The function and adaptive significance of tristyly in *Pontederia cordata* L. (Pontederiaceae)

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Darwin proposed that the adaptive significance of tristyly is to promote insect-mediated pollination among floral morphs with anthers and stigmas at equivalent levels (legitimate pollination). Strong pollen trimorphism in *Pontederia cordata* enables an evaluation of this hypothesis. In an investigation of pollen flow patterns in different parts of the North American range of the species, legitimate pollination of some morphs was observed in all but one population investigated. However at only one site (Ft. McCoy, Florida) was Darwin's hypothesis confirmed for the three floral morphs. The long-styled morph most frequently exhibited legitimate pollination, the mid-styled morph was intermediate, and the short-styled morph rarely experienced legitimate pollination.

Temporal variation in pollen flow was observed during the flowering season of *P. cordata*. Total stigmatic pollen loads decreased as the density of inflorescences and activity of pollinators declined. In spite of variable total loads the legitimate component was relatively constant in the long- and mid-styled forms, while in the short-styled form this measure increased as the season progressed. Little variation was detected between stigmatic samples collected at different times during a single day.

Legitimate pollination may be inhibited by local foraging of pollinators and the spatial segregation of floral morphs. At Paugh Lake (Ontario) over 75% of all bumble bee flights are among the five nearest neighbours of a particular inflorescence. In the same population, there is a probability of over 70% that the nearest three neighbours of an inflorescence are of the same floral morph. Despite these influences, legitimate pollination occurs in *P. cordata*, suggesting substantial pollen carryover.

KEY WORDS:—Pollination – *Pontederia cordata* – Pontederiaceae – tristyly.

CONTENTS

INTRODUCTION

Since Darwin's investigations of heterostyly over a century ago (summarized in Darwin, 1877), there has been considerable interest in the population biology of heterostylous plants. Species exhibiting this genetic polymorphism are composed of two (distyly) or three (tristyly) floral morphs which differ principally in the length of stamens and styles, pollen size, and incompatibility relationships. The taxonomic distribution and morphological characteristics of heterostylous species are well documented (Vuilleumier, 1967; Ganders, 1979), and there has been some theoretical consideration of the evolution of distyly (Charlesworth & Charlesworth, 1979; Muenchow, 1982) and tristyly (Charlesworth, 1979).

Darwin (1877) first proposed an hypothesis of the adaptive significance of heterostyly. He suggested that the reciprocal positioning of reproductive organs in the floral morphs was a mechanical device to promote insect-mediated pollination among anthers and stigmas at equivalent levels (legitimate pollination). This is phenotypic disassortative pollination between flowers of opposite forms (Ganders, 1974). The hypothesis has been examined in several species by studying the composition of pollen loads on naturally pollinated stigmas. The majority of these studies of pollen flow either do not support the Darwinian hypothesis or are inconclusive (see Ganders, 1979 for a review).

The purpose of this paper is to present the results of pollen flow studies of tristylous *Pontederia cordata* L. (Pontederiaceae). The paper documents the patterns of legitimate pollination in natural populations in different parts of the North American range of the species, examines factors which influence the pollination process, and enables an evaluation of Darwin's hypothesis of the functional significance of floral trimorphism.

TRISTYLY IN PONTEDERIA CORDATA

Pontederia cordata L., the Pickerelweed, is a perennial, emergent aquatic of eastern North America, Central America, Brazil and Argentina (Lowden, 1973). Populations usually contain the three floral morphs (Price & Barrett, 1982; Barrett, Price & Shore, 1983) and occur in marshlands, along drainage ditches, and at the periphery of lakes, rivers and streams. In North American populations the commencement and duration of the flowering period vary with latitude; populations in southern Florida commence flowering in January whereas those at the northern limit of the range in Ontario begin flowering in mid-June. The flowering season lasts from two (Ontario) to nine (Florida) months during which time the showy, blue entomophilous flowers attract a wide range of insect visitors (Hazen, 1918). A particular focus of our study was to investigate the function of tristyly under various pollinating regimes and during different periods of the day and of the flowering season. Previous pollen flow studies have usually involved samples collected from a single population during one day in the blooming period.

In *Pontederia*, controlled pollinations between anthers and stigmas of equivalent level (legitimate pollinations) result in significantly higher seed set in comparison with all other types of pollination (Ornduff, 1966; Barrett, 1977a, and unpubl. data). This is due to the presence of a sporophytic self-incompatibility system which regulates the mating pattern of populations.
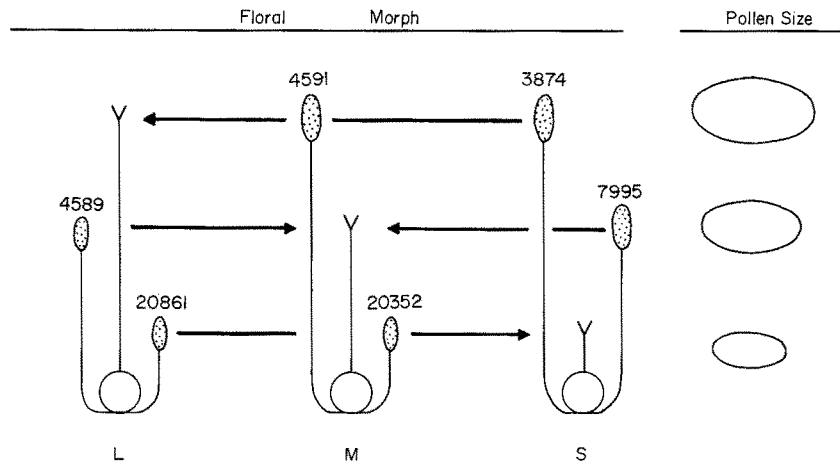
Figure 1. Generalized diagram of the relationship between pollen size, pollen production, and stamen-style polymorphism in *Pontederia cordata*. Legitimate pollinations are indicated by the arrows. Two size classes of anthers occur in *P. cordata*. Pollen production values for each anther level are average values for *P. cordata* (*n* = 20 populations). For details see Price & Barrett (1982).

Associated with trimorphic incompatibility in *P. cordata* is a striking pollen trimorphism with little overlap in the size distributions of pollen grains produced by the three anther levels (Figs 1, 2). Although pollen trimorphism is a feature of most tristylous species, considerable overlap between the pollen sizes of different anther levels occurs (Mulcahy & Caporello, 1970; Ornduff, 1972, 1975a; Barrett, 1977b, 1979). As a consequence, pollen grains cannot be identified unambiguously, and therefore examination of the levels of legitimate pollination in the three floral morphs of tristylous species has not been possible.
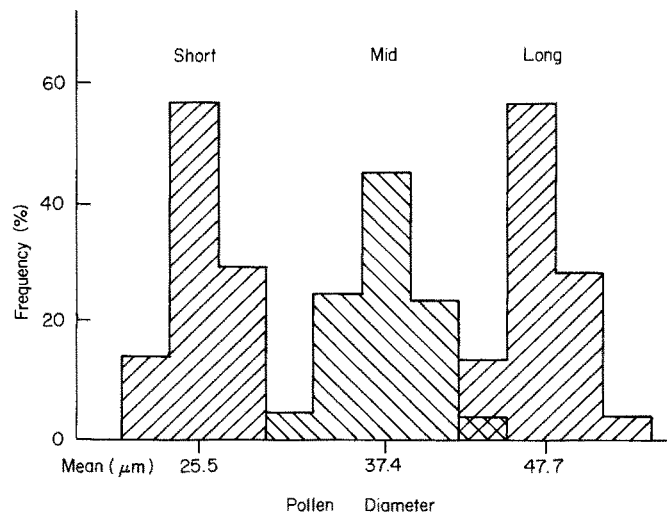

Figure 2. Pollen trimorphism in *Pontederia cordata*, following acetolysis (*n* = 100 pollen grains per anther level). Pollen size data from untreated, dry pollen grains are presented in Price & Barrett (1982).

MATERIALS AND METHODS

Analysis of pollen flow

According to Darwin's pollen transfer hypothesis, legitimate pollen should be preferentially transferred to stigmas. However, if the frequency of legitimate pollen on stigmas is equivalent to its production in the population, then it would appear that tristyly has not resulted in disassortative pollination, i.e. pollination would be random with respect to style length (Ornduff, 1970; but see Discussion and Ganders, 1979). To construct a random pollination model, the floral morph frequencies were determined for populations of *P. cordata* by counting the number of inflorescences of each morph within a population. An earlier study indicated that there were no significant differences in inflorescence production, flower number or flowering phenology among the floral morphs (Price & Barrett, 1981). A sample of buds was collected from each morph within the populations and preserved in 75% ethanol for measurements of pollen size and pollen production. Finally, to determine stigmatic pollen loads, stigmas were collected in late afternoon with fine forceps and preserved separately in 75% ethanol for each floral morph. Examination of the preservative following the removal of stigmas indicated that few pollen grains had been dislodged from stigmas. Sample sizes and sampling times are presented below. Inflorescences and flowers from which stigmas were collected were selected at random.

Bulk samples of stigmas of each floral morph were subjected to a modified acetolysis (Mulcahy & Caporello, 1970; Ganders, 1975). The acetolysis procedure digests all stigmatic tissue, but leaves the pollen grain wall intact. From the preserved buds, anthers were acetolysed to determine pollen size distributions for the three anther levels. In order to count and measure pollen grains in these standards and from acetolysed stigmas, samples of the acetolysate were displayed on a hemacytometer and measured with a compound microscope and ocular micrometer (Lloyd, 1965). Observed stigmatic pollen loads were tested against the predictions of the random pollination model by a G-test for goodness-of-fit (Sokal & Rohlf, 1969).

Variation in pollen flow

Patterns of geographical variation in pollen flow were examined by collecting a single sample of stigmas at peak flowering during 1978 or 1979 in each of four populations from different parts of the North American range of *P. cordata* (Table 1). Between one and ten stigmas were collected from 10 to 60 individuals for a total of 40 to 120 stigmas per morph. Seasonal variation in pollen flow patterns was investigated by collecting stigmas on each of five days (25 July; 4, 11, 21, 28 August) during the flowering season from a population at Paugh Lake NW (Ontario) in 1979. Variation during a single day was studied by examining collections made at four times on two days (3 August: 09.00, 11.00, 13.00, 15.00 hours EDST and 25 August: 12.00, 13.30, 15.00, 16.30 hours EDST) at the Paugh Lake NW population. The anthesis period of individual flowers begins in the morning and lasts for eight to twelve consecutive hours. Samples of 120 stigmas per morph were divided into three replicates of 40 stigmas, except on the fifth sampling date of the seasonal study. Only 50 stigmas

Table 1. Locality, habitat information, and insect visitors to *Pontederia cordata* populations examined for pollen flow.

Locality	Sampling dates	Habitat	Style morph frequency			Major insect visitors
			Long	Mid	Short	
Paugh Lake, Ontario (4°35'N, 77°45'W)						
a) SW	6 August, 1978	Lakeshore	0.37	0.25 (n=1094)	0.38	*Bombus vagans* *B. tenarius* *B. terricola*
b) NW	July–August, 1979	Lakeshore	0.30	0.27 (n = 274)	0.42	*B. borealis* *B. griseocollis*
Elizabeth City, North Carolina (36°16'N, 76°29'W)	30 June, 1978	Roadside ditch	0.21	0.37 (n = 530)	0.42	*Bombus pennsylvanicus* Anthophorids & Halictids
Osborn, S. Carolina (32°44'N, 80°22'W)	22 May, 1979	Marsh	0.23	0.31 (n = 297)	0.46	*Dialictus* sp. *Xylocopa virginica* *X. micans* *Bombus griseocollis* Anthophorids
Ft. McCoy, Florida (29°22'N, 81°53'W)	27 May, 1979	Pond	0.43	0.30 (n = 232)	0.27	*Melissodes* spp. *Bombus* spp.

per morph were collected at this time due to the decline of flowering in the population.

Pollinator behaviour

Since the functioning of tristyly is mediated by pollinator behaviour, some observations of foraging patterns were made. The most important pollinators in Ontario populations are the bumble bees (*Bombus borealis* Kirby, *B. griseocollis* [Degeer], *B. ternarious* Say, *B. terricola* Kirby, *B. vagans* F. Smith). At Paugh Lake NW, *B. vagans* was the predominant visitor throughout the 1979 blooming period. In one section of this population the flight patterns of bumble bees were analysed by recording the floral morph on consecutive inflorescences encountered during 125 individual flights. The data provide information on the extent of near-neighbour foraging, which in conjunction with data on the spatial segregation of floral morphs within the population enable an evaluation of factors influencing pollen transport. In the same section, the floral morph of the three nearest inflorescences was determined for all inflorescences.

RESULTS

All populations of *Pontederia cordata* examined in the study contained the three floral morphs and exhibited strong pollen trimorphism. Acetolysis did not alter the relative sizes of pollen grains and therefore the anther level from which pollen grains originated could be determined unequivocally. The contribution of the three pollen types to the pollen pool was markedly different in all populations

sampled. Pollen grains originating from short-level anthers were produced in far greater numbers at each of the four localities. Pollen grains from the long-level anthers were produced in the lowest numbers (Table 2). This striking difference in pollen production among the anther levels of *P. cordata* is associated

Table 2. Patterns of pollen deposition on stigmas of the floral morphs in four populations of *Pontederia cordata*

		Pollen			Total	G
		Long	Mid	Short		
Ft. McCoy, (Florida)						
Pollen production*		11.2	19.0	69.8	100.0	
Floral Morph	Grains per Stigma					
Long:	Observed { %	32.8	25.0	42.2	100.0	
	No	21	16	27	64	26.53†
	Expected No	7.2	12.2	44.6	64.0	
Mid:	Observed { %	11.0	31.5	57.5	100.0	
	No	16	46	84	146	13.42†
	Expected No	16.4	27.7	101.9	146.0	
Short:	Observed { %	4.9	16.7	78.4	100.0	
	No	7	24	113	144	8.47†
	Expected No	16.1	27.4	100.5	144.0	
Elizabeth City, (North Carolina)						
Pollen production*		16.2	19.6	64.2	100.0	
Floral Morph	Grains per Stigma					
Long:	Observed { %	41.3	27.6	31.1	100.0	
	No	121	81	91	293	149.93†
	Expected No	47.5	57.4	188.1	293.0	
Mid:	Observed { %	19.7	46.7	33.6	100.0	
	No	45	107	77	229	103.63†
	Expected No	37.1	44.9	147.0	229.0	
Short:	Observed { %	12.6	30.3	57.1	100.0	
	No	56	135	255	446	29.73†
	Expected No	72.3	87.4	286.3	446.0	
Osborn, (South Carolina)						
Pollen production*		14.9	22.9	62.2	100.0	
Floral Morph	Grains per Stigma					
Long:	Observed { %	43.7	21.3	35.0	100.0	
	No	58	28	47	133	66.77†
	Expected No	19.8	30.5	82.7	133.0	
Mid:	Observed { %	7.3	19.0	73.7	100.0	
	No	29	75	292	396	29.69†
	Expected No	59.0	90.7	246.3	396.0	
Short:	Observed { %	17.9	20.2	61.9	100.0	
	No	15	17	52	84	0.73**
	Expected No	12.5	19.2	52.3	84.0	

Table 2. —*Continued*

		Pollen			Total	*G*
		Long	Mid	Short		
Paugh Lake SW, Ontario						
Pollen production*		12.0	21.5	66.5	100.0	
Floral Morph	Grains per Stigma					
Long:	Observed { %	10.8	17.4	71.8	100.0	
	No	31	50	207	288	4.06**
	Expected No	34.6	61.9	191.5	288.0	
Mid:	Observed { %	5.7	16.5	77.8	100.0	
	No	23	68	319	410	29.93†
	Expected No	49.2	88.2	272.6	410.0	
Short:	Observed { %	4.5	23.1	72.4	100.0	
	No	15	77	241	333	22.66†
	Expected No	40.0	71.6	221.4	333.0	

*   Relative frequency of pollen in population.
**Not significant.
†$P<0.05$.

with the pollen size trimorphism exhibited by the species (Fig. 1). Further details of this association are published elsewhere (Price & Barrett, 1982).

Geographical variation in pollen flow

The patterns of legitimate pollination in *P. cordata* vary both among populations and among the floral morphs within populations (Table 2). At Ft. McCoy (Florida), all three floral morphs were pollinated legitimately. In the short-styled morph, 78.4% of the pollen load was composed of pollen grains from short-level anthers (legitimate pollen). This value is 12.4% higher than expectations of the random pollination model. Stigmas of the mid- and long-styled morphs at Ft. McCoy received considerably more legitimate pollen grains (66.1% and 192.0% respectively) than expected from random pollination (Table 2).

At Elizabeth City (North Carolina), both the mid- and long-styled morphs were pollinated legitimately whereas stigmas of the short-styled morph received 10.9% less legitimate pollen grains than would be expected from random pollination. At Osborn (South Carolina) only stigmas of the long-styled morph were pollinated legitimately. Stigmas of the mid-styled morph received less legitimate pollen than expected from random pollination whereas the short-styled morph experienced random pollination. At Paugh Lake SW (Ontario), legitimate pollination was not detected in the three floral morphs. Pollen loads of the long-styled morph did not differ significantly from the random model. In the mid-styled morph the legitimate component was 22.9% less than expectations of random pollination whereas in the short-styled morph pollen loads lay between the expectations of random and legitimate pollination.

If pollen from the three anther levels is deposited on different parts of a

pollinator's body, as Darwin proposed, then a prediction may be made concerning the relative levels of illegitimate pollen found on the stigmas of the short- and long-styled morphs. Mid-size pollen should be found on stigmas of the short-styled morph at a higher level, relative to its production in the population, than pollen from long-level anthers. Similarly a greater proportion of mid-size pollen might be anticipated on stigmas of the long-styled morph in comparison with pollen from short-level anthers. These expectations arise because of differences in the relative distances separating the reproductive organs in the two floral morphs and cannot be applied to the mid-styled morph where both the long- and short-level anthers are equidistant from mid-level stigmas. Examination of the data on illegitimate pollen loads presented in Table 2 reveals that these predictions are confirmed in three of the four populations for stigmas of the long- and short-styled morph.

Temporal variation in pollen flow

At the Paugh Lake NW population of *Pontederia cordata*, the total pollen loads per stigma of the floral morphs varied significantly during the flowering season (Short: $F = 26.535$, Mid: $F = 76.670$, Long: $F = 16.018$; d.f. $= 3,8$; $P < 0.001$). A decrease in the amount of pollen on stigmas was associated with a decline in flowering and pollinator activity as the season progressed. Stigmas of the mid-styled morph received over twice the number of pollen grains compared to the other floral morphs, except on the last sampling date, when all three morphs received similar amounts of pollen (Fig. 3).
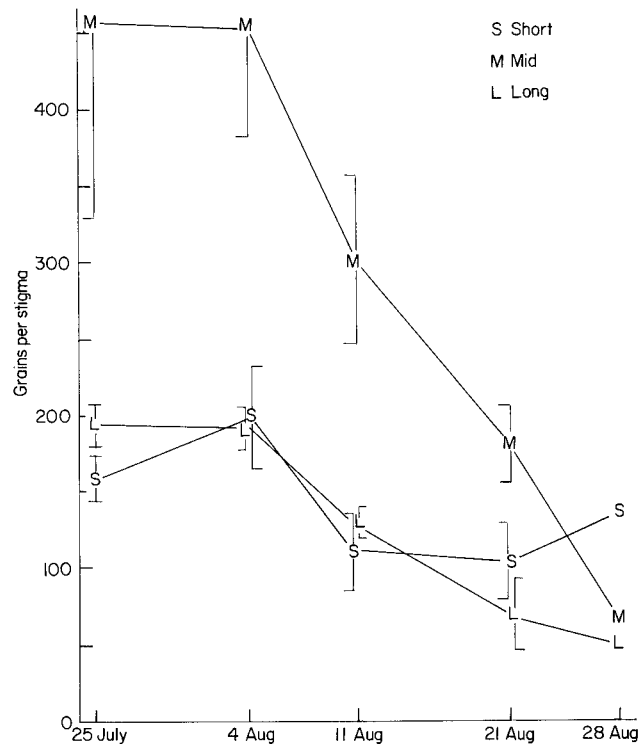

Figure 3. Total stigmatic pollen loads during the flowering season of *Pontederia cordata* at Paugh Lake NW, Ontario, 1979.

Table 3. Seasonal variation in legitimate pollen flow in *Pontederia cordata* at Paugh Lake NW (Ontario) in 1979*

Floral morph	Sampling dates				
	25 July	4 August	11 August	21 August	28 August
Long	45.9±3.2	38.7±7.1	27.9±6.4	26.3±9.6	34.7
Mid	29.3±3.1	23.5±1.7	21.5±3.1	25.3±1.5	20.6
Short	35.5±4.3	67.0±3.4	61.0±4.0	76.9±6.2	81.9

Summary of two-way analysis of variance

Source of variation	d.f.	MS	F	P
Among days	3.	11 131.7	4.254	<0.025
Among morphs	2	396 002.0	151.325	<0.001
Days × morphs	6	55 787.7	21.318	<0.001
Error	24	2 616.9		
Total	35			

*Percentage capture of legitimate grains by stigma (mean ±standard deviation).

Two-way analysis of variance of the proportion of legitimate pollen received by the three floral morphs indicates that significant variation occurs among sample dates during the season and among floral morphs (Table 3). Stigmas of the short-styled morph received increasing proportions of legitimate pollen during the season. Excess illegitimate pollen was evident on stigmas on the first sampling date, random loads were present in mid-season and excess legitimate pollen was received at the end of the season (Fig. 4). Throughout the flowering period of the mid-styled morph, pollen loads were similar to those based on random expectations (although legitimate pollination was observed on the first sampling date). Legitimate pollination of the long-styled morph was recorded on each of the five sampling dates.

The investigations of pollen loads during the anthesis period of individual flowers yielded variable results. On both sampling dates there was considerable variation in the total stigmatic pollen loads with a general trend of increased amounts of pollen being deposited as the day progressed (Fig. 5). In common with the findings of the seasonal study, the stigmas of mid-styled flowers received more total pollen grains than the other two floral morphs. Despite the variation in amounts of pollen captured by stigmas during different time intervals, the degree of legitimate pollination was remarkably constant throughout the day (Table 4). In addition the patterns observed for each morph were very similar to those recorded from the nearest sampling dates (4 and 28 August, Table 3) in the seasonal study reported above.

Pollinator behaviour

Illegitimate pollen flow may be promoted by non-random distribution of inflorescences of the three floral morphs and non-random foraging behaviour of pollinators. At Paugh Lake NW (Ontario), there is a high probability (approximately 75%) that neighbouring inflorescences will be of the same floral morph (Table 5). The likelihood that second- and third-nearest neighbours are
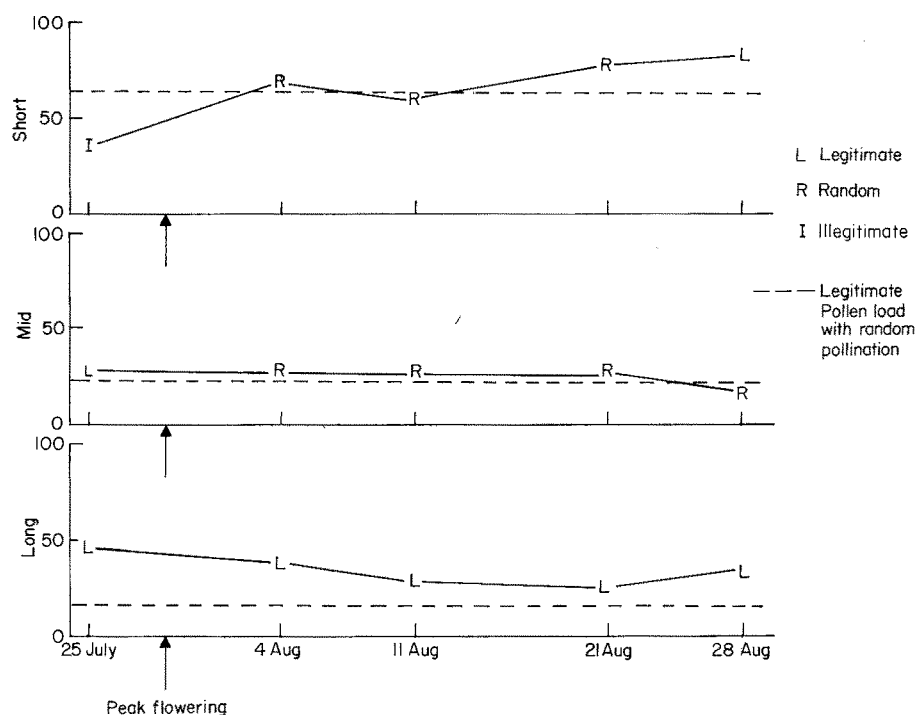
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Figure 4. Seasonal patterns of legitimate pollination in *Pontederia cordata* at Paugh Lake NW (Ontario) 1979.

of the same floral morph is almost as high (Table 5). Thus, inflorescences of the three floral morphs are spatially segregated. In other populations examined by us, similar and, in some cases, higher levels of spatial segregation have been observed. The major factor causing non-random distribution of inflorescences in *P. cordata* is the clonal habit of the species (Price & Barrett, 1982).

Table 4. Legitimate stigmatic pollen loads during the anthesis period of individual flowers of *Pontederia cordata* at Paugh Lake NW (Ontario) 1979*

Time (hours)	3 August			Time (hours)	26 August		
	Long	Mid	Short		Long	Mid	Short
09.00	43.2**	21.8†	48.7‡	12.00	25.4**	14.8‡	79.9**
11.00	46.8**	27.6†	51.5‡	13.30	22.9**	12.2‡	78.0**
13.00	44.0**	20.8†	50.8‡	15.00	28.6**	18.3‡	75.5**
15.00	45.1**	24.3†	47.6‡	16.30	23.5**	13.6‡	81.3**
Random (=100.0)	12.0	21.5	66.5	Random (=100.0)	12.0	21.5	66.5

*Percent legitimate capture.
**Legitimate pollination.
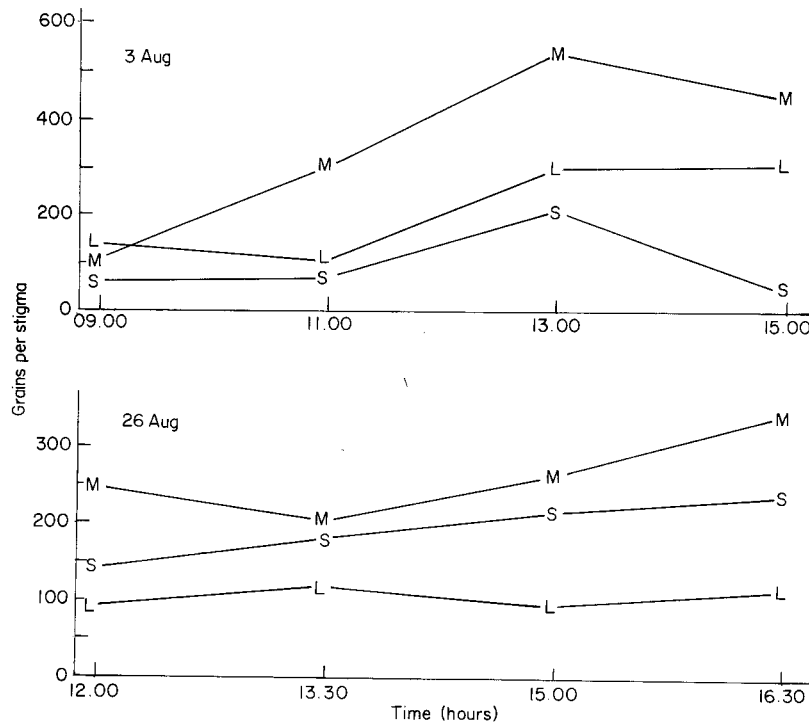†Random pollination.
‡Illegitimate pollination.

Figure 5. Total stigmatic pollen loads during the anthesis period of flowers of *Pontederia cordata* at Paugh Lake NW (Ontario) 1979.

Observations of bumble bee foraging behaviour indicate that one in three flights at Paugh Lake is between neighbouring inflorescences (Fig. 6). Flights to the nearest five neighbours account for over 75% of all flights. The relationship of consecutively-visited inflorescences departs significantly from a normal distribution and is skewed strongly towards nearest neighbours ($g_1 = +1.052$, $N = 120$, $P < < 0.001$).

Table 5. Nearest-neighbour analysis of inflorescences in *Pontederia cordata* at Paugh Lake NW (Ontario) on 22 August, 1979

Base	Short				Mid				Long			
Neighbour	S	M	L	n	S	M	L	n	S	M	L	n
1st	76.0	14.7	9.3	75	17.9	71.4	10.7	28	15.0	7.5	77.5	40
		$G = 20.629*$				$G = 35.122*$				$G = 42.354*$		
2nd	77.3	13.3	9.3	75	14.3	71.4	14.3	28	17.5	12.5	70.0	40
		$G = 22.064*$				$G = 35.884*$				$G = 31.485*$		
3rd	70.6	13.3	16.0	75	21.4	60.7	17.9	28	7.5	7.5	85.0	40
		$G = 10.565*$				$G = 23.168*$				$G = 58.102*$		

*Goodness-of-fit of observed morph frequencies to expected frequencies based upon the proportion of each morph in the population (random distribution). All tests: $P < < 0.001$ (excess of SS, MM and LL neighbours).
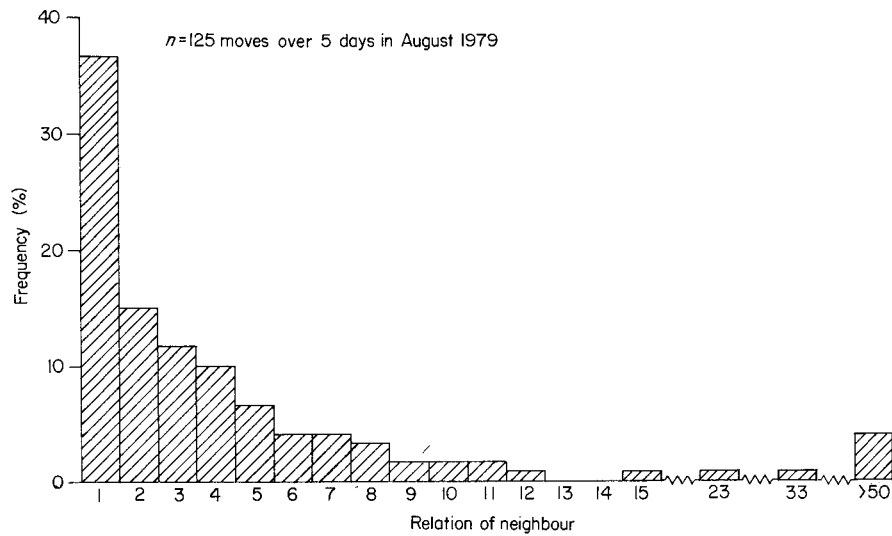
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Figure 6. Bumble Bee foraging on *Pontederia cordata*. Relation of consecutively-visited inflorescences at Paugh Lake NW (Ontario) 1979.

DISCUSSION

During the past decade, population biologists have investigated the efficacy of heterostyly in promoting legitimate pollination by using the pollen hetero-morphism which characterizes many heterostylous species. The majority of these studies have been undertaken on distylous plants and are reviewed by Ganders (1979). Examination of pollen flow patterns in tristylous species has been complicated by the overlap in size of pollen from different anther levels in the taxa examined. In nearly all studies of distylous plants, the stigmas of long-styled flowers received more total pollen than those of short-styled flowers. However, the short-styled morph was more likely to be pollinated legitimately. In most studies, large amounts of illegitimate pollen were observed on stigmas, particularly of the long-styled morph. Despite the consistent nature of much of the pollen flow data the factors influencing asymmetric patterns of pollination in heterostylous plants are poorly understood.

Legitimate pollination may not be as infrequent as most studies suggest. The reciprocal nature of the reproductive organs in the floral morphs of heterostylous species cannot influence the rate of intra-flower self-pollination to any greater extent than a monomorphic separation of anthers and stigmas (Ganders, 1974, 1979). Hence, the portion of the total stigmatic pollen load attributable to self-pollination may obscure the legitimate component of cross-pollination. The required measurement to fully test Darwin's hypothesis of legitimate pollination is the fraction of the outcrossed pollen load that is legitimate, and this can only be obtained by examining stigmas of emasculated flowers. However, emasculation of flowers of heterostylous species has proven difficult and may cause contamination of stigmas (Mulcahy & Caporello, 1970) or considerable modifications in the appearance of flowers (Ganders, 1974).

In *Pontederia cordata*, significant levels of legitimate pollination occurred in all three morphs in a population near Ft. McCoy, Florida. In all but one (Paugh

Lake SW, Ontario) of the other four populations examined, legitimate pollination of one or two morphs was observed. In our studies pollen loads were examined from intact rather than emasculated flowers and therefore stigmatic pollen loads designated as random almost certainly contained a significant selfing and geitonogamous component (see below). This obscures the illegitimate component of outcrossed pollen. Thus our estimates of legitimate pollination can be considered conservative since emasculation could only reveal *greater* levels of legitimate pollination.

The variation in pollen flow patterns observed among sites, and during the flowering period of *P. cordata*, cautions against generalization about the effectiveness of heterostyly at promoting legitimate pollination based on examination of single samples from one site. The full spectrum of behaviour from random pollination, through variation among morphs in the levels of legitimate pollination to legitimate pollination in all morphs, was recorded from samples collected in different parts of the North American range of the species. To what extent the patterns observed at each site on a single day are representative of the behaviour of the pollination system during the flowering season is not known. Results from the temporal study at Paugh Lake SW (Ontario) indicate significant variation between the sampling dates in legitimate pollination. In contrast, temporal variation in legitimate pollination during the period of anthesis of individual flowers was minimal.

It seems most probable that the variation in pollen flow patterns among the four localities is associated with differences in their pollinator regimes. In general, southern populations of *P. cordata* are visited by a more diverse pollinator fauna than those in the north where *Bombus* spp. are the most important pollinators. Bumble bees rarely visit tristylous Pontederiaceae in lowland tropical South America where tristyly in this family probably evolved (Barrett 1977a,b and unpublished). It seems unlikely, given their broad preferences, that they are highly co-adapted to the floral syndrome of *P. cordata*. Thus, bumble bees may not visit flowers in a manner that can regularly mediate legitimate pollination. Southern populations are visited by a range of long-tongued solitary bees of which species of *Melissodes* (Anthophoridae) may be the most important (Barrett, unpublished data). Species of Anthophoridae are regular visitors to tristylous Pontederiaceae, including *Pontederia*, in South America (Barrett, 1977a,b, 1980).

Ornduff (1970, 1971) has suggested that a loss or decrease in the service of co-adapted pollinators may result in the disruption of legitimate pollination. Under some circumstances this may result in the breakdown of heterostyly and the establishment of self-pollinating homostyles (Barrett, 1978, 1979; Charlesworth, 1979). However, no such modifications in floral biology of *P. cordata* were observed in any of 74 North American populations surveyed for floral morph frequencies (Price & Barrett, 1982). This suggests that the levels of legitimate pollination in natural populations are sufficient to maintain the tristylous genetic polymorphism.

Although the levels of legitimate pollination were variable among the samples collected in this study, several clear patterns emerged. The frequency of legitimate pollination was highest in the long-styled morph, intermediate in the mid-styled morph, and lowest in the short-styled morph. The cause(s) of this pattern is unknown, but may be associated with differences in the surface area of

the pollinator's body which contact the reproductive organs of *P. cordata*. Observations of the foraging behaviour of bees visiting flowers indicated that long-level organs regularly contacted the venter; mid-level organs, the face and proboscis base; and short-level organs, the proboscis tip. Thus, there is a considerably greater surface area available on the bee's body for deposition of large- and mid-sized pollen grains. The infrequent observation of legitimate pollination of the short-styled morph may result from the small surface area of the tip of the pollinator's proboscis. In addition, the tip may become quickly contaminated with illegitimate pollen while passing long- and mid-level anthers. Detailed analysis of pollen deposition on the bodies of bees, and the effects of grooming and other types of behaviour may reveal whether differences in surface area are important in legitimate pollination.

The traditional approach in examinations of the effectiveness of heterostyly at promoting legitimate pollination is to compare the observed pollen loads on naturally pollinated stigmas with those predicted from random pollination. However, in some cases random pollination among the floral morphs may be an unrealistic model. In *P. cordata* several factors appear to favour illegitimate pollination. These include: (1) the cloning habit of the species, which results in spatial segregation of morphs; (2) the large number of open flowers present on a single inflorescence (see Price & Barrett, 1982) and; (3) the nearest-neighbour foraging patterns of pollinators. These factors strongly favour geitonogamous pollination. Furthermore as pollinators frequently forage within a patch of one floral morph, legitimate pollen would be expected to become progressively less frequent on the bee and therefore less likely to be transferred to a stigma. The presence of significant amounts of legitimate pollen on stigmas of *P. cordata* suggests that there has been substantial 'pollen carryover' (Thomson & Plowright, 1980; Waser & Price, 1982). Presumably, in *P. cordata,* pollen adheres to the bodies of bees and does not mix extensively, in spite of grooming. Controlled experiments with bees, which exploited the marked pollen heteromorphism of *P. cordata*, should enable measurement of the extent of pollen carryover.

Darwin (1877) suggested that legitimate pollination might reduce pollen wastage by lowering the amount of incompatible pollen deposited on stigmas and thus conserving biomass. However, Ganders (1974) has argued that legitimate pollination would also result in the higher fecundity of flowers, especially in situations where ovules are pollen limited. The applicability of the 'fecundity maximization hypothesis' to a species such as *P. cordata,* which has uniovular flowers, is unclear. However, legitimate pollination may be of selective advantage for at least two reasons. First, the number of compatible grains per stigma necessary to achieve seed set with regularity may be greater than the legitimate component of random pollen loads. Second, illegitimate pollen may fill the interstitial spaces of the stigmatic papillae or stylar tissue down which pollen tubes grow. Legitimate pollination could decrease the clogging of these spaces with incompatible pollen. In view of the amounts of illegitimate pollen present on stigmas of heterostylous plants, these factors may be significant. Investigation of the relationships between fecundity and the number of legitimate and illegitimate pollen grains deposited on stigmas would be of interest.

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