

POLLEN REMOVAL FROM TRISTYLOUS *PONTEREDERIA CORDATA*: EFFECTS OF ANTHOR POSITION AND POLLINATOR SPECIALIZATION¹

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Abstract. Stamens produce male gametophytes and expose them to the pollination process, so that stamen characteristics should reflect selection on male function. For animal-pollinated plants, restricting removal in favor of using all available pollinators should generally promote pollen dispersal, so that staminal characteristics should limit pollen removal by individual pollinators. Here we describe the influences of anther position on pollen removal from *Pontederia cordata*, a tristylous plant with anthers at three distinct positions: exerted from the flower (long-level anthers), at the mouth of the flaring tubular perianth (mid-level anthers), and near the perianth base (short-level anthers).

To measure pollen removal, we presented unvisited flowers to freely foraging bees and counted the number of pollen grains left in flowers after 1-4 visits. The pollinators included two generalist bumble bees (*Bombus impatiens* and *B. vagans*: Apidae) and *Melissodes apicata* (Anthophoridae), a specialist with morphological and behavioral adaptations for collecting pollen from *P. cordata*. Analysis of pollen removal incorporated the effects of bee species, anther position, pollen size, flower length, and the number and cumulative duration of bee visits.

Anther position and bee species significantly influenced pollen removal, although the differences between bee species were not related to pollinator specialization. Long-level anthers dehisced early, lost an average of 39% of their pollen during their first visit, and did not consistently contact small pollinators, such as *M. apicata*. Short-level anthers restricted pollen removal more (28% lost) than long-level anthers, but dehisced more slowly and were subject to unpredictable pollen removal. Mid-level anthers combine the advantages of more exerted and inserted positions without suffering the corresponding disadvantages as they restricted removal (24% lost), dehisced relatively early, and seemed to consistently contact all pollinators. The overall benefits of positioning anthers within the perianth mouth, the position occupied by mid-level anthers in *P. cordata*, may explain the prevalence of this morphology among bee-pollinated plants with flaring, tubular, monomorphic flowers.

Key words: anther position; bumble bee; floral evolution; heterostyly; *Melissodes apicata*; pollen removal; pollinator specialization; *Pontederia cordata*.

INTRODUCTION

Flowers serve relatively few functions (gamete production, pollination, and fertilization) and yet they exhibit the greatest interspecific variation in morphology of any plant structure. This remarkable floral diversity apparently results from adaptive radiation in response to dissimilar pollination environments (Grant and Grant 1965) and reflects the spectrum of alternative mechanisms that can promote reproductive success. However, not all morphologies foster equivalent success (e.g., Horovitz and Harding 1972, Bell 1985, Abbott and Irwin 1988, Nilsson 1988, Galen and Stanton 1989, Murcia 1990, Young and Stanton 1990, Campbell et al. 1991, Johnston 1991, Stanton et al. 1991,

Kohn and Barrett 1992), so that some character combinations evolve more frequently than others (Grant and Grant 1965, Fægri and van der Pijl 1979, Armbruster 1990).

The recent realization that selective enhancement of male function has played an important role in the evolution of flower form (Willson and Price 1977, Charnov 1982, Lloyd and Yates 1982, Sutherland and Delph 1984, Bell 1985, Stanton et al. 1986) raises the question of how the structure and arrangement of stamens, the organs of male gametophyte production, promote pollen dispersal. If pollen dispersal by individual pollinators suffers diminishing returns with increasing pollen removal (see Harder and Thomson 1989), then animal-pollinated plants would maximize total pollen dispersal by restricting pollen removal by individual pollinators and using the services of all available pol-

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linators (Lloyd 1984, Harder and Thomson 1989; L. D. Harder and W. G. Wilson, *unpublished manuscript*). This hypothesis therefore predicts that stamen characteristics should restrict removal and Harder and Thomson reviewed several mechanisms that serve this function, including poricidal anthers, "spring-loaded" stamens, and secondary pollen presentation. However, most plants do not possess these specialized mechanisms, but rather present their pollen passively on longitudinally dehiscing anthers. Are there features of these anthers (or the stamens of which they are a part) that could also serve to optimize pollen dispersal?

An animal-pollinated plant can influence the dispersal of its pollen through two processes: pollinator attraction and pollen removal. Stamens probably do not play a major role in pollinator attraction, although they may act as short-range orientation cues (Lunau 1991), and the pollen they produce contains phagostimulants (Schmidt 1985) and odors that assist host-plant recognition by specialist pollinators (Dobson 1987). In contrast, stamens can affect pollen removal directly through two mechanisms. First, anther position can govern pollen removal during single visits (Murcia 1990), because removal depends on the likelihood and extent of direct contact with the pollinator (Harder 1990). Second, anther position determines the placement of pollen on a pollinator's body, which influences the likelihood of pollen loss through grooming (Fægri and van der Pijl 1979). Both of these mechanisms influence pollen dispersal by modifying the impact of the diminishing returns that characterize animal pollination (Harder and Thomson 1989).

Although pollen removal from longitudinally dehiscing anthers is passive from a plant's perspective, the amount of pollen removed during single visits can depend on characteristics of the pollinator (Strickler 1979, Harder 1990, Murcia 1990, Wilson and Thomson 1991). The size of the pollinator relative to the distance between anthers and the object of the pollinator's visit (nectar, fragrances, resin, oil) will affect contact between the pollinator and anthers (Grant and Grant 1965, Armbruster 1985, 1988, Harder 1990, Murcia 1990, Wilson and Thomson 1991). In addition, pollinators that specialize in collecting pollen from particular species often possess morphological and/or behavioral traits that promote pollen removal (Strickler 1979, Thorp 1979) and possibly reduce pollen dispersal. These pollinator characteristics could modify the effectiveness of particular anther positions in mediating successful pollen dispersal.

Here we examine three general problems concerning pollen removal. First, does anther position consistently affect pollen removal, as would be expected if anther position plays a major role in governing pollen dispersal? Second, does the influence of anther position on pollen removal depend on whether flowers are visited by generalist pollinators, or pollinators with behavioral and morphological specializations for remov-

ing pollen from the specific plant species? Third, is the effect of anther position on pollen removal modified by intraspecific variation in floral characteristics, such as flower size and pollen size? To address these problems we have taken advantage of the extreme floral variation found in heterostylous plants.

Heterostylous plants exhibit more intraspecific variation in anther placement than any monomorphic species. This condition involves two (distyly) or three (tristyly) floral morphs and has evolved independently in at least 25 families (Ganders 1979, Lloyd and Webb 1992). Each flower produces sexual organs at two (distyly) or three positions (tristyly), with the stigma at one position and anthers at the other(s). The different morphs represent all possible combinations of stigma and anther positions, with a particular morph being identified by the position of its stigma. Darwin (1877) proposed that heterostyly evolved through selection for increased efficiency of cross-pollination. Lloyd and Webb (1992) recently refined this hypothesis and also suggested that heterostyly resolves the conflict between selection for placement of stigmas and anthers in similar positions, which should promote efficient transfer of pollen between plants, and selection for the separation of these organs, which should discourage self-fertilization and self-interference.

To assess the role of anther position in pollen removal, we studied a tristylous species, *Pontederia cordata* L. (Pontederiaceae). *Pontederia cordata* is a clonal, emergent aquatic, which ranges from southern Canada to northern Argentina (Lowden 1973). Each day during the flowering season inflorescences produce 5–40 flowers, which open for ≈ 6 –8 h before wilting (Wolfe and Barrett 1987). The moderately zygomorphic flowers have flaring, tubular perianths and are visited by a variety of pollinators, primarily large-bodied bees (Price and Barrett 1984, Wolfe and Barrett 1988). Because each flower produces stamens at two distinct levels and each anther level produces pollen of different sizes (Price and Barrett 1982), we could study simultaneous pollen removal from anthers in two different positions. By examining all three morphs, we could therefore conduct all possible pairwise comparisons between long-, mid-, and short-level anthers.

During our study two bumble bees, *Bombus impatiens* Cresson and *B. vagans* Smith, and an anthophorid bee, *Melissodes apicata* Lovell and Cockerell, dominated the pollinator fauna of *P. cordata* and collected both nectar and pollen. Like most bumble bees, *B. impatiens* and *B. vagans* are opportunistic generalists and we observed them feeding from a variety of plant species in the vicinity of our study site. In contrast, *M. apicata* relies on *P. cordata* as its sole source of pollen and it possesses several adaptations that reflect this specialization (LaBerge 1956). The impact of pollinator specialization on pollen removal can therefore be assessed through comparison of pollen removal by *M. apicata* and the bumble bees.

Wolfe and Barrett (1989) previously studied pollen removal from *P. cordata* to assess Darwin's (1877) hypothesis that the reciprocal arrangement of stigmas and anthers promotes cross-pollination between morphs. To measure pollen removal, they observed single bee visits to each of 20 flowers and then pooled anthers from all flowers and counted the remaining pollen. Wolfe and Barrett found that, during single visits, bumble bees (*Bombus griseocollis*) removed a greater proportion of pollen produced by exerted long-level anthers (68.5%), than from mid-level anthers, which are located in the mouth of the perianth tube (50%), or short-level anthers, which are hidden at the narrow base of the perianth tube (37.5%). In contrast to Wolfe and Barrett, we measured the amount of pollen remaining in individual *P. cordata* flowers, so that we could assess the effects of visit duration, flower size, and pollen size on removal by different pollinators.

Our primary objective in this study was to take advantage of a tristylous species to inform our understanding of the functional significance of anther position in monomorphic species. We will generally interpret our results as though all factors, other than anther position, are unimportant or remain constant or controlled. Of specific relevance is L. D. Harder and W. G. Wilson's (*unpublished manuscript*) theoretical conclusion that a flower's pollen production should not affect the optimal pattern of pollen removal, which depends instead on the proportion of remaining pollen removed during any visit. Paradoxically, this approach may be less effective for elucidating the role of anther position in heterostylous plants. In particular, frequency-dependent selection on the constituent morphs should promote evolutionary adjustments in stigma position and relative pollen production by different anther levels, which should result in equivalent siring success. We will consider the complete pollen transfer process for *P. cordata* elsewhere.

METHODS

We studied *P. cordata* along a creek between Opinicon and Hart Lakes in eastern Ontario, Canada (44°32' N, 76°20' W) from 12 to 15 August 1989. Each morning we collected inflorescences before flowers opened, inserted their stems into water-filled vials, and placed them in a screen enclosure, which excluded insect visitors. Once flowers opened, we selected an inflorescence from the enclosure to measure pollen removal. From the upper and lower halves of each inflorescence we removed an unvisited flower for later measurement of pollen production: these flowers were stored in separate vials containing 70% ethanol. To facilitate viewing of the sequence of bee visits, additional flowers were removed so that not more than 10 flowers remained on an inflorescence and they were all visible from one side.

The prepared inflorescence was then mounted in a water-filled vial at the end of a stick and presented to bees as they foraged freely in the *P. cordata* stand.

When a bee moved onto the experimental inflorescence, an observer recorded the sequence of flower visits on a tape recorder, noting when the bee entered and left each flower. In many cases, the same inflorescence was presented to additional bees of the same species to accumulate multiple visits (≤ 4) to individual flowers. Immediately after an inflorescence had been visited, we removed all visited flowers and stored them individually in labelled vials with 70% ethanol. The tape recording was also transcribed at this time, so that timings could be associated with individual flowers.

Pollen produced by unvisited flowers and pollen remaining in visited flowers were counted with an electronic particle counter (Particle Data Elzone 180XY; see Harder 1990). The contents of a vial were washed into a 30-mL glass counting chamber filled with 0.63% NaCl solution and sonicated for 1 min. The flower was then removed and stored in ethanol for later measurements. The particle counter counted the number of particles in 1-mL subsamples and assigned them to 128 logarithmic size (diameter) classes. Differences in the sizes of pollen grains produced by the three anther levels of *P. cordata* (Fig. 1) allowed us to determine the amount of pollen remaining in each anther level within a flower. Estimates of total pollen number and pollen size are typically based on three 1-mL subsamples. For each visited flower we estimated the cumulative proportion of pollen removed by bees (P_{removed}) based on the number of grains left in the flower (L) and the average pollen production (A) by the two control flowers for the same inflorescence ($P_{\text{removed}} = 1 - L/A$). This study is based on 11 long-styled inflorescences (78 flowers), 8 mid-styled inflorescences (72 flowers), and 7 short-styled inflorescences (57 flowers).

To determine whether the timing of anther dehiscence differed among stamen levels, we observed five inflorescences of each morph during a single morning. The inflorescences were collected before flowers opened, and placed in water-filled vials in a sunlit, screened enclosure. At 10-min intervals, we inspected the inflorescences and recorded whether at least one flower was open and, after flower opening, whether at least one flower had fully dehisced anthers (different anther levels were assessed separately).

Statistical analyses of pollen removal

We used analyses of covariance to assess the influences of various categorical and continuous variables on the cumulative proportion of pollen removed from a flower. Initially we included all possible interactions between covariates and main effects in the analysis. We then used a backward elimination procedure to exclude covariates and their interactions that did not explain significant ($P < .05$) portions of the variation in the dependent variable. A term could only be excluded if it was not involved in higher order interactions. All analyses of covariance were conducted with the GLM procedure of SAS (SAS 1987).

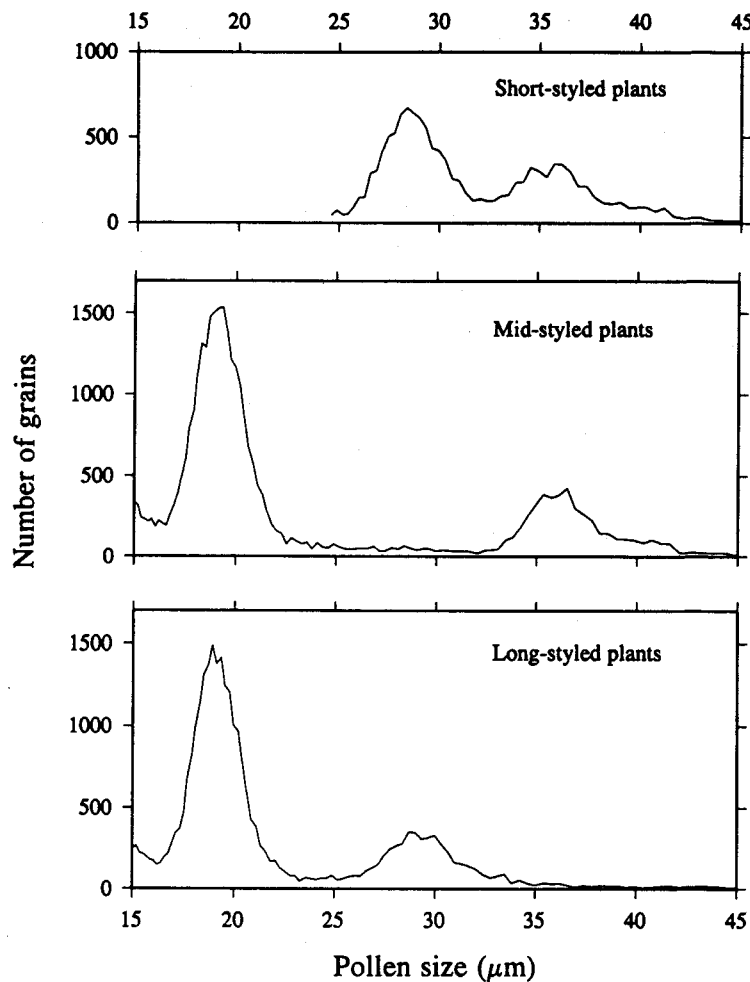


FIG. 1. Average size (diameter) frequency distributions of pollen from long-, mid-, and short-styled flowers of *Pontederia cordata* ($n = 5$ flowers for each morph).

Analyses of covariance often identified significant interactions between main effects and a covariate, which imply that the slope for the covariate differed among factor levels. In discussing these interactions, we present the partial regression coefficient and its standard error ($b \pm s_b$) for the covariate at each factor level as well as the results of a single-sample t test of the hypothesis that the regression coefficient did not differ significantly from zero. A posteriori pairwise comparisons of the regression coefficients for different factor levels involved Tukey-Kramer multiple comparisons to control the experimentwise Type I error to $\alpha = .05$ (Kirk 1982). We generally do not discuss the effect of any main effect or covariate in isolation if it was also involved in a significant interaction.

We adopted two perspectives in applying analyses of covariance to examine pollen removal from *P. cordata* flowers: removal from each anther level, and removal from each morph. These perspectives required different designs, which, due to their complexity, warrant detailed description.

Analysis by anther level.—This analysis involved two hierarchical units of observation, inflorescences and flowers within inflorescences. Inflorescences (=plant) can be viewed as individual replicates in a fully crossed three-factor design, with floral morph (two morphs contain each anther level), bee species (each inflorescence was visited by only one species), and time of day (before or after noon) as the main effects. Because one of the bee species (*Melissodes apicata*) stopped foraging shortly after noon (Harder and Barrett 1992), our sampling was incomplete with respect to the Morph \times Bee \times Time interaction. This interaction was therefore excluded from the analysis.

Two features of the Plant within Morph \times Bee \times Time effect (denoted Plant[Morph \times Bee \times Time]) warrant comment. First, in the ANCOVAs we have treated between-plant variation as a random effect. Second, because the same individual bee visited all flowers on an inflorescence, the so-called Plant[Morph \times Bee \times Time] effect involves the influences of both

TABLE 1. Characteristics of flowers produced by the three floral morphs of *Pontederia cordata*. The means \pm 1 SE are based on the mean of two flowers per inflorescence for the number of inflorescences given in parentheses. Superscript letters indicate the results of analyses of variance comparing morphs (within anther levels for pollen characters): morphs with different letters differ significantly ($P < .05$).

Anther level	Morph		
	Long-styled	Mid-styled	Short-styled
	a) Flower length (mm)		
	7.9 \pm 0.11 ^{a,b} (22)	8.0 \pm 0.12 ^a (19)	7.5 \pm 0.12 ^b (17)
	b) Maximum anther height above base of pistil (mm)		
Long		14.5 \pm 0.23 ^a (19)	14.2 \pm 0.24 ^a (17)
Mid	9.5 \pm 0.13 ^a (22)		9.2 \pm 0.15 ^a (17)
Short	4.9 \pm 0.10 ^a (22)	4.9 \pm 0.14 ^a (19)	
	c) Pollen number		
Long		4692 \pm 157.2 ^a (19)	4635 \pm 194.8 ^a (17)
Mid	5822 \pm 176.6 ^a (22)		8377 \pm 261.9 ^b (17)
Short	21 741 \pm 865.2 ^a (22)	20 630 \pm 1013.0 ^a (19)	
	d) Pollen size (diameter, μ m)		
Long		35.6 \pm 0.24 ^a (19)	35.5 \pm 0.18 ^a (17)
Mid	28.5 \pm 0.15 ^a (22)		28.0 \pm 0.13 ^b (17)
Short	18.8 \pm 0.07 ^a (22)	18.7 \pm 0.07 ^a (19)	
	e) Pollen volume per flower (mm ³)		
Long		0.037 \pm 0.0015 ^a (19)	0.037 \pm 0.0015 ^a (17)
Mid	0.024 \pm 0.0006 ^a (22)		0.032 \pm 0.0014 ^b (17)
Short	0.026 \pm 0.0012 ^a (22)	0.024 \pm 0.0013 ^a (19)	

between-plant variation and variation among bees of a given species.

In addition to pollen removal, we measured pollen size and flower length (from the flower base to the divergence of the upper and lower perianth lobes) for each flower visited within an inflorescence. These latter effects, as well as the number of visits that a flower received and their cumulative duration, were included as covariates in the statistical analyses.

Analysis by morph.—This analysis included plants as replicates within a two-factor design (Bee species \times Time of day) and flowers as replicates within plants (both random effects). In addition, each morph includes two anther levels, so that the pollen remaining in each anther level can be considered as a repeated measure for each flower. Hence, the analysis by morph involved a repeated-measures design with three between-flower factors (Bee species, Time of day, and Plant[Bee \times Time]) and one within-flower factor (an-

ther level). We also included flower length, number of visits, and cumulative visit duration as covariates in this analysis. Pollen size was not included as a covariate in the analysis by morph because it is largely confounded with anther level as anthers at different levels produce distinctly different pollen (see Fig. 1).

RESULTS

Floral characteristics

The three floral morphs of *P. cordata* differed in the placement of anthers and stigmas; otherwise their flowers were quite similar. Long- and mid-styled flowers did not differ significantly in length, but short-styled flowers were significantly shorter than mid-styled flowers (Table 1a; $F_{2,55} = 4.39$, $P < .025$; specific conclusion based on Tukey's multiple comparisons). The ratio of anther heights for long-, mid-, and short-level stamens was roughly 3:2:1, with no significant differences be-

TABLE 2. Selected correlations between flower length and the number and size of pollen grains from different anther levels in the floral morphs of *Pontederia cordata*. Correlations in bold type relate comparisons between flowers on the same inflorescence; the remaining correlations involve within-flower comparisons. The sample size below each correlation indicates the number of inflorescences sampled.

Anther level	Pollen production by anther level			Flower length (mm)
	Long	Mid	Short	
		a) Pollen production		
Long	0.711*** (36)	0.081 (18)	0.247 (20)	0.250 (37)
Mid		0.813*** (37)	-0.181 (23)	-0.380* (40)
Short			0.608*** (37)	-0.091 (42)
		b) Pollen size (μm)		
Long	-0.246 (37)			-0.254 (37)
Mid		-0.398* (40)		0.051 (40)
Short			0.020 (42)	0.067 (42)

* $P < .05$; *** $P < .001$.

tween morphs in anther height for corresponding stamens (Table 1b). Notice from Table 1 that mid-level anthers slightly exceed the length of the perianth tube, whereas, short-level anthers are well inserted and long-level anthers are greatly exerted.

Pollen characteristics differed greatly among anther levels. In general, long-level anthers produced relatively few large grains, short-level anthers produced many small grains, and mid-level anthers produced intermediate numbers of medium-sized pollen (Fig. 1, Table 1c, d). Differences in pollen production do not merely reflect a size-number trade-off, as long-level anthers produced a greater volume of pollen than mid- and short-level anthers in short- and mid-styled flowers, respectively (see Table 1e: repeated-measures ANOVA; short-styled flowers, $F_{1,16} = 6.72$, $P < .025$; mid-styled flowers, $F_{1,18} = 99.52$, $P < .001$). Mid- and short-level anthers produced equivalent pollen volumes in long-styled flowers ($F_{1,21} = 2.38$, $P > .1$). Mid-level anthers from short-styled flowers produced more, smaller pollen than corresponding anthers in long-styled flowers. Pollen production by one anther level was not correlated with production by the other level within the same flower; however, different flowers on the same inflorescence exhibited strong correlations for pollen production by a given anther level (Table 2). The average size of pollen grains generally increased through the day ($F_{1,49} = 21.58$, $P < .001$) at a rate ($0.2 \pm 0.04 \mu\text{m/h}$) that did not differ significantly between anther levels ($F_{2,47} = 1.89$, $P > .1$).

The different positions of stamens within *P. cordata* flowers significantly influenced the timing of anther dehiscence. On average (± 1 SE), long-level anthers opened completely 1.5 ± 0.05 h after the flower bud began opening, mid-level anthers opened after 2.6 ± 0.14 h, and short-level anthers opened after 3.2 ± 0.16

h ($n = 10$ in all cases). The timing of dehiscence for a particular anther level did not differ significantly between morphs ($P > .05$ in all cases). Within a morph, the lower anthers in a flower dehiscence significantly later than the upper anthers ($P < .025$ in all cases).

Pollinator characteristics

Melissodes apicata differs from the bumble bees in several relevant aspects. First, it is 50–65% smaller and has a shorter tongue (Table 3). This difference resulted in longer flower visits than *B. impatiens*, but not *B. vagans* ($F_{2,24} = 5.74$, $P < .01$; specific conclusion based on Tukey's multiple comparisons), probably because of slower nectar ingestion (Table 3). Second, unlike the proboscides of bumble bees (and related *Melissodes* spp.), the galea of *M. apicata* bears long, recurved hairs that apparently facilitate pollen removal from short-level anthers of *P. cordata* (LaBerge 1956). Third, *M. apicata* visited *P. cordata* only until ≈ 1300 , whereas the bumble bees visited *P. cordata* until the flowers wilted (≈ 1630 ; see Harder and Barrett 1992). Finally, while visiting long- or short-styled *P. cordata* flowers, *M. apicata* rapidly raked the mid-level anthers with their prothoracic legs.

The two bumble bee species also differ from each other. *Bombus impatiens* is larger, but has a shorter tongue than *B. vagans* (Table 3). As noted above, *B. impatiens* spent less time in *P. cordata* flowers than *B. vagans*. While visiting flowers, *B. impatiens* often rapidly vibrated its wing muscles, producing an audible buzz. This species apparently has a propensity for buzz-pollination as it seems to visit plants with poricidal anthers more commonly than other *Bombus* species in eastern North America (Sanford and Hanneman 1981; L. D. Harder, personal observations).

TABLE 3. Characteristics (mean \pm 1 SE) of the three common pollinators of *Pontederia cordata* at Hart Lake Channel, Ontario. Except for probing time data, the bees involved were collected for other studies (Harder 1983, 1985, 1986). Ingestion rates involved 30% (mass of solute/mass of solution) sucrose solutions. Probing times are the time to enter and leave a *P. cordata* flower.

Character	<i>Bombus impatiens</i>	<i>Bombus vagans</i>	<i>Melissodes apicata</i>
Body mass (mg)	145 \pm 5.6* (31)	108 \pm 4.6* (30)	69 \pm 11.8† (5)
Glossa length (mm)	5.8 \pm 0.08* (32)	6.7 \pm 0.09* (32)	4.5 \pm 0.14† (5)
Ingestion rate (μ L/s)	1.52 \pm 0.249‡ (3)	1.01 \pm 0.119‡ (2)	0.34 \pm 0.049† (5)
Probing time (s)§	1.3 1.15–1.57 (10)	2.3 1.94–2.63 (9)	2.7 2.31–3.05 (8)

* Bees collected 60 km southwest of study site (Harder 1983).

† Bees collected 1 km from study site (Harder 1985).

‡ Bees collected on Long Island, New York (Harder 1986).

§ Statistics based on log-transformed data, hence asymmetric standard errors.

Pollen removal from anthers at different levels

Anther level had several significant effects on pollen removal: it influenced the proportion of pollen removed by pollinators (Table 4), the diversity of influences on removal (Table 5), and the predictability of removal. During the first visit to a flower, bees removed equivalent proportions of the available pollen from short- (28%) and mid-level anthers (24%) and a significantly higher proportion of pollen from long-level anthers (39%; see Table 4). Wolfe and Barrett (1989) reported essentially the same relation of removal to anther level for a different bee species (*B. griseocollis*), although they estimated greater removal overall.

Short-level anthers.—Pollen removal from short-level anthers appears to be largely stochastic. Little of the observed variation in pollen removal from these anthers could be explained by measured variables ($R^2 = 0.253$). Furthermore, differences between plants (and/or bees of a given species) was the only identifiable source of variation in pollen removal (Table 5).

Mid-level anthers.—In contrast to short-level anthers, more of the observed variation in pollen removal from mid-level anthers could be explained by variation in measured variables ($R^2 = 0.442$). Although some of

this variation resulted from differences between plants, a larger component of variation was associated with the number of visits that a flower received (Table 5). In particular, cumulative pollen removal from mid-level anthers increased with successive visits ($b \pm s_b = 0.058 \pm 0.015$). This influence of visit number was not observed for either of the other anther levels.

Long-level anthers.—The relatively large proportion of variation explained in the amount of pollen remaining in long-level anthers ($R^2 = 0.568$) is associated with diverse influences (Table 5). The proportion of pollen removed from long-level anthers by *Melissodes apicata* generally declined with increasing flower length; however, this effect was only significant for short-styled flowers (short-styled flowers, $b \pm s_b = -0.247 \pm 0.061$, $t = 4.06$, 109 df, $P < .001$; mid-styled flowers, $b \pm s_b = -0.014 \pm 0.038$, $t = 0.38$, 109 df, $P > .5$). This difference between morphs and the absence of a flower length effect for either *Bombus* species resulted in the significant Morph \times Bee \times Length interaction for long-level anthers (Table 5).

All three bee species removed less pollen from long-level anthers of short-styled flowers than from the corresponding anthers of mid-styled flowers (Table 2). This morph difference was greater for *Melissodes* than for the bumble bees, resulting in a significant Morph \times

TABLE 4. Proportion of pollen removed from different anther levels of *Pontederia cordata* during first visits by three bee species (means \pm 1 SE). Numbers in parentheses indicate number of flowers sampled.

Bee species	Long-level anthers	Mid-level anthers	Short-level anthers
<i>Melissodes apicata</i>	0.38 \pm 0.04 (30)	0.31 \pm 0.03 (29)	0.32 \pm 0.03 (25)
<i>Bombus impatiens</i>	0.36 \pm 0.02 (34)	0.19 \pm 0.03 (37)	0.30 \pm 0.03 (35)
<i>Bombus vagans</i>	0.43 \pm 0.02 (36)	0.22 \pm 0.04 (32)	0.24 \pm 0.02 (32)
Combined	0.39 \pm 0.02 (100)	0.24 \pm 0.02 (98)	0.28 \pm 0.02 (92)

TABLE 5. Results of analyses of covariance for the proportion of pollen grains removed from anthers at different levels in *Pontederia cordata* flowers during 1–4 bee visits. The cumulative duration of bee visits and pollen size were initially included as covariates in these analyses, but they were later excluded because neither covariate (nor their interactions with main effects) significantly affected pollen removal from any anther level.

Effect	Long-level anthers	Mid-level anthers	Short-level anthers
Morph	$F_{1,111} = 0.20$	$F_{1,7} = 3.19$	$F_{1,10} = 0.05$
Bee species	$F_{2,111} = 7.18^{**}$	$F_{1,7} = 0.24$	$F_{2,10} = 0.17$
Morph × Bee	$F_{2,111} = 4.02^*$	$F_{2,7} = 1.52$	$F_{2,10} = 3.06$
Time of day	$F_{1,6} = 1.72$	$F_{1,7} = 0.12$	$F_{1,10} = 0.003$
Morph × Time	$F_{1,6} = 1.70$	$F_{1,7} = 2.95$	$F_{1,10} = 0.03$
Bee × Time	$F_{2,6} = 0.43$	$F_{2,7} = 0.10$	$F_{1,10} = 1.23$
Plant[Morph × Bee × Time]	$F_{5,109} = 2.82^*$	$F_{8,124} = 2.73^{**}$	$F_{10,142} = 2.01^*$
Number of visits		$F_{1,124} = 14.80^{***}$	
Flower length	$F_{1,109} = 2.09$		
Morph × Length	$F_{1,109} = 1.44$		
Bee × Length	$F_{2,109} = 5.05^{**}$		
Morph × Bee × Length	$F_{2,109} = 5.15^{**}$		

* $P < .05$; ** $P < .01$; *** $P < .001$.

Bee interaction (Table 5). Overall, bumble bees removed more pollen from long-level anthers than did *Melissodes* (Fig. 2, Table 4). Note that these conclusions apply specifically to flowers of average length: because of the Morph × Bee × Length interaction described above the difference between morphs for *Melissodes* was greater than illustrated in Fig. 2 for longer flowers and less for shorter flowers.

Pollen removal from floral morphs

In general, pollen removal from a particular morph should reflect the combined influences on removal for the two anther levels produced by that morph. As we have already demonstrated, the factors affecting removal often differed between anther levels, so that pollen removal from entire flowers should commonly involve statistical interactions with anther level.

Short-styled flowers.—As described above, flower length affected pollen removal from the long-level anthers of short-styled flowers by *Melissodes*, but not by bumble bees. In contrast, flower length did not influence removal from mid-level anthers by any bee species. The combination of these disparate influences within short-styled flowers resulted in a significant interaction between anther level, bee species, and flower length (Table 6).

The significant interaction between anther level and bee species (Table 6) reflects pollen removal as though all flowers were of average size. Given this condition, *Melissodes* removed equivalent proportions of pollen from both anther levels of short-styled flowers (long-level, 0.233 ± 0.025 ; mid-level, 0.239 ± 0.026). In contrast, the bumble bees generally removed more pollen from long-level anthers (*B. impatiens*, 0.350 ± 0.021 ; *B. vagans*, 0.347 ± 0.029) than from mid-level anthers (*B. impatiens*, 0.228 ± 0.022 ; *B. vagans*, 0.307 ± 0.030), although the difference was significant only for *B. impatiens*.

Mid-styled flowers.—Although mid-styled flowers also produce long-level anthers, flower length did not

influence pollen removal from them as it did from long-level anthers in short-styled flowers (see *Results: Pollen removal from anthers at different levels: Long-level anthers*). As a result, mid-styled flowers did not exhibit the Level × Bee × Length interaction observed for short-styled flowers. The significant main effect of anther level (Table 6) resulted because bees removed more pollen from long-level anthers (0.417 ± 0.016) than from short-level anthers (0.278 ± 0.019). Averaged over both anther levels, *B. impatiens* removed more pollen from mid-styled flowers (0.414 ± 0.019 , $n = 28$) than either *B. vagans* (0.322 ± 0.026 , $n = 29$) or *M. apicata* (0.292 ± 0.022 , $n = 20$).

Long-styled flowers.—Differences between anther levels of long-styled flowers were reflected in the influences of visit number and flower length on pollen removal. As outlined earlier, cumulative pollen removal

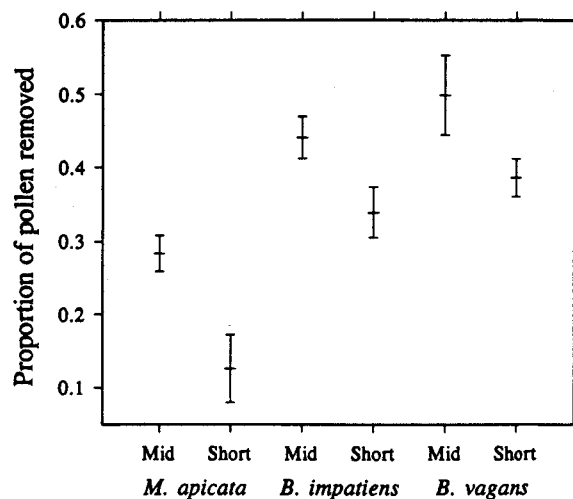


FIG. 2. Proportion of pollen removed (means \pm 1 SE) from long-level anthers of mid- and short-styled flowers of *Pontederia cordata* by each of three bee species (*Melissodes apicata*, *Bombus impatiens*, and *B. vagans*).

TABLE 6. Results of analyses of covariance for the proportion of pollen grains removed from anthers of different floral morphs of *Pontederia cordata* during bee visits. The cumulative duration of bee visits was initially included as a covariate in these analyses, but it was later excluded because neither it nor its interactions with main effects significantly affected pollen removal from any morph.

Effect	Long-styled flowers	Mid-styled flowers†	Short-styled flowers‡
Between-flower effects			
Bee species	$F_{2,25} = 0.18$	$F_{2,13} = 4.95^*$	$F_{2,47} = 1.16$
Time of day	$F_{1,27} = 0.26$	$F_{1,73} = 0.65$	
Bee × Time	$F_{1,70} = 0.69$		
Plant[Bee × Time]	$F_{6,30} = 0.48$	$F_{4,6} = 0.36$	$F_{1,34} = 0.003$
Number of visits	$F_{1,65} = 3.85$		
Flower Length	$F_{1,65} = 0.74$		$F_{1,47} = 5.31^*$
Bee × Length			$F_{1,47} = 3.83^*$
Within-flower effects			
Anther level	$F_{1,66} = 2.97$	$F_{1,4} = 17.71^*$	$F_{1,47} = 0.20$
Level × Bee	$F_{2,6} = 1.61$	$F_{2,4} = 0.07$	$F_{2,47} = 4.65^*$
Level × Time	$F_{1,6} = 2.57$	$F_{1,4} = 2.61$	$F_{1,1} = 8.34$
Level × Bee × Time	$F_{1,6} = 0.79$		$F_{2,1} = 5.71$
Level × Plant[Bee × Time]	$F_{6,65} = 4.68^{***}$	$F_{4,69} = 3.60^*$	$F_{1,47} = 0.11$
Level × Number of visits	$F_{1,65} = 7.19^*$		
Level × Length	$F_{1,65} = 4.78^*$		$F_{1,47} = 0.11$
Bee × Level × Length			$F_{2,47} = 4.86^*$

* $P < .05$; ** $P < .01$; *** $P < .001$.

† Bee × Time and Bee × Time × Level could not be tested for this morph because of incomplete sampling.

‡ Appropriate error mean squares could not be estimated to test Time of day and Bee × Time for this morph.

increased with successive visits for mid-level anthers, but not for short-level anthers (Table 5). This difference resulted in the significant interaction between anther level and visit number for long-styled flowers (Table 6).

In contrast, the significant interaction between anther level and flower length (Table 6) was unexpected as flower length did not significantly affect pollen removal from either mid- ($b \pm s_b = -0.017 \pm 0.041$, $t_{65} = 0.41$, $P > .05$) or short-level anthers ($b \pm s_b = 0.080 \pm 0.044$, $t_{65} = 1.79$, $P > .05$). However, these partial regression coefficients for the two anther levels were sufficiently different from each other to result in a significant interaction. In particular, bees tended to remove more pollen from the short-level anthers of large, long-styled flowers.

The nonsignificant F test for differences between anther levels reported in Table 6 compares levels as though they were produced by average-sized flowers that received the average number of visits. Because of the heterogeneous effects of flower size and visit number, removal could differ among anther levels in flowers of more extreme size or flowers that received few or many visits. A posteriori contrasts revealed that pollen removal did not differ between mid- and short-level anthers of long-styled flowers over the entire observed range of flower size and visit number.

DISCUSSION

Pontederia cordata possesses two floral characteristics that enabled this study: anthers occur at different levels and each level produces different-sized pollen, thereby identifying the position of production. Unfortunately this combination of traits also complicates

interpretation of our results, as dissimilar removal could have resulted from differences in either anther position or pollen size. Therefore, before considering the various effects of anther position on male success, we clarify which trait predominantly influenced pollen removal from *P. cordata*.

We interpret our results in relation to anther position primarily because of the mechanics of pollen removal. Removal from flowers with exposed, longitudinally dehiscent anthers results from contact between pollen and pollinator and should vary depending on the pollinator's texture at the point of contact and the force and duration of contact. Anther position obviously determines the point of contact with the pollinator's body and should therefore directly influence the likelihood of pollen removal. In contrast, it is not obvious how pollen size could affect the contact between pollen and pollinator to the same extent as anther position. This is not to deny functional significance to differences in pollen size in *Pontederia*; rather their significance probably lies in post-pollination processes, especially pollen-stigma complementarity, pollen tube growth, and the operation of heteromorphic incompatibility (Anderson and Barrett 1986, Glover and Barrett 1986, Scribailo and Barrett 1991).

Direct effects of anther position on pollen removal

A plant's ability to sire seeds on other plants depends, in part, on its effectiveness in employing pollinators to disperse pollen to stigmas and the success of dispersed grains in competing for unfertilized ovules. A particular trait may affect these components of male success differently, so that the most advantageous floral

morphology may reflect an evolutionary compromise between conflicting demands. Our results with *P. cordata* suggest that the most beneficial position for anthers within a monomorphic flower may be determined by such a compromise. Before we propose which position might promote male success most effectively, we will outline the expected implications of our results from *P. cordata* for pollen dispersal.

Restriction of pollen removal.—The pollen loss associated with animal pollination can be reduced if pollen removal during individual pollinator visits is restricted so that many pollinators are involved in pollen dispersal (Harder and Thomson 1989; L. D. Harder and W. G. Wilson, *unpublished manuscript*). Furthermore, the extent to which removal should be restricted to maximize dispersal should depend on pollinator availability, but not on initial pollen production. The observed differences in pollen removal between anther levels of *P. cordata* (Table 4) are therefore unexpected if all anther positions promote pollen dispersal equally. Because a diverse pollinator fauna intensively visits *P. cordata* at our study site and neighboring sites (Wolfe and Barrett 1988, Harder and Barrett 1992), pollen removal should be quite restricted during individual visits. Mid- and short-level anthers experienced lower pollen removal than long-level anthers (e.g., Table 4). Hence, anther exertion may often compromise effective pollen dispersal for bee-pollinated species with monomorphic flowers.

Relative value of dominant pollinators.—Grant and Grant (1965) proposed that anthers positioned within the floral tube would influence pollen dispersal more effectively than exerted anthers for butterfly-pollinated plants that are visited by a variety of pollinators. In particular, although the proboscis of any pollinator that can contact a flower's nectar will also pass anthers within the tube, small butterflies, or those with shorter tongues, may be less likely to contact exerted anthers. This argument implies that the most beneficial anther position for pollen dispersal should involve all common pollinators equally in pollen removal; otherwise the effort of attracting them has been misspent.

Our results with *P. cordata* indicate that bee-pollinated plants with exerted anthers would also experience variable removal, depending on the pollinator involved, as pollen removal from long-level anthers differed between bee species (Table 5, Fig. 2), with the specialist *Melissodes apicata* removing less pollen than bumble bees. This lower removal likely resulted because *M. apicata* is too small to consistently contact the exerted long-level anthers, especially in short-styled flowers whose hooked, mid-level anthers support the bee, lifting it from constant contact with the long-level anthers. Consequently, the dominant pollinators of *P. cordata* probably do not disperse pollen from long-level anthers with equal effectiveness.

Predictability of removal.—Removal from short-level anthers seems to be largely unpredictable, as it was

unaffected by all of the measured variables (Table 5). These anthers are so deeply recessed within the flower that their pollen is removed by the upper surface of the bee's proboscis. The proboscis of apid and anthophorid bees is a composite structure that includes a hairy glossa surrounded dorsally by a pair of galeae and ventrally by a pair of expanded labial palps (Winston 1979). Both the galeae and labial palps are chitinous plates that are typically quite smooth (except for *M. apicata*, LaBerge 1956) and provide little purchase for a pollen grain. As a consequence, pollen removal from short-level anthers may depend on whether, and to what extent, they contact the glossa, rather than the galeae or labial palps. The unpredictability of removal may therefore have resulted from variation in the path traversed by the proboscis. Wolfe and Barrett (1989) found that this position also resulted in unpredictable pollen receipt, as 40% of short-styled flowers received no legitimate pollen during single bee visits, as opposed to 26 and 21% for long- and mid-styled flowers, respectively.

Pollen removal schedule.—Maximal pollen dispersal requires a specific schedule of pollen removal with successive pollinator visits (L. D. Harder and W. G. Wilson, *unpublished manuscript*). Although no plant is likely to achieve the optimal removal schedule, even approximation to this schedule would require that cumulative removal increase with each new visit. However, for *P. cordata* the number of visits that a flower received affected pollen removal from mid-level anthers only (Table 5). The insensitivity of pollen removal from long- and short-level anthers to the cumulative number and duration of visits distinguishes *P. cordata* from all other species with longitudinally dehiscing anthers for which pollen removal has been measured (see Galen and Stanton 1989, Harder 1990, Young and Stanton 1990). This recalcitrance to removal during subsequent visits does not persist throughout anthesis, as <5% of a flower's pollen production remains after 1330 in naturally visited flowers at our study site (Harder and Barrett 1992).

Staggered anther dehiscence.—Anther position can affect pollen removal through its influence on the timing of anther dehiscence. Tubular flowers typically produce an intrafloral humidity gradient, with humidity increasing above ambient levels towards the flower base (Corbet et al. 1979). Because anther dehiscence largely involves dehydration (Keijzer 1983), this humidity gradient should retard the opening of anthers placed deeply within the flower, as we observed for *P. cordata*. This relation of dehiscence to anther position could influence a plant's male reproductive success if fertilization probabilities vary with the order of pollen arrival on stigmas (see Marshall and Ellstrand 1985, Epperson and Clegg 1987, Graham and Barrett 1990). In particular, monomorphic flowers with inserted anthers would generally donate pollen later and, therefore, suffer a competitive disadvantage relative to flowers with

longer anthers. Clearly, such effects will be most significant for species with relatively synchronous anthesis.

Although anther level affected the timing of dehiscence for *P. cordata*, our study of pollen removal did not detect corresponding significant effects of time of day (Tables 5 and 6). This apparent contradiction undoubtedly resulted because we presented experimental inflorescences to bees only after the anthers had dehisced to ensure equal opportunity for pollen removal from both included anther levels.

Which anther position is most beneficial?—The preceding discussion demonstrates that no single anther position is consistently the most beneficial for all of the diverse effects on pollen removal. If *P. cordata* is representative of bee-pollinated plants with flaring, tubular flowers, then exerted anthers should dehisce early, but they do not restrict pollen removal or consistently contact small pollinators. Inserted anthers restrict pollen removal more, but dehisce slowly and may be subject to unpredictable removal because they contact the bee's proboscis, rather than hairy structures more suited to carrying pollen. Overall, anthers placed in the opening of the perianth mouth seem to combine the advantages of more exerted and inserted positions without suffering the corresponding disadvantages as they restrict removal, dehisce relatively early, and seem to consistently contact all pollinators. Indeed, this is the prevalent anther location for bee-pollinated species with tubular flowers, as typified by members of many angiosperm families, including the Liliaceae, Boraginaceae, Scrophulariaceae, Labiatae, and zygomorphic Ranunculaceae.

In contrast to the foregoing conclusion, plants pollinated predominately by animals other than large bees often do not position their anthers in the flower mouth. For example, bird-pollinated flowers typically have greatly exerted anthers, whereas flowers pollinated by lepidoptera and long-tongued flies often have inserted anthers. Clearly the advantages of particular anther positions depend on the morphological and behavioral characteristics of a plant's dominant pollinators. For example, pollen may adhere poorly to a bird's smooth bill, promoting the evolution, in bird-pollinated species, of long stamens that place pollen more proximally on the head (also see *Relative value of dominant pollinators* above for Grant and Grant's [1965] hypothesis for inserted anthers and lepidoptera pollination).

Anther position and floral evolution

The greater impact of variation in floral characters on pollen removal and dispersal than on pollen receipt (Willson and Price 1977, Lloyd and Yates 1982, Bell 1985, Stanton et al. 1986, Cruzan et al. 1988, Galen and Stanton 1989, Murcia 1990, Young and Stanton 1990, Campbell et al. 1991) suggests an evolutionary priority for selective adjustment of the placement of floral organs for outcrossing species. In particular, an-

ther position should be modified to maximize pollen dispersal and that position then determines the location of the stigma to reduce self-pollination and self-interference. Studies of the relation of male fertility to stamen position, which complement the growing literature on the dependence of selfing on stigma-anther separation (Breese 1959, Ennos 1981, Thomson and Stratton 1985, Barrett and Shore 1987, Barrett and Husband 1990, Murcia 1990, Robertson and Lloyd 1991), would clearly be instructive.

In hypothesizing an evolutionary priority for anther placement over stigma placement we implicitly assume that pollination, although necessary, does not influence variation in seed production. However, when pollen receipt does limit female function, this priority may be relaxed in the face of stronger selection to ensure genetic contributions to offspring through any sexual route. Because a plant is probably more likely to receive than to donate pollen as a result of a single pollinator visit, such selection will probably favor stigma placement over anther placement. Results presented by Kohn and Barrett (1992) suggest that, as with pollen removal, the most advantageous position for pollen receipt is the opening of the perianth mouth. In particular, they found considerably less pollen limitation of seed production for mid-styled *Eichhornia paniculata* in monomorphic arrays than for either long- or short-styled plants. Therefore evolutionary displacement of the stigma from this position is only expected when resources limit seed production and self-pollination and self-interference are disadvantageous.

Comment on anther position and the functioning of heterostyly

The preceding discussion and the specific conclusion concerning the most beneficial placement of anthers rely on the assumption that all factors, other than anther position, are equal. However, heterostylous plants do not satisfy this assumption. In particular, the presence of flowers with stigmas at different levels and a heteromorphic incompatibility system that favors matings between anthers and stigmas at the same level must alter the relative advantages of different anther positions for heterostylous plants. For example, the dependence of the timing of anther dehiscence on anther position may be less significant in a functional heterostylous population because the pollen from short-level anthers does not compete with pollen from other anther levels for access to short-level stigmas.

To maintain the balanced polymorphism that characterizes heterostyly, the different anther levels must be equally effective at dispersing their pollen to corresponding stigmas. Such equality could arise because either each pollinator visit leads to equivalent pollen dispersal, or dissimilar pollen production by different anther levels compensates for unequal pollen dispersal by individual pollinators. Production of a greater volume of pollen by long-level anthers (Table 1e) suggests

that the latter explanation may be more appropriate for *P. cordata*.

Pollinator specialization and pollen removal

Unlike generalist bumble bees, *Melissodes apicata* specializes on *P. cordata* and displays several associated behavioral and morphological adaptations. The impact of these adaptations on pollen removal and the resulting dissimilar removal compared to *Bombus* spp. apparently differed between anther levels. Unlike the bumble bees, the ability of *M. apicata* to remove pollen from long-level anthers declined with increasing flower size (Table 5, Length \times Bee); however, this difference likely reflects developmental variation in flower size (Table 3) and is not a consequence of specialization. *Melissodes apicata* raked mid-level anthers with their prothoracic legs and, during a flower's first visit, seemed to remove a greater proportion of pollen from mid-level anthers than did bumble bees (Table 4). However, the difference between bee species for mid-level anthers was not evident when additional variables were considered (Table 5), perhaps because phenological differences between bees resulted in limited sampling of *M. apicata* during afternoons. Finally, LaBerge (1956) suggested that the hooked hairs on the galea of *M. apicata* represented an adaptation to increase pollen removal from short-level anthers. Our analysis did not detect greater removal from these anthers by *M. apicata* relative to bumble bees; however, the pertinent (unconsidered) comparison for the relative advantage of these hairs would involve related *Melissodes* species.

For animal-pollinated plants, differences between pollinators in pollination effectiveness (Schemske and Horvitz 1984, Herrera 1987, Ordway et al. 1987, Snow and Roubik 1987, Ramsey 1988, Armbruster et al. 1989, Murcia 1990, Wilson and Thomson 1991) could favor different suites of characters depending on the composition of the pollinator fauna (Schemske 1983, also see Schemske and Horvitz 1989). Restricted host-plant preferences, with attendant behavioral and morphological adaptations, improve the foraging abilities of specialist pollinators (Strickler 1979, Wolfe and Barrett 1987, Laverty and Plowright 1988, this study); however, pollinator specialization has apparently had little impact on floral evolution for plants with diverse pollinators (reviewed by Schemske 1983). This lack of reciprocal specialization results for two reasons. First, although specialist pollinators may remove pollen more efficiently than generalists, their effectiveness at dispersing that pollen to other plants need not differ (e.g., Motten et al. 1981) and may even be lower for specialists. Second, floral specialization on particular pollinators would require consistent abundance of those pollinators throughout the season and between years, a requirement that may seldom be satisfied (Wolfe and Barrett 1988, Schemske and Horvitz 1989). In general, pollinator specialization seems to be an asymmetrical process.

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