single bumble bee visit. Background was darkened for contrast. Grains can be seen on the lower left stigmatic lobe. (B) Pollen grains of *Mimulus* (false-colored blue) and *Lythrum* (false-colored green) nestled among the stigmatic papillae. Note germinated *Mimulus* grains in lower left and germinated *Lythrum* grains in upper right.

To quantify the influence of intervening competitors on conspecific and heterospecific pollen deposition, we collected the stigma of each tagged *Mimulus* flower 48 h after pollination. We used fluorescence microscopy to count the number of *Mimulus* and *Lythrum* grains on *Mimulus* stigmas resulting from a *Mimulus* to *Mimulus* transition (N = 17) vs. a transition in which there were visits to an intervening *Lythrum* plant (N = 23). Four additional stigmas were lost during the collection process and therefore were excluded from the analysis. Pollen grains of *Mimulus* and *Lythrum* can readily be distinguished due to differential autofluorescence under UV excitation (*Mimulus* autofluoresces blue, while *Lythrum* autofluoresces pale yellow). *Lythrum* and *Mimulus* grains also have distinctive morphologies (Fig. 1B). To prepare pollen for fluorescence microscopy, we placed stigmas collected in the screenhouse directly into FAA (95% ethanol, distilled water, 40% formaldehyde, glacial acetic acid, 10 : 7 : 2 : 1). Stigmas were then soaked in 5 N NaOH for ~1 h to soften the stigmatic tissue (Kearns and Inouye, 1993). One bumblebee probe can deposit thousands of *Mimulus* pollen grains, often in several layers on the papillose stigma (Bell et al., 2005). As a result, a simple squash generally leaves pollen grains in multiple focal planes, making them difficult to count. To create a monolayer of pollen, we used a stainless steel needle to spread softened stigmas across several grid-imprinted slides. The needle was then immediately washed in ethanol and the wash fluid dried down on an additional slide to ensure that no pollen was lost on the needle. We also examined the needle with a dissecting scope to verify that it was completely free of pollen and stigmatic tissue.

To quantify the relationship between pollen deposition and *Mimulus* reproductive success, 41 fruits on tagged flowers were left to ripen and then harvested in early September. Prior studies indicate that stigma removal in this manner allows normal seed maturation (R. Flanagan, unpublished data). A total of three fruits were lost during the collection process. Once the fruits had air dried, we counted seed number per fruit using a dissecting microscope.

Hand pollinations—To study the effect of heterospecific pollen on *Mimulus* seed production, we performed controlled crosses in a pollinator-free screenhouse. Crosses were performed on three ramets of each of six *Mimulus* genets. On all 18 plants, we pollinated a control flower with a mixture of *Mimulus* pollen from each of three unrelated donors (mean number of grains per stigma for five subsampled stigmas ± 1 SE = 11 309 ± 709). This pollen load was double the amount deposited by bees during single probes of flowers in our linear arrays. We pollinated treatment flowers with *Lythrum* pollen (mean number of grains for five subsampled stigmas = 950 ± 157, a quantity higher than we observed on stigmas in the previous experiment), followed by a mixture of *Mimulus* pollen from each of three unrelated donors (mean number for the five subsampled stigmas = 10 758 ± 1482). We tagged each pollinated flower and then counted seeds produced by each fruit with a dissecting microscope.

Pollinator constancy and grooming—Because our pollen transfer experiments were specifically designed to encourage pollinator movement between species, we conducted a separate series of pollinator observations to better understand the likelihood for pollen loss under more typical plant spatial arrangements. We made these observations in two-dimensional arrays over three fair-weather days in August 2007. Arrays consisted of 15 *Mimulus* and 15 *Lythrum* plants, regularly spaced and arranged in a checkerboard fashion.

Before each observation day, we stored *Mimulus* and *Lythrum* plants in a pollinator-free screenhouse to ensure that all plants had newly opened, unvisited flowers. We manipulated floral displays of all plants at 0500 hours, before pollinators were active. We trimmed *Mimulus* displays to eight flowers, a number which reflects the size of an intermediate floral display in nature (Karron et al., 2009). We chose not to manipulate *Lythrum* displays so that the number of stems resembled display sizes in nature.

We collected pollinator foraging data by following the first pollinator to enter the array at the start of each observation period and recording the species and sequence of all flowers visited by that pollinator. Pollinator observations began with the first pollinator visit after sunrise until *Mimulus* stigmas had closed (~1300 hours). We recorded pollinator foraging sequences every 10 min for the entire observation day (sunrise to *Mimulus* stigma closure). We used these pollinator foraging sequences to assess pollinator preference and constancy to these species.

We also used pollinator observations from our two-dimensional arrays to understand how grooming by inconstant foragers influences deposition of conspecific pollen on *Mimulus* stigmas. On two of the three observation days, we recorded both the foraging sequences of individual pollinators and the frequency of grooming events during each pollinator's entire foraging bout. A groom was recorded if the pollinator brushed her legs over the top of her head and face or against the sides of her body in a conspicuous manner. The grooms we report occurred either on flowers or during pollinator hovering and crawling between the closely spaced flowers on *Lythrum* spikes. We were not able to include in-flight grooms between plants. Therefore, we likely underestimated the number of grooms between plant species.

Pollen loss to heterospecific flowers—To characterize the loss of *Mimulus* pollen to *Lythrum* stigmas and petals in the two-dimensional arrays, we followed pollinators that probed several *Mimulus* flowers (mean = 6.83 ± 1.87) before foraging on a *Lythrum* spike. Because this collection was destructive to flowers and required us to immediately stop observations to collect floral material, we conducted these trials on different days than our observations of constancy and grooming. Immediately after each pollinator foraged on several *Lythrum* flowers, we removed the first four *Lythrum* flowers probed by the pollinator and placed them together in a single vial with fixative. We replicated this procedure for each of 10 pollinator foraging bouts. We examined collected flowers for evidence of *Mimulus* pollen loss to *Lythrum* stigmas, petals, or sepals. For each of our 10 samples, we excised *Lythrum* stigmas, squashed them, and counted *Mimulus* grains using an epiflourescence microscope. We washed the other floral material in each vial three times with ethanol. After each wash, we spun the used ethanol at 16 700 RCF (relative centrifugal force) for 15 min and resuspended the resulting pellet in 0.1 ml distilled water. We then dried the resuspended pellet onto a glass slide and counted the number of *Mimulus* grains present in each sample under an epifluorescence microscope.

Data analysis—We ran all analyses with the program JMP version 5.1.2 (SAS Institute, 2004) statistical software. We used a *t* test to compare the number of *Mimulus* pollen grains deposited when foragers interrupted a series of *Mimulus* probes with a visit to *Lythrum* vs. the number of pollen grains deposited when foragers only visited *Mimulus*. We also used *t* tests to compare the number of seeds per fruit resulting from these two patterns of pollinator foraging. In addition, we used *t* tests to assess the effect of heterospecific *Lythrum* pollen on *Mimulus* seed production and the number of *Mimulus* flowers probed in inconstant vs. *Mimulus* only runs. We used linear regression to assess the relationship between the number of *Mimulus* grains deposited by pollinators and seed number resulting from these pollen loads. We tested for pollinator preference by comparing the proportion of pollinator visits to *Mimulus* to the null expectation of 0.5 using a one-sample *t* test, with foraging bout as a replicate (Meléndez-Ackerman et al., 1997; Aldridge and Campbell 2007). To determine if pollinators visiting our arrays exhibited floral constancy, we performed *G*-tests of independence to test whether the next plant species visited by foragers was independent of the species last visited. This analysis excluded foraging bouts where the pollinator only occasionally visited a second species (<10%) (Aldridge and Campbell, 2007). Because we did not control for flower number on *Lythrum*, measures of preference and constancy are based on pollinator movements to entire plants, rather than to flowers.

RESULTS

Pollen transfer experiments—*Bombus vagans* workers were the predominant pollinators visiting the linear arrays, accounting for 95% of visitation sequences ($N = 42$). The remaining visitation sequences were by *Bombus impatiens* ($N = 2$). In 45% of the visitation sequences, bees foraged only on *Mimulus* plants. In the remaining 55% of visitation sequences, bees foraged both on *Mimulus* and *Lythrum*. When pollinators moved between *Mimulus* and *Lythrum*, the bright white stripe of *Mimulus* pollen on each bee's face diminished as the bee probed consecutive *Lythrum* flowers (Fig. 2).

Bees foraging only on *Mimulus* probed the same number of *Mimulus* flowers as did bees foraging on both species ($t = -1.02$, df =42, $P = 0.16$). In foraging sequences with only *Mimulus* visits, bees probed a mean of 3.80 ± 0.34 *Mimulus* flowers before probing the target flower. In foraging sequences with heterospecific moves, bees probed a mean of 3.33 ± 0.31 *Mimulus* flowers then probed 7.13 ± 1.54 *Lythrum* flowers before visiting the final *Mimulus* flower used in pollen and seed analysis.

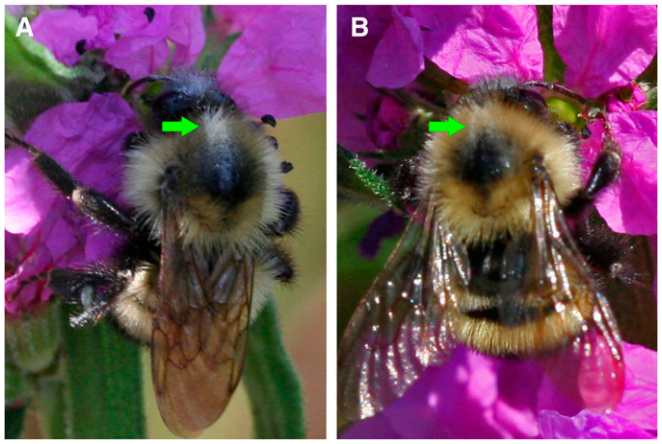

Fig. 2. Sequence of photographs to show that *Mimulus* pollen on a *Bombus vagans* worker diminishes as the bee forages on consecutive *Lythrum* flowers. (A) Worker probing a *Lythrum* flower immediately after visiting several *Mimulus* flowers. Green arrow indicates white stripe of *Mimulus* pollen. (B) The same bee probing the seventh consecutive *Lythrum* flower, 31 s later.

Bees that foraged on both species deposited significantly fewer *Mimulus* pollen grains on conspecific stigmas ($t = -5.08$, df = 38, $P < 0.0001$) (Fig. 3A). When foragers interrupted a series of *Mimulus* probes with a transition to an intervening *Lythrum* competitor, 2952.43 ± 273.63 grains were deposited. By contrast, when pollinators only visited *Mimulus* plants, 5083.29 ± 318.27 grains were deposited. Very little *Lythrum* pollen was deposited on *Mimulus* stigmas (mean = 24.93 ± 5.85).

Mimulus flowers pollinated by bees visiting both species had significantly fewer *Mimulus* seeds per fruit ($t = -4.16$, df = 39, $P < 0.001$) (Fig. 3B). Mean seed number following a transition to an intervening competitor (1551.67 ± 124.19) was 34% lower than mean seed number when bees foraged only on *Mimulus* (2354.59 ± 147.56).

There was a significant positive relationship between conspecific pollen deposition and seed number in *Mimulus* fruits when visitors probed only *Mimulus* flowers (linear regression model $F_{1,12} = 6.94$, $P = 0.02$). The best fitting equation was seeds = 946.47 + 1.8107 × pollen; $r^2 = 0.37$ (Fig. 4A). However, the relationship changed when bumble bees visited an intervening competitor. When *Lythrum* was present, the linear regression of *Mimulus* pollen and *Mimulus* seeds was not significant (model $F_{1,21} = 0.05$, $P = 0.83$; Fig. 4B). Inclusion of the number of *Lythrum* pollen grains on *Mimulus* stigmas in a multiple regression did not improve the fit.

Hand pollinations—Sequential application of *Lythrum* pollen followed by *Mimulus* pollen did not significantly influence *Mimulus* seed set. Control flowers receiving *Mimulus* pollen produced 1369.41 ± 239.09 seeds. Flowers that received *Lythrum* pollen prior to *Mimulus* pollen produced 912.64 ± 263.47 seeds. This difference was not statistically significant ($t = -1.284$, df =29, $P = 0.21$).

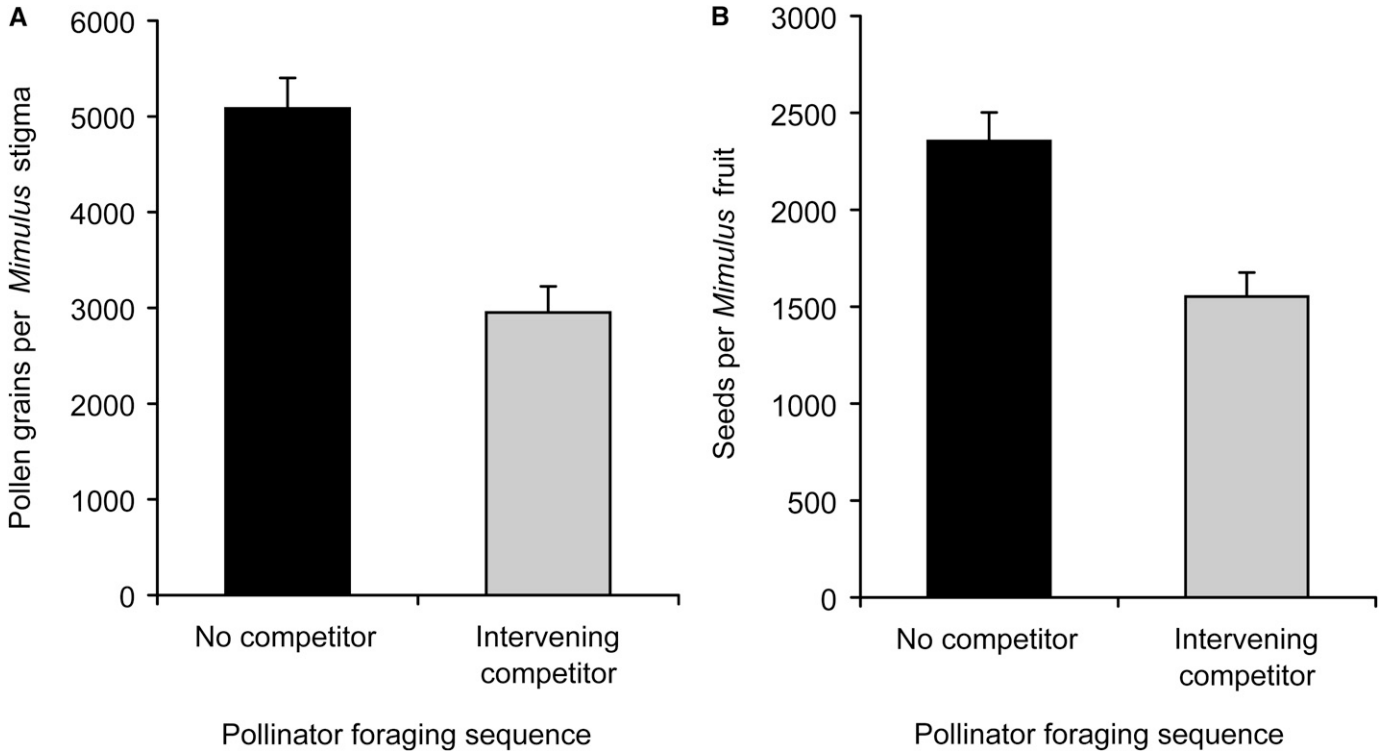

Fig. 3. The effect of pollinator foraging sequence on (A) the mean number of *Mimulus* pollen grains deposited per *Mimulus* stigma and (B) the mean number of *Mimulus* seeds per fruit.

Pollinator constancy and grooming—In two-dimensional arrays, we recorded 66 pollinator foraging sequences by *Bombus vagans* and *B. impatiens*. These two species had similar foraging preferences, so we pooled data across species for analyses of preference and constancy. The proportion of pollinator visits to *Mimulus* and *Lythrum* plants differed significantly from 0.5 ($t = -3.94$, df = 65, $P < 0.001$), with *Lythrum* receiving a higher proportion of visits (68%) than did *Mimulus* (32%). In the 66 pollinator foraging sequences recorded, 38 (58%) were by pollinators that exclusively visited *Lythrum* plants, 12 (18%) were by foragers that exclusively visited *Mimulus,* and 16 (24%) were by foragers that visited both species. When pollinators visited both species within a single foraging bout, the plant species visited was independent of the species visited previously ($G = 2.179$, df = 118; $P = 0.14$).

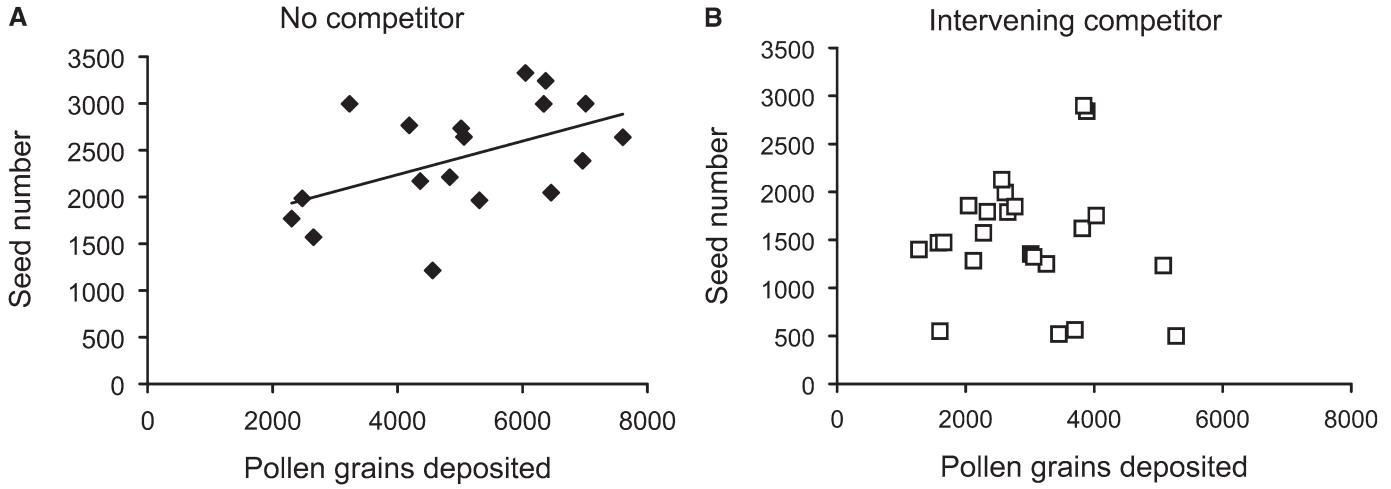

Fig. 4. The relation between the amount of *Mimulus ringens* pollen deposited during a single pollinator probe and the resulting number of *Mimulus* seeds per fruit. (A) No competitor. Pollinators foraged only on *Mimulus* plants and did not visit an intervening *Lythrum* plant. Model $F_{1,12} = 6.94$, $P = 0.02$. The best fitting equation was seeds = 946.41 + 1.8107 × pollen; $r^2 = 0.367$. (B) Intervening competitor. Foraging sequences on *Mimulus* were interrupted by a transition to a *Lythrum* plant. Model $F_{1,21} = 0.05$, not significant.

We collected data on grooming frequency for 45 foraging sequences. In 12 of these foraging bouts, pollinators moved between *Mimulus* and *Lythrum*. These foragers made a total of 30 movements from *Mimulus* to *Lythrum*. During these transitions, foragers visited 2.46 ± 0.26 *Mimulus* flowers and then moved to *Lythrum* and probed 13.92 ± 4.70 flowers. In the field, we noted that as foraging bees crawled up *Lythrum* spikes, they frequently groomed pollen off their heads and bodies. When a pollinator made a transition from *Mimulus* to *Lythrum*, the white stripe of *Mimulus* pollen on the bee's head and thorax visibly diminished with subsequent visits to multiple *Lythrum* flowers, despite the fact that the stripe did not appear to contact the *Lythrum* flowers (Fig. 2). Bumble bees which foraged on *Lythrum* after visiting *Mimulus* typically groomed 0.29 ± 0.05 times per *Lythrum* probe. During this grooming, the white stripe of *Mimulus* pollen on the bee was repeatedly scraped by the front legs, diminishing the pollen available for subsequent deposition on *Mimulus* stigmas.

Pollen loss to heterospecific flowers—A total of 40 *Lythrum* flowers probed during 10 transitions from *Mimulus* to *Lythrum* were analyzed for the presence of *Mimulus* pollen. Most *Lythrum* flowers showed no evidence of *Mimulus* pollen, either on the stigma or on other floral parts. In the few cases in which *Mimulus* pollen was detected, heterospecific pollen transfer was minimal with a mean of 5.35 ± 2.22 *Mimulus* grains on *Lythrum* stigmas. The mean number of *Mimulus* pollen grains found on *Lythrum* flowers was 32.24 ± 4.41.

DISCUSSION

Pollinator movements between *Mimulus* and *Lythrum* plants significantly reduced pollen export from donor flowers, the amount of conspecific pollen deposited on *Mimulus* stigmas, and the number of seeds in *Mimulus* fruits. These findings, in combination with the observation that *Mimulus* pollen carried by bees diminishes during consecutive probes of *Lythrum* flowers (Fig. 2), suggests that pollen loss may explain much of the reduction in *Mimulus* seed set. By contrast, very little *Lythrum* pollen was deposited on *Mimulus* stigmas, making it unlikely that heterospecific pollen clogged the stigmatic surface or blocked germination of *Mimulus* pollen.

Pollen loss—The term "interspecific pollen loss" typically refers to deposition of pollen onto the stigma or petals of competing species (Campbell, 1985; Feinsinger et al., 1988; Feinsinger and Tiebout, 1991; Inouye et al., 1994; Murcia and Feinsinger, 1996; Rademaker et al., 1997). However, substantial pollen losses may also occur during pollinator grooming (Thomson, 1986; Inouye et al., 1994; Harder and Wilson, 1998; Johnson et al., 2005). When grooming occurs during a visit to an interspecific competitor, there is no opportunity for pollen load replenishment. Therefore, when the pollinator returns to the focal species, little conspecific pollen may be available for deposition, leading to lowered seed set for the focal species. This reduced pollen delivery also lowers the siring success of the previous flower visited.

When pollinators foraging on *Mimulus* encountered a *Lythrum* plant in our pollen transfer experiments, they typically probed 7–9 *Lythrum* flowers. We found that ~32 *Mimulus* grains were lost to each *Lythrum* flower; therefore, fewer than 300 grains would be lost to competitor floral structures. By contrast, the first *Mimulus* flower probed following a *Lythrum* visit had ~2100 fewer conspecific grains than *Mimulus* flowers visited prior to the *Lythrum* visit. This finding suggests that only a small fraction (~1/7) of the pollen loss is attributable to deposition on *Lythrum* flowers.

In bumblebee-pollinated systems, very little pollen removed from one flower reaches the stigma of the next flower visited (Harder and Wilson, 1998; Harder and Thomson, 1989; Inouye et al., 1994; Johnson et al., 2005). Pollen may be removed from the pollinator's body passively by wind, movement, or contact with flowers or incompatible stigmas (Inouye et al., 1994; Murcia and Feinsinger, 1996; Rademaker et al., 1997; Johnson et al., 2005). A visit to a competitor plant may exacerbate such transport-related losses by allowing more opportunity for both passive loss and losses due to pollinator grooming. Our results suggest that 6/7ths of *Mimulus* pollen lost is due to such transport-related losses during interspecific foraging. Such transport-related losses may be especially likely on competitor plants with very large floral displays such as *Lythrum*, which may encourage pollinators to spend a long time and visit many flowers before moving to another plant.

Mimulus was less preferred by pollinators and received a lower proportion of pollinator visits in our arrays. Although a favored species may receive higher quality visits as a result of pollinator preference, the opposite may be true for a less-favored species. Very few bees visited *Mimulus* exclusively—in fact, more than half of the foragers that visited *Mimulus* also visited *Lythrum*. In addition, foragers that visited both species showed no pattern of floral constancy. The species pollinators visited was independent of the species previously visited in a foraging bout. Taken together, these results suggest that interspecific foraging movements are not uncommon with respect to overall *Mimulus* visitation and that interspecific pollen transfer may be likely to occur between *Mimulus* and *Lythrum*.

Stigma pollen loads and seed set—When pollinators foraged only on *Mimulus*, there was a significant positive relationship between stigma pollen load and the number of seeds per fruit (Fig. 4A). Interestingly, the positive relationship between stigma pollen load and seed set did not hold when foraging sequences on *Mimulus* were interrupted by a transition to *Lythrum* (Fig. 4B). This result was surprising and suggests some sort of postpollination mechanism limiting seed production as a result of interspecific foraging. One possible cause may relate to differences in the precision of pollen placement on *Mimulus* stigmas, such that pollinators leaving a *Lythrum* plant may deposit pollen in less suitable locations on *Mimulus* stigmas, perhaps because the foraging posture of the bee changed (see Heinrich, 1979). However, quantification of *Mimulus* pollen loads on stigmas was destructive so we were not able to evaluate this hypothesis. The lack of a relationship could also be spurious, due to high variability in seed production and/or due to a relatively low sample size.

Heterospecific pollen on Mimulus ***stigmas***—Other studies have shown that inconstant foragers may deposit foreign pollen onto a focal plant's stigma, which may clog the stigma or block germination of conspecific pollen (Waser, 1978; Waser and Fugate, 1986; Galen and Gregory, 1989; Randall and Hilu, 1990; Caruso and Alfaro, 2000; Brown and Mitchell, 2001). However, even if interspecific movements occur, in many cases heterospecific pollen deposition on stigmas is minimal, with little influence on seed production (Campbell and Motten, 1985;

Murcia and Feinsinger, 1996; Jakobsson et al., 2008). Although *Lythrum* pollen grains can fit among *Mimulus* papillae and in some cases germinate (Fig. 1B), the mean number of *Lythrum* grains transferred to *Mimulus* stigmas was very low (25). In our hand cross experiments, we applied much more *Lythrum* pollen than is typically found in the field but still observed no significant influence on *Mimulus* seed production.

An unexpected result of these hand cross experiments is that flowers which were hand-crossed with *Mimulus* pollen set fewer seeds per fruit than did flowers which received a single bumble bee visit, despite the fact that we applied much more *Mimulus* pollen on stigmas than these bees deposited. We believe this result may reflect differences in pollen placement by bumble bees and hand pollination, and that seed set in *Mimulus* may depend on location of pollen placement.

Conclusion—We found that inconstant pollinator movements significantly reduced the amount of pollen exported by donor flowers and significantly lowered *Mimulus* seed production. Although pollinators deposited small amounts of *Lythrum* pollen on *Mimulus* stigmas, such deposition did not appear to influence reproductive success. Instead, the reduction in seed set appears to be due to pollen loss, which most likely resulted from pollinator grooming as bees foraged on *Lythrum* flowers. To our knowledge, earlier studies of interspecific pollen loss have not explicitly addressed pollen loss due to grooming. However, our novel findings suggest that grooming loss may have a much greater impact on patterns of pollen delivery than more traditional types of interspecific pollen loss. Such grooming losses may even occur when competitor flowers make no contact with pollen on the vector's body. This scenario has received little consideration in the context of competition for pollination and may be important to a full understanding of the influence of coflowering competitors on patterns of pollen delivery and plant reproductive success.

LITERATURE CITED

AGREN, J. 1996. Population size, pollinator limitation, and seed set in the self-incompatible herb *Lythrum salicaria. Ecology* 77: 1779–1790.

ALDRIDGE, G., AND D. R. CAMPBELL. 2007. Variation in pollinator preference between two *Ipomopsis* contact sites that differ in hybridization rate. *Evolution* 61: 99–110.

BELL, J. M., J. D. KARRON, AND R. J. MITCHELL. 2005. Interspecific competition for pollination lowers seed production and outcrossing rate in *Mimulus ringens. Ecology* 86: 762–771.

BLOSSEY, B. L., C. SKINNER, AND J. TAYLOR. 2001. Impact and management of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation* 10: 1787–1807.

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. Pollinator sharing and seed set of *Stellaria pubera*: Competition for pollination. *Ecology* 66: 544–563.

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., AND M. ALFARO. 2000. Interspecific pollen transfer as a mechanism of competition: effect of *Castilleja linariaefolia* pollen on seed set of *Ipomopsis aggregata. Canadian Journal of Botany* 78: 600–606.

DARWIN, C. 1877. The different forms of flowers on plants of the same species. D. Appleton, New York, New York, USA.

FEINSINGER, P., W. H. BUSBY, AND H. M. TIEBOUT III. 1988. Effects of indiscriminate foraging by tropical hummingbirds on pollination and plant reproductive success: Experiments with two tropical treelets (Rubiaceae). *Oecologia* 76: 471–474.

FEINSINGER, P., AND H. M. TIEBOUT III. 1991. Competition among plants sharing hummingbird pollinators: Laboratory experiments on a mechanism. *Ecology* 72: 1946–1952.

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.

GEGEAR, R. J., AND T. M. LAVERTY. 2005. Flower constancy in bumblebees: A test of the trait variability hypothesis. *Animal Behaviour* 69: 939–949.

GRANT, A. L. 1924. A monograph of the genus *Mimulus. Annals of the Missouri Botanical Garden* 11: 99–389.

HAGER, H., AND K. D. MCCOY. 1998. The implications of accepting untested hypotheses: A review of the effects of purple loosestrife (*Lythrum salicaria*) in North America. *Biodiversity and Conservation* 7: 1069–1079.

HARDER, L. D., AND M. B. ROUTLEY. 2006. Pollen and ovule fates and reproductive performance by flowering plants. *In* L. D. Harder and S. C. H. Barrett [eds.], Ecology and evolution of flowers, 61–80. Oxford University Press, New York, New York, USA.

HARDER, L. D., AND J. D. THOMSON. 1989. Evolutionary options for maximizing pollen dispersal of animal-pollinated plants. *American Naturalist* 133: 323–344.

HARDER, L. D., AND W. G. WILSON. 1998. Theoretical consequences of heterogeneous transport conditions for pollen dispersal by animals. *Ecology* 79: 2789–2807.

HEINRICH, B. 1979. "Majoring" and "minoring" by foraging bumblebees, *Bombus vagans*: An experimental analysis. *Ecology* 60: 245–255.

HUSBAND, B. C., AND S. C. H. BARRETT. 1992. Pollinator visitation in populations of tristylous *Eichhornia paniculata* in northeastern Brazil. *Oecologia* 89: 365–371.

INOUYE, D. W., D. E. GILL, M. R. DUDASH, AND C. B. FENSTER. 1994. A model and lexicon for pollen fate. *American Journal of Botany* 81: 1517–1530.

JAKOBSSON, A., B. PADRÓN, AND A. TRAVESET. 2008. Pollen transfer from invasive *Carpobrotus* spp. to natives—A study of pollinator behaviour and reproduction success. *Biological Conservation* 141: 136–145.

JOHNSON, S. D., P. R. NEAL, AND L. D. HARDER. 2005. Pollen fates and the limits on male reproductive success in an orchid population. *Biological Journal of the Linnean Society* 86: 175–190.

KARRON, J. D., K. G. HOLMQUIST, R. J. FLANAGAN, AND R. J. MITCHELL. 2009. Pollinator visitation patterns strongly influence among-flower variation in selfing rate. *Annals of Botany 103: in press, AoB*Preview*:* doi:10.1093/aob/mcp030.

KARRON, J. D., R. J. MITCHELL, AND J. M. BELL. 2006. Multiple pollinator visits to *Mimulus ringens* (Phrymaceae) flowers increase mate number and seed set within fruits. *American Journal of Botany* 93: 1306–1312.

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. 1995a. 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. 1995b. Comparison of pollinator flight movements and gene dispersal patterns in *Mimulus ringens. Heredity* 75: 612–617.

KEARNS, C. A., AND D. W. INOUYE. 1993. Techniques for pollination biologists. University Press of Colorado, Niwot, Colorado, USA.

KOHN, J. R., AND N. M. WASER. 1985. The effect of *Delphinium nelsonii* pollen on seed set in *Ipomopsis aggregata*, a competitor for hummingbird pollination. *American Journal of Botany* 72: 1144–1148.

LEEBENS-MACK, J., AND B. G. MILLIGAN. 1998. Pollination biology in hybridizing *Baptisia* (Fabaceae). *American Journal of Botany* 85: 500–507.

LEVIN, D. A., AND W. W. ANDERSON. 1970. Competition for pollinators between simultaneously flowering species. *American Naturalist* 104: 455–467.

MAL, T. K., AND B. P. HERMANN. 2000. Quantitative evaluation of pollen polymorphism in a tristylous weed, *Lythrum salicaria* (Lythraceae). *Canadian Journal of Botany* 78: 1086–1094.

MAL, T. K., J. LOVETT-DOUST, L. LOVETT-DOUST, AND G. A. MULLIGAN. 1992. The biology of Canadian weeds. 100. *Lythrum salicaria. Canadian Journal of Plant Science* 72: 1305–1330.

MELÉNDEZ-ACKERMAN, E., D. R. CAMPBELL, AND N. M. WASER. 1997. Hummingbird behavior and mechanisms of selection on flower color in *Ipomopsis. Ecology* 78: 2532–2541.

MITCHELL, R. J., R. J. FLANAGAN, B. J. BROWN, N. M. WASER, AND J. D. KARRON. 2009. New frontiers in competition for pollination. *Annals of Botany* 103: In press, Annals of Botany: doi:10.1093/aob/mcp062.

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: 885–890.

MORALES, C. L., AND A. TRAVESET. 2008. Interspecific pollen transfer: Magnitude, prevalence, and consequences for plant fitness. *Critical Reviews in Plant Sciences* 27: 221–238.

MUCHHALA, N., AND M. D. POTTS. 2007. Character displacement among bat-pollinated flowers of the genus Burmeistera: Analysis of mechanism, process, and pattern. *Proceedings of the Royal Society of London, B, Biological Sciences* 274: 2731–2737.

MURCIA, C., AND P. FEINSINGER. 1996. Interspecific pollen loss by hummingbirds visiting flower mixtures: Effects of floral architecture. *Ecology* 77: 550–560.

RADEMAKER, M. C. J., T. J. DE JONG, AND P. G. L. KLINKHAMER. 1997. Pollen dynamics of bumblebee visitation on *Echium vulgare. Functional Ecology* 11: 554–563.

RANDALL, J. L., AND K. W. HILU. 1990. Interference through improper pollen transfer in mixed stands of *Impatiens capensis* and *I. pallida* (Balsaminaceae). *American Journal of Botany* 77: 939–944.

SAS INSTITUTE. 2004. JMP version 5.1.2, statistical software. SAS Institute, Cary, North Carolina, USA.

SHORE, J. S., AND S. C. H. BARRETT. 1984. The effect of pollination intensity and incompatible pollen on seed set in *Turnera ulmifolia* (Turneraceae). *Canadian Journal of Botany* 62: 1298–1303.

STONE, G. N., P. WILLMER, AND J. A. ROWE. 1998. Partitioning of pollinators during flowering in an African *Acacia* community. *Ecology* 79: 2808–2827.

THOMSON, J. D. 1986. Pollen transport and deposition by bumble bees in *Erythronium*: Influences of floral nectar and bee grooming. *Journal of Ecology* 74: 329–341.

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* C. E. Jones and R. J. Little [eds.], Handbook of experimental pollination biology, 277–293. Van Nostrand Reinhold, New York, New York, USA.

WASER, N. M. 1983b. The adaptive nature of floral traits: Ideas and evidence. *In* L. A. Real, [ed.], Pollination biology, 241–285. Academic Press, New York, New York, USA.

WASER, N. M. 1986. Floral constancy: Definition, cause and measurement. *American Naturalist* 127: 593–603.

WASER, N. M., AND M. L. FUGATE. 1986. Pollen precedence and stigma closure: A mechanism of competition for pollination between *Delphinium nelsonii* and *Ipomopsis aggregata. Oecologia* 70: 573–577.