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The effect of wild radish floral morphology on pollination efficiency by four taxa of pollinators

Abstract The effects of floral morphology on rates of pollen removal and deposition by different pollinators in generalist plant species are not well known. We studied pollination dynamics in wild radish, *Raphanus raphanistrum*, a plant visited by four groups of pollinators: honey bees, small native bees, butterflies, and syrphid flies. The effects of anther position and other factors on pollen removal during single visits by all four pollinator taxa were measured. Flowers with high anther exsertion (i.e., anthers placed higher above the opening of the corolla tube) tended to have the highest numbers of pollen grains removed, but this effect was strongest for honey bees and butterflies. For all pollinator taxa, pollen removal increased with the number of pollen grains available on a flower and showed a positive, decelerating relationship with the duration of the visit. The effects of stigma position and other factors on pollen deposition during single visits by honey bees and butterflies were also studied. The nectar-feeding butterflies had a higher pollination efficiency (percentage of pollen grains removed from anthers that were subsequently deposited on a stigma) than the nectar- and pollen-feeding honey bees. Flowers with intermediate stigma exsertion had the highest numbers of pollen grains deposited on their stigmas by butterflies, but stigma exsertion had no effect on deposition by honey bees. For both butterflies and honey bees, pollen deposition on the recipient flower increased with the amount of pollen removed from the donor flower, and there was a positive, decelerating relationship between deposition and time spent at the flower; these results are analogous to those for pollen removal. The effects of anther and stigma exsertion on pollen removal and deposition did not fit predictions based on patterns of floral correlations, but results for morphology, pollen availability, time spent per visit, and pollinator efficiency are in broad agreement with previous studies, suggesting the possible emergence of some general rules of pollen transfer.

Key words *Raphanus raphanistrum* · Pollination efficiency · Floral morphology · Floral correlations · Anther and stigma exsertion

Introduction

Since Darwin, biologists have been interested in the evolution of floral morphological traits as adaptations for effective animal pollination. Much of this interest has focused on the effect that floral morphology has on the efficiency of pollen removal and deposition during pollinator visits. Pollen removal has been measured far less frequently than pollen deposition, in part due to the difficulty of accurately estimating pollen removal, except in plants where pollen is packaged into pollinia (e.g., orchids: Darwin 1877; Nilsson 1988). There have been only a few quantitative estimates of the effect of morphology on both pollen removal and deposition (e.g., Nilsson 1988; Galen and Stanton 1989; Murcia 1990; Young and Stanton 1990). Most of these were studies either of relatively specialized plant species, that is, those pollinated by a few related pollinator species, or have only measured pollen removal and deposition by one pollinator taxon (but see Murcia 1990). Here we report the effects of floral morphology in a generalist species, wild radish, on efficiency of pollination by a variety of pollinator taxa.

One aspect of floral morphology that has received attention from pollination biologists is the position of the anthers and stigma relative to the corolla tube. Most of this attention has been directed at plant species exhibiting heterostyly, in which anthers and stigmas can be found at two or three distinct positions relative to the corolla tube (see Barrett 1992 for a review). Studies on the heterostylous species *Pontederia cordata* (Wolfe and Barrett 1989; Harder and Barrett 1993) have shown that

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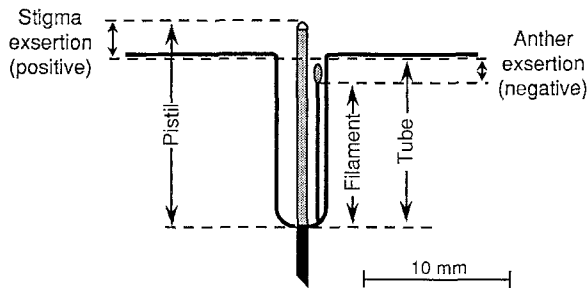
Fig. 1 Schematic cross-section of a wild radish flower, showing the traits measured. Anther exsertion was calculated as filament length minus corolla tube length, and stigma exsertion was calculated as pistil length minus corolla tube length. A positive stigma exsertion and a negative anther exsertion are shown for illustration only; both positive and negative values of both stigma and anther exsertion are found in wild radish flowers (see Figs. 4, 5)

anther and stigma position do affect pollen removal and deposition patterns, as have studies of several non-heterostylous species (Galen and Stanton 1989; Murcia 1990; Campbell et al. 1994). Several hypotheses have been advanced to explain these patterns (Grant and Grant 1965; Barrett 1992; Harder and Barrett 1993; Campbell et al. 1994).

Another set of hypotheses concerning the effect of floral morphology on pollen removal and deposition were generated by studies of patterns of correlations among floral traits (Conner and Via 1993; Conner and Sterling 1995). These studies examined four species in the Brassicaceae and one *Phlox*, and showed that the phenotypic correlations between filament and corolla tube lengths were significantly higher than the correlations among the rest of the floral traits in some of the study species. This led to the hypothesis that the high correlations were the result of selection for intermediate anther position in those species exhibiting the higher correlations: anthers that were placed either too high or too low were hypothesized to be less likely to contact pollinators.

Anther exsertion is a measure of the position of the anthers relative to the opening of the corolla tube, and is defined as the difference between the filament and corolla tube lengths (Fig. 1). Stabilizing selection for an intermediate value of the difference between two traits (e.g., exsertion) is equivalent to selection to increase the correlation between the two traits (Brodie 1992; P. Phillips, personal communication). Selection for intermediate exsertion does not necessarily mean that the optimum occurs when the two trait means are equal (i.e., zero exsertion), only that there is some optimum exsertion within the typical phenotypic distribution.

In contrast to the correlations between filament and corolla tube lengths, the correlation between the pistil and corolla tube lengths was not significantly greater than the rest of the floral correlations in any species (Conner and Via 1993; Conner and Sterling 1995). Therefore, it was hypothesized that selection for intermediate stigma exsertion has not occurred. Stigma exsertion

describes the position of the stigma relative to the opening of the corolla tube, and is defined as the difference between the corolla tube and pistil lengths (Fig. 1).

In the study reported here, we test these hypotheses using wild radish (*Raphanus raphanistrum*), a weedy annual in the family Brassicaceae. The effects of floral morphology on pollination was studied using representatives of all four major taxa of wild radish pollinators in North America: cabbage butterflies, honey bees, small bees, and syrphid flies (one study of wild radish in Europe listed bumble bees as an important pollinator also; Kay 1976). While the relative numbers of these pollinators varies greatly among populations, all of these groups are major components of the fauna (i.e., comprising at least 30% of all visitors) in some populations (Stanton et al. 1989, 1991, 1992; Conner et al. 1996; J. Conner, unpublished data). All of these taxa are effective pollinators of wild radish (see Results).

We measured one component of male fitness, pollen removal from the anthers, by all pollinator taxa, and one component of male and female fitness, pollen deposition on the stigma, by butterflies and honey bees only. These are the fitness components that are predicted to be affected by anther and stigma exsertion. Since wild radish, like all members of the Brassicaceae, has four long stamens and two short stamens, each flower has two levels of anther exsertion, each of which varies continuously. Therefore, we measured the effect of both long and short stamen anther exsertion on pollen removal from each type of anther individually.

Finally, we measured additional factors that studies of other species have suggested might be important for pollination success: numbers of pollen grains available on the anthers (for pollen removal), numbers of pollen grains removed from flowers visited previously by the pollinator (for pollen deposition), time spent at the flower by the pollinator, and corolla tube length.

Materials and methods

Study organisms

Plants used were grown in a pollinator-free greenhouse from field-collected seeds (collected near Binghamton, NY; see Conner and Via 1993), or seeds produced by greenhouse-propagated descendants of that population. One plant from each maternal parent was used. For details of greenhouse growing conditions, see Conner and Via (1993).

The cabbage butterfly (*Pieris rapae*) is common throughout the U.S. (Scott 1986) and is the dominant pollinator of wild radish in some populations (Stanton et al. 1989). Butterflies used in this study were from a laboratory colony established from eggs and pupae provided by J.A. Renwick (Cornell University). Butterflies were housed in 0.61 × 0.61 m cages and maintained on 10% sucrose solution. Larvae were reared on greenhouse-grown cabbage plants. All butterflies used in the experiments were naive: they had not visited flowers previously, and they were housed only with other naive butterflies to ensure that they did not pick up pollen from experienced butterflies.

Honey bees are also dominant pollinators of wild radish, especially in large plant populations (Stanton et al. 1992). Honey bee experiments were conducted in two indoor 2.5 m³ flight cages,

each containing an active honey bee hive. A variety of small bees and syrphid flies are common visitors to wild radish, especially in smaller populations (Conner et al. 1996). Experiments with these pollinators were done using natural pollinator populations at the Phillips Tract Natural Area of the University of Illinois near Urbana, Illinois.

Experimental design – honey bees and cabbage butterflies

To test the effect of morphology on pollination efficiency in honey bees and cabbage butterflies, the caged insects were presented with two single flowers from two different plants. One, the donor flower, was used to measure pollen removal. The other, the recipient flower, was used to measure pollen deposition. Each plant provided at most one flower used as a pollen donor and one flower used as a pollen recipient in different trials.

Donor and recipient flowers were prepared the day before an experimental trial. Large buds with sealed sepals were selected to be donor flowers, to ensure that the pollen had not yet dehisced. All open flowers on the plant were removed; thus, any flowers found open on the plant the next morning would be newly opened (and therefore have a full pollen load), and would be approximately the same age.

Buds with partially open sepals (i.e., part of the corolla was visible) were chosen to be recipient flowers. To ensure that pollen found on the stigma of this flower was from the donor flower, these buds were then emasculated (all anthers removed). The emasculation procedure was tested prior to this experiment; the results showed negligible pollen on stigmas after emasculation (mean = 6.1 grains/stigma, SD = 13.5, n = 10), so most pollen found on the recipient flower after visitation is from the donor flower.

To further check that any pollen found on the recipient flower was transferred from the donor flower by the pollinator during visitation, another preliminary experiment was performed in which the anthers from both the recipient and donor flower were removed prior to visitation. Control trials were performed in which a honey bee or a naive butterfly visited the two emasculated flowers. Control trials were conducted in exactly the same manner as experimental trials. Very little pollen was found on the recipient flowers' stigmas (mean = 6.0 grains/stigma, SD = 12.1, n = 10 for butterflies; in five honey bee control trials, no more than two pollen grains were found on each stigma).

Immediately prior to a trial, single donor and recipient flowers were prepared by cutting the stems containing the flower and buds from the plant and placing each stem in a separate water-filled vial. A trial consisted of placing one donor and one recipient flower in a cage with a butterfly or honey bees, and recording total visitation time at each flower. Visits to the recipient flower made before any visits to the donor flower were ignored, as there was no chance for pollen transfer. These visits may have depleted nectar and thus affected the duration of the recipient visit after the donor visit. These possible effects were accounted for by including visit duration in the statistical analyses (see below). Once the recipient was visited after the donor, the flowers were removed from the observation cage. This procedure was repeated 67 times with butterflies and 72 times with honey bees, each time with a unique pair of donor and recipient flowers.

Experimental design – small bees and syrphid flies

Since small bees and syrphids are not readily maintained in the laboratory, experiments with these pollinators were conducted in the field. Ninety-seven plants were raised in a pollinator-free greenhouse. Each plant was used only once in the study. Groups of plants were brought to the Phillips Tract Natural Area near Urbana, Illinois, from 8 July to 6 August 1993 and placed in a shed so that flowers were not exposed to pollinators. An array of 16–20 potted wild radish plants were maintained at the site to attract pollinators to the area. Individual experimental plants were then placed in the field and observed until a pollinator visited a flower. As soon as the pollinator left the flower the plant was returned to the shed to prevent further pollination. Small bees and syrphid flies visited a total of 45 and 52 flowers respectively. To estimate pollen removal, anthers were collected from both the visited and an adjacent unvisited flower (see below).

Small bees and syrphid flies could not be induced to visit emasculated flowers, probably because they forage primarily for pollen. Therefore, pollen deposition could not be measured directly as in the butterfly and honey bee experiments. To determine whether small bees and syrphids deliver outcross pollen to wild radish stigmas, and to determine the relative effectiveness of the two taxa, the following experiment was performed in 1994. An array of 20 potted wild radish plants was placed in the field, with a plant that had never been visited before placed at the center. The center plant was observed until a pollinator made a single visit to one flower. The visited flower was marked, and the taxon of the pollinator (small bee or syrphid) was recorded.

The visited plant was then returned to the greenhouse to prevent further visitation. After 3 weeks, the marked flower was checked to see if it set fruit, and if so, the number of seeds produced by that fruit was counted. Small bees and syrphid flies visited 76 and 61 flowers, respectively, in this experiment. Since pollinators were not followed, it is possible that in some trials the marked flower was the first wild radish flower visited by the insect; therefore, fruit set values may be underestimates. Flowers that were pollinated received only a single visit, and the plants were maintained in the greenhouse with ample light, water, and nutrients. Therefore, it seems likely that fruit and seed set were limited primarily by pollen in this experiment.

Pollen counting and floral morphology

To measure the amount of pollen removed from donor flowers in all experiments, two pollen counts were used. First, the amount of pollen on four of the six donor flower's anthers (one from a short stamen and three from long stamens) was counted with a Coulter counter (the other two stamens were left intact for morphological measurements; see below). This count measured the amount of pollen left on the donor flower after visitation. To determine how much pollen was on the donor flower prior to visitation, pollen from four anthers was counted on the next bud to open on the donor flower stem. Preliminary tests showed that the two counts from adjacent flowers obtained in this manner (without any pollinator visitation) were similar. Pollen was collected from one pair of adjacent flowers on each of 40 plants. The results showed that 75% of the variance among pollen counts was among plants, 11% was among unvisited adjacent flowers on the same plant, and 14% was measurement error. As in other species, therefore, adjacent unvisited flowers provide a good estimate of the amount of pollen on the donor flower itself prior to visitation (e.g., Harder 1990; Young and Stanton 1990; Galen 1992; Harder and Barrett 1993).

Therefore, the amount of pollen removed by the pollinators was estimated as the difference in pollen counts between the two adjacent flowers, one that was visited and one that was not. In some cases this estimate was negative. The negative values were caused by measurement error that should be random with respect to variables of interest. The measurement error had two sources: variability in pollen counts between adjacent flowers and machine error.

To measure the number of pollen grains deposited on the recipient stigma in the honey bee and butterfly experiments, the stigma was removed from the recipient flower and stained with a fuchsin jelly stain (Beattie 1972) with malachite green added (Alexander 1980). The number of pollen grains on the stigma was counted with the aid of an eyepiece grid at 40x and 100x using a compound microscope.

Finally, the morphology of the donor and recipient flowers was measured using digital calipers. The traits measured were corolla tube length (= claw length; wild radish does not have a true fused corolla tube, but it is a functional tube), the length of one of the

long and one of the short filaments, and pistil length. For details on traits and measurement methods, see Conner and Via (1993). For the donor flowers in all experiments and the recipient flowers in the honey bee experiment, morphology was measured on the flower used in the trial. For the recipient flower in the butterfly experiment, the next flower to open on the stem was used, because we had not yet perfected the technique (used in the honey bee experiments) of measuring the pistil and collecting the stigma for pollen counts from the same flower. An earlier study showed that adjacent flowers of wild radish plants grown in the greenhouse were very similar morphologically (between 83% and 89% of variance in the traits listed above was among plants; Conner, unpublished data). However, because the pistil elongates throughout the life of the flower, care was taken to measure the next open flower when it was as close to the age of the recipient flower as possible.

Data analysis

Pollen removal from long and short stamens was analyzed using separate multiple regressions for each pollinator taxon, with pollen removed from the donor as the response (dependent) variable. Pollen removal from the three long and the one short stamen collected were measured separately for all pollinators with one exception: the four stamens were measured together for butterflies. Pollen deposition was also used as the response variable in two additional multiple regressions for the butterfly and honey bee experiments. Relative values of these response variables were created by dividing all values by the overall mean. All predictor (independent) variables were standardized to $\bar{x} = 0$ and SD = 1. Anther and stigma exsertion were calculated from raw data and then standardized. Both linear and quadratic (squared) terms were fited for each predictor variable. Pollen deposition was ln transformed to improve normality, after which all variables were acceptably normal and residual plots showed no evidence of heteroscedasticity. Variance inflation factors (Neter et al. 1985) were calculated and were all less than three, indicating no multicollinearity problems. JMP (1994) on a Macintosh computer was used for all analyses.

To determine the factors that affected pollen removal from the donor flower, the relative number of pollen grains removed was regressed on the following predictor variables: the number of pollen grains available before visitation (pollen available), the total time spent by the pollinator at the donor flower, donor corolla tube length, and anther exsertion.

To determine the factors that affected pollen deposition on the recipient flower by butterflies and honey bees, the natural log of the number of pollen grains deposited on the stigma of the recipient flower was regressed on the following predictor variables: the number of pollen grains removed from the donor flower, the time spent at the recipient flower, and recipient stigma exsertion. Donor anther exsertion and recipient corolla tube length were included in preliminary regression models, but the regression coefficients for these variables were never significant and their inclusion reduced the fit of the models, so they were dropped from the final models presented below.

Results

Overall pollination efficiency

The number of pollen grains produced by the flowers and removed by single pollinator visits are presented in Table 1. Assuming that the four anthers were representative, the estimated number of pollen grains available on all six anthers ranged between 72,000 and 92,000 for the four experiments, and the estimated number removed from all six anthers ranged from approximately 13,000 (butterflies) to 48,000 (honey bees). Thus, the rates of pollen removal in these single visits were high: the estimated percentage of pollen removed from six anthers ranged from 18% to 52%. In all cases studied the percentage removal from long stamens was considerably greater than removal from short stamens (Table 1), indicating that the increased exsertion of long stamen anthers leads to higher removal overall.

Despite the large difference in the number of pollen grains removed by butterflies and honey bees, the median number of pollen grains deposited on stigmas by the two insects was similar: the median deposition by butterflies was 72 (quartiles: 11, 159), and by honey bees was 61 (quartiles: 30, 108). Therefore, the pollination efficiency of cabbage butterflies, estimated as the median percentage of pollen grains removed that were deposited on recipient stigmas, was over 4 times as great as the efficiency of honey bees (0.54% versus 0.13%, respectively).

In the pollen deposition experiment with small bees and syrphid flies, fruit set was 42% and 38%, respectively, after single visits by these pollinators, indicating that both are effective pollinators of wild radish. These proportions are not significantly different (chi-square = 0.12, 2 df, P > 0.9). On visits that did result in a fruit, small bee visits resulted in slightly more seeds per fruit (2.0 ± 0.17 versus 1.4 ± 0.21, mean ± SEM, t = 2.09, P = 0.04). Given that small bees removed slightly more pollen per visit than syrphid flies did (Table 1), this result suggests that these two pollinators have similar efficiencies.

Pollen removed

The overall regression models for pollen removal were highly significant, with the four predictor variables explaining 50–80% of the variance in the number of pollen

Table 1 Means ± SEM for number of pollen grains available before visitation and the number removed by the four taxa of pollinators. Long and short stamen values are for three and one anther(s), respectively (see Methods); these were not counted separately in the butterfly experiment

	Pollen available	Pollen removed	Average% removed
Butterflies	48,103 ± 1,584	8,828 ± 1,660	18
Honey bees – long stamens	38,791 ± 1,512	24,291 ± 1,555	63
Honey bees – short stamen	20,442 ± 750	7,844 ± 995	38
Small bees – long stamens	34,628 ± 1,336	13,873 ± 1,974	40
Small bees – short stamen	16,706 ± 845	1,530 ± 1,229	9
Syrphid flies – long stamens	31,788 ± 1,742	9,282 ± 1,825	29
Syrphid flies – short stamen	14,921 ± 980	2,505 ± 1,291	17

Table 2 Factors affecting pollen removal. Values represent standardized partial regression coefficients with significance levels. Each column represents the results of a separate multiple regression of the relative number of pollen grains removed from anthers by each pollinator on the four predictor variables listed. Both linear and quadratic (denoted by ^{2}) coefficients from a quadratic regression are shown. *Long* and *Short* refer to pollen removed from the three long stamens or one short stamen collected, respectively (see Methods). The r^2 value gives the amount of variance in pollen removal explained by all the independent variables combined; all regression models were significant at $P<0.0001$

	Butterflies	Honey bees Long	Honey bees Short	Small bees Long	Small bees Short	Syrphid flies Long	Syrphid flies Short
Pollen available	0.78****	0.46****	0.58****	0.39***	3.03****	1.04****	2.30****
Pollen available^2	0.11	0.01	-0.01	-0.24**	-0.62	-0.06	0.47
Visit duration	1.34****	0.18****	0.56****	0.79****	2.70**	0.86***	-0.04
Visit duration^2	-0.18***	-0.07*	-0.18*	-0.22***	-0.88*	-0.22	0.32
Tube length	-0.04	0.04	0.16	0.08	0.56	-0.11	-0.06
Tube length^2	0.03	-0.01	-0.06	0.02	-0.64	0.09	-0.03
Anther exsertion	0.40**	0.08*	0.05	0.04	1.20	-0.01	0.74
Anther exsertion^2	0.30***	0.02	-0.02	-0.06	1.04*	0.06	0.11
Total r^2	0.64	0.80	0.61	0.65	0.63	0.69	0.50
	n=67	n=72	n=71	n=45	n=45	n=52	n=52

$* P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001$

grains removed by single pollinator visits (Table 2). The amount of pollen available had a strong positive effect on pollen removed by all pollinators and from both anther heights (Table 2; Fig. 2). For example, the standardized regression coefficient of 0.78 for butterflies can be interpreted as follows: an increase of 1 SD in pollen available resulted in a 78% increase in relative pollen removal. The relationship between pollen available and pollen removed was roughly linear in all cases but one, since the quadratic regression coefficients were not significant. The only exception to this was the result for small bees, in which there was a positive decelerating relationship (Fig. 2D,E), especially for the long stamens.

The time that the pollinator spent at the flower also had a strongly positive effect on the number of pollen grains removed from the flower, except in the case of syrphid flies on short stamens (Table 2; Fig. 3). Overall, the standardized linear slopes were roughly comparable to those for pollen available, suggesting that these two variables have effects on pollen removal that are similar in magnitude. The time spent at the flower had a positive decelerating relationship with pollen removal for all pollinators except syrphid flies, as shown by the significantly negative quadratic coefficients. This is in contrast with the mainly linear relationships between pollen available and pollen removed.

The length of the corolla tube had no effect on the number of pollen grains removed from flowers, but anther exsertion had a strong effect for butterflies and weak effects for the two bees (Table 2; Fig. 4). For butterflies, both the linear and quadratic coefficients for anther exsertion were significantly positive, indicating a minimum of pollen removal at intermediate exsertion with the greatest removal occurring with highly exserted anthers (Fig. 4A); this is the opposite of what was predicted based on the correlation patterns (see Introduction). The linear slope for honey bees on long stamens was weakly positive, suggesting increased removal with increased exsertion (Fig. 4B) There was little relationship between

pollen removal and exsertion for small bees at short stamens over most of the range of exsertion, but then an increase in removal at greatest (least negative) exsertion values (Table 2; Fig. 4C).
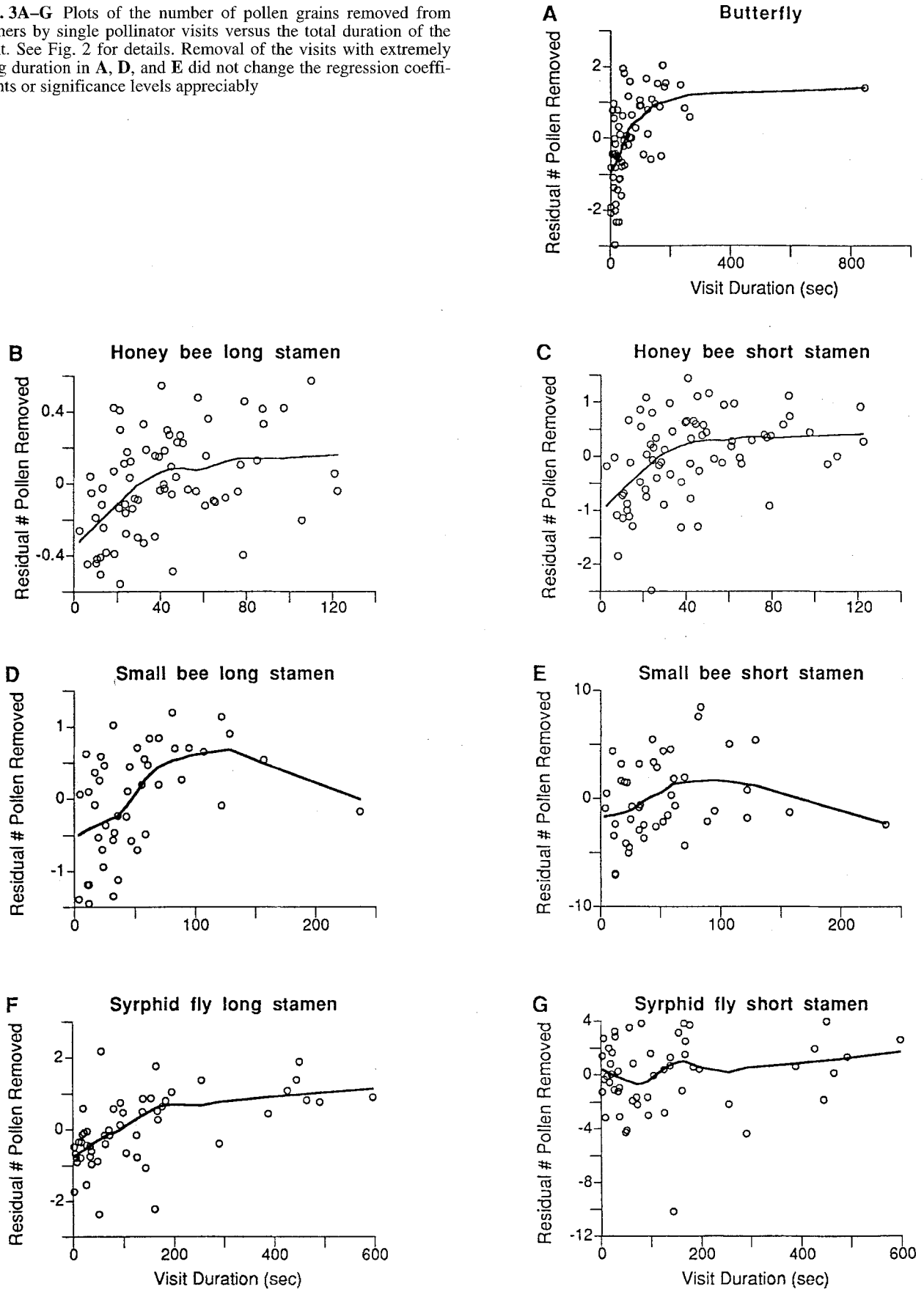
Pollen received

The multiple regression models for number of pollen grains received on the stigma of the recipient flower in visits by butterflies and honey bees explained 45% and 21% of the variance in pollen deposition, respectively (Table 3). Compared to the pollen removal regressions, the proportion of variance explained is less and the standardized slopes tend to be smaller for pollen received. The relationships between pollen deposition and the predictor variables are similar for the two pollinators, but are much stronger for butterflies than they are for honey bees (Table 3). There is a positive relationship between pollen removed from the donor flower and pollen deposition on the recipient (Table 3); this relationship is linear, especially for butterflies (Fig. 5A) and thus does not show any evidence for diminishing returns with increased pollen removal. This lack of diminishing returns for butterflies was not due to ln transformation of pollen deposition; the untransformed relationship showed an accelerating, not decelerating, positive relationship. There was some evidence for diminishing returns for honey bees (Fig. 5B), but the quadratic coefficient was not significant (Table 3). There was a positive relationship between pollen deposition and the time both pollinators spent on the flower, with evidence for diminishing returns for both taxa, although the quadratic coefficient was not significant for honey bees (Table 3; Fig. 5C,D). These relationships were similar to, but weaker than, the relationships between visit time and pollen removal (cf. Table 2).

There was no linear relationship between stigma exsertion and pollen deposition for either pollinator, but the

Fig. 2A–G Plots of estimated number of pollen grains removed from anthers by single pollinator visits versus the number of pollen grains available before the visit. The y-axis is the residual number of pollen grains removed from a multiple regression of relative number of grains removed versus the other three predictor variables (see Table 2). This provides a visual depiction of the relationship that the partial regression coefficients for pollen available in Table 2 describe. Unstandardized values are presented on the x-axis for ease of interpretation (standardized variables were used in the multiple regression analyses; see Methods). Lowess (locally weighted regression scatter-plot smoothing; Chambers et al. 1983) curve fits are shown (smoothing factor = 66%)
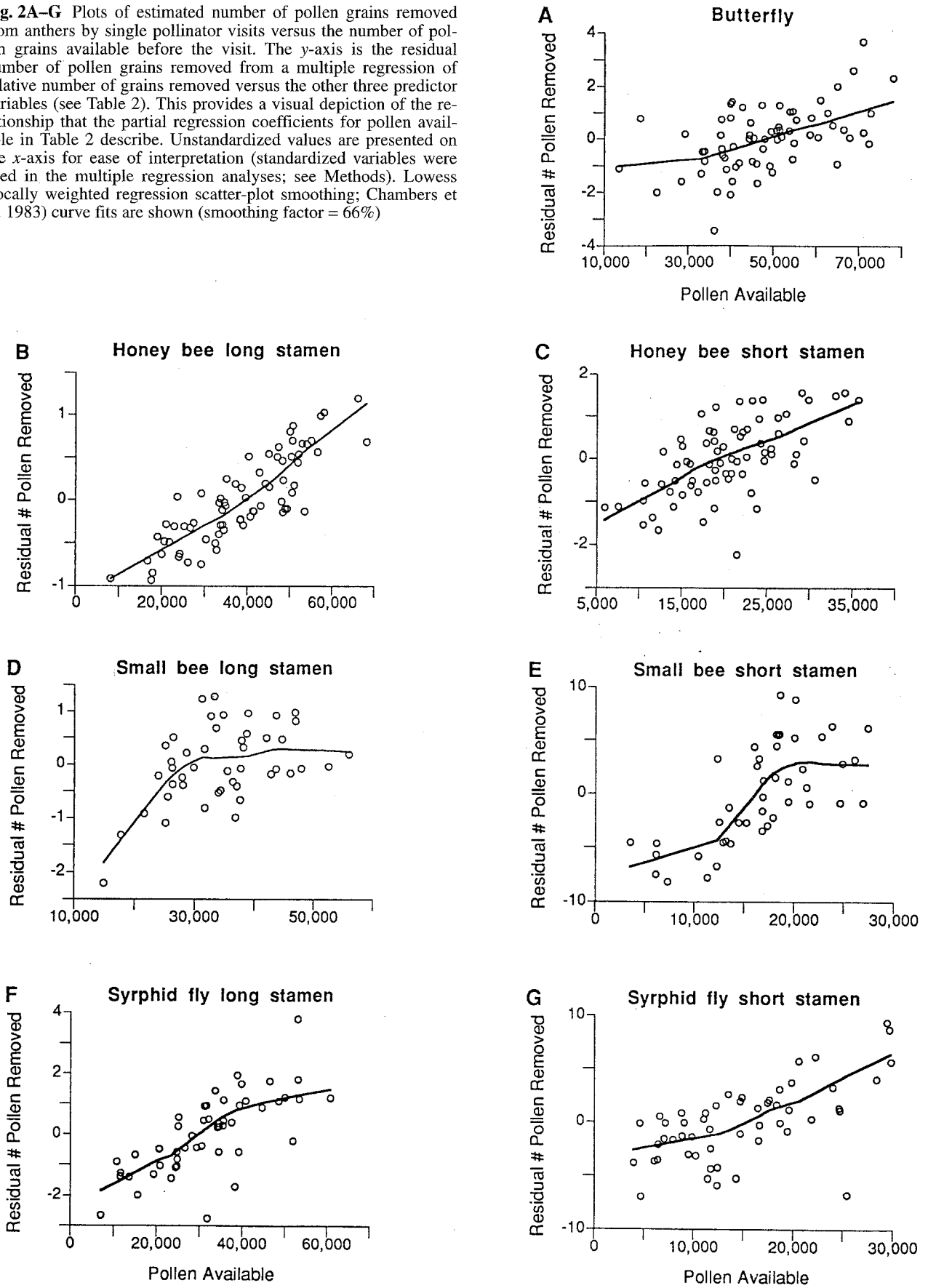
Fig. 3A–G Plots of the number of pollen grains removed from anthers by single pollinator visits versus the total duration of the visit. See Fig. 2 for details. Removal of the visits with extremely long duration in **A**, **D**, and **E** did not change the regression coefficients or significance levels appreciably

A **Butterfly**

B **Honey bee long stamen**

C **Small bee short stamen**
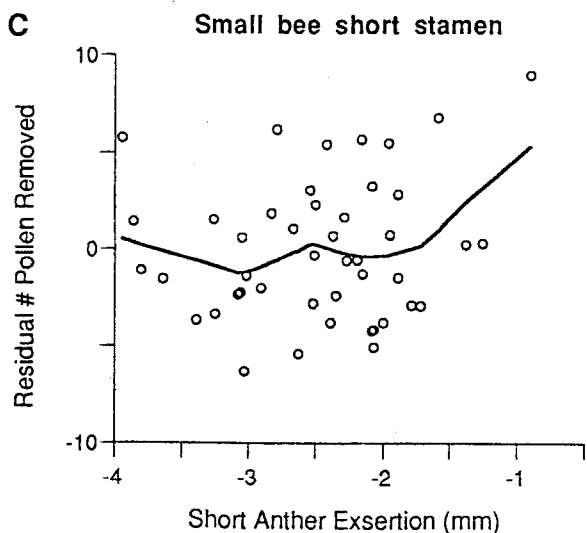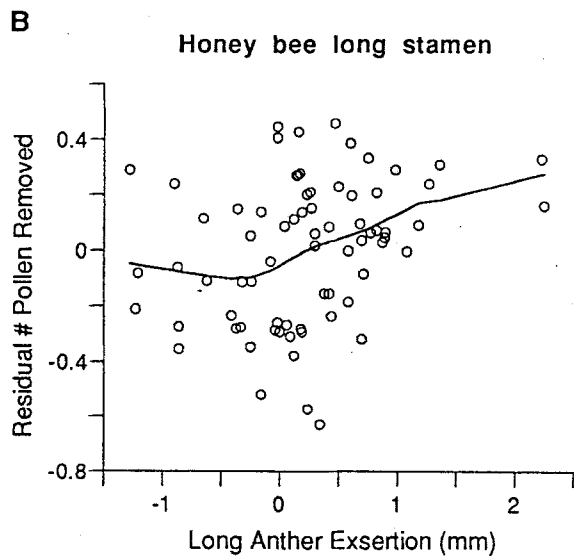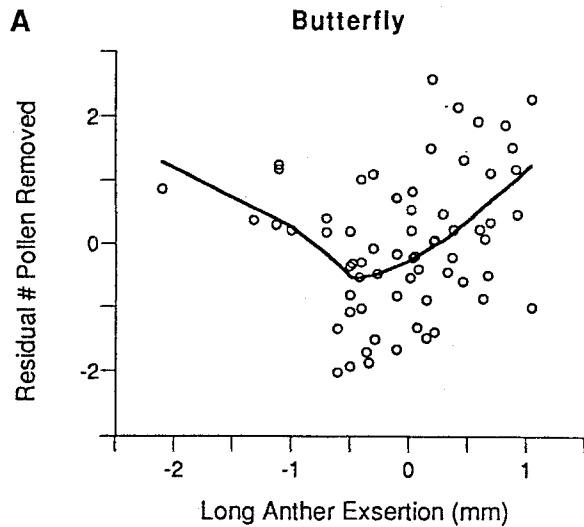
Table 3 Factors affecting pollen deposition. Standardized partial regression slopes with their standard errors and significance levels (P), and overall model fit for the amount of pollen received by the recipient flower. Both linear and quadratic (denoted by ^{2}) coefficients from a quadratic regression are shown. All variables refer to the recipient flower except where noted. Number of pollen grains received was ln transformed. The r^2 value gives the amount of variance in pollen removal explained by all the predictor variables combined, with the significance level of the overall model. Sample sizes are slightly smaller than the total number of trials performed due to missing values.

	Butterflies	Honey bees
Pollen removed from donor	0.20***	0.09*
Pollen removed from donor^2	0.03	−0.06
Visit duration	0.35**	0.17*
Visit duration^2	−0.07**	−0.02
Stigma exsertion	0.01	−0.04
Stigma exsertion^2	−0.11**	−0.04
r^2	0.45****	0.21*
	$n = 62$	$n = 68$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$

quadratic regression coefficient for butterflies was significantly negative, indicating an intermediate optimum (Table 3; Fig. 5E). This result was not predicted based on the correlation patterns (see Introduction).

Discussion

Anther and stigma exsertion

This study was designed to examine the effects of floral morphology on the efficiency of pollen removal and deposition. Based on patterns of correlation among floral traits in several species (Conner and Via 1993; Conner and Sterling 1995), we predicted that *R. raphanistrum* plants with intermediate anther exsertion would have the highest fitness, but that plants with intermediate stigma exsertion would not (see Introduction). Our results did not support these predictions: in no case did flowers with intermediate anther exsertion have the greatest levels of pollen removal (Table 2; Fig. 4), and flowers with intermediate stigma exsertion had the highest levels of pollen deposition by cabbage butterflies (Table 3; Fig. 5E). Only the results for pollen deposition by honey bees fit the prediction of no relationship with stigma exsertion. The simplest explanation for these results is that the hypotheses are wrong, and that the correlation patterns are caused by something other than selection exerted by pollinators (e.g., developmental relationships; see Conner

Fig. 4A–C Plots of the number of pollen grains removed from anthers by single pollinator visits versus anther exsertion, calculated as the difference between the corolla tube and the filament lengths (see Fig. 1). See Fig. 2 for details. Only cases in which regression coefficients were significant (see Table 2) were included. Removing the plant with exsertion of −2 in **A** or the two plants with long stamen exsertion greater than two in **B** did not change the regression coefficients or significance levels appreciably

A **Butterfly**

B **Honey Bee**

C **Butterfly**

D **Honey Bee**
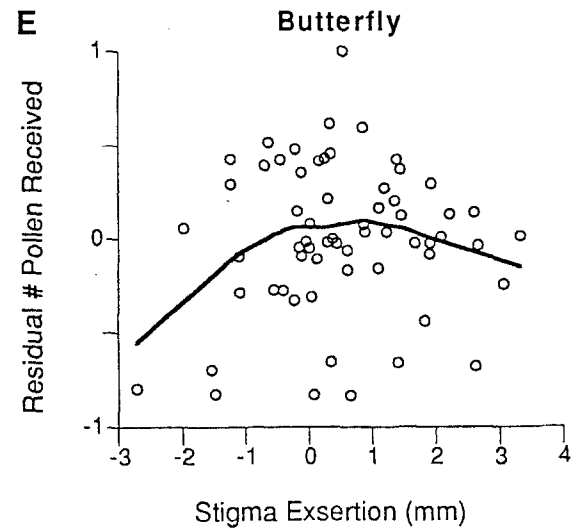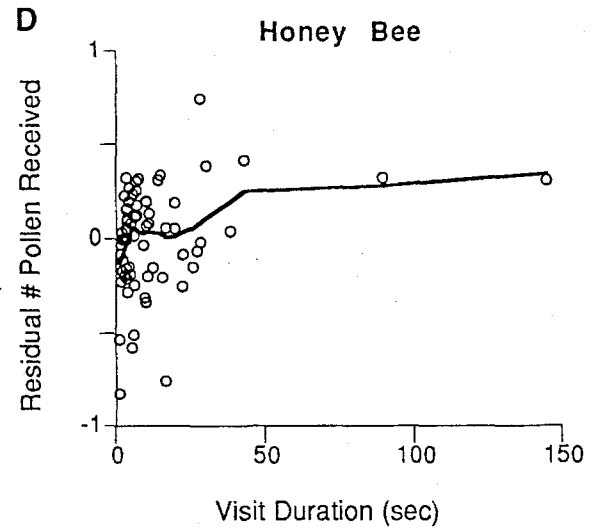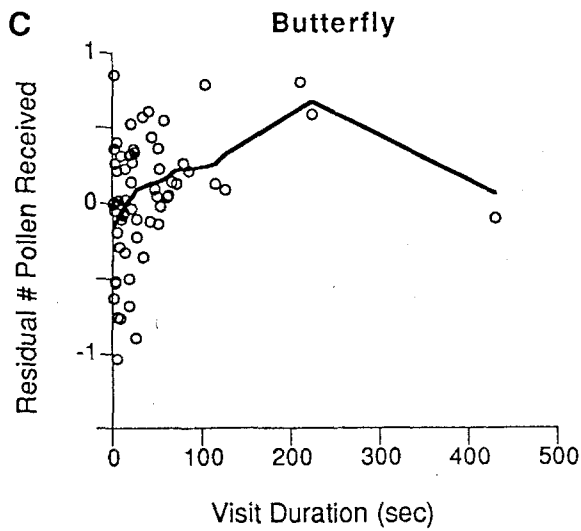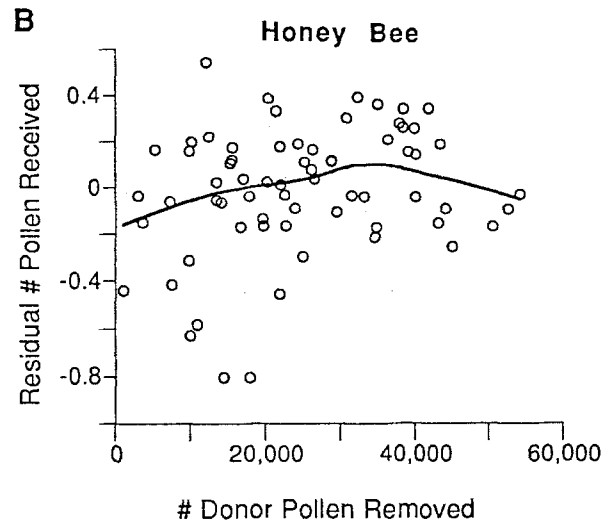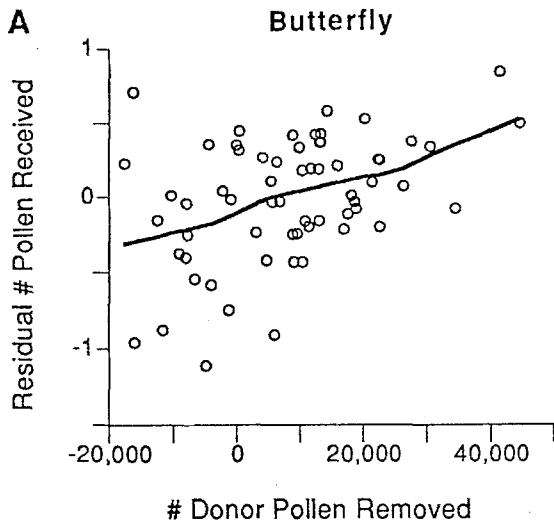
Fig. 5A–E Plots of number of pollen grains deposited on stigmas of recipient flowers by single pollinator visits versus the three predictor variables that were significant in the regression models (Table 3). See Fig. 2 for details. Removing the plants with extremely long visit times in **C** and **D** or with stigma exsertion less than −2 in **E** did not change the regression coefficients or significance levels appreciably

E **Butterfly**

and Via 1993; Conner and Sterling 1995). Before rejecting the selection hypotheses, however, it is important to note that our experiments are only part of a complete test of these hypotheses.

First, since our experiments measured components of fitness, not total fitness, it may be that these fitness components are not directly related to total fitness. Maximum pollen deposition may not translate into maximum female fitness if there is a correlation between self and outcross pollen deposition (see below) or if seed set is not pollen-limited, as has been shown in another population of *R. raphanistrum* (Stanton et al. 1986; Stanton et al. 1989). In fact, in three field seasons there was no relationship between stigma exsertion and lifetime female fitness (Conner et al. 1996).

Similarly, maximum pollen removal per visit may not translate into maximum male fitness if the relationship between pollen removal and deposition exhibits diminishing returns (Harder and Thomson 1989). Our experiments did not show diminishing returns for pollen deposition (see Results and below). Even without diminishing returns in deposition, if visitation rates are high and pollen is removed quickly (as is true in *R. raphanistrum*; Rush et. al. 1995), then placing smaller amounts of pollen on each of a large number of pollinators might lead to more seeds sired and a more diverse set of seeds sired than placing large amounts of pollen on fewer pollinators (cf. Lloyd 1984). We are currently estimating male fitness from field populations to test this possibility.

Second, it is possible that the variation in anther exsertion in present-day populations is not adequate to detect an effect. If stabilizing selection on anther exsertion has occurred in the past, then this could have eliminated genetic and phenotypic variation for this trait. The high correlation between filament and corolla tube lengths means that there is little variation in anther exsertion. In future work we will attempt to modify anther exsertion further using artificial selection and hormonal treatments.

Hypotheses based on observed floral correlation patterns are only one set of a number of hypotheses concerning the functional significance of anther and stigma exsertion during pollination. In our study, maximum pollen removal from long stamens occurred with highly exserted anthers in visits by butterflies and honey bees, but variation in exsertion within each stamen type had little effect on removal by small bees and syrphid flies. However, in small bees, syrphid flies and honey bees, the proportion of pollen removed from short stamens was less than that from long stamens (Table 1), indicating that the increased exsertion exhibited by long stamen anthers leads to increased pollen removal by all pollinator taxa. This further suggests that the lack of a relationship between exsertion and removal within each stamen length for small bees and syrphid flies was due to a lack of variability in exsertion, because the larger differences in exsertion between short and long stamens does lead to a difference in removal.

To our knowledge only three other studies have examined the effect of anther exsertion on pollen removal, and all three found that maximum anther exsertion resulted in maximal pollen removal, in agreement with our results (Wolfe and Barrett 1989; Murcia 1990; Harder and Barrett 1993). However, predictability of pollen removal was greatest with intermediate anther exsertion in one of these studies (Harder and Barrett 1993), apparently because small pollinators did not always contact high anthers and proboscis pollen removal of pollen from the highly inserted anthers was inefficient (see also Grant and Grant 1965; Levin and Berube 1972). Therefore, total male fitness (number of seeds sired across all recipients) may be greatest with intermediate anther exsertion, as suggested above.

Our results from butterfly visits showed an intermediate optimum in stigma exsertion: those plants that placed their stigmas slightly above the opening of the corolla tube received the highest numbers of pollen grains, while plants with either more inserted or more exserted stigmas had lower deposition (Fig. 5E; Table 3). Two studies of heterostylous species have also provided evidence for highest pollen deposition on plants with intermediate stigma exsertion (Wolfe and Barrett 1989; Kohn and Barrett 1992). In contrast, several other studies of non-heterostylous species have reported negative relationships between stigma exsertion and pollen deposition in single visits (Galen and Stanton 1989; Murcia 1990; Campbell et al. 1994). The differences in the findings of the two sets of studies may be due to differences among plant species in pollinators, average exsertion, or other aspects of floral morphology.

Therefore, across a number of plant and pollinator species, there seems to be a pattern emerging: maximum anther exsertion results in maximum pollen removal, but intermediate or low stigma exsertion results in maximum pollen deposition. This may be due to two different types of selection acting on these traits: one to reduce self-pollen loads, and the other to maximize pollen removal and outcross pollen deposition. Wild radish flowers exhibit approach herkogamy (Webb and Lloyd 1986), with stigmas placed above anthers on average (i.e., mean stigma exsertion greater than mean long stamen anther exsertion). Approach herkogamy has been hypothesized to be the result of selection for stigma-anther separation to reduce the deposition of self pollen. The stigma-anther separation may have been produced, at least in part, by the evolution of decreased anther exsertion, so that the average value for exsertion is below its optimum for maximum pollen removal. This would in turn result in the observed pattern of maximum removal with maximum exsertion.

Other factors

Overall efficiency

We found that cabbage butterflies were 4 times as efficient in transferring pollen than honey bees were (0.54% versus 0.13% of removed pollen deposited). At least part of the reason for this is that almost all of our honey bees

were foraging for pollen as well as nectar, so that much of the removed pollen was likely groomed away from areas of the bees' bodies that contacted stigmas. Also, bees spent much less time on the emasculated recipient flowers than on the intact donor flowers, since they could only forage for nectar on the recipients.

The efficiency of nectar-foraging honey bees on *R. sativus*: (0.6%; Young and Stanton 1990) was more similar to our nectar-foraging butterflies than to our pollen- and nectar-foraging honey bees. Other studies of nectar foragers have also found fairly high efficiencies (2.9%, Galen and Stanton 1989; 0.6%, Harder and Thomson 1989), while at least one study of pollen foraging bees reported much lower efficiency (Snow and Roubik 1987). One other study in addition to ours has directly shown that pollen foragers are less efficient than nectar foragers (Wilson and Thomson 1991), although the recipients in that study were in female phase and lacked anthers. Therefore, the two studies that have shown lower efficiency of pollen foragers measured deposition on flowers that lacked pollen. Clearly, studies of the efficiencies of pollen foragers using pollen-laden recipient flowers are needed to rigorously test the generalization that nectar foragers are more efficient than pollen foragers; however, the difficulty of distinguishing self from outcross pollen deposition has caused these kinds of studies to be rare.

Pollen availability

Our experiments demonstrated a strong linear increase in the pollen removed from a flower with increasing pollen available on the stigmas for all taxa but small bees, which showed evidence for diminishing returns (Fig. 2; Table 2). Young and Stanton (1990) also found an essentially linear relationship between pollen removal and availability in either single or multiple visits by honey bees on *R. sativus* [while Young and Stanton (1990) used curvilinear fits in two cases, linear fits had r^2 within 2%]. Harder and Thomson (1989) report a linear relationship between pollen availability and removal by bees in *Erythronium*. Murcia (1990) also reported a positive relationship between pollen availability and removal by single bumble bee visits to *Ipomoea*, but the shape of this relationship was not reported. Therefore, available evidence suggests that pollen removal increases roughly linearly with increasing pollen availability, without strong evidence for diminishing returns in most cases.

Our results also showed a positive relationship between pollen removed from the donor flower and pollen deposition on stigmas of recipient flowers (Fig. 5; Table 3); this relationship was strong for nectar-foraging butterflies and weaker for pollen-collecting honey bees. Considering the small percentage of pollen removed that was actually deposited on stigmas by butterflies (median = 0.54%), the strength of the relationship between pollen removal and deposition that we found is surprising. This seems to be a general pattern, however, because

other studies have found strong relationships between number of pollen grains removed and deposited with similar small percentage efficiencies (Harder and Thomson 1989; Galen 1992; but see Galen and Stanton 1989). The weaker relationship for the pollen-foraging bees is likely to be due to the same reasons as those discussed above for reduced efficiency.

The strong linear relationship between pollen removal and deposition for butterflies suggests that there may not be diminishing returns associated with increased pollen removal during butterfly pollination of wild radish, as has been shown with some bee-pollinated plants (Harder and Thomson 1989). The difference may be due to differences in grooming behavior, since grooming of pollen off the body by bees may be a major cause of diminishing returns (Young and Stanton 1990; L.D. Harder, personal communication). Our quadratic term for honey bees was negative but non-significant, suggesting the possibility of diminishing returns (see Fig 5B). Note also that our study examined deposition on only one flower; it is possible that diminishing returns with butterflies could occur if we measured deposition on a series of flowers visited, as Harder and Thomson (1989) did.

Time at flower

The length of pollinator visits to *R. raphanistrum* flowers had a strong positive effect on pollen removal and a somewhat weaker positive effect on pollen deposition in our study (Tables 2, 3; Figs. 3, 5). At least two other studies have also shown that the length of bee visits was positively related to both pollen removal and deposition (Galen and Stanton 1989; Young and Stanton 1990). The relationship between increasing time at the flower and both removal and deposition showed diminishing returns in our study, in contrast to our results for pollen available.

Corolla tube length

The lack of a significant relationship between radish corolla tube length and the number of pollen grains removed by any pollinator (Table 2) differs from an earlier study in which orchids with longer tubes had higher rates of pollinium removal by hawk moths (Nilsson 1988). Murcia (1990) reported increasing pollen deposition by hawk moths with increasing corolla tube length, but in bumble bee visits there was no relationship between corolla tube length and pollen deposition and a negative relationship between tube length and pollen removal. Therefore, tube length appears to be critical in hawk moth pollination; this is probably due to the extremely long proboscides found in hawk moths. Tube length seems to be less important in pollination by cabbage butterflies and bees, which have shorter proboscides and are more likely to contact anthers and stigmas even in flowers without extremely long corolla tubes.

Summary

Our results did not support predictions of the effects of anther and stigma exsertion on pollination efficiency based on patterns of correlation among floral traits, but did agree with other studies showing maximum pollen removal with increased anther exsertion and maximum pollen deposition with intermediate stigma exsertion. Our results on relative pollination efficiency of nectar and pollen foragers and the effects of pollen availability and pollinator visit duration on pollen transfer are in broad agreement with most other studies of different plants and different pollinators, suggesting the possible emergence of some general rules of insect pollination.

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