

Jeffrey K. Conner · Scott Rush

Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*

Received: 25 May 1995 / Accepted: 7 September 1995

Abstract Plant traits that increase pollinator visitation should be under strong selection. However, few studies have demonstrated a causal link between natural variation in attractive traits and natural variation in visitation to whole plants. Here we examine the effects of flower number and size on visitation to wild radish by two taxa of pollinators over 3 years, using a combination of multiple regression and experimental reductions in both traits. We found strong, consistent evidence that increases in both flower number and size cause increased visitation by syrphid flies. The results for small bees were harder to interpret, because the multiple regression and experimental manipulation results did not agree. It is likely that increased flower size causes a weak increase in small-bee visitation, but strong relationships between flower number and small-bee visitation seen in 2 years of observational studies were not corroborated by experimental manipulation of this trait. Small bees may actually have responded to an unmeasured trait correlated with flower number, or lower small-bee abundances when the flower number manipulation was conducted may have reduced our ability to detect a causal relationship. We conclude that studies using only 1 year, one method, or measuring only one trait may not provide an adequate understanding of the effects of plant traits on pollinator attraction.

Key words Pollinator attraction · *Raphanus raphanistrum* · Corolla size · Flower number · Experimental manipulation

Introduction

For plants that rely on animals for pollination, the ability to attract the animals to their flowers can be a crucial component of fitness (Darwin 1877; Waser 1983; Stanton et al. 1986; Galen 1989). Therefore, plant traits that

increase visitation rates may be under strong selection. A large number of studies have examined the effects of floral color, scent, and nectar on pollinator attraction, using both artificial and natural flowers (reviewed in Clements and Long 1923; Waser 1983; Kearns and Inouye 1993, chap. 7). Two other traits that have been shown to affect visitation are flower number (e.g., Willson and Rathcke 1974; Willson and Bertin 1979; Augspurger 1980; Thomson et al. 1982; Bell 1985; Geber 1985; Cruzan et al. 1988; Thomson 1988; Eckhart 1991) and corolla size (Clements and Long 1923; Bell 1985; Galen and Newport 1987; Stanton and Preston 1988; Galen 1989; Young and Stanton 1990; Campbell et al. 1991; Eckhart 1991). The majority of these studies suggest that increased flower number or corolla size increases visitation rates.

Many studies have measured only one floral trait in unmanipulated plants and related those measurements to visitation rates. This does not provide strong evidence that the measured trait is causing differences in visitation or that the trait is under direct selection, because the differences in visitation could be due to some other trait that is correlated with the measured trait. For example, corolla size and pollen production are correlated in some cases (Stanton and Preston 1988), so that a study that showed increased visitation with increasing pollen production without taking corolla size into account could lead to erroneous conclusions.

There are two complementary methods to address the problem of correlations among traits. The first is to use multiple regression (or a related technique) on observational data to correct statistically for correlations among traits. Ideally, visitation rates would be regressed on as many of the plant traits likely to affect visitation as possible. The advantage of this technique is that it can be used on undisturbed plants in natural populations, so that the relevance of the results to the natural situation is assured. The drawback is that purely observational studies like these can never demonstrate causation definitively, because there is always the possibility that another unmeasured trait that is correlated with the measured traits is causing the differences in visitation (cf. Lande and Ar-

J.K. Conner (✉) · S. Rush
Department of Ecology, Ethology, and Evolution,
University of Illinois, Shelford Vivarium, 606 E. Healey Street,
Champaign, IL 61820, USA

nold 1983; Waser 1983). The seriousness of this problem can be diminished by measuring as many traits as possible, focusing on traits that are likely to affect visitation. Multiple regression has rarely been used in studies of the effects of flower size and number on visitation (but see Willson and Bertin 1979; Eckhart 1991).

To demonstrate that a given floral trait causes differences in visitation, experimental manipulation of the trait is necessary. If treatment and control groups are randomly chosen, the appropriate controls are included, and the manipulation alters only the trait of interest (this is sometimes difficult), then differences in visitation between treatment and control groups can reliably be attributed to the manipulated trait and not some correlated trait. However, such experiments cannot determine how much of the natural variation in visitation is due to natural variation in that trait as well as multiple regression on observational data can. It is also usually easier to explore non-linear relationships among variables using continuous natural variation. Experimental manipulation has been used several times in studies of the effects of flower number on visitation (Willson and Rathcke 1974; Thomson et al. 1982; Bell 1985; Geber 1985; Schmid-Hempel and Speiser 1988; Thomson 1988), but much less frequently in studies of the effects of floral size (but see Clements and Long 1923; Bell 1985).

Waser (1983) has argued that the most powerful approach to understanding ecological relationships in general, and adaptation of floral traits specifically, is to combine observational studies using natural variation within or among populations or species (called "comparative" studies in Waser 1983) with manipulative experiments; this takes advantage of the strengths of both approaches. We would add that it is crucial to measure many plant traits and use multiple regression when doing observational studies. This combination of multiple regression and experimental manipulation has rarely, if ever, been applied to studies of pollinator visitation.

In this study we use a combination of multiple regression and experimental manipulation to examine simultaneously the effects of flower number, corolla size, and pollen production on pollinator visitation. We divide visitation into three components: the number of pollinators visiting a plant in a fixed amount of time, the average number of flowers probed during an insect's visit, and the average time spent per flower. The product of these components equals the total amount of time spent on a plant by all pollinators. The first two components are similar to the first two components of visitation of Waser (1983). It seems reasonable to predict that floral attractants such as flower number and size would affect the first component, while floral rewards such as the number of pollen grains per flower should have a greater effect on the last two components. Flower number is also likely to affect the second component, number of flowers visited (e.g. Hodges and Miller 1981; Geber 1985; Schmid-Hempel and Speiser 1988; Thomson 1988; Eckhart 1991).

In addition, we tested for differences among pollinator taxa in their response to flower number and size.

Variation among pollinator taxa in responses to flower size and number has rarely been studied (but see Thomson et al. 1982; Eckhart 1992), and to our knowledge, differences in these responses among pollinator taxa have never been tested statistically.

Materials and methods

Plants and sites

Wild radish (*Raphanus raphanistrum*) is a weedy annual of disturbed sites. Since it is self-incompatible (Sampson 1964), wild radish relies on insect pollinators for nearly all reproduction. Pollinators of wild radish include butterflies, honey bees, smaller bees (mainly Halictidae) and syrphid flies (Kay 1976; Stanton et al. 1989; Conner et al. 1996; see Results below); all of these taxa are effective pollinators (Conner et al. 1995). The pollinators in this study foraged almost exclusively for pollen (see Results).

Pollinators were observed at the Phillips Tract natural area of the University of Illinois, located approximately 5 km northeast of Urbana, Ill. This site is a mixture of mature forest, restored prairie, and abandoned farmland in various successional stages. In 1992 an experimental garden of 64 wild radish plants was planted in a square grid with 1-m spacing between plants. In 1993 and 1994 potted plants were grown in a greenhouse and placed in square grids with 1-m spacing at the same field site. Seeds for all experiments were collected from a single large population in upstate New York containing only the yellow flower morph (see Conner and Via 1993 for details). Wild radish is present naturally in central Illinois, but usually in extremely small populations. All observations were conducted between 0800 and 1700 hours.

Observational studies (1992 and 1993)

Observations

All observations were conducted as follows. Individual plants were observed for a fixed period of time (see below), and all visitors to that plant were recorded as belonging to one of three categories: small bees, syrphid flies, and other (primarily Lepidoptera, honey bees, and bumble bees). Only visitation by small bees and syrphid flies are considered in this paper, because these two groups together accounted for 80% of all visits in 1992, and over 90% of all visits in 1993 and 1994 (Conner et al. 1996); no other groups of insects made more than 6% of the total visits. A sample of small bees and syrphids were collected for identification (see below). In addition, on a subset of visitors the total time spent at the plant and the number of flowers probed by the visitor were recorded.

In 1992, 53 plants were observed for one hour each between 16 July and 10 August. Seven sets of 16 potted plants (112 total) were used in 1993; sets did not differ in average flower size. Each set was brought to the field on a given day, and plants were observed for 10–40 min each. This procedure was repeated six times for each set; i.e., the sets were cycled through until all sets had been in the field six times. Plants were observed in a different random sequence on each day. Not all plants were observed each time, but 93% of the plants were observed five or six times. These observations were conducted from 8 July to 29 August, which spans the majority of the normal wild radish flowering season.

Variables measured

Each time a plant was observed, the length of the showy part of one petal (the "limb") was measured with digital callipers as an estimate of corolla size, the number of flowers open on the plant was counted, and the height of the plant was measured. Medians

Table 1 Medians and ranges for flower size (petal limb length) and number in each year. The *two columns for 1994* refer to different sets of plants: those listed in the *petal length column* are from the petal length manipulation experiment, and those listed in the *flower number column* are from the flower number manipulation experiment

| | Petal length | Flower number |
|-------------------|----------------|---------------|
| 1992 | 8.1 (6.1–10.7) | 55 (3–327) |
| 1993 | 7.9 (4.2–11.1) | 22 (1–107) |
| 1994 Control | 7.7 (6.0–11.0) | 17 (6–70) |
| 1994 Experimental | 5.5 (4.4–7.1) | 8.5 (3–35) |

and ranges for petal length and flower number are given in Table 1. Temperature data were obtained from hourly readings at an Illinois State Water Survey weather station located approximately 10 km from the field site. The temperature at the reading time closest to the start of the observation period was used.

Pollen production by flowers was measured only in 1992 as follows. Flower buds were covered with a bridal-veil net bag the day before observations to prevent visitation to the flowers that opened inside. At the start of the observation period pollen was collected from one open flower immediately upon bag removal. Pollen was collected by removing the anthers with forceps and placing them in a vial. The pollen sample was later counted using a Coulter counter (Harder et al. 1985; see Rush et al. 1995 for details). This procedure provided a good estimate of pollen production per flower, but not of pollen available per flower at the time of observations, because most of the flowers on the plant were not bagged and therefore had experienced variable levels of pollen depletion.

Analyses

Data were analyzed using multiple regression. Separate regressions were performed for each of the 2 years, and within each year for each of the two main taxa of pollinators (small bees and syrphid flies). Within each year and pollinator taxon, separate regressions were performed for three dependent variables: total number of visits (standardized to a per hour basis), the average number of flowers probed per visit, and the average time spent per flower probed. The correlations among the six dependent variables in each year were generally low. In 1992, all but 1 of the 15 correlations were <0.31 , and 7 were <0.15 . The one exception was correlation of 0.51 between the number of flowers probed per visit by the two taxa of pollinators. The 1993 correlations were even lower, all being <0.30 and 11 being <0.15 . Therefore, independent regressions are appropriate.

Each of the 12 regressions included corolla size (petal length), number of open flowers, date, time of day, and air temperature as independent variables. The number of pollen grains produced per flower was also included in the 1992 regressions. Plant height was included in all initial regressions, but later dropped because it was never significant. This made no major changes to the results for the rest of the variables, but did improve the model fit in several cases. In addition, the 1993 regressions included a categorical variable for plant identity, since plants were observed multiple times. None of the interactions between plant identity and the other variables were significant, so these were dropped from the final model. Quadratic (squared) terms were included for all continuous variables in 1993 to test for curvature in the relationships. Due to the smaller samples sizes in 1992, quadratic terms were fit in initial models, but non-significant quadratic terms were dropped for final models to keep closer to the rule of thumb of at least 10 observations per independent variable (Mitchell 1993 and references therein).

To test explicitly whether syrphid flies and bees differed in their responses to flower size and number, we compared the slopes

of the regressions of the three visitation variables on the plant traits across the two taxa. To do this, we calculated the difference between syrphid flies and small bees for each plant, and regressed this difference on the variables listed above. A significant slope for either flower size or number indicates that the two taxa of pollinators respond differently to that plant trait, that is, their individual slopes are different (C. McCulloch, personal communication).

All dependent variables and flower number in 1992 were \ln -transformed to improve normality and reduce heteroscedasticity. Continuous independent variables were standardized before analysis (mean = 0 and variance = 1) to facilitate comparisons among variables measured on different scales, pollinator taxa, and years. Independent variables were not strongly correlated, with no correlation greater than 0.6, and 85% less than 0.3. Variance inflation factors (Neter et al. 1985) were less than 2 for all regressions, also indicating little multicollinearity. All analyses were performed using JMP (SAS Institute 1994) on a Macintosh computer.

Experimental manipulations (1994)

Two experiments were performed in 1994 to test further the roles of corolla size and flower number in pollinator attraction. Plants were used only once in each experiment. In the corolla size experiment, the petals of half of the flowers on each plant were experimentally shortened as follows. Plants with relatively long petals were used. If the plant had an odd number of racemes (inflorescences), one raceme was removed. The remaining racemes were assigned to two groups of equal number of racemes, and then flowers were trimmed from the group with more flowers so that there were equal numbers of flowers in the two groups. The two groups of racemes were then assigned to either control or experimental treatment by a coin-flip. The distal ends of all petals on all flowers in both groups were then trimmed using fingernail trimmers, which roughly simulated the curved ends of natural petals. In the control group, only a small amount of petal was removed (<1 mm) to control for the effects of trimming. In the experimental group, petals were trimmed to a median of 5.5 mm in length (Table 1); 5 mm is the length of the smallest natural petals in field populations (J.K. Conner, unpublished work). Therefore, the experimental and control flowers were paired within a plant, which controlled for all other traits that vary among plants.

Within each of the two groups of racemes on a plant (experimental and control) the flower with the longest and the flower with the shortest petals were chosen by eye in an attempt to capture the full range of variability within each group; the length of one petal from each of these flowers was measured with digital calipers. The average petal length was significantly greater in control racemes compared to experimental racemes (average difference = $2.3 \text{ mm} \pm 0.12 \text{ SEM}$; $P < 0.0001$, Wilcoxon signed-rank test; see Table 1). Because the wild radish petal narrows proximally, petal width was also reduced by the experimental reduction in petal length (average difference $0.45 \text{ mm} \pm 0.10 \text{ SEM}$; $P < 0.0001$, Wilcoxon signed-rank test).

Since in most cases the observer in the field could not easily distinguish the experimental from the control racemes, in the greenhouse each raceme was marked with a small piece of green tape, and a black dot was placed on one group of racemes (chosen randomly). The plants were then taken to the field site and observed for one to three 10-min periods. Visitors to both groups of racemes (marked with black dot or not) were recorded during these periods. Therefore, the observations were largely blind. The two groups of racemes on a plant were treated as separate plants for the observations, because there were often too many pollinators present to follow the flights of individual insects. Thus, a pollinator that moved from the control to the experimental group of racemes was recorded as having visited both. Since wild radish often grows in dense patches, discrimination between plants in nature often may occur over similar short distances. Observations were conducted on seven days from 21 June to 29 July, and then another set on 5 days from 1 September to 5 October to sample seasonal variability in pollinators. A total of 64 plants were used in the experiment.

In the flower number experiment, we reduced the number of flowers on a plant to half the number found on a paired control plant as follows. Pairs of plants were chosen with similar corolla sizes. One member of the pair was chosen to be the experimental plant by coin flip. Flowers were removed from this plant by cutting the pedicels until it had half the number of flowers as the control plant. An equal number of pedicels of recently wilted flowers were cut on the control plant to control for the effects of cutting. Control and experimental plants had a median of 17 and 8.5 flowers respectively; this is at the small end of the natural range observed in 1992 (Table 1). Plant pairs were placed next to each other in the array and observed simultaneously by a pair of observers. Observers did not exchange information about visitation during observations. After all pairs of plants had been observed once, they were observed again in the same order, but with observers assigned to the opposite members of each pair. This procedure controlled for possible observer bias, and sampled the temporal variation in visitation more widely. Observations were conducted on seven days from 31 August to 13 September on a total of 32 pairs of plants.

Data analyses for the two experimental studies were similar. Data from all 10-min observation periods for each plant were combined, and the number of visits by small bees and syrphid flies were analyzed separately. Data were paired either within plants (flower size experiment) or across pairs of plants (flower number experiment). This procedure controls for temporal variation in visitation, because pairs were observed simultaneously (see above). The Wilcoxon signed-rank test for paired data was used to test for differences in visitation rates between experimental and control groups.

To test for differences between syrphids and bees in their responses to the two plant traits, the number of each pollinator visiting the control member of a pair was subtracted from the number of the same pollinator visiting the experimental member of the pair. These new variables represent the magnitude of the response of each pollinator to the treatment. To correct for differences in overall abundance of the two taxa of pollinators, each response was divided by the median number visits made to control plants by that pollinator taxon over the course of the experiment. The relative response magnitude for syrphids was then compared to the relative response magnitude for bees, again using a Wilcoxon signed-rank test.

Insect collection

In 1993 and 1994, insects visiting the potted plants at the study site were collected for identification. All insects that were observed foraging on wild radish flowers were collected, and collections were done at various times of day on several dates spanning the period during which observations were conducted each year. Collecting was not done during observation periods, however, to minimize disturbance. A total of 127 small bees and syrphid flies were collected, evenly divided between years (63 in 1993 and 64 in 1994).

Results

Insect visitors

Twelve species of small bees from three families and five genera and nine species of syrphid flies from nine genera were collected while visiting wild radish in 1993 and 1994 (Table 2). In both years, the majority of small bees collected were in the family Halictidae, with the vast majority of these consisting of seven species of *Dialictus*, particularly *D. rohweri* and *D. imitatus*. The major difference between the two years was that the non-halictid visitors were from different families: *Ceratina dupla*

Table 2 Small bee and syrphid fly species collected while visiting wild radish at the study site in 1993 and 1994, with numbers of individuals collected and percentages of the total within each taxon. Bee families are in **boldface**, as are the combined numbers for all species of *Dialictus*. One *Dialictus* specimen could not be identified to species due to poor preparation, and three genera of syrphids could not be identified to species

| | 1993 | | 1994 | |
|-------------------------------------|-----------|-----------|----------|-----------|
| | No. | % | No | % |
| A. Small bees: | | | | |
| Anthophoridae | | | | |
| <i>Ceratina dupla</i> | 6 | 21 | 0 | 0 |
| Colletidae | | | | |
| <i>Hylaeus grossicornis</i> | 0 | 0 | 1 | 6 |
| <i>H. illinoiensis</i> | 0 | 0 | 8 | 44 |
| Halictidae | | | | |
| <i>Augochlora pura</i> | 2 | 7 | 0 | 0 |
| <i>Dialictus atlanticus</i> | 1 | 4 | 0 | 0 |
| <i>D. hartii</i> | 0 | 0 | 1 | 6 |
| <i>D. imitatus</i> | 5 | 18 | 1 | 6 |
| <i>D. obscurus</i> | 1 | 4 | 0 | 0 |
| <i>D. rohweri</i> | 8 | 29 | 7 | 39 |
| <i>Dialictus</i> sp. | 1 | 4 | 0 | 0 |
| <i>D. viridulus</i> | 2 | 7 | 0 | 0 |
| <i>D. zephyrus</i> | 1 | 4 | 0 | 0 |
| All <i>Dialictus</i> species | 19 | 68 | 9 | 50 |
| <i>Heriades carinata</i> | 1 | 4 | 0 | 0 |
| Total | 28 | 100 | 18 | 100 |
| B. Syrphid flies: | | | | |
| <i>Allograpta obliqua</i> | 4 | 11 | 8 | 17 |
| <i>Eristalis tenax</i> | 1 | 3 | 2 | 4 |
| <i>Melanostoma pictipes</i> | 0 | 0 | 3 | 7 |
| <i>Mesograpta polita</i> | 1 | 3 | 0 | 0 |
| <i>Platycheirus hyperboreus</i> | 1 | 3 | 0 | 0 |
| <i>Sphaerophoria</i> sp. | 3 | 9 | 0 | 0 |
| <i>Syrpitta pipiens</i> | 1 | 3 | 0 | 0 |
| <i>Syrphus</i> sp. | 7 | 20 | 10 | 22 |
| <i>Toxomerus</i> sp. | 17 | 49 | 23 | 50 |
| Total | 35 | 100 | 46 | 100 |

(Anthophoridae) in 1993 and two *Hylaeus* species (Colletidae) in 1994. The syrphid flies collected were dominated by *Toxomerus*, *Syrphus* and *Allograpta* in that order, with similar proportions of each across years.

All of the species foraged primarily for pollen. The syrphid flies foraged for pollen exclusively, as did the smaller bees. *Ceratina dupla*, the only long-tongued species collected, was able to crawl part way into the corolla tube and forage for nectar as well as pollen on some visits.

Observational studies (1992 and 1993)

Number of visits

The multiple regression analyses for 1992 and 1993 explained between 45 and 66% of the variance in the num-

Table 3 Factors affecting the number of pollinator visits to plants. Shown are the results of four separate multiple regressions of the number of visits made by the two categories of pollinators in 1992 and 1993 (ln-transformed) on the independent variables listed. Standardized partial regression slopes are shown. Quadratic (squared) terms that were not significant were dropped in 1992 due to the smaller sample size that year. The 1993 regressions also included a categorical variable for plant identity that was not significant. Pollen production was measured in 1992 only. The overall R^2 line gives the proportion of variance explained by all the predictor variables combined, with the significance of the overall regression model. Total df were 49 in 1992 and 586 in 1993

| | Small bees | | Syrphid flies | |
|---------------------|---------------------|---------------------|---------------------|---------------------|
| | Linear | Quadratic | Linear | Quadratic |
| A. 1992: | | | | |
| Corolla size | 0.04 | – | 0.27* ² | – |
| No. flowers open | 0.63* ⁴ | –0.12* ² | 0.18 ^a | – |
| No. pollen grains | –0.01 | – | 0.01 | – |
| Date | 0.16 | – | 0.37* ³ | – |
| Time | 0.03 | –0.44* ³ | –0.39* ³ | – |
| Temperature | 0.42* ³ | – | 0.02 | – |
| Overall model R^2 | 0.66* ⁴ | | 0.57* ⁴ | |
| B. 1993: | | | | |
| Corolla size | 0.10 ^a | –0.03 | 0.19* ² | –0.07* ¹ |
| No. flowers open | 0.39* ⁴ | –0.11* ⁴ | 0.33* ⁴ | –0.02 |
| Date | –0.45* ⁴ | –0.28* ⁴ | 0.47* ⁴ | 0.09 ^a |
| Time | –0.25* ⁴ | –0.08* ¹ | –0.14* ² | 0.04 |
| Temperature | 0.45* ⁴ | –0.01 | –0.42* ⁴ | –0.01 |
| Overall model R^2 | 0.45* ⁴ | | 0.48* ⁴ | |

^a $P < 0.15$, *¹ $P < 0.05$, *² $P < 0.01$, *³ $P < 0.001$, *⁴ $P < 0.0001$

ber of visits by the two taxa of pollinators, and all of the regression models were highly significant (Table 3). Results for flower number and size were quite consistent across years, but differed across taxa. Small bee visitation increased strongly with increasing flower number in both years, but there was little or no effect of corolla size. The standardized partial regression coefficient of 0.63 for the relationship between flower number and small bee visitation in 1992 means that a 1 SD increase in ln-flower number was associated with a 63% increase in ln-small-bee visitation after correcting for the other variables in the model. There were significant negative quadratic terms for the effect of flower number on small bee visitation in both years, suggesting diminishing returns to the plant for increasing flower number.

In contrast, syrphid fly visitation increased with increased flower number and size in both years, although the 1992 result for flower number was only marginally significant. In contrast to small bees, the relationships between syrphid fly visitation and flower number were linear, but there was evidence for diminishing returns for increased flower size in 1993 (negative quadratic coefficient). The linear regression slopes for bees and flies are significantly different for flower number in both years ($P = 0.0002$ and 0.03 , respectively) and marginally significantly different for corolla size in 1992 only ($P = 0.09$

and 0.86 , respectively). Pollen production per flower did not affect the number of visits by either category of pollinator in 1992, the only year that pollen was measured.

The effect of date, time, and temperature varied between pollinator categories and somewhat between years (Table 3). Syrphid fly visitation increased later in the season (date) in both years. Date did not affect small bee visitation significantly in 1992, but in 1993 small bee visitation declined in an accelerating fashion later in the season (negative linear and quadratic terms in Table 3). Visitation by syrphids in both years and small bees in 1993 declined later in the day, while in 1992 small bee visitation was lowest at mid day (positive quadratic coefficient with no linear component). Small bee visitation increased with increasing temperature in both years; in contrast, syrphid visitation decreased with temperature in 1993 and was unaffected by temperature in 1992.

There were highly significant differences between the slopes for bees and flies for both date and temperature in 1993 (both $P < 0.0001$) and a marginally significant difference for temperature in 1992 ($P = 0.07$). There were no differences in slopes for time in either year or date in 1992. Thus, in 1993 the two pollinator categories responded in opposite directions to date and temperature and in the same direction to time of day. Recall that each of these partial regression coefficients represents the effect of the independent variable on visitation after the effects of other independent variables have been removed.

Flowers probed/visit and time spent/flower

There were few significant relationships between the variables we measured and flowers probed per visit or time spent per flower (Tables 4 and 5). All the variables together explained between 13 and 40% of the variance, less than the variance in number of visits explained by these variables, and two of the eight regression models were not significant. Neither corolla size nor pollen production per flower had any effect on either of these visitation variables for either pollinator category. The lack of a relationship with pollen production could be due to the fact that observations were made at different times of day when the flowers had been depleted by different amounts. The negative relationship between time of day and time spent per flower by syrphid flies in both years (marginally significant in 1993) could reflect the effects of pollen depletion.

Not surprisingly, the number of flowers open on a plant had a positive effect on the number of flowers probed per visit for small bees in both years and for syrphid flies in 1993, but had no effect on the time spent per flower (Tables 4 and 5). The relationship between flower number and number of flowers probed showed diminishing returns for both taxa of pollinators in 1993. Both flowers probed per visit and perhaps time spent per flower seemed to decline with date; seven of the eight regression coefficients were negative, with five of these at least marginally significant. Temperature had little effect

Table 4 Results of multiple regressions of the average number of flowers probed per pollinator visit on the independent variables listed (see Table 3 for details). Total *df* were 47 in 1992 and 488 and 440 for small bees and syrphid flies, respectively, in 1993

| | Small bees | | Syrphid flies | |
|-------------------------------------|---------------------|---------------------|---------------------|---------------------|
| | Linear | Quadratic | Linear | Quadratic |
| A. 1992: | | | | |
| Corolla size | -0.02 | - | 0.02 | - |
| No. flowers open | 0.13* ¹ | - | 0.04 | - |
| No. pollen grains | -0.05 | - | -0.03 | - |
| Date | -0.25* ² | - | -0.16 ^a | - |
| Time | -0.03 | - | 0.07 | - |
| Temperature | 0.12 ^a | - | -0.12 | - |
| Overall model <i>R</i> ² | 0.36* ² | | 0.13 | |
| B. 1993: | | | | |
| Corolla size | -0.04 | -0.01 | -0.02 | -0.02 |
| No. flowers open | 0.28* ⁴ | -0.07* ³ | 0.29* ⁴ | -0.08* ³ |
| Date | -0.20* ⁴ | -0.05 | -0.10* ¹ | -0.06 |
| Time | -0.03 | -0.03 | -0.07 ^a | 0.03 |
| Temperature | 0.02 | 0.02 | 0.07 ^a | 0.03 |
| Overall model <i>R</i> ² | 0.36* ⁴ | | 0.37* ³ | |

^a $P < 0.15$, *¹ $P < 0.05$, *² $P < 0.01$, *³ $P < 0.001$, *⁴ $P < 0.0001$

Table 5 Results of multiple regressions of the average time spent by pollinators per flower on the independent variables listed (see Table 3 for details). Total *df* were 47 in 1992 and 487 and 440 for small bees and syrphid flies, respectively, in 1993

| | Small bees | | Syrphid flies | |
|-------------------------------------|---------------------|---------------------|---------------------|-----------|
| | Linear | Quadratic | Linear | Quadratic |
| A. 1992: | | | | |
| Corolla size | 0.00 | - | -0.01 | - |
| No. flowers open | 0.00 | - | 0.02 | - |
| No. pollen grains | 0.04 | - | 0.13 | - |
| Date | 0.04 | - | -0.09 | - |
| Time | -0.05 | - | -0.38* ³ | - |
| Temperature | -0.17 ^a | - | 0.03 | - |
| Overall model <i>R</i> ² | 0.14 | | 0.35* ² | |
| B. 1993: | | | | |
| Corolla size | 0.01 | -0.01 | 0.00 | -0.03 |
| No. flowers open | 0.04 | -0.04 ^a | 0.02 | 0.00 |
| Date | -0.19* ⁴ | 0.02 | -0.06 | 0.07 |
| Time | 0.00 | -0.08* ¹ | -0.07 ^a | 0.05 |
| Temperature | -0.03 | -0.06 ^a | -0.31* ⁴ | 0.03 |
| Overall model <i>R</i> ² | 0.30* ¹ | | 0.40* ⁴ | |

^a $P < 0.15$, *¹ $P < 0.05$, *² $P < 0.01$, *³ $P < 0.001$, *⁴ $P < 0.0001$

on these visitation variables, except for a negative relationship between temperature and time spent per flower for syrphid flies in 1993.

Finally, the slopes for small bees and syrphid flies did not differ significantly for either flowers probed per visit or time spent per flower in any year for any predictor variable.

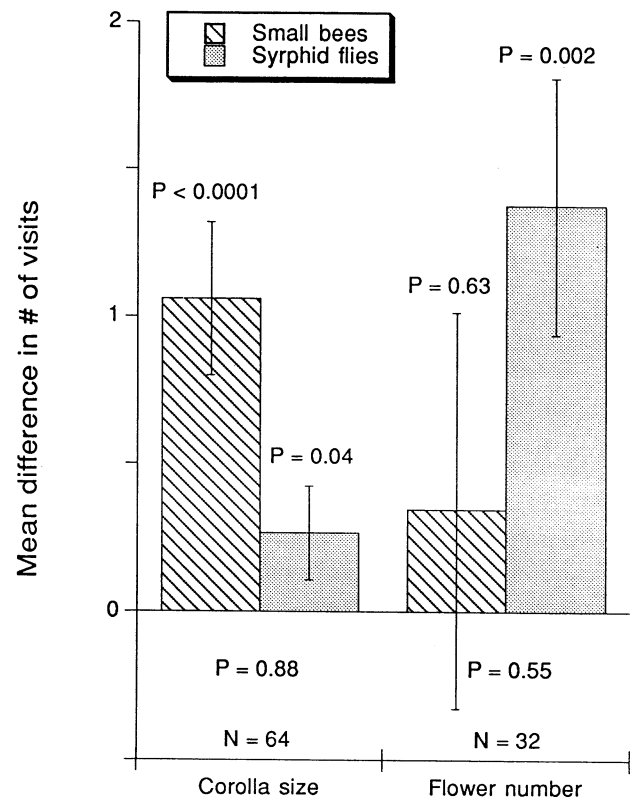


Fig. 1 Results of the experimental manipulations of flower size and number on visitation by the two main taxa of pollinators, small bees and syrphid flies. Bars represent the mean difference between the number of visits to control versus experimental inflorescences (petal size or flower number reduced) in the paired tests. Error bars are ± 1 SEM. *P*-values above each bar are from Wilcoxon signed-rank tests for differences between controls and treatments within each taxon, and *P*-values below each pair of bars are the probability of no difference between the two taxa in their responses to the treatments. *N*=64 plants for corolla size and 32 pairs of plants for flower number (see Methods)

Experimental manipulations (1994)

The results of the corolla size manipulation experiment showed that both small bees and syrphid flies preferred flowers with longer petals, and this preference did not differ between taxa after correcting for the higher abundance of bees (Fig. 1). In contrast, the flower number manipulation experiment showed a strong preference of syrphid flies for plants with more flowers, but no discrimination on the basis of flower number by small bees. These preferences were not significantly different from each other after correcting for the greater number of syrphids, however, due to the large standard errors (Fig. 1).

Discussion

Clearly, increases in both corolla size and flower number caused increases in pollinator visitation in wild radish, in agreement with most studies of other species (see Introduction). The results for syrphid flies were very consistent across the three years and different methods used in this study: increased flower size and number clearly have

a causal relationship with syrphid fly visitation. The small bee results were more variable. The multiple regression results from 1992 and 1993 indicated little or no increase in small bee visitation with increasing flower size, but a strong response to increased flower number. The experimental manipulation in 1994 showed the opposite pattern: a strong response of small bees to flower size, but no effect of flower number.

It seems likely that the experimental manipulation gave us more power than the observational results to detect subtle effects of flower size on small bee visitation, and a causal relationship seems likely. Conversely, the strong effect of flower number in 1992 and 1993 may have been due to an unmeasured correlated trait, since experimental manipulation of flower number in 1994 had little effect on bee visitation. However, it is not obvious what this other trait might be, and there is an alternative explanation: the flower number manipulation was done later in the season when there were fewer small bees foraging on wild radish (median = 4.5 visits/h compared to 24 visits/h in 1992 and 1993), so there may have been too few bees present to produce a significant difference.

In some cases the responses of the two taxa of pollinators differed significantly within the same year: bees responded more strongly than flies to flower number in 1992 and 1993, and flies responded marginally more strongly to corolla size in 1992.

While flower number and size were clearly important in determining the number of pollinators visiting the plant, in most cases they did not affect the number of flowers probed and time spent per flower during a given visit. However, increasing flower number was usually associated with increased number of flowers probed per visit, in agreement with previous studies (Hodges and Miller 1981; Geber 1985; Schmid-Hempel and Speiser 1988; Thomson 1988; Eckhart 1991). Corolla size did not affect either number of flowers probed or time spent per flower, also in agreement with previous studies (Young and Stanton 1990; Eckhart 1991).

At first glance, it was somewhat surprising that the number of pollen grains produced by flowers did not affect any aspects of visitation by pollinators that were feeding almost exclusively on pollen. However, since we measured pollen production, not pollen available at the time of observations, any effect of differential pollen production by plants may have been masked by differences in pollen depletion. Pollen is removed very rapidly from wild radish flowers at our study site (Rush et al. 1995). In fact, the length of the first visit to a previously unvisited flower was much longer than the average visit recorded in this study (J.K. Conner and S. Rush, unpublished work), suggesting that available pollen does influence time spent per flower. Stanton et al. (1991) found that visits by small bees (mainly anthophorids and halictids, as in our study) to *Raphanus sativus* increased with increased pollen production.

Future studies of the effect of pollen on visitation should use either measurements of pollen available at time of visit (which might be difficult to estimate accu-

rately in a plant such as wild radish with many flowers) or experimental manipulation of pollen number. Both experimental increases and decreases in pollen number should be feasible in many plants. For example, Buchmann and Cane (1989) showed that both experimental removal of pollen and glueing the anther pore closed significantly reduced the time that bees spent per flower. The most extreme form of experimental pollen decrease, complete emasculation, had no effect on visitation in one study (Geber 1985), but decreased both the number of visits and the time spent per flower in another study (Young and Stanton 1990).

In conclusion, the combination of multiple regression on unmanipulated plants with experimental manipulation of both flower number and size has clearly shown that both these plant traits are important to pollinator attraction. However, the importance of these two traits varied among the two categories of pollinators observed, and experimental manipulations suggested that the apparent increased attractiveness of plants with more flowers to small bees may actually be due to an unmeasured correlated trait.

Acknowledgements S. Strauss provided invaluable discussion and assistance with the corolla size experiment. We thank A. Winn and C. Fenster for discussion, C. West and S. Kercher for conducting much of the corolla size and flower number experiments, and many University of Illinois undergraduates for help with pollinator observations. C. Augspurger, K. Paige, B. Silverman, and S. Strauss made many useful comments on earlier versions of this paper. C. McCulloch helped with statistics, and W. LaBerge and D. Webb graciously identified the bees and flies, respectively. This material is based upon work supported by the Research Board and the Howard Hughes program for undergraduate research at the University of Illinois, and the National Science Foundation under Grant No. DEB 93-18388.

References

- Augspurger CK (1980) Mass-flowering of a tropical shrub (*Hybanthus prunifolius*): influence on pollinator attraction and movement. *Evolution* 34:475–488
- Bell G (1985) On the function of flowers. *Proc R Soc Lond B* 224:223–265
- Buchmann SL, Cane JH (1989) Bees assess pollen returns while sonicating *Solanum* flowers. *Oecologia* 81:289–294
- Campbell DR, Waser NM, Price MV, Lynch EA, Mitchell RJ (1991) Components of phenotypic selection: pollen export and flower corolla width in *Ipomopsis aggregata*. *Evolution* 45:1458–1467
- Clements FE, Long FL (1923) Experimental pollination: an outline of the ecology of flowers and insects. Carnegie Institution of Washington, Washington, DC
- Conner J, Via S (1993) Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47:704–711
- Conner JK, Davis R, Rush S (1995) The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators. *Oecologia* 104:234–245
- Conner JK, Rush S, Jennetten P (1996) Measurements of natural selection on floral traits in wild radish (*Raphanus raphanistrum*). I. Selection through lifetime female fitness. *Evolution*, in press
- Cruzan MB, Neal PR, Willson MF (1988) Floral display in *Phyla incisa*: consequences for male and female reproductive success. *Evolution* 42:505–515

- Darwin C (1877) The different forms of flowers on plants of the same species. University of Chicago Press, Chicago
- Eckhart VM (1991) The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). *Evol Ecol* 5:370–384
- Eckhart VM (1992) Spatio-temporal variation in abundance and variation in foraging behavior of the pollinators of gynodioecious *Phacelia linearis* (Hydrophyllaceae). *Oikos* 64:573–586
- Galen C (1989) Measuring pollinator-mediated selection on morphometric traits: bumblebees and the alpine sky pilot, *Polemonium viscosum*. *Evolution* 43:882–890
- Galen C, Newport MEA (1987) Bumble bee behavior and selection on flower size in the sky pilot, *Polemonium viscosum*. *Oecologia* 74:20–23
- Geber MA (1985) The relationship of plant size to self-pollination in *Mertensia ciliata*. *Ecology* 66:762–772
- Harder LD, Thomson JD, Cruzan MB, Unnasch RS (1985) Sexual reproduction and variation in floral morphology in an ephemeral vernal lily, *Erythronium americanum*. *Oecologia* 67:286–291
- Hodges CM, Miller RB (1981) Pollinator flight directionality and the assessment of pollen returns. *Oecologia* 50:376–379
- Kay QON (1976) Preferential pollination of yellow-flowered morphs of *Raphanus raphanistrum* by *Pieris* and *Eristalis* spp. *Nature* 261:230–232
- Kearns CA, Inouye DW (1993) Techniques for pollination biologists. University Press of Colorado, Niwot
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Mitchell RJ (1993) Path analysis: pollination. In: Scheiner SM, Gurevitch J (eds) Design and analysis of ecological experiments. Chapman and Hall, New York, pp 211–231
- Neter J, Wasserman W, Kutner MH (1985) Applied linear statistical models, 2nd edn. Irwin, Homewood
- Rush S, Conner J, Jennetten P (1995) The effects of natural variation in pollinator visitation on rates of pollen removal in wild radish, *Raphanus raphanistrum* (Brassicaceae). *Am J Bot* 82:1522–1526
- Sampson DR (1964) A one-locus self-incompatibility system in *Raphanus raphanistrum*. *Can J Genet Cytol* 6:435–445
- SAS Institute (1994) JMP, version 3. SAS Institute, Cary
- Schmid-Hempel P, Speiser B (1988) Effects of inflorescence size on pollination in *Epilobium angustifolium*. *Oikos* 53:98–104
- Stanton ML, Preston RE (1988) Ecological consequences and phenotypic correlates of petal size variation in wild radish, *Raphanus sativus* (Brassicaceae). *Am J Bot* 75:528–539
- Stanton ML, Snow AA, Handel SN (1986) Floral evolution: attractiveness to pollinators increases male fitness. *Science* 232:1625–1627
- Stanton ML, Snow AA, Handel SN, Berezky J (1989) The impact of a flower-color polymorphism on mating patterns in experimental populations of wild radish (*Raphanus raphanistrum* L.). *Evolution* 43:335–346
- Stanton ML, Young HJ, Ellstrand NC, Clegg JM (1991) Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. *Evolution* 45:268–280
- Thomson JD (1988) Effects of variation in inflorescence size and floral rewards on the visitation rates of traplining pollinators of *Aralia hispida*. *Evol Ecol* 2:65–76
- Thomson JD, Maddison WP, Plowright RC (1982) Behavior of bumble bee pollinators of *Aralia hispida* Vent. (Araliaceae). *Oecologia* 54:326–336
- Waser NM (1983) The adaptive nature of floral traits: ideas and evidence. In: Real L (eds) Pollination biology. Academic Press, Orlando, pp 241–285
- Willson MF, Bertin RI (1979) Flower-visitors, nectar production, and inflorescence size of *Asclepias syriaca*. *Can J Bot* 57:1380–1388
- Willson MF, Rathcke BJ (1974) Adaptive design of the floral display in *Asclepias syriaca* L. *Am Midl Nat* 92:47–57
- Young HJ, Stanton ML (1990) Influences of floral variation on pollen removal and seed production in wild radish. *Ecology* 71:536–547