

# The influence of *Mimulus ringens* floral display size on pollinator visitation patterns

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## Summary

1. Pollinators visiting large floral displays may probe several flowers in sequence, leading to geitonogamous (among-flower) self-pollination. To investigate the relationship between floral display size and patterns of pollinator movement, we studied foraging by several pollinator species in four replicate arrays of *Mimulus ringens* (Scrophulariaceae). In each array displays were trimmed to two, four, eight and 16 flowers per plant.

2. Bees preferred large displays, and probed more flowers in sequence on large than on small displays. However, the proportion of available flowers probed decreased with display, resulting in nearly equal floral visitation rates across treatments.

3. Because pollinators probed more flowers in sequence on large displays, plants with numerous flowers should experience more geitonogamous self-pollination than plants with small displays.

4. In all four treatments, pollinators frequently visited only one flower before leaving the plant. As the first flower probed on a plant cannot receive geitonogamous pollen, this potentially reduces selfing rates for those flowers, compared to flowers probed late in a long visitation sequence on a plant. Such differences among flowers in pollination history should increase variation in geitonogamous self-pollination among fruits within plants.

5. The three most abundant pollinator species differed significantly in behaviours that could influence plant mating patterns, including number of flowers probed per plant; interplant movement distances; and grooming. Variation in foraging patterns was also evident among individuals within species. These subtle differences in response should affect the pollination services provided to plants.

*Key-words:* *Bombus*, inflorescence size, monkey flower, pollination, pollinator foraging

*Functional Ecology* (2004) **18**, 116–124

## Introduction

Plants with large floral displays may attract more pollinators than plants with few open flowers (Schmid-Hempel & Speiser 1988; Klinkhamer & de Jong 1990; Eckhart 1991; Mitchell 1994; Galloway *et al.* 2002). Once at a plant with a large display, visitors are likely to probe more flowers in sequence (Klinkhamer *et al.* 1989; Barrett *et al.* 1994; Ohara & Higashi 1994; Harder & Barrett 1995; Robertson & Macnair 1995; Snow *et al.* 1996; Goulson *et al.* 1998; Vrieling *et al.* 1999), but may probe a decreasing proportion of the flowers in the display (Klinkhamer *et al.* 1989; Robertson 1992; Harder & Barrett 1995; Robertson & Macnair 1995). The result of these countervailing factors is that per flower visitation rates may be unaffected by floral display (Kadmon

1992; Robertson & Macnair 1995; Goulson *et al.* 1998; Vrieling *et al.* 1999).

These changes in pollinator foraging patterns can have important effects on plant mating. As floral display size increases, the tendency of pollinators to visit more flowers in sequence on a single plant increases the opportunity for geitonogamous self-pollination (movement of pollen among flowers within plants; Dudash 1991; de Jong *et al.* 1993; Harder & Barrett 1995; Snow *et al.* 1996). Changes in pollinator flight distance in response to floral display (Pyke 1978; Ohashi & Yahara 1998) may also influence plant mating patterns (Karron *et al.* 1995a).

Although most studies of pollinator response to floral display do not distinguish among pollinator taxa, species-specific responses were detectable in those few that have done so (Goulson *et al.* 1998). Differences in pollinator response can have important ecological and evolutionary consequences, including changes in

mating patterns and altered selection on floral traits (Pettersson 1991; Wilson & Thomson 1991). A full understanding of the interaction requires recognition of those differences among pollinators, and an important goal of our work is to quantify this variation.

The simplest approach to studying response to floral display is to document how visitation patterns differ for plants that vary naturally in display. Unfortunately, although such observational studies provide valuable information on pollinator responses, they do not control for effects of variables that are frequently correlated with floral display, such as population density or plant health. Although various statistical techniques can be used to take account of some correlated traits (e.g. Mitchell 1994), directly controlling for these confounding variables by experimental manipulation of floral display is a much more powerful approach (Schmid-Hempel & Speiser 1988; Harder & Barrett 1995; Brody & Mitchell 1997). Nonetheless, studies manipulating size of floral display have rarely controlled other factors that may influence patterns of pollinator visitation, such as plant vigour, plant spacing, floral morphology, and pollen and nectar production. To control for the effects of these variables on patterns of pollinator visitation, we manipulated floral display in replicate arrays with uniform spacing and a common genetic background.

To better understand how several species of bumble bee respond to flower number, we manipulated floral displays of a wild plant species. Using these data we address the following questions: (1) How does floral display size influence the number of flowers probed sequentially on a plant? (2) Do pollinators prefer plants with large floral displays? (3) Do closely related pollinator species differ in their behaviour and in their response to floral display? We also report new findings about the pollination biology of this species, discovered while answering these questions.

## Methods

*Mimulus ringens* L. (Scrophulariaceae) is a perennial herb native to wetlands of central and eastern North America. Our observations over the past 10 years indicate the following details of its natural history in Wisconsin and Illinois. Populations tend to be small, typically with fewer than 50 individuals. Daily floral display size varies from one to more than 15 flowers per plant. Flowers are produced in pairs in the nodes of opposite leaves, and the paired flowers often open on different days. The large and showy purple hermaphroditic flowers open and anthers dehisce during the night, and corollas fall by mid-afternoon (<14 h after opening; unpublished data), so that all open flowers on a plant are the same age. Flowers are also scattered across several separate stems, so there is no discernible spatial gradient in nectar or pollen rewards. Nectar contents of flowers at 6 am are small (mean  $\pm$  SE =  $0.73 \pm 0.08 \mu\text{l}$ ; concentration =  $26\% \pm 1\%$  sucrose

equivalents wt/wt;  $N = 32$  flowers on 16 genets), and almost no nectar is secreted after that time.

Visitation to *M. ringens* is primarily by worker bumble bees (*Bombus* sp.; Karron *et al.* 1995a, 1995b). When handling flowers, both pollen and nectar-foraging bees first contact the stigma, then the anthers, with their faces. Pollinator activity is highest in the morning, and decreases around midday when nectar and pollen standing crops are depleted. The stigmas of *M. ringens* are plate-like and bi-lobed, and close slowly (15–90 min after pollination).

*Mimulus ringens* is self-compatible and has a mixed-mating system that varies considerably within and among populations, due in part to effects of plant population density on bee visitation patterns (Karron *et al.* 1995b, 1997). This species also reproduces vegetatively by stoloniferous rhizomes (Karron *et al.* 1995b), allowing the use of different ramets of the same group of genets in each of the experimental gardens (see below).

This study was conducted at the University of Wisconsin-Milwaukee Field Station (Saukville, Wisconsin, USA; N 43°23'29.4", W 88°01'25.0") in the summer of 2000. On 7 June we planted four replicate arrays of 36 individual cloned plants in four isolated gardens. Each garden was separated from all others by at least 75 m of vegetation that included a high abundance of unrelated bumble bee-pollinated species.

The 36 ramets in each array were planted on a regular grid with 0.8 m spacing. Each array included 16 different genets that differed significantly in floral morphology and reproductive characters (unpublished data). The 15 central plants in each array were single ramets of each of 15 different genets. These core 15 genets were surrounded with a buffer row of 21 ramets of the 16th genet. This design was implemented for a related study of mating systems and patterns of paternity (Karron *et al.* 2004). In each array, the central 15 ramets were arranged in a different random order. All ramets in each array, including the buffer row, were observed and manipulated.

## FLORAL DISPLAY TREATMENTS

To determine the effects of daily floral display on pollinator behaviour (and plant mating patterns), display sizes in all four arrays were manipulated on each of four separate days in August 2000. Excess flowers on each plant were removed with scissors in the early morning (5:00 am local time) before pollinators became active at  $\approx$  6:30 am. These treatments were imposed on 4 days during peak bloom (9, 11, 13 and 15 August, hereafter referred to as days 1, 2, 3 and 4, respectively). During this time, plants in our arrays consistently produced 25 or more new flowers every day (these plants grew larger than those in natural populations). Flowers were removed by thinning randomly across the plant, which generated displays that matched the general appearance of natural displays. On each treatment day in all four arrays, floral displays for all plants were

trimmed to one of four floral display sizes. These treatments (two, four, eight or 16 flowers) spanned the range typically observed in field populations of *M. ringens*. A regular spatial arrangement of floral display sizes was used to ensure every genet was surrounded by two plants with each of the four floral display sizes. By rotating flower number treatments in the four arrays among genets in a Latin square design, each genet experienced all four floral display sizes on each day, and each ramet experienced all display sizes over the 4 days. Because traplining bees may exhibit visitation patterns related to the previous day's floral display (Thomson 1988, 1999; Thomson & Chittka 2001), each treatment day was preceded with an acclimation day, during which plants were trimmed to the floral display for the following day's treatment, but pollinator behaviour was not recorded. Note that floral rewards (pollen and nectar production per flower) should be independent of floral display because of the random assignment and temporal rotation of floral display treatments among genets.

#### POLLINATOR OBSERVATION

On each treatment day, pollinator observations began immediately after floral manipulations. Two teams, each with two to four observers, recorded patterns of pollinator visitation in all four arrays. During each 20 minute observation period the exact sequence of floral probes and plant visits was recorded, noting each visited plant's identity and spatial position, and the number of flowers probed on that plant. Individual bees were followed for as long as possible. Each team was responsible for observing two of the four arrays. After the team randomly chose the array in which to begin observations, it alternated between the two arrays until pollinator activity declined, and >90% of stigmas were closed (determined using approximately hourly non-destructive observations of 16–32 flowers in each array; see Results). Because most stigmas were closed by the time observations ended, all effective pollinator visits occurred during the first 4–6 h of observation. This limited pollination window allowed a fairly complete record of visitation to flowers – we estimate that >40% of all effective floral probes in the arrays were observed and recorded (arrays were observed for half of the pollination window, but probably slightly less than half of all probes were recorded because of observer travel time between gardens, and a few times when several bees were active at once).

From these data, pollinator species composition; pollinator visitation rate to flowers and to plants; number of flowers probed per plant; distance travelled between plants; and frequency of among-plant movements were determined.

#### DATA ANALYSIS

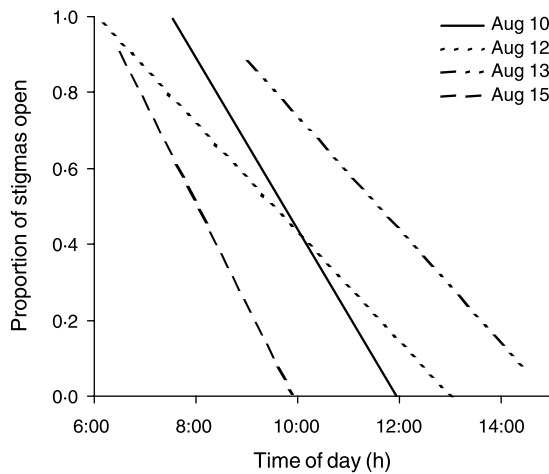
Many response variables (e.g. number of consecutively probed flowers on a plant) were small integer counts

(e.g. 1–16), violating an assumption of ANOVA. These variables were analysed using a generalized linear model (PROC GENMOD of SAS 8.2; SAS Institute 2000) with Poisson errors, a log-link function and type III significance tests (Crawley 1993; Agresti 1996). Examination of diagnostics (e.g. deviance : df ratios) indicated that the data met assumptions of this analysis. Patterns of significance from GENMOD analyses closely matched those from analogous ANOVA (unpublished results). Proportions (e.g. proportion of flowers probed) were analysed using logit modelling under PROC GENMOD (binomial errors, logit link; Agresti 1996). For response variables that fit the assumptions of standard ANOVA (e.g. visitation rate per plant and per flower), fixed-effects ANOVA or ANCOVA with type III (simultaneous) sums of squares (PROC GLM; SAS Institute 2000) was used. In all analyses floral display treatment was considered a categorical variable. To test for effects of floral display on movement patterns, PROC FREQ (SAS Institute 2000) was used for likelihood ratio  $\chi^2$  analysis (see also Karron *et al.* 1995a,b).

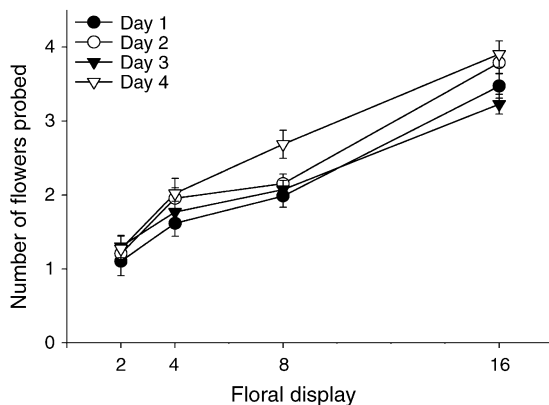
#### Results

A total of 255 bee foraging bouts and 4402 floral probes were observed during >30 h of observations. *Bombus fervidus* (Fabricius) was by far the most common floral visitor, accounting for 80.5% of floral probes (69.8% of foraging bouts). *Bombus impatiens* Cresson (7.9% of probes, 15.7% of bouts) and *Bombus griseocollis* (Degeer) (7.6% of probes, 9.4% of bouts) were regular but much less common visitors. The remainder (<5%) of all probes and bouts was by *Bombus nevadensis* Cresson, *Bombus bimaculatus* Cresson, and other insects. For reasons of sample size, in the analyses of interspecific differences in patterns of visitation presented below, only the three most common species (*B. fervidus*, *B. impatiens*, *B. griseocollis*) were compared. There were small but significant differences among gardens in pollinator species composition based on the number of bee foraging bouts (likelihood ratio  $\chi^2_3 = 19$ ,  $P < 0.03$ ), but *B. fervidus* was the most common visitor in all gardens. By contrast, differences in pollinator species composition among days were not significant (likelihood ratio  $\chi^2_3 = 8.3$ ,  $P > 0.4$ ).

Availability of open stigmas declined sharply and significantly over the day (Fig. 1), paralleling changes in availability of pollen and nectar (R.J.M., personal observation). Most stigmas were closed before noon, restricting the period of effective pollinator visitation to the morning hours. ANCOVA revealed a significant effect of time of day ( $F_{1,25} = 91$ ,  $P < 0.0001$ ). Day and interaction effects were not significant ( $P = 0.3$ ,  $0.06$ , respectively). Note that there was a morning rainstorm on day 3 (August 13) which delayed both pollinator activity and stigma closure. *Bombus fervidus* activity on *M. ringens* usually peaked in mid- to late morning, just before stigmas closed and pollen supplies dwindled.



**Fig. 1.** Temporal decline in proportion of stigmas open to receive pollen. Each line represents a linear regression plot for a single date, and extends from the time of the first sampling period to that of the last. The number of sampling periods and  $r^2$  for each date are: August 10 (eight, 0.90); August 12 (three, 0.98); August 13 (13, 0.67); August 15 (nine, 0.91). Pollinators were observed on 9, 11, 13 and 15 August. Stigma closure was not recorded on 9 and 11 August because of logistic constraints; instead data collected 1 day after each of those days, taken in the same fashion, are presented.



**Fig. 2.** Effect of floral display on number of consecutively probed flowers on a plant. Data are shown for each of four separate days. Values are back-transformed from log-linked LS means  $\pm 1$  SE from a generalized linear model analysis (Table 1).

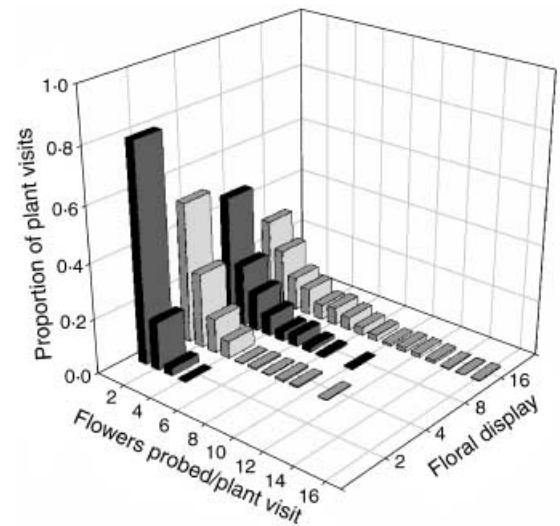
In contrast, visitation by *B. griseocollis* and *B. impatiens* was generally highest before 8 am. Indeed, in the early morning the combined number of bee bouts for *B. griseocollis* and *B. impatiens* was often greater than that for *B. fervidus*, as was total probes  $\times$  flower $^{-1} \times$  h $^{-1}$  (unpublished analyses).

Bees responded strongly to floral display treatments (Fig. 2; Table 1), increasing the number of consecutive intraplant (geitonogamous) movements on plants with large floral displays. This response was significant across gardens and across days (NS interaction of display  $\times$  garden or display  $\times$  day). However, the significant three-way interaction indicates that the response to floral display varied among day–garden combinations.

**Table 1.** Generalized linear model analysis of number of consecutively probed flowers on a plant (Poisson errors, log link)

Source	df	$\chi^2$	<i>P</i>
Garden	3	4.8	0.19
Day	3	9.6	<b>0.023</b>
Display	3	495.1	<b>&lt;0.0001</b>
Garden $\times$ day	9	17.3	<b>0.0443</b>
Garden $\times$ display	9	4.2	0.89
Day $\times$ display	9	7.4	0.60
Garden $\times$ day $\times$ display	27	42.1	0.032

Deviance = 2077, 1668 df. Significant values are in bold type.

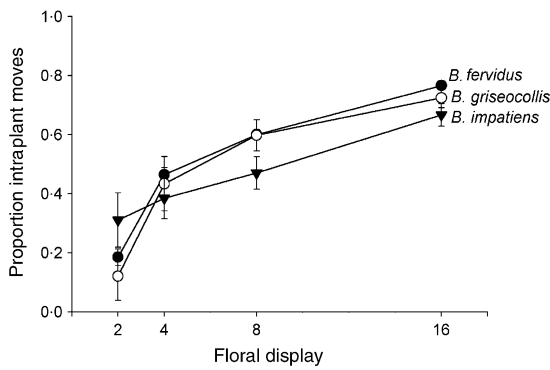


**Fig. 3.** Distribution of number of consecutively probed flowers as a function of floral display. *N* plant visits contributing to the total for two-, four-, eight- and 16-flowered displays are, respectively, 239, 335, 478 and 680. The 16 probes per plant visit category also includes the one occasion when a bee probed more than 16 consecutive flowers on a single ramet.

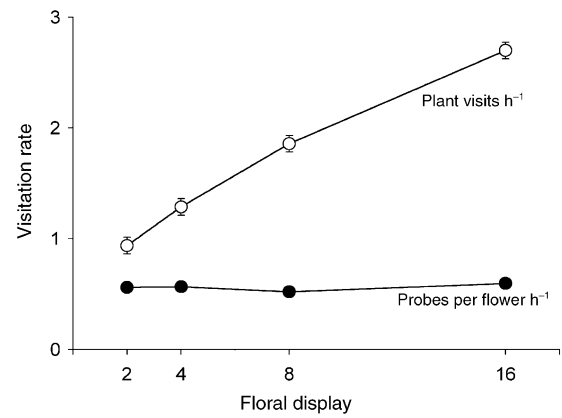
These differences, although significant, were generally small (usually within 0.3 probes of the means shown in Fig. 2), and do not change the general conclusion that number of floral probes increases with floral display.

For all floral display treatments, bees most frequently probed one flower and then left the plant (Fig. 3). Over 79% of bee visits to two-flowered plants ended after a single floral probe, so that those plants seldom experienced intraplant (potentially geitonogamous) pollinator moves. Even for the 16-flowered treatment, over 30% of plant visits ended after a single probe. Supplemental analyses (unpublished; analogous to those in Table 1) revealed no difference in number of consecutively probed flowers as a function of genet ( $P > 0.4$ ), time of day ( $P > 0.1$ ), and plant location (on the border vs interior of the garden  $P > 0.6$ ).

Bee species differed significantly in their responses to floral display, as measured by proportion of inter-flower movements that were intraplant (Fig. 4; Table 2). In particular, *B. impatiens* was less responsive to



**Fig. 4.** Effect of floral display on intraplant moves by the three most abundant pollinator species. Values are proportion of interflower moves that are intraplant, back-transformed from LS mean logit values  $\pm 1$  SE.  $N$  for each point = 123–332 for *Bombus fervidus*; 14–34 for *B. griseocollis*; 20–48 for *B. impatiens*. SE for *B. fervidus* is smaller than the symbol.



**Fig. 5.** Per flower and per plant visitation rate as a function of floral display treatment (LS means  $\pm$  SE; in some cases SE is smaller than the symbol).  $N = 144$  garden–display–day combinations for each point.

**Table 2.** Generalized linear model analysis (PROC GENMOD; binomial errors, logit link) of frequency of movements between flowers that are intraplant vs interplant

Source	df	$\chi^2$	$P$
Pollinator species	3	10.3	<b>0.0058</b>
Floral display	2	438.9	<0.0001

Deviance = 4761 with 3980 df. The interaction was not significant ( $P = 0.3$ ), and deleting it from the model did not significantly reduce log likelihood ( $\chi^2 = 6$ ,  $df = 3.4$ ,  $P > 0.05$ ). Significant values are in bold type.

display than the other species, tending to probe fewer flowers on large displays than did *B. fervidus* and *B. griseocollis*.

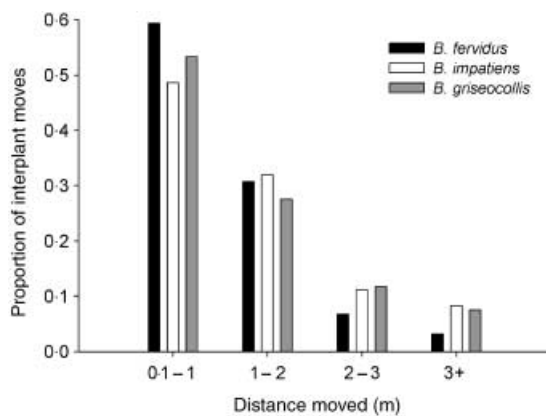
Plants with large floral displays were preferred by bees, as indicated by the steady increase in plant visits per h with floral display (Fig. 5). This significant increase in plant visitation rate (Table 3) is not directly in proportion to floral display (display increases by a factor of 8, while plant visitation increases only by a

factor of 2.9). When combined with the concurrent (and less than proportionate) increase in flowers probed per plant visit (Fig. 2; three times more), the outcome is that flowers on plants with different displays receive nearly equal per flower visitation rates (probes  $\times$  flower<sup>-1</sup>  $\times$  h<sup>-1</sup>, Fig. 5; NS effect of display in Table 3). For both plant and flower visitation rates, the significant interaction of garden and day indicates that overall differences among gardens varied from day to day. The lack of significant two-way interactions involving floral display indicates that response to display was broadly consistent across days and gardens. However, for plant visits per hour the significant three-way interaction reflects minor differences in the effect of display across day–garden combinations. This is because plant visits increased monotonically with display in all day–garden combinations, but with slightly different slopes (unpublished results). This interaction does not change our general conclusion that plants with larger displays were visited more often. These measures slightly underestimate visitation rates (by <10%) because of the few time periods when only one of two bees active in a plot

**Table 3.** ANOVA for per flower visitation rate (probes  $\times$  flower<sup>-1</sup>  $\times$  h<sup>-1</sup>) and plant visitation rate (plant visits per h)

Source	df	Probes $\times$ flower <sup>-1</sup> $\times$ h <sup>-1</sup>			Plant visits per h		
		MS	$F$	$P$	MS	$F$	$P$
Garden	3	2.391	19.2	<b>&lt;0.0001</b>	14.174	17.7	<b>&lt;0.0001</b>
Day	3	1.068	8.5	<b>&lt;0.0001</b>	6.828	8.5	<b>&lt;0.0001</b>
Display	3	0.135	1.1	0.4	83.790	104.7	<b>&lt;0.0001</b>
Garden $\times$ day	9	0.331	2.6	<b>0.006</b>	3.435	4.3	<b>&lt;0.0001</b>
Garden $\times$ display	9	0.072	0.6	0.8	0.697	0.8	0.6
Day $\times$ display	9	0.073	0.6	0.8	0.626	0.7	0.6
Garden $\times$ day $\times$ display	27	0.154	1.2	0.2	1.221	1.5	<b>0.045</b>
Error	512	0.123			0.800		

Plant–day combinations are used as the unit of observation ( $N = 576$ ). Significant values are in bold type.



**Fig. 6.** Interplant movement patterns for the three major visitors to *Mimulus ringens*. Movement patterns are significantly different among species (likelihood ratio  $\chi^2 = 19.3$ ,  $df = 6$ ,  $P < 0.004$ ). Nearest neighbour plants were 0.8 m apart. Total  $N$  moves for *Bombus fervidus* = 1178; *B. impatiens* = 144; *B. griseocollis* = 120. Mean  $\pm$  SD movement distances for *B. fervidus* =  $1.18 \pm 0.68$  m; *B. griseocollis* =  $1.35 \pm 0.82$  m; *B. impatiens* =  $1.44 \pm 0.90$  m; median  $\pm$  interquartile ranges are, respectively,  $0.8 \pm 0.33$ ,  $0.80 \pm 0.80$ ,  $1.13 \pm 0.99$  m.

could be followed. Unlike the case for intraplant moves (Fig. 4), plant visits per h, or probes  $\times$  flower $^{-1} \times$  h $^{-1}$  did not differ significantly among bee species (analyses not shown; see Fig. 5).

There was also no evidence that visitation rates (per flower or per plant) varied among genets ( $P > 0.44$ ), or with flower attributes such as floral morphology (corolla width, corolla height, floral tube length, floral tube width), pollen production, or nectar production rate (analyses not shown; in all cases  $P > 0.13$  in multiple regressions including each trait and floral display as predictors;  $N = 256$ ). Visitation rates (plant visits per h and probes  $\times$  flower $^{-1} \times$  h $^{-1}$ ) did not differ significantly among plants on the border vs interior of the garden ( $P > 0.29$ ; analysis similar to Table 3).

More than half of all interplant movements by bees were between nearest neighbours (0.8 m), and these movements showed the same leptokurtic pattern exhibited by most flower visitors (Fig. 6; Waser 1982). Interplant movement distances differed significantly but subtly among bee species (likelihood ratio  $\chi^2 = 19.3$ ,  $df = 6$ ,  $P < 0.004$ ), with *B. fervidus* making proportionally more short movements ( $< 2$  m) than either of the other two species (Fig. 6).

All bee species at least occasionally groomed pollen from their heads immediately after exiting a flower, by scraping a foreleg from the top to front of their heads. Mean rates of grooming differed significantly among species (GENMOD analysis;  $\chi^2_3 = 78$ ,  $P < 0.0001$ ), with *B. impatiens* (mean of 3.2 flowers probed between groomings) and *B. fervidus* (mean of 4.8 flowers probed between groomings) grooming more frequently than *B. griseocollis* (mean of 14.3 flowers probed between groomings).

The proportion of observed foragers with visible loads of *M. ringens* pollen in their corbiculae differed

significantly among bee species ( $\chi^2_2 = 14.5$ ,  $P < 0.0008$ ). *Bombus fervidus* and *B. impatiens* frequently carried pollen (51 and 42% of foragers, respectively), while *B. griseocollis* seldom had corbicular loads (12%;  $N = 24$ –178 bee bouts per species), although it was most active early in the day when pollen was plentiful.

During each foraging bout, individual bees exhibited distinct foraging styles (R.J.M., personal observation), with some foraging very thoroughly (probing many flowers on each plant), while others moved frequently between plants (probing only one or a few flowers before moving on to another plant). This was in part related to foraging bout length; the mean number of flowers probed per plant increased significantly with the amount of time each bee was observed in the plot (linear regression  $F_{1,191} = 38.6$ ,  $P < 0.0001$ ; mean flowers probed for a bout =  $0.175 \times$  (time observed) + 1.91, duration of observation was recorded for 193 of 255 visitors, mean observed bout length  $\pm$  SE = 2.5 min  $\pm$  0.2). This result could conceivably arise as an artefact of sampling if bees on long bouts chose plants with larger displays than did bees on short bouts. To test this possibility, we used regression to examine the relationship between mean size of displays visited and foraging bout length. This relationship was not significant ( $F_{1,191} = 2.3$ ,  $P = 0.13$ ), and tended in the opposite direction (mean floral display for a bout =  $-0.12 \times$  (time observed) + 10.3). Thus bees did not differ in their preference for many-flowered plants as a function of bout length. Therefore the relationship between flowers probed per plant and bout length was not an artefact, and individual bees appear to be behaving differently.

## Discussion

Experimental manipulation of *M. ringens* floral display strongly affected bumble bee foraging patterns. Furthermore, pollinator species and even individuals within species differed subtly but significantly in their response. These differences in behaviour have the potential to alter plant mating patterns, especially rates of geitonogamy.

### NUMBER OF FLOWERS PROBED

Bumble bees visiting *M. ringens* probed more flowers on plants with large displays, dramatically increasing the potential for geitonogamous self-pollination. Elsewhere (Karron *et al.* 2004) we report that intraplant pollinator movements on day 2 were positively correlated with the rate of self-fertilization ( $r = 0.993$ ). The selfing rate on 16-flower displays was 1.6 times the selfing rate on two-flower displays, in accordance with expectations based on the pollinator behavioural changes reported here.

There are at least two explanations for why bumble bees probed more flowers but a declining proportion of flowers on plants with large floral displays (Ohashi

& Yahara 1998, 2001, 2002; Pappers *et al.* 1999; Goulson 2000). First, on large displays the odds of revisiting a flower are initially lower, increasing the mean reward value of flowers. Second, for plants with loose inflorescence architecture such as *M. ringens*, transit costs for intraplant moves on large displays are lower than on small displays. Both explanations probably play a role in our system, but determination of the relative importance of these two explanations awaits further investigation (Pyke 1978; Hodges 1985; Cresswell 1990; Goulson 2000; Harder *et al.* 2001; Ohashi & Yahara 2001).

For all floral display treatments, bees visiting *M. ringens* most commonly probed a single flower before departing from a plant. This striking pattern has been documented for several other plant and bee species (Waser 1982; Hodges 1985; Cresswell 1990; Dudash 1991; Burd 1994). The implications and causes of this common behaviour have not, to our knowledge, been discussed in any detail, from either the plant or pollinator points of view (but see Lloyd & Schoen 1992; Snow *et al.* 1996). Indeed, many studies of pollinator response to floral display report only means and standard errors. However, because of the non-normal distribution of number of flowers probed per plant visit (Fig. 3), mean values may obscure biologically important information about the experience of individual flowers. In our study, >30% of the visits to 16-flowered displays concluded after a single probe, behaviour that strongly reduces potential geitonogamy for those particular flowers. In contrast, other flowers on the same plant (e.g. the 10th flower in a long plant visit) should experience a pollination environment dominated by geitonogamous self-pollen. From the plant's point of view, this may cause a wide variation in outcrossing rates among individual fruits. This variation might be lessened as pollen accumulates on stigmas over several distinct pollinator foraging bouts. Nonetheless, genetic analysis of progeny from our arrays confirms the prediction of large fruit-to-fruit variation in selfing rates (Karron *et al.* 2004). From the bee's point of view, there are several proximal and ultimate mechanisms that may explain why they so often probe a single flower on a plant (Hodges 1985; Cresswell 1990; Kadmon 1992; Snow *et al.* 1996; Ohashi & Yahara 2001); we cannot distinguish among these hypotheses using our data.

#### INTERSPECIFIC DIFFERENCES IN BUMBLE BEE FORAGING PATTERNS AND IMPLICATIONS FOR POLLEN TRANSPORT

Pollinator species differed significantly in their behaviour, both in ways that may directly affect selfing (likelihood of intraplant movements), and in ways that could affect pollen transfer among plants (interplant movement distances and pollen grooming). Although such differences in behaviour are well known among distantly related pollinator species (Waser 1982; Thompson 2001), there is scant evidence for or against such differences among species within an insect genus (but see

Wilson & Thomson 1991; Cresswell *et al.* 1995; Goulson *et al.* 1998; Stout 2000). In our study, *B. impatiens* and *B. griseocollis* were more likely to promote outcrossing than was their more abundant congener (*B. fervidus*) for two reasons: (1) *B. impatiens* probed fewer flowers than did its congeners; (2) *B. impatiens* and *B. griseocollis* tended to move further between plants. In a natural population with spatial genetic structure, increased movement distances would reduce the likelihood of pollen dispersal between clones of a single genet and close relatives (Eckert 2000). Note also that, although *B. impatiens* and *B. griseocollis* were minority visitors, both were most active early in the morning when stigmas were open and pollen was plentiful, and so probably transferred more pollen than expected based solely on their low overall abundance. Their distinctive response and activity patterns, along with differences in the extent of grooming and pollen foraging, and the consequent potential for differences in pollen transfer properties, indicate that the pollination service provided to *M. ringens* may vary among closely related pollinator species.

There also were notable differences in foraging behaviour among individuals of the three most common species. Some bees moved rapidly among plants, while others foraged much more thoroughly. We infer that those bees that visited only a few flowers on a few plants and then quickly exited the array were sampling plants and patches. Probes by these bees should generate more outcrossing than would probes by thorough bees, assuming the rapidly moving bees deposited and exported as much pollen per probe as thorough foragers. Similar arguments about how differences in pollinator behaviour might affect mating patterns have been made concerning bird-pollinated systems (Linhart 1973; Craig 1989). Again, in such situations individual differences in foraging behaviour are likely to have strong effects on the plant's mating system (Stout 2000; Thomson & Chittka 2001).

#### VISITATION RATES

Bumble bees preferred to visit *M. ringens* ramets with large displays, but probed a smaller proportion of flowers on large displays (24% on 16-flowered displays) than on small displays (60% on two-flowered displays; Fig. 2). The net result of these behaviours is that visitation rates to flowers in the four floral display treatments were nearly equal (mean of 0.56 probes  $\times$  flower<sup>-1</sup>  $\times$  h<sup>-1</sup>), as has been shown in several other studies (Robertson & Macnair 1995; Goulson *et al.* 1998; Vrieling *et al.* 1999). This is consistent with the idea that bees achieve an ideal free distribution across flowers, which perhaps results from bees adjusting visitation rates to match rates of resource production by flowers (Kadmon 1992; Dreisig 1995). We do not yet know whether bees achieved this distribution through systematic foraging or optimal patch-departure rules (Dreisig 1995). We also do not know whether bees preferred large floral displays because they are easier to

detect, or because there are lower flight costs associated with them (Ohashi & Yahara 2001). As might be expected from the equality of visitation received by individual flowers, seed set per fruit did not vary significantly among floral display classes (Karron *et al.* 2004).

Flowers in these arrays were receptive to pollination for only a short time, and therefore we calculate that they should have received only two to three probes while stigmas are open (stigmas are open for  $\approx 4$ –6 daylight hours (Fig. 1) and are visited about once every 2 h; Fig. 5). There would probably be a large variance among flowers in number of probes they receive (Poisson, if flowers are chosen randomly), so that some flowers are never visited and others are visited much more often (Burd 1994). However, recent behavioural studies indicate that bees may use memory, traplines and scent marks in a manner that tends to equalize intervisit intervals for individual flowers (Kadmon 1992; Thomson 1999; Thomson & Chittka 2001). Direct measurement of the visitation history of individual flowers is needed to clarify the issue.

In conclusion, bumble bee pollinators of *M. ringens* responded strongly to floral display size. These pollinators generally preferred plants with numerous flowers, and probed more flowers in sequence on them. These behaviours increase the opportunity for geitonogamous self-pollination, and are consistent with foraging theory (Pyke 1978; Hodges 1985). However there are significant differences among bee species and individuals within species in response to floral display. This suggests that the pollination service provided to plants may differ among pollinators, and this variation may have important implications for plant mating patterns, especially the rate of geitonogamous self-fertilization. Further work is required to determine whether this result is common in other systems. Our findings support Thomson & Chittka (2001) in their call for researchers to quantify both intra- and inter-specific differences in pollinator foraging behaviour.

### Acknowledgements

We thank Jim Reinartz, Tom Schuck, Walt Macior, Forrest Meekins, Lori Artiomow, Nicole Poirier, Gretchen Meyer and Todd Egan for help, support and advice. Thanks to Nick Waser, Tia-Lynn Ashman, and several anonymous reviewers for comments on an earlier version of the manuscript, and to James Thomson for suggesting premanipulations of floral display. NSF Grants DEB 9816712 to J.D.K. and DEB 9903308 to R.J.M. supported this research.

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Received 30 May 2003; revised 25 September 2003; accepted 8 October 2003