

## BUMBLE BEE SELECTION OF *MIMULUS GUTTATUS* FLOWERS: THE EFFECTS OF POLLEN QUALITY AND REWARD DEPLETION

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**Abstract.** The ability of flower visitors to monitor returns when collecting pollen from flowers has been seldom studied despite the importance of pollen as a food resource, particularly for bees. Californian populations of *Mimulus guttatus* are polymorphic for pollen quality: many plants produce a high proportion of cytoplasmless pollen grains that render the grains incapable of fertilizing ovules or of supporting bees nutritionally. We found that different genotypes maintained a consistent proportion of inviable pollen within a genotype and over time. The number of pollen grains per flower was also consistent within a plant at each date but declined over time. We studied the ability of British bumble bees (*Bombus* spp.) to discriminate among plants of *Mimulus guttatus* on the basis of pollen quality and quantity at three scales: indoors with choices of two genotypes, in outdoor plots of several genotypes that varied in pollen quality, and outdoors at a whole-patch scale where two patches of plants differed in quality.

We found that bees could discriminate among plants on the basis of pollen quality provided that flowers still retained most of the pollen. In the two-genotype trials, bees chose genotypes primarily on the quantity of viable pollen, and nectar was much less important. Similarly, where patches of low- and high-pollen quality plants were established, bees responded by visiting the high-quality patch more often and by visiting more flowers within the patch. However, the results from the outdoor plots that contained genotypes of varying phenotypes were inconsistent. A meta-analysis of a large number of separate plots showed that the overall correlation between visitation rate and pollen quality was significant, but variation among plots was also significant. A possible explanation for this inconsistency was suggested in a greenhouse trial in which we showed that, when foraging density was high, depletion of the standing crop of pollen happened quickly, and this reduced the ability of the foragers to choose the higher-quality genotypes. The results have implications for the evolution of pollen production in *Mimulus guttatus* and reward production in other plants.

**Key words:** *Bombus*; *bumble bees*; *Mimulus guttatus*; *pollen number*; *pollen quality*; *pollinator behavior*; *pollinator-mediated selection*; *reward depletion*.

### INTRODUCTION

Nectar and pollen are the most common rewards offered by flowering plants to flower visitors in return for providing a pollination service (Simpson and Neff 1983). For many flower visitors, nectar is the primary source of easily assimilated energy, while pollen is a source of protein. The availability of either may limit the fitness of colonies of social bees and generate selection for behavior that maximizes the uptake of these resources (Charnov 1976). Plants, on the other hand, are under selection to secure regular visitation and an efficient pollen delivery service at minimal cost. The amount of reward that is offered by a plant may de-

termine both the rate of visitation and the subsequent behavior of flower visitors (Zimmerman 1988, Rathcke 1992) and therefore has consequences for pollen transfer. Several studies have shown that selection can act on plants through the effect of foraging preferences of nectar-gathering visitors (e.g., Pyke et al. 1988, Real and Rathcke 1991, Mitchell 1993, Hodges 1995).

Despite the wealth of studies demonstrating a relationship between nectar level and pollinator behavior (reviewed in Zimmerman 1988, Rathcke 1992), very little attention has been given to establishing whether the quantity and quality of the other major reward, pollen, has similar effects on pollinator behavior. Some workers have suggested that the ability of bees to detect differences in amounts of available pollen in flowers is limited (Hodges and Miller 1981, Haynes and Mesler 1984). Indeed, some evidence that pollen-collecting bees discriminate between flowers on the basis of pollen availability is equivocal. For example, a preference for the polleniferous flowers of some dioecious or

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gynodioecious species could be due to the greater levels of nectar these flowers also offer (e.g., Kay et al. 1984, Elmqvist et al. 1988, Ashman and Stanton 1991, Delph and Lively 1992 but see Eckhart 1991). Nevertheless, several papers report a preference for flowers based on visual signals of pollen availability; either phenological changes that correspond with the pollen donation phase (Wainwright 1978, Galen and Plowright 1985, Pellmyr 1988, Gori 1989, Cresswell and Robertson 1994) or differences between species in pollen production that can be visually assessed (Armbruster and Webster 1982, Armbruster and Herzig 1984). However, we know of only three papers that show that bees discriminate amongst flowers on the basis of pollen when the relative amounts of available pollen is disguised (Buchmann and Cane 1989, Gori 1989, Harder 1990).

When rewards are concealed, the ability to make foraging decisions on the basis of reward distribution may depend on the local density of foragers as they deplete the resource. Work on nectar-gathering bees has shown that where bees are numerous, the reward basis on which foraging decisions are made may be altered as the standing crops of nectar in highly rewarding plants is lowered by foraging activity. Heinrich (1976) showed that in meadows in Maine, favored species of plant became successively depleted of nectar following the heavy usage by bumble bees, and as a result, foragers switched their attention away from the high nectar producers to less rewarding species. If this phenomenon also occurs within a species at a site, it could limit the potential for pollinator-mediated selection on reward production (Zimmerman 1988).

Here, we report bumble bee discrimination on the basis of pollen quality among plants of the monkey flower *Mimulus guttatus* (Scrophulariaceae). This species produces little nectar but does produce large amounts of pollen of varying quality (Robertson et al. 1994) which is collected by bumble bees and honey bees. In this paper, we present the results of seven seasons of field and indoor trials on the foraging preferences of bumble bees. We investigated the variability of pollen production within and among genotypes and asked how this affected the foraging behavior of bumble bees at three scales: indoors, in two-genotype choice tests; outdoors within patches; and outdoors between patches that varied in pollen quality. Finally, we tested whether the ability of bumble bees to differentiate among plants is affected by the density of foragers.

#### METHODS

##### *Study system—Mimulus guttatus*

*Mimulus guttatus* is a hydrophilic annual or short-lived perennial, native to western North America, though it has become naturalized in many temperate areas worldwide. Vegetative reproduction can occur by layering in favorable conditions, but the primary mode of reproduction is through the production of numerous

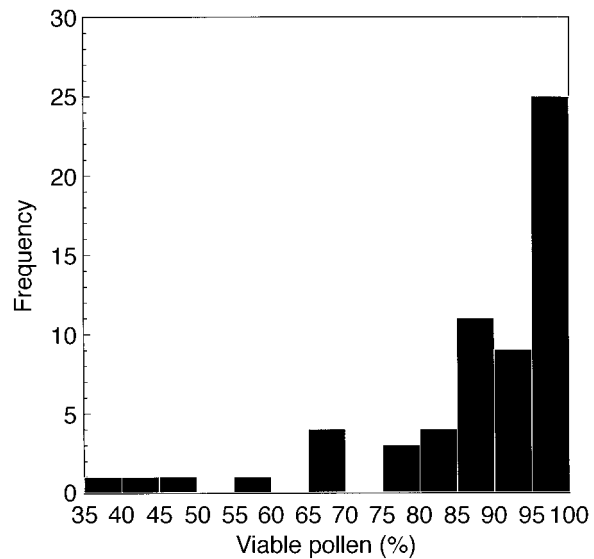


FIG. 1. Frequency distribution of pollen viability in 60 plants from Copperopolis Mine in California assessed from single buds collected in the field. Approximately 100 pollen grains were counted from each bud after crushing the anthers on a microscope slide.

small seeds in dry dehiscent capsules. The large yellow flowers (length ~30 mm, width ~25 mm) are funnel-shaped and are borne on erect stems and contain two pairs of anthers partially concealed in the corolla tube. Conspicuous red dots at the mouth of the funnel act as "nectar guides" but direct visitors, not to the nectar (which is minimal), but to pollen that is shed by the sequentially dehiscing anthers.

The materials used for this study were chosen from greenhouse-raised stock grown from seeds collected within the natural range, from tailings at Copperopolis Mine in Calaveras County, California, USA (37°58' N, 120°38' W) (for more details see Macnair et al. 1993). In this population the plants are pollinated by *Bombus californicus* and *Apis mellifera* (Macnair et al. 1989). In common with a number of other Californian populations (A. Robertson and M. Macnair, *unpublished results*), this population is polymorphic for pollen quality (Fig. 1). Some plants produce a mixture of normal pollen and pollen with no stainable contents. This variation has a strong heritable basis, although total pollen number per flower apparently does not (Robertson et al. 1994).

##### *Propagation*

Cuttings were made from stock plants of varying pollen quality and allowed to flower in a greenhouse at the University of Exeter, UK. When the plants were beginning to flower, they were transferred to plots either outdoors in the university grounds or into a 15 × 8 m polythene tunnel greenhouse. Plants were spaced 1 m apart in arrays of varying size. In 1985, plants to be used in plots were planted directly into the ground,

but in subsequent years they were maintained in 22.5-cm plastic pots.

#### *Variability of pollen production*

We examined the variability of pollen viability (proportion of grains with cytoplasm) and pollen production (number of pollen grains per flower) in a greenhouse study. Two mature flower buds (ready to open within 24 hr) were collected from each of 52 plants as the plants came into flower. Two more buds were collected a month later as the plants began to lose vigor and cease flowering. Buds were preserved in 3:1 ethanol:acetic acid. The four anthers were dissected out into 0.3 ml of 0.7M mannitol solution colored with a little aceto-carmin, and macerated with a glass rod to release the pollen. For each bud, four independently drawn samples were taken from each vial and counted on a haemocytometer and the numbers pooled. Typical samples contained 300–400 grains. Inviolate pollen, having no cytoplasm, appear transparent after staining and contrast strongly with the densely staining viable grains. We made counts of each category.

We used a repeated-measures analysis of variance of pollen number and pollen viability to partition the variation in pollen parameters into differences among plants and to temporal change. To improve the normality of the data and the homogeneity of the plant's variance, we transformed the proportion viable as:

$$\log(\text{viable pollen}/(\text{inviolate pollen} + 2500)).$$

In all the other experiments reported here, pollen quantity was either measured by direct counting with a haemocytometer, or by estimation from the size (length  $\times$  width) of anthers measured by an eyepiece micrometer. A regression of pollen number against anther size was highly significant ( $n = 261$ ,  $F = 637.72$ ,  $P < 0.001$ ,  $R^2 = 71.0\%$ ) showing that pollen production could be reliably measured in this way.

#### *Outdoor plot trials 1985–1991*

To test the effects of pollen quality on the foraging behavior of flower visitors, we set out nine plots of plants of varying pollen quality over five summers during May to September. From here on, the plots are coded with year and plot number, i.e., 91-1 indicates the first plot in 1991. Each plot was monitored for between one and three weeks. Most plants had between 50 and 300 flowers during the periods of observation. Unless it was raining, we spent several hours each day observing the plots, recording the numbers of bees visiting the plot and the number of flowers probed at each visit to a plant. The pollinators visiting the plots varied over the summer, but included the native British bumble bees, *Bombus pratorum*, *B. pascuorum*, *B. lucorum*, *B. hortorum*, *B. lapidarius*, *B. ruderals*, *B. terrestris* and the honey bee *Apis mellifera*. The most common visitors were *B. pascuorum* (worker body length 9–15 mm and tongue length 8.3 mm; data for British species

from Alford [1975] and Prŷs-Jones and Corbet [1987]) and *B. pratorum* (worker body length 9–14 mm, tongue length 7.1 mm), and these species, along with *B. terrestris* (worker body length 11–17 mm, tongue length 8.2 mm) were used in the captive bee trials below. For comparison, the bumble bee that visits *M. guttatus* in California, *B. californicus*, is smaller on average but overlaps with the British species (worker body length 7–12 mm, tongue length 7.1 mm [Lawrence Harder and David Inouye, *personal communication*]).

At the start of the trial and at the end of each week, we recorded the number of open flowers on each plant and rearranged the plants randomly in the grid. At this time, four buds were harvested and preserved for pollen scoring. In one plot (91-1), we measured the production of nectar over 24 hr prior to placing the plants outside. On each genotype, five flowers that were just beginning to open were tagged. Twenty-four hours later, the accumulated nectar was removed using small pieces of filter paper and total carbohydrate measured following the method of McKenna and Thomson (1988).

#### *Pairwise choice tests*

In the spring of 1991, we ran a series of choice tests on captive bumble bees. Small colonies of *Bombus pratorum* were obtained from bumble bee rearers in The Netherlands. The colonies were connected to a flight chamber by a tube that could be opened or closed to control access by the workers. The bees were fed supplementary honey and pollen during the course of the trials. Prior to the start of the trials, the bees were trained to forage from vases of *Mimulus* flowers for 2 d to accustom them to variation in pollen quality amongst flowers.

To test for bee preferences, "vases" of two genotypes were arranged randomly in a checkerboard fashion in the flight cage, and workers were allowed to forage one at a time on the vases. Each vase contained 10 open but previously unvisited flowers of one or other of two genotypes of *Mimulus*. There were 10 vases in all, five of each genotype. The total number of the flowers visited of each genotype was recorded. The trial was terminated when a bee rested after visiting more than ~50 flowers or by our intervention when ~100 flowers had been visited. Many visits were, in fact, revisits to previous flowers so that even after 100 visits, there were many unvisited flowers in some of the vases. Six trials were performed each with a different pair of genotypes. For each trial, the preference of three to five bees was tested. All the flowers used in the trials were obtained from large plants that were producing many flowers in the greenhouse. When a genotype was chosen for a trial, five buds were collected and preserved as above for pollen counting later. At the same time the nectar standing crop was sampled from five fresh flowers. Pollen production as well as the proportion and number of viable pollen grains were also recorded.

### *The effect of pollinator density*

In 1992, we established five plots in a polythene tunnel greenhouse from which wild bees were excluded. We reared colonies of *Bombus terrestris* from locally collected queens and placed them inside the tunnel with an exit tube that could be blocked if necessary. Two different sized colonies were used to provide different foraging bee densities. In the smaller colony, there were only one or two active foragers while the larger colony had up to 12 workers. We observed the visitation rates to the plants as described for the outdoor trials above. For each plot, foragers were monitored continuously for one 8-h period. The bees were allowed to forage on the plants for a day prior to measurements being made, except on plot 92-5, where the plants received no visits prior to observation. For this plot, we kept the data on the visits made in the first 4 h separate from the data gathered in the subsequent 4 h.

Pollen production was recorded as above by harvesting four unopened flower buds per plant at the start of each trial. In one plot (92-1) the same plants were measured under each pollinator density regime, the observation days separated by a week so that a new set of flowers had been produced and were therefore unvisited prior to the start of the second period. In this plot, the standing crop of pollen was recorded from four randomly chosen flowers at the end of the 8-h observation period. One plant had begun to stop flowering by the time of the second trial and it was removed from the analysis.

### *Assessing pollinator preferences*

Two kinds of data are presented here on pollinator preference. The parameter employed initially measured the number of flowers probed by a bee to a plant at each visit. To determine preferences, the average number of visits made to each plant when a bee visited that plant was regressed against the plant phenotype (either the number of viable grains per plant or the proportion of grains that were viable).

In later plots, a different parameter was preferred. By keeping track of all visits made to a plant during the period of observation and of the number of open flowers that plant had at the time, the number of times each flower was probed could be estimated. This parameter has two advantages over the previous one. First, account is taken of the rate at which plants are visited. If the plant is experiencing frequent but short visit sequences it is not necessarily scored as being of lower preference to a plant that receives few visits but long sequences. Second, we have shown that for bees visiting *Mimulus*, this parameter is relatively insensitive to variation in flower number (Robertson and Macnair 1995), except on plants with very few flowers, and such plants were not included in these plots.

### *Meta-analysis of plot correlations*

The nine plots each yielded separate correlations between the two dependent variables (mean number of

visits per plant and visits per flower per hour) and the independent variables (number of viable pollen grains per flower and the proportion of grains that were viable). To detect whether the plots were converging on a common mean effect for each variable, a meta-analysis of correlation coefficients was performed (Arnqvist and Wooster 1995). In order to ensure that the correlations were independent, we used only the visitation data for the bee species that was the most common visitor to each plot if more than one species had been recorded. To carry out each meta-analysis we followed the procedures of Hedges and Olkin (1985) for combining estimates of correlation coefficients. The sample correlation coefficients were transformed to  $z$ -values using (Fisher's (1921)  $z$ -transformation then combined and back-transformed to give an estimate of the population correlation using the sample size as weighting factors. The weighted mean  $z$ -score was then tested against zero using the standard normal distribution to test the significance of the overall estimated correlation. To check whether different plots were consistent with a single overall effect, the homogeneity of the plot correlations was tested using  $z$ -transformed sample correlations and calculating the sums-of-squares about the weighted mean tested against the chi-squared distribution (Hedges and Olkin 1985).

### *The effect of overall patch quality*

To test whether bees would discriminate between patches of plants on the basis of their average pollen quality, two further outdoor plots were set up as follows. Plants were categorized as high- or low-pollen viability. One plot consisted of 14 plants of high-pollen viability and two of intermediate quality (high-viability plot), while the other contained 14 poor quality plants with the same two intermediate genotypes (low-viability plot). The plants were arranged in a  $4 \times 4$  grid with the two intermediate plants occupying central positions along one diagonal of the grid. The two plots were  $\sim 50$  m apart.

Data were collected on the number of bees (*Bombus* spp. and *Apis mellifera*) that visited each plot and the numbers of flowers that the bees visited in the plot during 2 h of observation on each of 21 d spread throughout a 40-d period. Visits that were made to the intermediate-viability plants were recorded separately from visits to the other plants in the array. After 30 d, the positions of the two plots were swapped. We tested for heterogeneity of visitation patterns before and after the swap using rank sum tests before pooling the results. Over the course of the period of observation, it became necessary to substitute plants that had begun to stop flowering with fresh ones from the greenhouse of approximately the same pollen viability, thus maintaining the difference between plots in overall plot pollen quality. Contrasts between plots were made each day of the average number of visits made by bees per

TABLE 1. Pollen production in *Mimulus guttatus*. Analysis of variance of two components of pollen production: pollen number and pollen quality (ratio of full to empty pollen grains) in 52 greenhouse-grown plants measured on two occasions (time).

Effect	df	Mean square	F	P	R <sup>2</sup>
a) Pollen number (1000s of grains)					
Plant	51	172 418	5.37	<0.001	36.5
Time	1	8 140 940	253.52	<0.001	33.8
Plant × Time	51	83 135	2.59	<0.001	17.6
Error	104	32 111			13.9
b) Pollen viability (log (viable grains/(inviabile grains + 2500))					
Plant	51	3.267	35.96	<0.001	85.1
Time	1	2.925	32.20	<0.001	1.5
Plant × Time	51	0.326	3.59	<0.001	8.5
Error	104	0.0909			4.8

Notes: Two flower buds were measured from each plant both at the beginning and at the end of the flowering period. Type III sums-of-squares were used for hypothesis testing.

hour and the average number of flowers visited by each bee.

## RESULTS

### Variability of pollen production

Pollen production in greenhouse-grown *Mimulus guttatus* was variable in both number and quality and varied significantly with genotype and with the time at which the measurements were made (Table 1). Approximately equal amounts of variability in number was accounted by these two factors and between them they explained >70% of the variance. In the month between measurements, average pollen production dropped by a third (from a mean of ~35 300 grains to ~22 800 per flower). The rate of decline was variable among plants (as shown by the interaction). However, at any one time, pollen production was consistent within plants. This meant that only a small number of flowers were needed to be sampled in order to accurately characterize the pollen production of a plant.

Eighty-five percent of the variance in the proportion of pollen that was viable occurred among genotypes; over time genotypes remained very consistent in this proportion despite the reduction in pollen number that occurred between samples. In fact, genotypes that were grown on by clonal propagation maintained their phenotype for viable pollen ratio and could be reliably predicted to exhibit very similar ratios from one trial to the next (A. Robertson, unpublished data).

### Nectar production

The anthrone carbohydrate assay confirmed that *M. guttatus* produces little nectar (enclosed flowers averaged 50 µg of sucrose equivalents, while the standing crops of nectar in flowers on outdoor plots was ~20 µg). A flying bumble bee consumes between 0.27 and 0.54 calories per minute (depending on temperature) (Heinrich 1979) and would thus be required to visit three to seven flowers per minute to satisfy this requirement. Although we cannot rule out the possibility that nectar was an important goal of foragers, most bees

were observed to be actively gathering pollen and would spend longer than this on flowers that contained fresh pollen. Thus, the primary motivation for visits appeared to be for pollen gathering.

### Outdoor plot trials 1985–1991

The nine plots that were set outdoors to test for visitor preferences for viable pollen production yielded inconsistent results (Table 2). In some plots, bees were clearly discriminating in favor of plants that produced better quality pollen (e.g., plot 86-1, Fig. 2). In other plots, however, there was an insignificant relationship between viable pollen production and visitation rate, based either on the mean number of flowers visited, or visits per flower per hour. Such inconsistency could be either the result of an effect that truly varies in magnitude, or the result of Type II sampling error. We therefore carried out a meta-analysis of these correlations, and these comparisons, using only the most common bee species for each plot, are presented at the bottom of Table 2. For each combination of dependent (visits per flower per hour or flowers visited at a plant) and independent variables (viable pollen number or proportion viable), a combined estimate of the overall effect size ( $\rho$ ) and its statistical significance is presented along with a test of the heterogeneity of the plot correlations ( $Q$ ). These analyses reveal that, overall, there is a highly significant positive relationship between both measures of pollen production and the two measures of visitation rate. However, the results also show that the relationship between mean numbers of flowers visited per plant and the proportion of viable pollen varied significantly among plots. The results suggest that although there is a moderately strong relationship between this parameter and pollen production, its strength varies significantly among plots and that the variation is not due simply to Type II sampling error. Visits per flower per hour, which was measured only on later plots, showed an overall positive, significant and consistent relationship with both aspects of pollen production.

TABLE 2. Correlation coefficients (*r*) between pollen production or viability of *Mimulus guttatus* clones and visitation rates of bumble bees (*Bombus* spp.) and/or honey bees (*Apis mellifera*) on outdoor plots during 1985–1991.

Plot	Plants	Bee species	Mean flowers visited at a plant		Visits per flower per hour	
			No. viable pollen†	Proportion viable	No. viable pollen†	Proportion viable
85-1	6	<i>B. pascuorum</i>	<b>0.79</b> (c-r)	<b>0.44</b>	...	...
		<i>B. lucorum</i>	0.85* (c-r)	0.37	...	...
85-2	5	<i>B. pascuorum</i>	<b>0.58</b> (c-r)	<b>0.93*</b>	...	...
86-1	16	<i>B. pratorum</i>	<b>0.21</b> (c-r)	<b>0.75***</b>	...	...
87-1	11	<i>B. pratorum</i>	<b>0.53</b> (c)	<b>0.69*</b>	...	...
		<i>B. pascuorum</i>	0.52 (c)	0.54	...	...
		<i>B. lucorum</i>	-0.05 (c)	-0.01	...	...
		<i>B. hortorum</i>	0.60* (c)	0.70*	...	...
		combined	0.70* (c)	0.82**	...	...
90-1, Wk 1	14	<i>B. pratorum</i>	<b>0.56*</b> (c)	<b>0.48</b>	...	...
90-1, Wk 2	50	<i>B. pratorum</i>	-0.04 (as)	-0.04	...	...
90-1, Wk 3	50	<i>B. pratorum</i>	-0.11 (as)	-0.10	...	...
90-3, Wk 1	17	<i>B. pascuorum</i>	<b>0.57*</b> (as)	<b>0.61**</b>	<b>0.69**</b> (as)	<b>0.66**</b>
90-3, Wk 2	17	<i>B. pascuorum</i>	<b>0.45</b> (as)	<b>0.53*</b>	<b>0.48</b> (as)	<b>0.56*</b>
90-3, Wk 3	16	<i>B. pascuorum</i>	<b>0.56*</b> (as)	<b>0.64**</b>	<b>0.69**</b> (as)	<b>0.53**</b>
90-4, Wk 1	16	<i>B. pascuorum</i>	<b>0.27</b> (as)	<b>0.18</b>	<b>0.34</b> (as)	<b>0.38</b>
		<i>A. mellifera</i>	0.04 (as)	0.02	0.18 (as)	0.19
		combined	0.32 (as)	0.19	0.40 (as)	0.44
90-4, Wk 2	17	<i>B. pascuorum</i>	<b>0.02</b> (as)	-0.04	<b>0.20</b> (as)	<b>0.21</b>
		<i>A. mellifera</i>	0.12 (as)	0.05	0.10 (as)	0.30
		combined	-0.05 (as)	0.01	0.35 (as)	0.34
91-1	24	<i>B. pratorum</i>	<b>0.22</b> (as)	<b>0.30</b>	...	...
91-2	12	<i>B. pascuorum</i>	<b>0.19</b> (c)	<b>0.07</b>	<b>0.31</b> (c)	<b>0.27</b>
		<i>A. mellifera</i>	0.69* (c)	0.49	0.60* (c)	0.57
		combined	0.27 (c)	0.11	0.40 (c)	0.32
Meta-analysis						
Overall weighted correlation ( <i>ρ</i> )			0.20**	0.24***	0.48***	0.46***
Heterogeneity of plot coefficients ( <i>Q</i> )			20.14	33.79**	5.03	3.28

Notes: Only plants with >30 flowers were included in the analyses. The figures in bold show the correlations for the most frequent visitor to each plot and are used in the meta-analysis (see *Methods: Meta-analysis of plot correlations* for details). Significant coefficients are denoted by asterisks.

† Method (in parentheses): c, direct count; c-r, flowers scored on subsequent regrowth shoots; as, estimated from anther size.  
 \* *P* < 0.05; \*\* *P* < 0.01; \*\*\* *P* < 0.001.

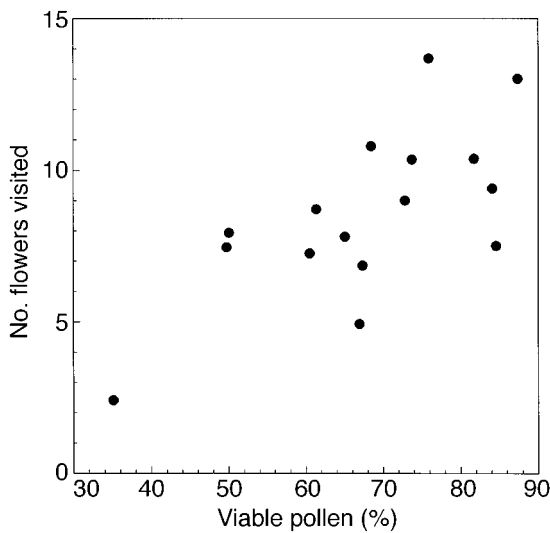


FIG. 2. Mean number of flowers visited on 16 clones of *Mimulus guttatus* by *Bombus pratorum* in Plot 86-1 plotted against mean pollen viability.

Pairwise choice tests

When individual workers of *Bombus pratorum* were presented with a pair of genotypes of *Mimulus*, 21 of the 26 bees discriminated significantly against one of the pair presented (binomial tests with an expected proportion of 50% visits to each genotype). To examine the relative importance of the four predictors of pollinator preference, a logistic regression was performed with pollinator preference as the dependent variable and the measures of pollen and nectar production as

TABLE 3. Logistic regression of the six two-genotype trials with the proportion of visits to the favored genotype as the dependent variable.

Effect	Coefficient	df	Deviance	<i>P</i>	<i>R</i> <sup>2</sup>
Viable pollen number	14.06	1	31.64	<0.001	20.4
Nectar	0.57	1	5.43	0.020	3.5
Percentage viable	-14.44	1	16.16	<0.001	10.4
Total pollen number	-2.45	1	8.33	0.004	5.4
Residual		1	93.56		

Note: Predictors are the number of viable pollen grains, the proportion of the pollen that is viable, the total pollen production, and the nectar standing crop.

TABLE 4. Correlation coefficients ( $r$ ) between viable pollen production of *Mimulus guttatus* clones and visits per flower per hour by bumble bees (*Bombus terrestris*) in the 1992 poly-tunnel trials.

Plot	Bee density	Plants	Correlation
92-1	Low	17	0.79***
92-1	High	18	0.02
92-2	Low	14	0.69**
92-3	High	21	0.21
92-4	Low	8	0.92**
92-5	High	8	0.80* (initial 4 h) 0.11 (subsequent 4 h)

#### Meta-analysis

Overall weighted correlation ( $\rho$ ) = 0.51\*\*\*

Heterogeneity of plot coefficients ( $Q$ ) = 18.25\*\*

Notes: For plots 1–4, observations were made for 8 h after a day of preconditioning by the bees; plot 5 had no preconditioning. Only plants with >30 flowers were included in the analyses. Significant coefficients are denoted by asterisks.

\*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .

independents (Table 3). The results for individual bees were pooled to obtain independent data points. This analysis was performed with S-PLUS (Mathsoft 1997) using a binomial distribution and a logit link function. The full model was highly significant and explained ~40% of the variance in the choices bees made. A stepwise removal procedure using Akaike Information Criterion (AIC) (Akaike 1974) did not remove any of the predictors from the final model, suggesting they all have unique predictive power. The number of viable pollen grains was the single most important predictor of bumble bee behavior (explaining ~20% of the variance in pollinator behavior). Nectar standing crop was positively associated with preference but explained only ~3% of the variance. The proportion of pollen that was viable, and the total pollen production were negatively correlated with preference, after the other two variables had been entered. This suggests that bumble bees preferentially visit genotypes that have high numbers of viable pollen but an otherwise low total pollen production. Our unpublished data show that the viable grains in a mixed pollen type genotype tend to be larger than pollen produced in flowers that have only viable pollen.

#### The effect of pollinator density

In 1992 trial plots were conducted inside a poly-tunnel where the density of foraging bees could be controlled. Plot 92-1 was exposed to both high and low bumble bee foraging densities. When a low density of foraging bees was given access to the plants for 1 d, the preference for plants with high levels of viable pollen was strong (Table 4, Fig. 3). One week later, however, when the plot was exposed to high rates of visitation, there was no evidence of a correlation between visitation rate and viable pollen number (Table 4).

For this plot, pollen standing crops initially and at

the end of the foraging day were also recorded. Under the low visitation regime, although between one-third and one-half of the pollen had been removed from the high viability plants by the end of the day, these plants still contained more viable pollen than the low-viability plants in the plot (Fig. 4a and b). The correlation between pollen production and pollen number at the end of the day was consequently high ( $r = 0.93$ ,  $P < 0.001$ ). Hence, it was still possible for a bee to discriminate between plants on the basis of the first few flowers visited, as plants could be reliably judged for pollen quality.

In contrast, when the plot was exposed to higher numbers of foragers, much of the pollen had been stripped from the flowers by the end of the day and the amount of viable pollen available on the previously highly rewarding flowers was not higher than that available on the low-viability plants (Fig. 4c and d). The correlation between initial production and final pollen standing crops had also been lost ( $r = 0.02$ , NS). Additionally, there was evidence that significant amounts of pollen were being moved from flower to flower as many of the plants that had previously contained little inviable pollen, contained quite high levels of it by the end of the day (Fig. 4d). Presumably this pollen had been picked up from low-viability plants and deposited in the flowers of high-viability plants. Some pollen was inevitably moved in the other direction, and this pollen movement would have further homogenized the pollen reward available on the plants.

Four further plots were studied in the poly-tunnel. In these plots, no measurements were made of pollen standing crops but observations were again made of pollinator visitation patterns. Again, when bees foraged at low densities, clear preferences for high-viability

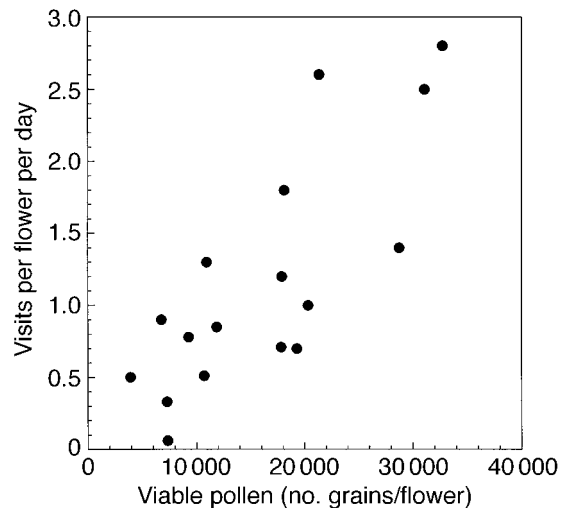


FIG. 3. Visitation rate to plants of *Mimulus guttatus* subjected to low bee density visitation (1–2 foraging workers only) plotted against the number of viable pollen grains per flower.

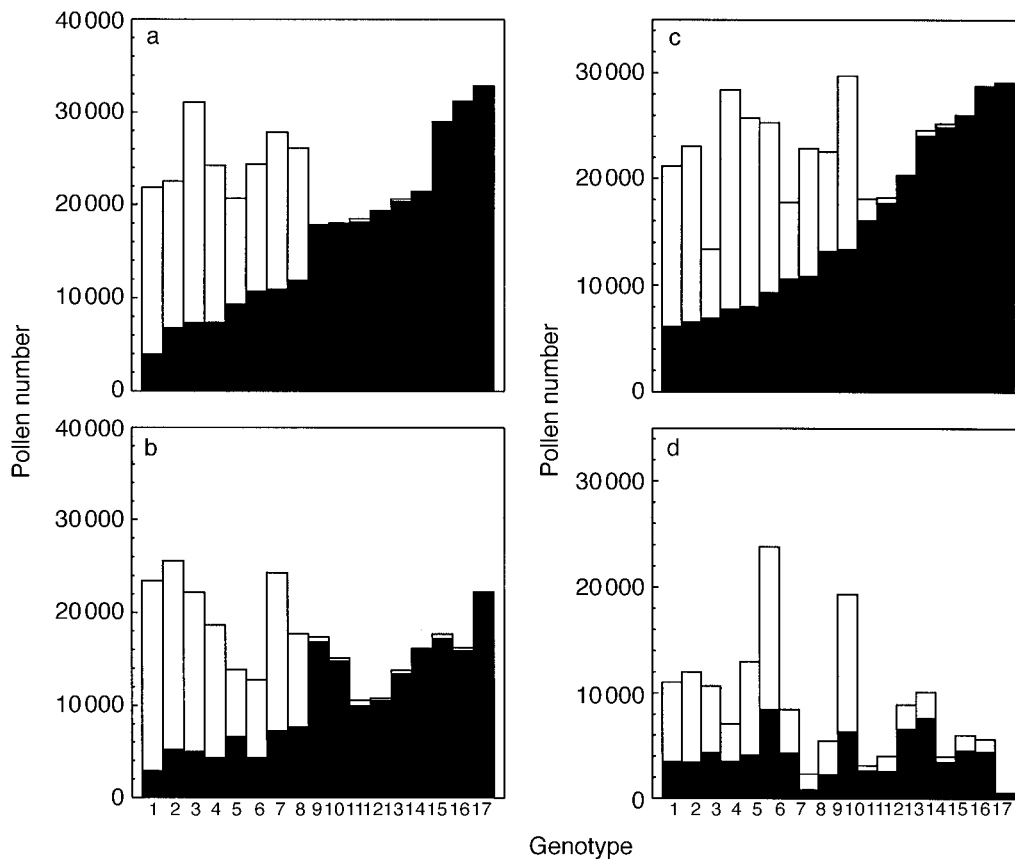


FIG. 4. Pollen depletion of *Mimulus guttatus* by foraging bumble bees *Bombus terrestris* in plot 92-1: (a) pollen production of plants during low-bee-visitation regime (1–2 foraging workers only), filled bars = viable pollen, unfilled bars = inviable pollen; (b) standing crop of pollen in open flowers following a day of low bee visitation; (c) pollen production during high-bee-visitation regime (10–12 workers); and (d) standing crop of pollen following a day of high bee visitation.

plants were shown (Table 4). At high bee densities, preferences were not detected. In one plot (92-5) which, unlike the other plots, had not received any visitation prior to the observation day and thus began the trial with all-virgin flowers, there was an initial preference for the high-viability plants but by the second half of the observation period, that preference had become insignificant. The meta-analysis of these correlations revealed that although there was a highly significant overall effect of pollen viability on visitation patterns by the bumble bees, the individual coefficients were heterogeneous suggesting that there was not a consistent effect size (Table 4). We can conclude, therefore, that the behavior of bees was dependent on the density of foragers, and that a high density of workers reduced the ability to discriminate between genotypes on the basis of their pollen production.

#### Variation in overall patch quality

We were also interested to know whether bees could discriminate between patches of plants that differed in pollen quality. More bees were observed foraging on *M. guttatus* patches that had higher pollen quality and,

once a bee arrived at that patch, the numbers of flowers visited on that foraging trip was higher than on trips made to the lower quality patch (Table 5). Both the length and frequency of the visits to the two patches was highly significantly different as shown by sign tests. This avoidance of the low-quality patch continued even when the positions of the two patches were reversed, suggesting that bees quickly re-learned the relative quality of the patches (Wilcoxon rank sum tests to test heterogeneity in proportion of bees seen at the high-viability plot before and after the plot positions were swapped,  $Z = -1.2687$ ,  $P = 0.2046$ ; proportion of flowers visited that were in the high-viability plot,  $Z = -0.4849$ ,  $P = 0.6277$ ).

The tendency to visit more flowers on the high-quality patch extended even to the two intermediate-quality plants embedded in the patches. Expressed as flowers visited per plant per hour, the rate of visitation to these plants was significantly greater in the high-quality patch than in the poor-quality patch (Table 5), which suggests that these plants were benefiting from the effect of the higher rate of bee-use of the high-quality patch. We had expected that bees would recognize the

TABLE 5. Bumble bee visitation to the two 1993 outdoor plots of *Mimulus guttatus* that contrasted in pollen viability.

Measure	High-viability plot	Low-viability plot
Mean no. bees visiting†	7.29	5.19***
Mean no. flowers visited†	13.54	8.27**
Mean flowers visited-plant <sup>-1</sup> ·h <sup>-1</sup> on intermediate plants†	14.71	11.07**
Mean visits to intermediate plants (%)‡	13.52	23.02**

Notes: The average number of bees recorded foraging per hour at each plot, the average number of flowers entered per visit to the plots, and the average visitation rates to two plants that had intermediate pollen quality are shown. Bees were recorded for two hours each day on 21 d on each plot. Asterisks denote significant differences between high- and low-viability plots.

† Tested by sign tests using each of the 21 observation days as replicates.

‡ Tested by Mann-Whitney *U* test.

\*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ .

poorer quality of these plants in the high-quality patch and make fewer visits to them compared to other plants in the array but in fact, the proportion of flowers that were visited in the patch that belonged to the intermediate plants was slightly higher than we would have expected if the bees were foraging at random (expected, 2/16 plants = 12.5%; observed = 13.5%, Table 5). In the low-viability plot, the departure from random visitation was even greater (observed 23.0%). If we compare the number of visits to intermediate plants to the expected number using a binomial distribution and an expected value of 0.125, in both plots the intermediate plants appear to be over-visited ( $P < 0.001$ ), but the departure from expected is much higher in the case of the low-viability plot. The proportion of visits made to these plants were again unaffected by the swapping of the plot positions (Wilcoxon rank sum test,  $Z = -0.0412$ ,  $P = 0.9671$ ).

#### DISCUSSION

##### *Bumble bee discrimination on the basis of pollen availability*

*Mimulus guttatus* genotypes differ markedly with respect to pollen quantity and quality. We have shown that when given a choice of genotypes with previously unvisited flowers in an indoor choice test, pollen-collecting bumble bees can readily discriminate among them on the basis of the quantity of viable pollen. In outdoor plots, however, the preference for the better pollen producers of an array was variable. During the 1985 to 1987 a series of poor summers meant that very few bumble bees used the plots. The summers of 1990 and 1991, on the other hand, were more favorable, and bumble bees were more common. Bees made ~2 visits/h to the 1985–1987 plots while they averaged 4.4 visits/h in 1990 and 1991 (range 1.3–6.4). The 1992 plots were an attempt to test the idea that such differences

in relative bee densities could affect the ability of bees to assess pollen quantity and quality. We postulated that the scarcity of bees during the earlier seasons allowed those that were present to be able to discriminate between genotypes on the basis of pollen. In later seasons, when bees were numerous and foraging could persist for several days, bees may have been rapidly depleting the resource and preventing preferences from being shown. Bumble bees harvest *Mimulus* pollen quite efficiently. *Bombus pratorum* workers remove ~50% of the total pollen production after one visit to virgin flowers (A. Robertson, unpublished data). In our outdoor plots, flowers were estimated to receive 1–2 visits/d on average in our 1990 and 1991 plots (Robertson and Macnair 1995) and given that the flowers last 3–5 d, it is likely that flowers received multiple visits by bumble bees. Under such a regime it seems plausible that many flowers had most or all of their pollen stripped, making it difficult for bees to discover or learn the position of consistently rewarding plants. The indoor trials of 1992 confirmed quite clearly that bees could only discriminate on the basis of pollen production when they were foraging at low densities. The reason was clear: the standing crops of pollen of open flowers only closely mirrored the production per flower when there were few bees removing pollen.

Not surprisingly, bumble bees had little trouble in recognizing and discriminating between patches of plants that contained either high- or low-quality plants. Both the number of foragers and the numbers of flowers visited was very much lower in the low-quality patch compared to the high. This kind of discrimination suggests that bees learn to associate patches with reward value. The behavior shown on the intermediate plants in these patches was less expected. In the low-quality patch, flowers on the two plants of relatively higher quality were visited much more often than flowers in the rest of the plot, as expected. However, in the high-quality patch, these plants, which should have been perceived as being of lesser quality than rest of the plants in the patch, were visited in greater numbers than their proportion of the plot suggests they should have been. In fact, the intermediate plants were achieving higher rates of visitation per flower when surrounded by good-quality plants than when they were the best plants in an otherwise low-quality patch. The failure of bees to discriminate against the poor producers in the high-quality plot may be due to pollen-stripping on the plants in the rest of the plot, or it could be that the bees fail to notice that there are two poor plants in amongst the otherwise high-quality patch. Such “mistakes” are suggested to account for the relatively high visitation to rewardless species when they grow in close association with highly rewarding species, the so-called “magnet effect” (Laverty and Plowright 1988, Laverty 1992, Dafni 1993).

Pollen limitation of the colony-development rate of social bees is at least as likely as nectar limitation

(Fisher 1987, Sutcliffe and Plowright 1990, Plowright et al. 1993), so there should be selective pressure on foragers to develop efficient pollen-harvesting behaviors. Our results for bumble bees feeding on *Mimulus* show, for the first time, that bees not only monitor the quantity but also the quality of the pollen they harvest from flowers. However, it is not clear how bees determine either the quantity or quality of the pollen they are collecting. Harder (1990) suggested that the sensory apparatus on honey bee corbiculae that are thought to sense the size of the growing corbicular loads (Ford et al. 1981) may also be involved in assessing the rate of pollen harvest by bumble bees. If this is the sensory system involved then it must also be able to differentiate between the full and empty pollen grains that *Mimulus* produces. It is possible that some property of the empty grains (perhaps the mass, electrostatic charge, or stickiness of the grains) means that they do not pack properly into the baskets. Alternatively, the odor of pollen may differ between full and empty grains. Pollen odor has been recently implicated in behavioral responses of honey bees to the presence of pollen including the ability to determine levels of available pollen in individual flowers (Dobson et al. 1996).

#### *Implications for plant fitness*

Our results also have implications for plant fitness. While other studies have shown that bees are aware of phenological changes that alter the availability of pollen in flowers and can recognize empty flowers from full (Wainwright 1978, Galen and Plowright 1985, Pellmyr 1988, Gori 1989, Eckhart 1991, Cresswell and Robertson 1994), our results suggest that bees may discriminate among genotypes on the quantity and quality of the pollen they produce. Thus, there may be opportunity for pollinator-mediated selection on floral phenotype in an analogous way to that suggested for nectar production (Zimmerman 1988). If seed or fruit set is limited by pollen receipt on stigmas (Bierzuchudek 1981, Burd 1994), female fitness of hermaphrodites might benefit from increased expenditure on pollen to increase attractiveness to pollen-collecting vectors. Even if sufficient visits occur to all genotypes to allow a full complement of seed, additional visits may benefit female fitness through allowing greater female choice of pollen genotype that fertilizes its ovules (Marshall and Folsom 1991).

Male fitness may also be affected by differences in pollen production. The greater visitation rates to genotypes with more viable pollen may lead to greater amounts of pollen export and therefore a larger share of paternity on recipient plants (Lloyd 1984). When enhanced pollinator attractiveness through pollen is added to models of sex allocation, an accelerating male gain curve (the rate of returns to fitness with an increase in male allocation) may result which could favor the evolution of separate sexes (Charnov 1979, Charlesworth and Morgan 1991). It might be tempting, there-

fore, to interpret the situation in *Mimulus guttatus* as a case of cryptic gynodioecy where plants with large amounts of inviable pollen act mostly as females. However, inefficient pollen transfer associated with an increased pollen number (Harder and Thomson 1989), pollen discounting through increased within-plant visits (Harder and Barrett 1996), and decreased pollen vigor associated with an increase in pollen number (Vonhof and Harder 1995) all may contribute to decelerating male gain curves and argue against expecting the low-viability plants to necessarily father less seed. Thus, these plants may be better regarded as employing an alternative strategy to ensure a share of paternity rather than being functionally more female in gender. Our unpublished results show that these kinds of *M. guttatus* genotypes are often quite successful in fathering seeds.

#### *Reward depletion and the strength of pollinator-mediated selection*

We have shown that the depletion of the standing crop of pollen eliminates bumble bee preferences for higher-quality pollen producers in plots of *Mimulus*. We suggest that this kind of reward depletion will be common in many species of plant that have high visitation rates and freely available rewards, and will reduce the opportunity for pollinator-mediated selection for reward quantity or quality. Unfortunately, few studies have measured both the average nectar production rate and the average nectar standing crop in the field. From the data available, however, it appears that the average standing crop is often only a fraction of the rate produced over 24 h, presumably because of rapid depletion by flower visitors (Pleasants and Zimmerman 1979, Zimmerman and Pyke 1986, Waser and Mitchell 1990, Delph and Lively 1992, but see Zimmerman and Pyke 1988 for an exception). Furthermore, restricted-area foraging and the recent history of visitation within a patch may further obscure differences in production (Pleasants and Zimmerman 1979, Zimmerman 1981, Waser and Mitchell 1990).

Much less is known of the dispersion of pollen rewards. Pollen production per plant is rarely measured nor is the standing crop of pollen in flowers. However, removal rates of pollen following a single visit from bees is typically 40–80% of the amount available (Strickler 1979, Wolfe and Barrett 1987, Galen and Stanton 1989, Harder 1990, Young and Stanton 1990, Thøstesen and Olesen 1996) and standing crops are often low (Wolfe and Barrett 1987, Wilson and Thomson 1991, Robertson and Lloyd 1993).

Foraging animals usually make decisions about the value of a plant on the basis of sampling flowers and are thought to leave a plant when one or a series of poor flowers is encountered (Pyke 1978, 1981, Hodges 1985, Cresswell 1990). This method of choosing plants will increase the efficiency of foragers only if rewards in the first few flowers correlate with the rewards that

can be expected within the rest of the plant. Where depletion rates are high, the only richly rewarding flowers will be those that have been missed by visitors. Foragers may have difficulty determining which are the rich flowers under these circumstances. Some plants signal old flowers with color changes (Gori 1983, Lunau 1996) but in many species, reward status is not visually apparent. Floral scent marks left by bees may aid in avoiding flowers that have already been visited (Schmitt and Bertsch 1990) although it is not clear how commonly these signals occur, and they may only work over short distances. Alternatively, bumblebees may learn the position of richly rewarding plants (Thomson 1988) which may allow them to ignore conflicting signals of patchy rewards, but this may not be reliable if these plants have subsequently become depleted. We believe that, while these foraging aids may help pollinators to choose rewarding flowers, they are insufficient to overcome the difficulties of highly depleted systems. As a result, whenever flower visitors are abundant, reward depletion will be a potent force in preventing pollinator-mediated selection on reward production rates. This may explain how polymorphisms in reward levels such as we have shown in *Mimulus guttatus* may persist and why rewardless species or sexes are common in nature (Dafni 1984, Willson and Ågren 1989).

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