

Tristyly in *Pontederia cordata* (Pontederiaceae)

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Pontederia cordata L. (Pontederiaceae) is a perennial, entomophilous, emergent aquatic and possesses a tristylous breeding system. A survey of 74 North American populations indicates that populations usually contain the three floral morphs. A strong pollen trimorphism is associated with differences in stamen and style length among the morphs. Pollen size is proportional to stamen height, while pollen production is inversely proportional. There are two size classes of anthers in *P. cordata*. Long-level anthers of mid- and short-styled plants and mid-level anthers of the short-styled morph are significantly larger than short-level anthers of long- and mid-styled plants and the mid-level anthers of the long-styled morph. The larger mid-level anthers of the short-styled morph produce nearly twice as many pollen grains as the equivalent level in the long-styled morph. It is suggested that this difference may be associated with varying patterns of stamen insertion and development in the floral morphs.

Comparisons of various reproductive parameters among the floral morphs in natural populations suggest that there are no significant differences in flowering patterns, inflorescence and flower production, seed production, and germination levels. In addition, bumble bees show no apparent preferences among the floral morphs during foraging activity. Tristyly appears to be a relatively stable breeding system in *P. cordata* in comparison with tristylous species of the related *Eichhornia*.

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Pontederia cordata L. (Pontederiaceae) est une espèce aquatique émergente, vivace et entomophile, qui a un système tristylique de croisements. Un relevé de 74 populations nord-américaines montre que les populations contiennent habituellement les trois morphes floraux. Un trimorphisme prononcé du pollen est associé aux différences entre les morphes dans la hauteur des étamines et la longueur du style. La dimension du pollen est directement proportionnelle, mais la production de pollen est inversement proportionnelle à la hauteur des étamines. Les anthères sont de deux dimensions. Les anthères hautes des plantes médiostylées et brévistylées et les anthères moyennes des plantes brévistylées sont significativement plus grosses que les anthères basses des plantes longistylées et médiostylées et que les anthères moyennes des plantes longistylées. Les plus grosses anthères moyennes de la plante brévistylées produisent presque deux fois plus de pollen que les anthères de même niveau de la plante longistylée. Les auteurs suggèrent que cette différence est associée aux différents modes d'insertion et de développement des anthères chez les morphes floraux.

Divers paramètres liés à la reproduction ont été comparés chez les trois morphes floraux dans des populations naturelles: il n'y a pas de différences significatives entre les morphes dans les patrons de floraison, la production d'inflorescences et de fleurs, la production de graines et les niveaux de germination. De plus, les bourdons ne semblent pas montrer de préférences pour l'un ou l'autre des morphes. La tristylye semble être un système de croisements relativement stable chez *P. cordata*, par comparaison avec les espèces tristyliées du genre apparenté *Eichhornia*.

[Traduit par le journal]

Introduction

Pontederia cordata L., the Pickerelweed, is a blue-flowered, perennial, emergent aquatic of eastern North and South America (Schulz 1942; Castellanos 1958; Lowden 1973). Populations occur in marshlands, along drainage ditches, and at the periphery of lakes, rivers, and streams. Adventive populations are reported from many parts of the Old World where the species is used as a pond ornamental (Täckholm and Drar 1950; Clapham *et al.* 1962; Aston 1973).

Pontederia is one of two genera in the Pontederiaceae that possess the rare form of floral heteromorphism known as tristyly (Solms-Laubach 1883; Hazen 1918; Ornduff 1966; Barrett 1977a). Tristyly, and the more widespread distyly, are complex floral mechanisms which promote outcrossing in flowering plants (Darwin 1877). Populations of tristylous species usually contain

three floral forms or morphs which differ in style length, anther height, pollen size, and incompatibility relationships (Vuilleumier 1967; Charlesworth 1979; Ganders 1979).

Comparative studies of tristylous members of the Pontederiaceae suggest that in *Eichhornia* floral trimorphism has become modified in each of the three tristylous species (*Eichhornia azurea* (Swartz) Kunth, *E. crassipes* (Mart.) Solms, *E. paniculata* (Spreng.) Solms) to give rise to self-fertilizing, homostylous forms (Barrett 1977b, 1978, 1979; S. C. H. Barrett, unpublished). To evaluate the nature and adaptive significance of tristyly in *Pontederia cordata* an intensive study of natural populations throughout the North American range was undertaken (Price 1981). This paper presents the results of part of this study, describes the general features of the tristylous syndrome

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of *P. cordata*, and compares various reproductive parameters among the three floral morphs in natural populations.

Materials and methods

Description of the tristylous syndrome

A total of 74 North American populations of *Pontederia cordata* were surveyed to examine the morphological expression of tristily. Populations were from Ontario (28), Wisconsin (1), North Carolina (4), South Carolina (11), Georgia (15), Florida (11), and Louisiana (4). Floral measurements were made on 10 individuals per morph selected at random from five populations. Stamen and style lengths were measured from the base of the ovary to the top of the anther or stigma. A survey of anther length was conducted in six populations by measuring one anther from each of the two anther levels per flower in each morph. A more detailed examination of anther size at Paugh Lake NW (Ont.) involved measurements of the length and width of anthers of 10 individuals per morph.

From Balsam Lake (Ont.), pollen was collected separately from the two anther levels of an open flower from 10 individuals of each morph. Pollen size was determined by measuring the equatorial and polar axes of 100 grains per anther level. The amounts of pollen produced by each of the two anther whorls per morph were estimated from buds due to open the following day. The hemacytometer method of Lloyd (1965) was employed. Detailed, replicated measurements of 15 flowers per morph were made at Paugh Lake SW (Ont.) and a more extensive nonreplicated survey of 20 populations was also undertaken using 15 flowers per morph.

Comparison of reproductive parameters among floral morphs

To determine the duration and pattern of flowering of the floral morphs, 20 individuals of each morph were examined in a population at Paugh Lake NW (Ont.). To ensure that discrete genets (clones) were sampled, plants of the same morph were selected at least 5 m apart, with plants of different morphs intervening. On alternate days throughout the flowering season (July 18 – September 6, 1979), the number of open flowers per inflorescence was recorded for each inflorescence produced per plant. In addition, a random sample of 30 inflorescences per morph was collected to compare the total number of flowers produced per inflorescence. In populations at Altamaha (GA) and Osborn (SC) 50 and 100 inflorescences per morph, respectively, were collected at random and scored for the number of open flowers per inflorescence during a single day's bloom.

To determine whether pollinators visit the three morphs with equal frequency, bumble bees (*Bombus borealis* Kirby, *B. griseocollis* [Degeer], *B. ternarius* Say, *B. terricola* Kirby, *B. vagans* F. Smith) were observed in a trimorphic section of the Paugh Lake NW (Ont.) population. All inflorescences were tagged according to floral morph and a total of approximately 2000 visits to flowers were observed during clear and overcast conditions on 10 days from August 4 to 13, 1979 between 0900 and 1700 eastern daylight saving time. A record was made of the floral morph of each visit and the number of flowers visited per inflorescence. To account for the unequal representation of the three morphs in the subpopulation (45.2% short, 30.0% mid, 24.8% long; $N = 303$ inflorescences), the data are

presented for each morph as a percentage frequency distribution of the number of flowers visited per inflorescence.

Inflorescences in the flowering phenology study described above were enclosed in bridal-veil bags after flowering to enable collection of fruits. In October 1979 and 1980, when fruits had matured, the bags were collected from the field and the seed fecundity of the floral morphs was estimated ($N = 45$ and $N = 20$ infructescences per morph in 1979 and 1980, respectively). An estimate of seed fecundity was also made at Balsam Lake 2 (Ont.) by counting all maturing fruits on 30 infructescences per morph in a nearly complete sample of the population.

To compare fruit weight and germination among 10 individuals of each of the floral morphs, six replicates of 10 fruits from two infructescences per morph were selected at random from the material collected in the fecundity comparison (Paugh Lake NW) described above. The fruits were air dried and stored at room temperature in the dark for 6 months (October 1979 – April 1980). A germination test was conducted beginning on April 6, 1980, in the glasshouse using a completely randomized design. Fruits were placed in vials containing tap water at 20–30°C. The number of seedlings per vial was recorded after 18 days, after which time little germination occurred.

Statistical analysis

Following each experiment, the variances of the morphs were analyzed by " F_{\max} " tests and the distributions of the variates were analyzed by measures of kurtosis and skewness to determine the appropriate statistical test to apply. Since nearly all of the variates were distributed normally and variances were homogeneous, *t*-tests and ANOVA were used. Comparisons of fecundity, fruit weight, and germination were analyzed by nested ANOVA (Sokal and Rohlf 1969).

Results

Floral trimorphism

The survey of stamen and style lengths in flowers of *Pontederia cordata* demonstrates that tristily is a consistent and stable feature of populations. Of the 74 populations surveyed, 69 were trimorphic. No semi-homostylous individuals were observed among the approximately 20 000 inflorescences which were sampled. Further details, concerning the relative frequency of morphs in natural populations, will be presented elsewhere.

In *P. cordata*, there is a close correspondence in height between the stigmas of long styles and the anthers of long-level stamens, mid stigmas and mid anthers, and short stigmas and short anthers (Table 1). There is no overlap among the three levels in the lengths of reproductive organs. The separation between the short and mid levels and between the mid and long levels is approximately 4 to 5 mm. There is minor variability among populations in stamen and style length but whether this variation has a genetic basis is not known.

Investigation of anther size in *P. cordata* at Paugh Lake NW (Ont.) revealed two length classes (Table 2).

TABLE 1. Mean height and standard deviation (millimetres) of stigmas and anthers in the three floral morphs of *Pontederia cordata*. Each value is based on 10 flowers per morph. Underlined figures are stigma heights; the remainder are anther heights

Population	Organ level ^a	Floral morph		
		Long	Mid	Short
Paugh Lake SW (Ont.)	<i>l</i>	14.0±1.2	14.1±1.0	14.6±0.9
	<i>m</i>	8.9±0.8	<u>8.6±0.5</u>	9.8±0.4
	<i>s</i>	4.5±0.7	4.2±0.4	<u>4.1±0.5</u>
McQuire Lake (Ont.)	<i>l</i>	<u>14.2±0.6</u>	13.6±0.8	13.7±0.8
	<i>m</i>	<u>9.0±0.4</u>	<u>7.7±1.0</u>	7.8±0.6
	<i>s</i>	4.6±0.3	<u>4.9±0.3</u>	<u>4.0±0.8</u>
Cache Lake (Ont.)	<i>l</i>	13.3±0.8	13.8±0.7	13.3±1.1
	<i>m</i>	<u>8.0±0.4</u>	<u>7.6±0.4</u>	8.3±0.6
	<i>s</i>	4.1±0.5	<u>4.5±0.5</u>	<u>3.4±0.4</u>
Oxtongue River (Ont.)	<i>l</i>	<u>12.3±0.8</u>	11.5±0.5	11.3±0.8
	<i>m</i>	<u>8.3±0.9</u>	<u>7.1±0.5</u>	8.1±0.9
	<i>s</i>	4.7±0.5	<u>4.7±0.5</u>	<u>3.7±0.5</u>
Palmdale (FL)	<i>l</i>	14.6±1.0	14.1±0.7	14.1±0.9
	<i>m</i>	<u>9.0±0.8</u>	<u>9.2±0.4</u>	10.1±0.5
	<i>s</i>	4.8±0.2	<u>4.9±0.5</u>	<u>4.0±0.2</u>

NOTE: Ontario populations *P. cordata* var. *cordata*, Florida population var. *lancifolia*.
^a*l*, long; *m*, mid; *s*, short.

TABLE 2. Mean length × width with standard deviation (millimetres) of anthers in floral morphs of *Pontederia cordata* from Paugh Lake NW (Ont.). Each value is based on 10 flowers per morph

Anther level ^a	Floral morph		
	Long	Mid	Short
<i>l</i>	—	1.06 ^a ±0.03 × 0.68 ^c ±0.09	0.98 ^a ±0.04 × 0.63 ^c ±0.10
<i>m</i>	0.85 ^b ±0.08 × 0.66 ^c ±0.03	—	1.00 ^a ±0.08 × 0.69 ^c ±0.07
<i>s</i>	0.87 ^b ±0.05 × 0.67 ^c ±0.06	0.83 ^b ±0.06 × 0.63 ^c ±0.08	—

NOTE: Means not significantly different if superscripts (*a*, *b*, *c*) are the same. Means compared by least significant difference following ANOVA (length: $F = 18.06$; $df = 2, 54$; $P < 0.001$; width: $F = 0.68$, $df = 2, 54$; $P > 0.5$).

^a*l*, long; *m*, mid; *s*, short.

Anthers of the long-level stamens (of mid- and short-styled flowers) and of the mid-level stamens of short-styled flowers are significantly longer than anthers of the short-level stamens (of long- and mid-styled flowers) and of the mid-level stamens of long-styled flowers. All anthers are approximately the same width. Thus, anthers of both long stamen sets, like the anthers of both short stamen sets, are similar in size, but the size of anthers of the two mid-level stamens differs. The survey of anther size in six populations indicates that this pattern is a

general feature of tristily in *P. cordata*. The average length of anthers of the large set of stamens is 1.02 ± 0.06 mm and that of the small set is 0.85 ± 0.05 mm. The means are significantly different ($F = 17.065$; $df = 1, 34$; $P < 0.001$).

Examination of gynoecial characters in plants collected from Driftwood Bay (Ont.) revealed differences among the morphs. The colour of the style just below the stigma is purple in long-styled flowers, lilac in mid-styled flowers, and pink in short-styled flowers. Stigmas

TABLE 3. Mean length \times width with standard deviation (micrometres) of pollen grains of the floral morphs of *Pontederia cordata* from Balsam Lake 1 (Ont.). Each value is based on measurements of 100 grains, 10 flowers per morph

Anther level ^a	Floral morph			<i>t</i>	<i>P</i>
	Long	Mid	Short		
<i>l</i>	—	63.0 \pm 5.0 \times 25.6 \pm 1.7	58.9 \pm 7.1 \times 28.8 \pm 2.7	4.72 10.03	<0.001 <0.001
<i>m</i>	46.7 \pm 4.0 \times 25.0 \pm 4.5	—	46.2 \pm 2.0 \times 22.2 \pm 3.2	1.19 5.07	>0.02 <0.001
<i>s</i>	29.0 \pm 2.1 \times 15.0 \pm 2.0	28.8 \pm 2.1 \times 14.1 \pm 1.7	—	0.67 3.40	>0.4 <0.001

^a*l*, long; *m*, mid; *s*, short.

TABLE 4. Mean pollen production per anther level per flower with coefficient of variation in floral morphs of *Pontederia cordata* at Paugh Lake SW (Ont.). Each value is based on five replicates, 15 flowers per morph

Anther level ^a	Floral morph			<i>t</i>	<i>P</i>
	Long	Mid	Short		
<i>l</i>	—	5070 (29.0)	3552 (21.0)	2.06	>0.05
<i>m</i>	4174 (14.0)	—	8418 (24.0)	20.18	<0.001
<i>s</i>	23 061 (15.0)	23 790 (23.0)	—	0.61	>0.5

^a*l*, long; *m*, mid; *s*, short.

are broadly cleft in longs, less cleft in mids, and not cleft at all in shorts. The density of stigmatic papillae is inversely correlated with style length and thus the size of the interstitial spaces is directly proportional to style length and the size of the pollen compatible with the stigma (see below). The papillae appear to differ only slightly in size. Stigmas of long styles exhibit the longest papillae while stigmas of short styles bear the smallest papillae. Stigmas of mid styles have papillae which are intermediate in size.

Pollen size and production

Populations of *P. cordata* exhibit a striking pollen trimorphism with very little overlap in the distributions of pollen size among the three anther levels. Pollen size is positively correlated with anther height (Table 3).

Pollen size and production measurements from the Driftwood Bay (Ont.) population indicate that pollen size increases with anther height, whereas pollen production per anther level decreases (Table 4). Long-level anthers produce the largest grains in the smallest numbers, mid-level anthers are intermediate in pollen size and production, and short-level anthers produce the smallest grains in the largest numbers. There is no significant difference between the pollen production of the long-anther levels of mid- and short-styled flowers or

between the productions of the short-level anthers of mid- and long-styled flowers. However, the mid-level anthers of short-styled flowers produce approximately twice as many pollen grains as the mid-level anthers of long-styled flowers (Table 4). This pattern of differential pollen production in mid-level anthers correlates with the anther size dimorphism noted above. The larger of the two mid anther sets produces the greater number of pollen grains.

Differential pollen production at mid-level anthers is a consistent feature of *P. cordata* populations (Table 5). In each of the 20 populations surveyed, pollen production of the mid-level anthers of short-styled flowers was greater than that of the equivalent anther level of long-styled flowers. If the pollen production of the mid-level anthers of longs is taken as 1, the average production of the mid-level anthers of shorts is 1.79 ± 0.341 , standard error = 0.076. There is no significant difference between the pollen production of the two short-level anthers. However, the production ratio of 1.22 to 1 between the long-level anthers of mid- and short-styled plants is marginally different ($P = 0.048$).

Flowering periods

At Paugh Lake, the flowering phenologies of the three morphs are similar (Fig. 1). In comparison to a normal

TABLE 5. Survey of pollen production in 20 populations of *Pontederia cordata*. Each value is based on 15 flowers per morph

Population	Anther level of style morph					
	l_s^a	l_m	m_s	m_L	m_S	s_L
<i>Var. cordata</i>						
Paugh Lake NW (Ont.)	5093	4800	7 796	4693	20 899	26 676
Paugh Lake SW (Ont.)	3552	5070	8 418	4174	23 790	23 061
Oxtongue River (Ont.)	3308	4648	8 285	4444	22 543	21 815
Cache Lake (Ont.)	3916	4462	8 767	5231	20 625	24 359
Cheddar (Ont.)	3889	5921	8 048	6352	24 889	30 029
Indian Point N (Ont.)	5115	5227	9 879	4718	25 200	26 000
Indian Point S (Ont.)	5314	5748	11 362	6133	27 970	23 830
Elizabeth City (SC)	2710	2879	5 608	2617	16 684	16 216
Osborn (SC)	2667	4578	6 471	4827	20 711	20 427
Brunswick 3 (GA)	3573	2516	7 067	3396	16 596	15 733
Brunswick 4 (GA)	2862	2667	6 427	3716	16 604	15 351
Greenville (FL)	5218	6204	8 329	5227	17 884	23 511
Immokalee (FL)	3258	5509	7 317	5418	23 509	21 481
<i>Var. lancifolia</i>						
Ridgeland (SC)	5307	5929	9 911	4933	22 684	20 738
Hardeeville (SC)	2382	4000	5 804	3271	15 004	11 793
Brunswick 1 (GA)	2800	3404	6 960	4622	20 000	21 449
Brunswick 2 (GA)	3333	4107	7 244	5271	13 547	22 560
Clyo (GA)	4729	4356	8 782	3333	21 618	13 840
Myakkahatchee R. (FL)	4520	5183	8 736	5200	18 727	20 318
Palmdale (FL)	3938	4604	8 693	4204	17 556	18 044
Mean	3874	4591	7 995	4589	20 352	20 861
SD	962	1056	1 405	943	3 670	4 472
<i>F</i>	4.788		77.034		0.148	
<i>P</i>	<0.05		<0.001		>0.7	

^a l_s , long-level anthers of short-styled morph.

distribution, the flowering curves of the three morphs are strongly skewed, the peaks occurring early in the season (g_1 -long = +0.953, $N = 7330$; g_1 -mid = +0.729, $N = 9221$; g_1 -short = +0.872, $N = 7757$; $P \ll 0.001$). Peak flowering was on July 28. By this time, about half of all the season's flowers had been produced, although the flowering season was less than one-third complete. Skewed flowering periods were also observed in several other populations of *P. cordata* in Ontario.

Individual inflorescences begin flowering at the bottom of the spike and finish near the top. Most flowers are borne before the midpoint of the flowering period of the inflorescence, hence individual inflorescences also exhibit a skewed flowering pattern.

The three morphs do not differ in the pattern of inflorescence flowering or in the individual period of anthesis of flowers. Flowers open early in the morning and wither by late afternoon. Anthesis occurs as the corolla opens. By noon, anthers have shed their pollen and the filaments have withered. Flower opening is

delayed in cool weather, although flowers usually close by sunset.

Insect visitation

There is no evidence that pollinators preferentially visit any of the three floral morphs. Of 1939 visits by bumble bees to flowers observed at Paugh Lake, 44.9% was to shorts, 30.1% was to mids, and 25.0% was to longs. The morph frequencies in the pollinator study area were 45.2% short, 30.0% mid, and 24.8% long ($N = 303$). The number of visits to each morph does not differ significantly from the number predicted by the morph frequencies ($G = 2.078$; $df = 2$; $P > 0.75$). Furthermore, bumble bees visit the three morphs with equal frequency over the entire range of number of flowers visited per inflorescence (Fig. 2).

Reproductive characters

Comparisons of the total number of flowers and inflorescences per genet at Paugh Lake NW (Ont.) demonstrate no significant differences associated with floral morph (Table 6). Furthermore, in the United

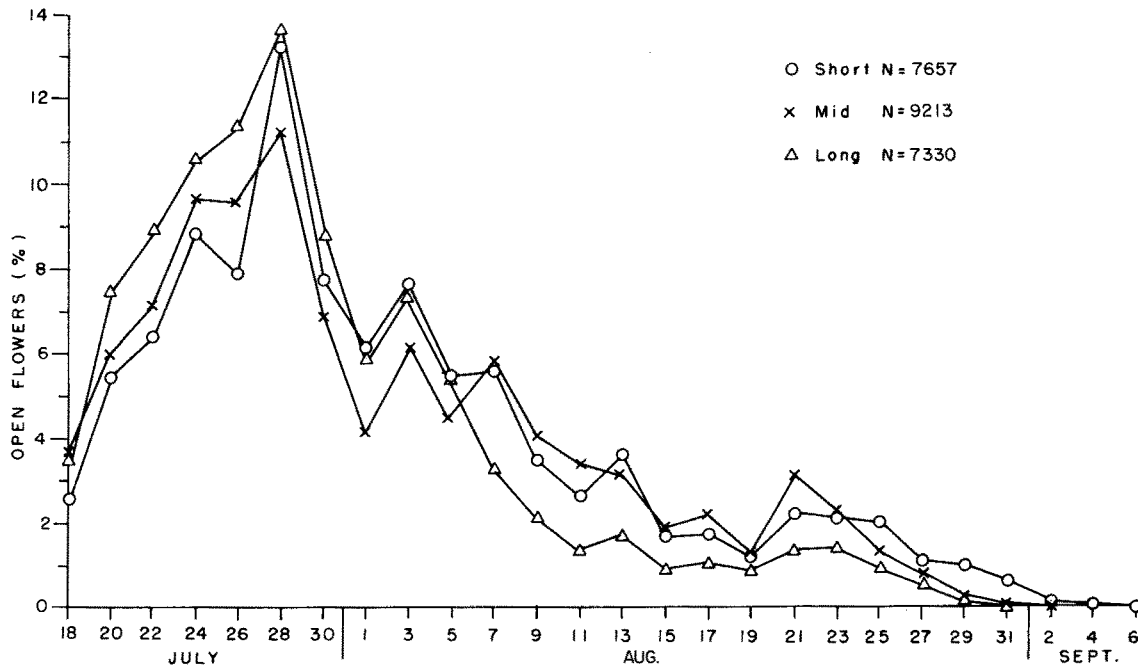


FIG. 1. Flowering phenology of floral morphs of *Pontederia cordata* at Paugh Lake, Ont., during 1979.

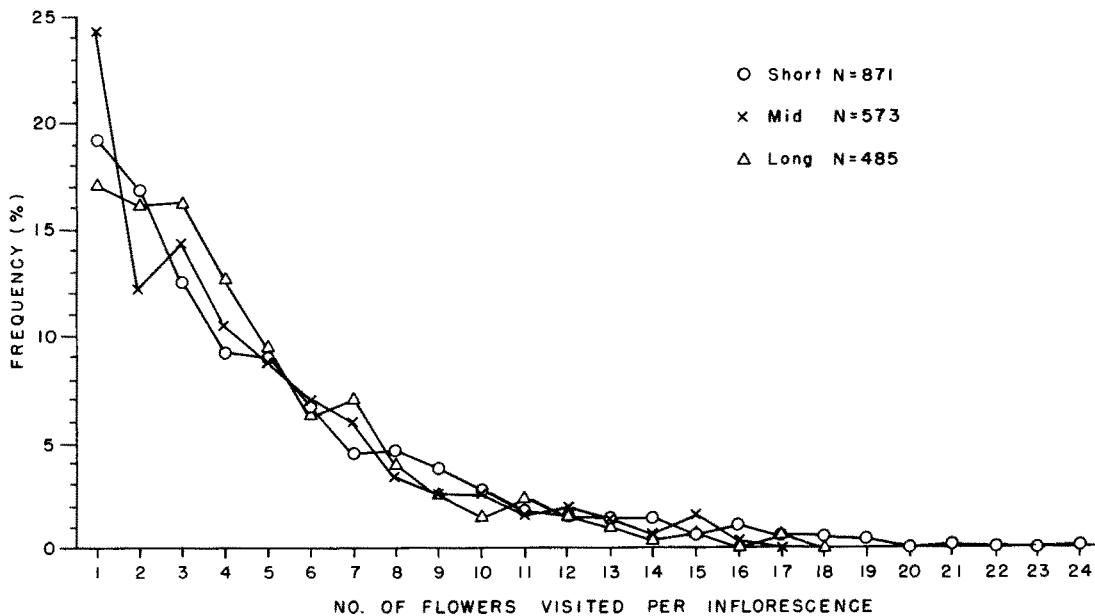


FIG. 2. Bumble bee foraging on floral morphs of *Pontederia cordata* at Paugh Lake, Ont., during 1979.

States, at Osborn (SC) and Altamaha N (GA), each morph exhibited a similar number of open flowers per inflorescence during a single day (Table 6). The two comparisons of fecundity (seed set per inflorescence) are consistent with the hypothesis that there are no signifi-

cant differences in reproductive potential among the floral morphs. At both Paugh Lake NW (1979 and 1980) and Balsam Lake 2 (1979), the mean number of fruits per infructescence does not differ significantly among the three morphs (Table 6). At Paugh Lake, nested ANOVA

TABLE 6. Comparison of reproductive parameters among the floral morphs in natural populations of *Pontederia cordata*

Population	Parameter	N/morph	Floral morph			F
			Long	Mid	Short	
Paugh L. (Ont.)	Inflorescences/individual	20	3.4±1.2	3.8±1.3	3.6±0.9	0.74 ^a
Paugh L. (Ont.)	Flowers/inflorescence	72	100.9±35.8	114.1±44.1	103.6±43.0	2.13 ^a
Paugh L. (Ont.)	Flowers/individual	20	366.5±203.5	461.7±199.3	393.6±178.7	1.28 ^a
Paugh L. (Ont.) 1979	Seeds/inflorescence	45	138.7±59.8	146.6±66.2	109.3±56.0	1.99 ^a
Paugh L. (Ont.) 1980	Seeds/inflorescence	20	158.8±36.8	154.5±35.2	139.1±32.4	1.67 ^a
Balsam L. (Ont.)	Seeds/inflorescence	30	151.8±55.3	165.5±52.4	143.1±53.1	1.29 ^a
Osborn (SC)	Open flowers/inflorescence	100	14.9±6.4	15.3±5.7	14.9±5.8	0.12 ^a
Altahama (GA)	Open flowers/inflorescence	50	18.8±8.1	19.8±7.9	19.4±7.5	0.19 ^a

^aNot significant.

on the 1979 data reveals that 5% of the variation in fecundity is attributable to floral morph, whereas 95% resides among genets (among morphs: $F = 1.99$; $df = 2$; $P > 0.10$; among genets: $F = 4.10$; $df = 37$; $P < 0.001$).

Seed germination

Although in the Paugh Lake NW population the average weight of fruits of the short morph was approximately 15% less than those from mid and long morphs (\bar{x} fruit weight \pm SD in milligrams: long = 157.9 ± 39.2 ; mid = 153.5 ± 52.8 ; short = 143.1 ± 33.1), this difference did not appear to influence seed germination. The percentage of germinated seeds did not differ significantly among the floral morphs in the germination test ($\bar{x}\%$ germination \pm SD: long = 34.7 ± 32.5 ; mid = 40.5 ± 36.7 ; short = 36.6 ± 30.1). Variation among floral morphs accounted for only 5.2% of the variance in germination levels. Most of the variation in germination (62.3%) resided in differences among infructescences within individuals.

Discussion

Tristyly is a stable feature of North American populations of *Pontederia cordata*. Although semihomostylous individuals and races are known from taxa within each of the three tristylous families (Lythraceae: Stout 1925; Oxalidaceae: Ornduff 1972; Pontederiaceae: Barrett 1978, 1979), no modifications in floral morphology of the three floral morphs were observed in this study. Elsewhere in the genus, *P. parviflora* is monomorphic for style form and is considered to be derived from tristylous ancestors (Lowden 1973).

The difference in anther length among the three morphs of *P. cordata* is the first demonstration of an anther size polymorphism in a tristylous species. In most distylous taxa where anther size has been measured, the anthers of the long level are larger than the anthers of the short level (Ganders 1979). The most interesting feature of the anther size polymorphism in *P. cordata* is the

difference in the size of anthers at the mid-anther level. The size difference is positively correlated with a pollen production difference. The larger mid-level anthers of short-styled flowers produce on average 1.8 times more pollen grains than the corresponding anther level in long-styled flowers. Whether anther size determines pollen production or vice versa is not known.

Ganders (1979) has suggested that the number of pollen grains produced per anther differs among the floral morphs in heterostylous species to counteract asymmetric patterns of pollen flow. In distylous species, selection may have resulted in more pollen grains per anther in short-level anthers of the long-styled morph because stigmas of the short-styled morph are less accessible than those of longs and thus receive less total pollen. Similar arguments could apply to tristylous species. If, as Ganders suggests, the probability of pollen transfer is dependent on anther and stigma height, the anthers of the same level in different morphs of tristylous species should produce the same number of pollen grains. The few measurements of pollen production which have been undertaken in tristylous species have indicated that similar amounts of pollen are in fact produced within anther levels of the floral morphs (Weller 1976; Ornduff 1975). Why *P. cordata* differs in this respect is therefore of some interest.

The presence of a mid-level anther pollen production difference in each of the 20 populations surveyed strongly suggests that it is not an adaptive response to local selective forces associated with a specific pollinator fauna. A wide range of insect visitors including bumble bees, solitary bees (*Melissodes* spp., *Dialictus* spp., *Xylocopa virginica* (L.), *X. micans* Lepeletier) and butterflies were observed in varying frequencies at different populations (and see Hazen 1918). Other explanations would seem to be required.

It is possible that the pollen production difference has no direct adaptive value but is instead an inevitable outcome of differences in floral development among the floral morphs. In tristylous species the insertion patterns

of the two sets of stamens within a flower differ (Barlow 1923). In the Pontederiaceae the shortest set is positioned on the upper side of the perianth tube and the longest set is inserted on the lower side. Developmental studies of the related *Eichhornia paniculata* (J. H. Richards and S. C. H. Barrett, unpublished data) indicate that two of the three anthers of the shortest level are associated with narrow tepals, with the remaining anther inserted on a broad tepal. The insertion patterns are reversed in the longest anther level, with two anthers associated with broad tepals and one anther inserted on a narrow tepal. A similar pattern is evident in *P. cordata*. Thus, while the long-level anther of mid- and short-styled flowers and the short-level anther of long- and mid-styled flowers have identical insertion patterns, the arrangement in mid-level anthers differs between the short- and long-styled morphs. This anomaly in mid-level anther insertion in tristylous species was first observed by Barlow in *Lythrum salicaria* (1923). In short-styled flowers of *P. cordata* the mid-level anthers are the shortest anther set in the flower and are therefore inserted on the upper side of the perianth tube, whereas in long-styled flowers the mid-level anthers are the longest set and arise from the lower portion of the perianth tube. It is quite possible that this pattern is associated with differences in the timing and development of anthers in the two floral morphs and this may result in the differences in anther size and pollen production observed at the mid-level. Clearly a detailed analysis of stamen development in *P. cordata* would help to resolve this problem.

Although the basis of the difference in pollen production at the mid-level anthers is not known, it may have an important influence on the structure of natural populations of *P. cordata*. Surveys of floral morph frequency over a wide geographical range indicate that the short-styled morph is in excess and the long-styled morph is reduced in comparison with expectations based on an isoplethic (1:1:1) equilibrium (morph frequencies: short = 0.399, mid = 0.346, long = 0.255, $N = 74$ populations). Survey data and experimental studies to be reported elsewhere suggest that, as a result of the difference in production at the mid-level anthers, pollen from the short-styled morph fertilizes more ovules of mid-styled plants than pollen from the long-styled morph.

In virtually all other comparisons which were made among the floral morphs of *P. cordata*, no significant differences were observed. The patterns of flowering, inflorescence production, flower production, fecundity, fruit weight, and germination levels all varied considerably more among individuals of the same morph than among floral morphs. In addition, bumble bees showed no particular preference for floral morphs during foraging activity. Although rates of cloning of the floral morphs were not measured directly, several lines of

evidence suggest that they also do not differ significantly among the morphs (Price 1981). At the Paugh Lake NW (Ont.) population, flowering individuals averaged 3.6 reproductive shoots per season (range 1 to 8) but there were no significant differences among morphs. Secondly, at Balsam Lake 1 (Ont.) a complete marking of inflorescences revealed that the morph frequencies based on distinct individuals (presumably genets) were identical to those based on inflorescence (ramet) sampling. Lastly, nearest neighbour analysis of inflorescences in four Ontario populations indicated that, while the probability that neighbouring inflorescences were of the same morph varied among populations, there was relatively little difference among the floral morphs within populations. These data all suggest that inflorescence production and clone sizes among the morphs are similar. Elsewhere, differences in cloning ability among floral morphs have not been demonstrated in heterostylous plants.

There are no a priori reasons why the floral morphs of tristylous species should differ in reproductive potential. Where differences do occur, they are frequently associated with the breakdown of tristily owing to selection for distyly (Ornduff 1964; Weller 1976) or increased selfing (Barrett 1977b, 1979). In *P. cordata* the similarities among the three morphs in reproductive characteristics are associated with self-incompatibility, strong pollen trimorphism, and the occurrence of all three morphs in most natural populations. These data, accompanied by the lack of observations of floral modification, suggest that the tristylous syndrome is a well canalized and stable feature of *P. cordata* populations.

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