Pollinator foraging behavior and pollen collection on the floral morphs of tristylous *Pontederia cordata* L.

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Summary. The foraging behavior of the pollinators of tristylous *Pontederia cordata* was studied to determine if differences in floral morphology would lead to preferential visitation of the floral morphs. Although nectar production is not different in the three floral morphs, differences in the production and size of pollen grains produced by the three anther levels results in the morphs offering variable amounts of resources to pollen-collecting insects. Bumblebees (*Bombus* spp.) and the solitary bee *Melissodes apicata* used *P. cordata* primarily as a nectar source and therefore did not seem to exhibit any morph preference. In contrast, honeybees visited flowers mainly for pollen and preferred to forage on long-level anthers of the short- and mid-styled morphs. An analysis of the composition of corbicular pollen loads indicated that, relative to the frequency of production in the population: 1) honeybees collected an excess of pollen from long-level anthers; 2) bumblebees collected the three types of pollen without any apparent preference; and 3) *M. apicata* preferentially collected pollen from the short-level anthers – presumably because their proboscides are modified by the presence of tiny hairs. The results suggest that *P. cordata* in Ontario is serviced by a diverse, unspecialized pollinator fauna which is not co-adapted to the tristylous floral polymorphism.

Key words: *Pontederia cordata* – *Bombus* – Tristyly – Pollination – Behavior

For outcrossing plants pollinated by animals, variation in floral traits is likely to influence pollination. In recent years many studies have documented intrapopulation variation in traits such as flower color (Harding and Mankinen 1967; Waser and Price 1981; Stanton 1987) and stigma-anther separation (Ennos 1981; Thomson and Stratton 1985) and their effects on either pollinator behavior or seed production. While these studies have examined characters that vary in a continuous fashion, heterostylous plants offer systems in which the influence of discrete variation in reproductive characters may be examined. However, very little is known about the pollination biology of heterostylous plants.

Heterostyly is a rare, genetically controlled floral polymorphism in which populations are composed of two (distyly) or three (tristyly) floral morphs that differ in style

and stamen length, pollen size, and incompatibility behavior (Ganders 1979). Darwin (1877) originally proposed an explanation of the functional significance of tristyly by hypothesizing that the reciprocal arrangement of stamens and styles promotes insect-mediated pollination among morphs with anthers and stigmas at equal heights (compatible pollination). He reasoned that due to the spatial organization of reproductive parts, pollen from different stamen levels would adhere to parts of a pollinator's body corresponding to the position where compatible stigmas would contact the insect. It is important to stress that since outcrossing in a tristylous species is enforced by a self-incompatibility system, the floral heteromorphism is a mechanism to influence the directionality of pollen transport by pollinators (Ganders 1979). Indeed, studies on tristylous *Pondederia* spp. (Glover and Barrett 1983; Price and Barrett 1984; Barrett and Glover 1985; Barrett and Wolfe 1986) and *Lythrum* spp. (Mulcahy and Caporello 1970; Ornduff 1975) have shown that the floral polymorphism influences pollen flow in natural populations.

In order for equilibrium frequencies of the three morphs of a tristylous species to be maintained in a population, pollinators should not exhibit any marked morph preference in foragin behavior. Thus we would expect selection to have minimized differences among morphs in floral rewards that might lead to preferential visitation. The basis for preferential visitation could be generated by differences in nectar production among style morphs. Alternatively, differences in the positioning, and production, of pollen by anthers within flowers might allow for specialization on certain morphs by pollen collecting insects. In fact, Ornduff (1975) reported that honeybees preferentially collected pollen from the long-level anthers of *Lythrum junceum*. In contrast, pollinators of *Oxalis alpina* (Weller 1981) and *Pontederia cordata* (Price and Barrett 1982) were found to forage randomly with respect to floral morph. However, in these two studies the investigators focused on only the most common species that was observed visiting flowers. Since pollinator species are known to vary in their foraging behavior and pollination effectiveness (Primack and Silander 1975; Schemske and Horvitz 1984), it is possible that while the major pollinator species did not exhibit any preference, other members of the pollinator fauna might.

The purpose of this paper is to investigate several features concerned with the foraging behavior of pollinators of *Pontederia cordata* L. (Pontederiaceae), a tristylous plant. We were particularly interested in examining whether floral morph choice and pollen collection was random in

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the most common pollinator taxa. The principal objectives of our study were to: 1) compare the amount of nectar produced by flowers of the three morphs to see if this could serve as a basis for preferential visitation; 2) determine whether pollinator species differ in their behavior while visiting inflorescences (e.g.: time necessary to visit a flower, number of flowers visited per inflorescence; 3) experimentally determine whether pollinators show any preference for particular morphs; and 4) determine whether pollinators preferentially collect pollen from different anther levels.

Tristyly in Pontederia cordata

Pontederia cordata is a perennial, emergent aquatic and populations usually contain all three floral morphs (Barrett et al. 1983). Individuals produce spicate inflorescences that bear purple-blue, zygomorphic, univovulate flowers. As in other tristylous species, flowers of each morph have three positions, or levels, and of these, two are occupied by anthers and the third by a stigma. Thus, the long-styled morph has long styles and mid- and short-level stamens; the mid-styled morph has medium length styles and long- and short-level stamens; and the short-styled morph has short styles and long- and mid-level stamens. The long-, mid-, and short-styled morphs will hereafter be referred to as the *L, M,* and *S* morphs, respectively. Unique among flowering plants is the fact that anthers at different heights within a flower produce pollen in significantly different amounts and sizes: long-level stamens produce relatively few, large-sized pollen grains, short-level stamens produce many, small-sized grains, and the mid-level stamens produce grains of intermediate size and number (Price and Barrett 1982; Wolfe 1985). Thus from the perspective of a pollen collecting insect, flowers of the three morphs offer pollen in varying sizes, amounts, and accessibility. Finally, the polymorphism is linked with a sporophytically-controlled incompatibility system that regulates matings between stigmas and anthers of the same height (Ornduff 1966; Barrett and Anderson 1985).

In Ontario, where this study was conducted, flowering commences in early July and lasts approximately two months. Individual inflorescences produce about 200 flowers of the same morph, and on average, 20 are open per day (Wolfe 1985). Flowers of *P. cordata* are serviced by a diverse suite of pollinator species including hummingbirds, flies, butterflies, and many bee species. However, at this study site, bumblebees (*Bombus* spp.), honeybees (*Apis mellifera*) and *Melissodes apicata* (hereafter referred to as *Melissodes*), a solitary bee, were the most abundant visitors (Wolfe 1985).

Study site and methods

This study was conducted at Pothole Lake, Leeds and Grenville County, Ontario. Pothole Lake is connected to the Rideau Lake System and is situated about 50 km north of Kingston, Ontario. The lake is approximately 800 m long and varies from 50 to 300 m in width. *Pontederia cordata* is the most abundant emergent plant in the littoral zone and grows along the shoreline and in shallow areas in the middle of the lake.

The volume of nectar in flowers was measured with 1 µl micropipettes (Drummond microcaps) by removing nectar that accumulates around the base of the ovary. Individual flowers were sampled once because complete removal of nectar permanently damages flowers. Nectar concentration was measured with a pocket refractometer (Bellingham and Stanley) calibrated in percent sucrose equivalents (g sucrose/100 g solution). Twenty flowers were sampled per morph. An additional set of flowers were sampled at hourly intervals to determine the daily patterns of nectar production.

Four attributes of the foraging behavior of pollinators were examined in the commonest floral visitors: 1) the position on an inflorescence at which visitors arrived (inflorescences were divided into thirds: top, middle and bottom); 2) total number of flowers visited per inflorescence; 3) direction of vertical movements on an inflorescence; and 4) an estimate of the time required to visit a flower. The latter was determined by dividing the time spent on an inflorescence by the number of flowers visited. As a result, the time per flower includes both time spent probing a flower and travelling between flowers within an inflorescence. Travel time was much less than probing time.

In order to determine if bumblebees and honeybees preferred any floral morph, an array of nine inflorescences (three per morph) was constructed by placing inflorescences in jars containing water in a latin square design on a floating piece of plywood (50 × 50 cm). All inflorescences were trimmed to 20 flowers. The array was placed in the population and naturally foraging bees were allowed to visit. Four foraging behaviors were recorded: 1) the morph first visited upon entering the array (arrival morph); 2) the last morph visited prior to departing the array; 3) the number of inflorescences of each morph visited during a visit to the array; 4) the number of flowers of each morph visited. An inflorescence was recorded as having received a visit if at least one flower was contacted. A total of 494 inflorescence-visits and 1859 flower-visits were recorded. Data were analyzed with goodness-of-fit tests to determine if the foraging patterns of pollinators were independent of floral morph.

Pollen loads collected by bumblebees (*Bombus bimaculatus*), honeybees and *Melissodes* were examined to determine if there was evidence of preferential collection of the three pollen types. Preferential collection may be detected by comparing the frequency of pollen types in corbicular loads (pollen baskets) with the frequency of production. This population pollen pool was calculated by taking into account the morph ratio ($L = 0.31$; $M = 0.27$; $S = 0.42$; $n = 332$ inflorescences) and number of pollen grains produced at each anther level which was determined by replicated pollen counts using a hemacytometer. Bees were captured while on inflorescences and killed rapidly with ethyl acetate. Pollen composition was analyzed by removing the pollen load from one leg per bee, mounting in aniline blue-lactophenol on a hemacytometer, and classifying the first 100 grains encountered on the grid by size. In addition, the possibility that pollen load composition varied through the day was explored by analyzing loads of bumblebees and honeybees captured during each of three time intervals: 0900–1100, 1100–1300, and 1300–1500 h. The composition of corbicular pollen loads was later analyzed by goodness-of-fit tests to determine if the frequency of pollen types deviated from their frequency in the population.

Results

Nectar production in flowers of *P. cordata* begins as flowers open (~0900 h) and continues throughout the 6–8 h anthe-

Table 1. The production and concentration of nectar secreted by flowers of the three morphs of *Pontederia cordata*. Values are mean ± S.D. of 20 flowers per morph. There was no significant differences among the morphs in either volume or concentration (ANOVA)

	Floral morph			
	L	*M*	*S*	*F*
Volume (μl)	0.035 ± 0.015	0.042 ± 0.023	0.038 ± 0.018	0.559
Concentration (% sucrose)	55.6 ± 20.4	52.3 ± 14.9	56.6 ± 13.2	0.260

Table 2. Arrival position and intra-inflorescence foraging behavior of common pollinators of *Pontederia cordata*. Values are mean ± S.D.

Pollinator	N	No. of flowers visited per inflorescence	Estimate of time spent per flower (seconds)	Percent of moves upwards	Percent of visits starting on bottom third of inflorescence
Bombus spp.	303	8.2 ± 5.9	1.0 ± 0.2	89 ± 16	88
Apis mellifera	41	5.1 ± 3.2	2.2 ± 0.4	61 ± 21	63
Melissodes apicata	46	8.2 ± 2.1	2.8 ± 2.6	84 ± 17	84

Table 3. Foraging behavior with respect to floral morph by bumblebees and honeybees on an experimental array of nine inflorescences (three per morph). Values are percent of visits that arrived and departed on a particular morph and percent of all inflorescence and flower visits received by each morph. χ^2 tests deviation from equality

Bee species	N	Foraging parameter	Floral morph			χ^2
			L	*M*	*S*	
Apis mellifera	63	Arrival morph	29	30	41	1.83
	63	Depature morph	49	27	24	7.32*
	397	Inflorescence visits	30	37	33	2.99
	1268	Flower visits	9	47	44	330.62***
Bombus spp.	27	Arrival morph	33	30	37	0.23
	27	Departure morph	37	26	37	0.68
	97	Inflorescence visits	41	27	32	3.15
	591	Flower visits	43	27	30	25.62***

$P = * < 0.05; *** < 0.001$

sis period. Owing the high pollinator densities, the standing crop of nectar is usually extremely low (< 0.05 μl). Neither the amount of nectar produced, nor the concentration of nectar varied significantly among the morphs (Table 1).

Several patterns were evident in the behavior of pollinators visiting *P. cordata*. Most species, especially bumblebees and *Melissodes* began feeding on the bottom third of an inflorescence (Table 2). Few insects arrived at the top of an inflorescence. Pollinators usually moved up an inflorescence and probed less than 10 flowers per inflorescence (Table 2). Pollinators tended to visit more flowers on large inflorescences ($r = 0.344$, df = 294, $P < 0.001$). On average, approximately 20% of open flowers on an inflorescence were probed during a visit. The time spent per flower differed almost three-fold with bumblebees being much faster than honeybees or *Melissodes* (Table 2).

Most pollinator species entered the flowers to collect nectar. Although bumblebees and *Melissodes* were often seen grooming pollen placed on their bodies during nectar feeding, they were never observed collecting only pollen. In contrast, honeybees usually collected only pollen from *P. cordata* flowers. Furthermore, these bees rarely entered

flowers and most often foraged on pollen from the exserted long-level stamens, and on occasion, from other anther levels. Based on this observation it was predicted that honeybees would visit *L* inflorescences and flowers of the *L* morph less frequently than those of other morphs. Indeed, results from the nine-inflorescence array indicated that honeybees started a visit on the array without regard to floral morph; however they visited significantly fewer flowers of the *L* morph than flowers of the *S* and *M* morphs (Table 3). Discrimination against the *L* morph occurred once honeybees had landed on an inflorescence since inflorescences of all three morphs were visited at equal frequency. Additionally, honeybees left the array more often after encountering the *L* morph. In contrast, bumblebees arrived, departed, and visited inflorescences with no significant regard to morph, although they visited significantly more flowers on *L* inflorescences (Table 3).

Most corbicular pollen loads collected from bees captured contained primarily *P. cordata* pollen. Only two bumblebees had heterospecific pollen and this alien pollen made up less than 5% of the total pollen load. This suggests that pollinators are very constant on *P. cordata*. The rela-
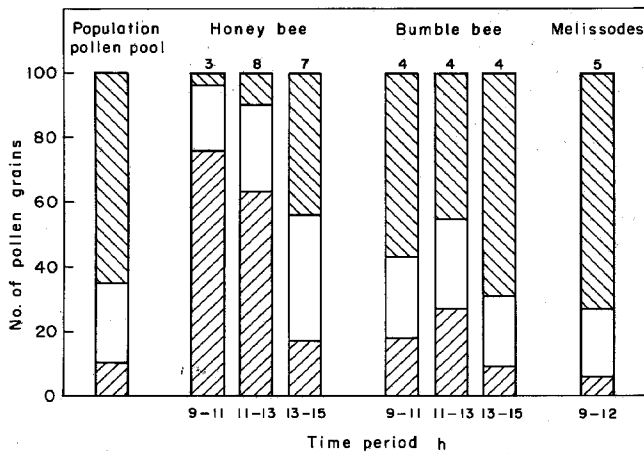
Fig. 1. Composition of corbicular pollen loads of three bee groups captured while foraging on flowers of *Pontederia cordata* at Pothole Lake. Pollen counts represent a subsample of one pollen basket per bee. Bars are mean number of grains of each pollen type. Sample size is given on top of each bar. Pollen type legend: bottom diagonal hatch = large-sized pollen; empty space = mid-sized pollen; top diagonal hatch = small sized pollen. Honeybees and bumblebees were captured at different times through the day and time is denoted under each bar. The population pollen pool represents the relative abundance of each pollen type in the population and was calculated from morph frequency and pollen production values. The composition of each corbicular load was compared to the population pollen pool using goodness-of-fit tests (χ^2). All loads were significantly different ($P<0.001$) from the population pollen pool except bumblebees captured betwen 1300 and 1500 h ($P>0.5$)

tive number of pollen grains from each size class in the corbicula varied among the three bee species (Fig. 1). In addition, most loads differed from the population pollen pool (long-level = 0.10; mid-level = 0.25; short-level = 0.65). *Melissodes* pollen loads contained more pollen from short-level anthers than expected based on its frequency in the population. Only bumblebees collected between 1300–1500 h had pollen loads that were not significantly different from the population pollen pool. Although the frequency of pollen from different anther levels varied through the day in bumblebee corbicular loads, no patterns were evident. In contrast, the composition of honeybee pollen loads displayed a daily pattern. Honeybees captured between 0900 and 1300 h had an excess of pollen from long-level anthers and a deficiency of pollen from short-level anthers relative to expected numbers. Later in the day, however, the number of pollen grains from long-level anthers decreased while the fraction of pollen from mid- and short-level anters increased. At no time in the day did honeybees have pollen loads in the same proportion as the population pool, suggesting preferential pollen collection.

Discussion

The most common visitors to *P. cordata* at Pothole Lake are bumblebees (Wolfe 1985). These bees selected inflorescences without any significant regard to floral morph. In fact, none of the many types of visitors that used this plant primarily as a nectar source seemed to exhibit any preference (unpublished work). This is not surprising since the morphs do not vary in the quality or quantity of nectar produced. Unlike other insects, honeybees probe flowers

of *P. cordata* mainly for pollen and they preferentially collected pollen from the long-level anthers of the *S* and *M* morphs. Further evidence of the difference in foraging behavior of honeybees and bumblebees is that 90% of randomly collected honeybees ($n=40$) had corbicular pollen loads compared with 62% of bumblebees ($n=118$; Wolfe 1985).

The finding that pollen baskets removed from *Melissodes* contained an excess of pollen from short level anthers is interesting in light of the fact that this bee apparently restricts its feeding to *Pontederia* species (Laberge 1956). These bees have numerous hooked hairs on their proboscides which Laberge considered to function in removing pollen from the narrow tubular flowers. Thus, while honeybees specialize on pollen from long-level anthers, *Melissodes* preferentially collects short-level anther pollen. Despite this specialization, *Melissodes* is probably not as important a pollinator to *P. cordata* at Pothok Lake since it is not as abundant as bumblebees throughout the entire flowering period of *P. cordata* (Wolfe 1985).

As expected, based on their foraging behavior, honeybees had an excess of pollen from long-level anthers in their pollen baskets while bumblebees did not preferentially collect any pollen type. We have shown elsewhere that pollen from the long-level anthers is removed at a much faster rate through the day than pollen from other anther levels (Barrett and Wolfe 1986). Rapid removal of this pollen is probably due to several factors including the preferential collection by honeybees, and that these anthers contact the large surface area of bumblebee abdomens which cary more pollen than other body parts (Barrett and Wolfe 1986). This rapid removal of large pollen probably explains the decrease in relative frequency of long-level anther pollen in honeybee pollen baskets through the day. As their primary choice of pollen is removed from the population pollen pool, honeybees are more likely to enter flowers more deeply to collect pollen from the mid- and short-level anthers. This suggests that honeybees adjust their behavior over short time periods in response to pollen availability.

At least two possibilities exist that may explain why honeybees exhibited a pollen preference. First, a preference for long-level anther pollen may result form the greater accessability of these anthers. Since the anthers are not concealed in the perianth tube as are the other two sets of anthers, less time and a lower energetic output would be required to collect pollen. In addition, preferential collection may be due to some feature of the large grains. For example, Ornduff (1975) found that honeybees preferentially collected pollen from the long-level anthers in *Lythrum junceum* and suggested that this may be related to its higher nutritional value due to the presence of starch (Dulberger 1970). Whether nutritional differences exist among the pollen types of *P. cordata* is unknown.

Does the non-random foraging behavior of honeybees have any consequences for reproductive success in *P. cordata*? In the latter part of the 1982 and 1983 flowering seasons honeybees were very common visitors and this period coincided with a decrease in the level of percent seed set in all morphs, but most dramatically in the *L* morph (Wolfe 1985). Therefore, the avoidance of the *L* morph, coupled with preferential collection of pollen from the long-level anthers in the *M* and *S* morphs (which effectively removes this pollen from the pool available for deposition on stigmas of the *L* morph), may have had a depressing

effect on seed production. However, an alternative explanation for this occurrence may be morph-specific physiological effects due to the cool temperatures at the end of August in both years (Wolfe 1985).

Seed production in the self-incompatible, tristylous *P. cordata* is dependent on bees transporting pollen between morphs. While it seems likely that for tristyly to evolve, a specialized relationship with a specific group of pollinators would be required, in *P. cordata* the polymorphism appears to be evolutionarily stable despite the diversity in size, shape and foraging patterns of its pollinators. It has been suggested that a loss or decrease in service of co-adapted pollinators in a tristylous species may result in the disruption of compatible pollination leading to a breakdown of the floral polymorphism (Ornduff 1971; Charlesworth 1979). In addition, spatial segregation of floral morphs due to vegetative growth (Price and Barrett 1982), and near-neighbor foraging behavior of bees (Wolfe 1985) would tend to favor the deposition of incompatible pollen on stigmas. However, at Pothole Lake and other Ontario populations levels of seed set are very high suggesting that levels of compatible pollination in natural populations are sufficient to maintain tristyly (Price and Barrett 1982; Barrett and Wolfe 1986). This effective pollination is due to: 1) high pollinator densities through the entire flowering period, 2) the uniovulate flowers requiring very few compatible pollen grains (3–5) to ensure seed set with regularity, and 3) that most visits by bumblebees deposit sufficient pollen to set seed (Barrett and Wolfe 1986). Also, the common visitors usually bear all three pollen types on their body so that most insects, regardless of body size probably serve as effective pollinators (Barrett and Wolfe 1986). These observations indicate that despite the possession of a complex pollination mechanism, there is no evidence that populations of *P. cordata* in N. America are serviced by a specialized pollinator fauna co-adapted to the tristylous floral polymorphism.

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