

# Pollen Heteromorphism as a Tool in Studies of the Pollination Process in Pontederia cordata L.

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## 1 Introduction

Heterostylous breeding systems provide useful experimental material for studies of mating system evolution and the influence of natural selection on floral form. Recent work has focused on the functional significance of the syndrome of traits that comprise the heterostylous syndrome (Ganders 1979). The conspicuous size differences among pollen types in heterostylous species enables a distinction to be made between compatible and incompatible pollen present on stigmas. Species studies of stigmatic pollen loads have been undertaken to evaluate whether the stamen-style polymorphism promotes phenotypic disassortative pollination among the floral morphs as Darwin (1877) originally proposed. Studies of deposition patterns have largely ignored other components of the pollination process and as a result little quantitative information is available on the pollination biology of most heterostylous species.

As part of a comprehensive study of tristylous breeding systems in the monocotyledonous family Pontederiaceae, we have examined in detail the pollination biology of several Pontederia species. In this chapter we review some of this work and then describe several field experiments concerned with the dynamics of pollen transport in P. cordata. We organise our discussion by addressing three questions: 1) How effective are insect visitors at removing pollen from the

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three stamen levels of tristylous flowers? 2) Does the complementary placement of sexual organs in tristylous flowers result in significant pollen partitioning on the bodies of pollinators? 3) How much compatible and incompatible pollen is deposited on individual stigmas by pollinators?

## 2 Floral Biology of *Pontederia*

*Pontederia* is composed of five species of long-lived, clonal, emergent aquatics native to the New World. Details of the tristylous breeding systems and reproductive biology of populations can be found in Barrett (1977), Price and Barrett (1982), and Glover and Barrett (1983). Most work has been conducted on N. American populations of *P. cordata*. Figure 1 illustrates salient features of the tristylous syndrome.

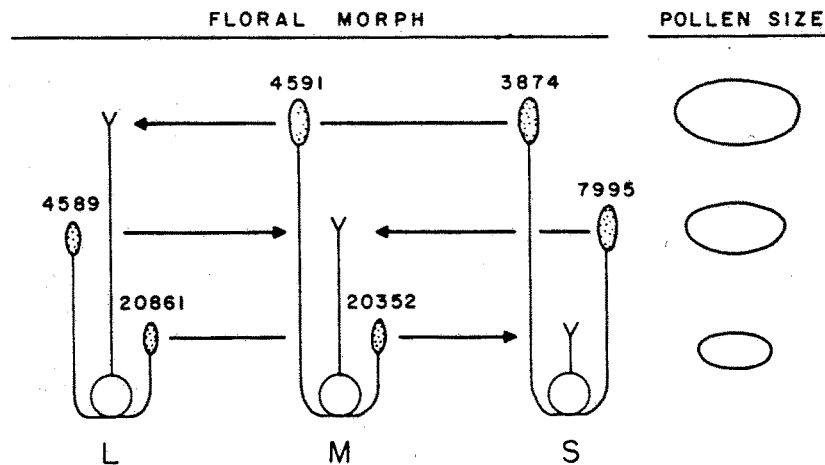


Fig. 1 Schematic diagram of the relationship between pollen size, pollen production, and stamen-style polymorphism in *Pontederia cordata*. After Price and Barrett (1984).

Three points are worth noting: 1) There is virtually no overlap in the size of pollen produced by the three stamen levels. 2) Large pollen production differences occur among anthers and morphs with approximately 2/3 of all pollen produced in populations originating from short-level anthers

and mid-level anthers of the S morph producing twice as much pollen as the corresponding anthers of the L morph (Price and Barrett 1982; Barrett et al. 1983). 3) There is variation in the expression of trimorphic incompatibility among morphs with the M morph considerably more self-compatible than the L and S morphs (Barrett and Anderson, 1985). These three peculiarities seem to be general features of the tristylous syndrome of Pontederia species.

Pontederia flowers are pollinated by a range of bees especially long-tongued solitary bees in tropical regions (Ancyloscelis and Florilegus spp.) and Bombus spp. and Melissodes apicata in North America. The blue, mauve, or white, uniovulate flowers are produced in showy spicate inflorescences, and anthesis usually lasts for 6-8 hours. A study of the dynamics of pollination in Ontario populations of P. cordata (Wolfe 1985) revealed that the daily events associated with pollination occur very rapidly. At peak flowering virtually all flowers in populations are pollinated between 2-3 hours from the beginning of anthesis and pollen tubes can be detected at the base of the style soon after. Experimental study of the number of compatible pollen grains required to ensure seed set with regularity indicate that 3-5 per flower are sufficient. The low pollen requirement and high bumblebee densities that service populations in many N. American populations result in near maximal seed set. Attempts to elevate seed set above open-pollinated controls by hand cross-pollinations have been unsuccessful, suggesting that ovules are rarely pollen limited at least where bumblebees are the primary pollinators.

### 3 Pollen Removal

Notwithstanding the growing awareness of the importance of male function to plant fitness, few studies have quantified pollen removal from stamens. Such considerations may be particularly significant in P. cordata since early bee visits to flowers are likely to be important in increasing male mating success. We examined how stamen position influences the quantity of pollen removed from previously unvisited flowers following a single bumblebee (B. griseocollis) visit. Pollen

grain counts to visited and unvisited flowers were made using a hemacytometer. The results (Table 1) indicate that 1/3-1/2 of the total number of pollen grains produced by flowers are removed by the first bee visit. Relative to the total number of pollen grains in unvisited flowers, significantly different fractions of pollen are removed from the three stamen levels (l 68.5%, m 50.0%, s 37.5%). Measurements of the rate of pollen removal during the anthesis period of flowers indicate that within 60 minutes of anther dehiscence more than 1/2 of all pollen within a population is removed from stamens by pollinators.

**Table 1** Pollen removal and pollen deposition in previously unvisited flowers of *Pontederia cordata* following a single visit by *Bombus*. Removal data based on 20 flowers per morph, values are the mean percentage of pollen grains removed from each stamen level. Deposition data from 60, 85, and 58 stigmas of the L, M, S morph, respectively (L.M. Wolfe and S.C.H. Barrett unpublished data).

	Floral Morphs		
	<u>L</u>	<u>M</u>	<u>S</u>
A) <u>Pollen removal</u>			
<u>l</u> anthers	-	64	73
<u>m</u> anthers	61	-	39
<u>s</u> anthers	32	43	-
Total	36	45	52
B) <u>Pollen deposition</u>			
% visits with no deposition	26	21	39
Mean number of pollen grains deposited/stigma	38	68	18
% compatible pollen grains	59	22	25

#### 4 Pollen Partitioning on Bees

The three stamen levels in *Pontederia* flowers may be expected to contact different parts of a bee's body during nectar feeding. The idea of pollen partitioning is in fact

central to Darwin's hypothesis on the function of the stamen-style polymorphism. A critical issue in evaluating the hypothesis is whether significant partitioning is maintained during foraging activity.

To verify that pollen is initially located on different parts of an insect's body simple observations on the distribution of pollen on dead bees inserted into flowers can be undertaken. When this was done using bumblebees and P. cordata flowers a high degree of segregation of the pollen types was observed with most l, m, and s pollen on the abdomen, head and proboscis, respectively. Under field conditions, grooming activities as well as variable body orientations during entry and exit from flowers are likely to disturb these patterns. The amount of mixing of pollen types was investigated in three taxa of bees that commonly visit flowers of P. cordata (Bombus spp., Apis mellifera, Melissodes apicata). Pollen counts were made by sampling portions of the body using a uniform size cube of fuchsinglycerine jelly mounted on a pin. The cube was pressed onto different parts of a bee's body and then melted on a microscope slide and the pollen types counted. Fifteen free-foraging individuals of each taxon were sampled in this manner; Figure 2 illustrates the results. As can be seen the

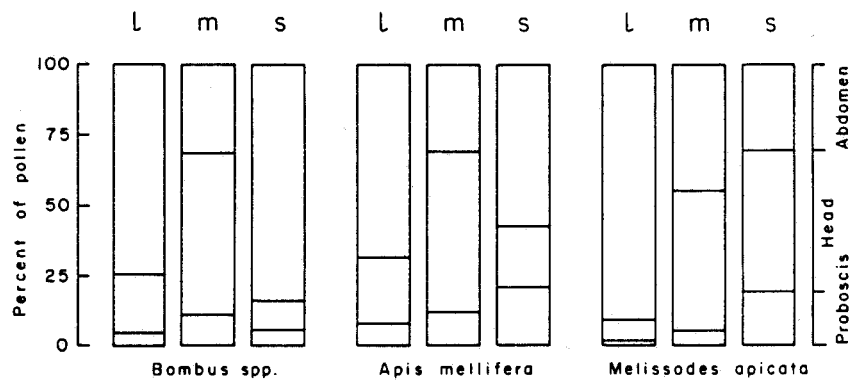


Fig. 2 Percentage of Pontederia cordata pollen types on body parts of three pollinator groups. N = 15 bees per taxon. (L.M. Wolfe and S.C.H. Barrett, unpublished data.)

pollen types are not deposited similarly on each body part. Pollen from long-level anthers (l) was most abundant on the abdomen, and m pollen on the head; s pollen differed in abundance with bee group. In bumblebees and honeybees s pollen was most common on the abdomen, whereas in Melissodes apicata it was most frequent on the head. The data indicate that l and m pollen tends to remain in greatest concentration on the body parts where it is initially deposited, whereas s pollen tends to be displaced backwards from the proboscis to more posterior parts of the bee. The total number of pollen grains deposited on the proboscis was much lower than on the other regions sampled. This is probably due to the smaller surface area of the proboscis, for adherence of pollen, as well as the tendency for bees to remove pollen from it during cleaning activities.

Despite differences in the size, morphology and behavior of the three bee taxa some degree of pollen partitioning is maintained during foraging. This suggests that close co-adaptation between Pontederia and its pollinators is not a prerequisite for pollen stratification and compatible pollen transfer to occur.

##### 5 Pollen Deposition

Surveys of stigmatic pollen loads in natural populations of P. cordata indicate that despite considerable amounts of incompatible pollen on stigmas, sufficient compatible pollen is deposited by pollinating insects to ensure seed set (Price and Barrett 1984; Barrett and Glover 1985). The question arises as to how effective the first bee visit to a flower is in bringing about pollination. In a uniovulate flower early visits are of some importance and may result in most of the successful fertilizations. Results from a study of the composition of stigmatic pollen loads of previously unvisited flowers following a single bumblebee visit are presented in Table 1. While virtually all visits to flowers are successful at removing pollen a significant number result in no pollen deposition. The reduced frequency of pollinating visits in the S morph may be associated with the lower pollen load carried by the proboscis and the

smaller contact areas involved in pollination. Analyses of the stigmatic pollen loads indicate that the M and S morphs receive mostly incompatible pollen from single visits whereas in the L morph compatible pollen comprises the largest fraction of the pollen load. Data from multiple-visited flowers indicate that the L morph is most likely to experience legitimate pollination whereas the M and S morphs frequently display random pollination (Barrett and Glover 1985).

## 6 Conclusion

The marked size differences among pollen types in P. cordata enables examination of fine scale micropollination events not normally investigated in homomorphic species. In addition to being able to determine the number of compatible and incompatible pollen grains deposited on stigmas in natural populations, field experiments can be designed to examine pollen carryover, the magnitude of self- and geitonogamous-pollination, as well the effects of pollen clogging on the seed set of floral morphs (Wolfe 1985; Barrett and Glover 1985). Together, these studies indicate that the pollination process in P. cordata is characterized by large asymmetries in the amount of pollen that is produced, transported and deposited on stigmas of the floral morphs. Despite this variation the seed fecundity of morphs is usually similar because of reliable pollinator service and low pollen requirements for seed set. Although differences in the fecundity of morphs are not generally evident, morph-specific male fertility differences appear to be important in regulating the relative frequency of floral morphs in natural populations (Barrett et al. 1983).

Since P. cordata is an outcrosser with high fecundity, possesses uniovulate flowers, and experiences little seed abortion, we may anticipate strong gametophytic selection if genetic variation for pollen tube growth occurs within populations. To what extent style length differences among the morphs influence the intensity of pollen tube competition remains to be determined. Detection of male fertility variation and selective effects at the gametophytic level

requires controlled crosses with genetic markers and extensive progeny testing of the floral morphs in natural populations.

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