

## Variation in expression of trimorphic incompatibility in *Pontederia cordata* L. (Pontederiaceae)

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**Summary.** *Pontederia cordata* L. (Pontederiaceae), a perennial diploid, possesses the rare genetic polymorphism tristylous. A controlled pollination programme was conducted over a three year period, under glasshouse conditions, on 36 clones of *P. cordata* var. *cordata* to examine the nature of the self-incompatibility system. The three major findings of the pollination study were: (1) the three floral morphs display different levels of self-incompatibility, (2) pollen from the two anther levels within a flower exhibits different compatibility behaviour in self-pollinations, (3) considerable individual genetic variation in the expression of self-incompatibility is evident among clones within floral morphs. Similar results were also obtained from a smaller study on 15 clones of *P. cordata* var. *lancifolia* conducted over a 6 month period. In common with other *Pontederia* species the mid-styled morph (M) of *P. cordata* produces large amounts of seed when self-pollinated with pollen from long-level anthers. A developmental model is proposed to explain the high level of self-compatibility of the M morph in *Pontederia* species. Self-pollination of segregating progenies from M and S morphs of known incompatibility status demonstrated that the expression of incompatibility is closely associated with style length. It is suggested that overall differences in incompatibility behaviour among the floral morphs may be due to the pleiotropic effects of major genes controlling sub-characters of the tristylous syndrome, rather than linked modifier genes. However, the variable expression of trimorphic incompatibility within floral morphs suggests that this variation may be polygenic in origin.

**Key words:** Trimorphic incompatibility – *Pontederia cordata* L.

### Introduction

Trimorphic incompatibility is a genetic polymorphism in which flowering plant populations contain three mating groups that differ in style length, anther height, pollen size and incompatibility relationships. A unique feature of tristylous plants is the production, by alternate anther levels within a flower, of two distinct pollen phenotypes that differ in their incompatibility behaviour and size.

Although the developmental and biochemical basis of pollen differentiation is unknown, the consequences for mating were first established by Darwin (1877). By controlled self- and cross-pollinations of the floral morphs in *Lythrum salicaria* and *Oxalis* spp. he revealed a complex pattern of self- and cross-compatibility. Pollinations between anthers and stigmas of the same relative level yield abundant seed whereas all remaining pollinations, irrespective of whether they are self, intramorph, or intermorph, result in little to no seed. Subsequent work indicates that these patterns characterise the polymorphism in each of the three tristylous families (e.g., Lythraceae – Dulberger 1970; Oxalidaceae – Weller 1980; Pontederiaceae – Barrett 1977 a).

In the Pontederiaceae tristylous occurs in four species of *Pontederia* and three species of *Eichhornia*. Comparative studies indicate that the expression of tristylous in the two genera is markedly different. In *Pontederia* floral trimorphism is associated with self-incompatibility, strong pollen trimorphism, and populations which usually contain the three floral morphs (Barrett 1977 a; Price and Barrett 1982; Barrett et al. 1983; Glover and Barrett 1983). In *Eichhornia* tristylous is associated with high levels of self-compatibility, weak pollen trimorphism and monomorphic population structure. Self-pollinating semi-homostylous variants are reported in each of the tristylous species of *Eichhornia* (Barrett 1977 b, 1978, 1979, 1985 a; Barrett and Forno 1983). While in *Eichhornia* tristylous is susceptible to evolutionary modifications favouring selfing, the level of outcrossing in *Pontederia* is sufficient to maintain the genetic polymorphism.

In *Eichhornia* the relaxation and loss of self-incompatibility appears to have preceded the develop-

ment of modifications in floral structure that favour selfing. For natural selection to alter the compatibility relationships of tristylous populations genetic variation in the expression of trimorphic incompatibility must occur. Earlier work on *Pontederia* spp. demonstrated differences in the levels of self-incompatibility among the floral morphs (Ornduff 1966; Barrett 1977 a; Glover and Barrett 1983). To investigate this phenomenon further a detailed examination of the expression of self-incompatibility in *P. cordata* L. was undertaken. The specific questions we addressed were: (1) Do the floral morphs differ in their levels of self-incompatibility? (2) How do the two pollen types produced within flowers behave in self-pollinations? (3) Are individual genetic differences in the expression of self-incompatibility evident? (4) Do offspring from self-fertilization display the self-incompatibility phenotype of their parents? Answers to these questions were sought by controlled pollinations of clones of *P. cordata*, from two natural populations, at the extremities of the N. American range. The two populations constitute distinct taxonomic varieties of the species.

## Materials and methods

### Plant material

Experimental work was undertaken on clones of *Pontederia cordata* originating from seed collected in two natural populations. Thirty-six clones of *P. cordata* L. var. *cordata* were obtained from seed families collected in September 1978 from a population at Paugh Lake, Ontario, Canada. Each clone was the descendant of a different maternal parent. A smaller collection of 15 clones of *P. cordata* L. var. *lancifolia* (Mohl.) Torrey was derived from a bulk collection of seed obtained from a population 35 km south-west of Miami, Florida, USA, in January, 1982. Clones were grown in plastic pots submerged in waterfilled tubs placed on a single glasshouse bench under uniform growth conditions.

### Pollination experiments

Two separate pollination programmes were carried out on the varieties of *P. cordata*. The largest involved *P. cordata* var. *cordata* and was undertaken for three consecutive years (1981–1983) during the flowering period (March–September). Clones were all two years old at the commencement of pollinations and hence were of similar size and developmental status. Pollinations of *P. cordata* var. *lancifolia* were carried out from January–March 1983 when clones were one year old. Temperature during the pollination programmes averaged 25 °C (range 20–35 °C) and the glasshouse was kept pollinator-free.

To determine the compatibility status of each clone of *P. cordata*, controlled self- and cross-pollinations were undertaken using fine forceps. Three treatments were applied to all clones: 1) cross-pollination between anthers and stigmas of equivalent height (legitimate pollinations), 2) the two classes of self-pollination using alternate anthers levels within a flower e.g., self-pollination of the long-styled morph with pollen from mid-level anthers ( $L \times m/L$ ) and pollen from

short-level anthers ( $L \times s/L$ ). Since the two classes of self-pollination yielded different results in each floral morph we refer to these pollinations as either the most compatible self-pollinations ( $L \times m/L$ ,  $M \times 1/M$ ,  $S \times m/S$ ) or the least compatible self-pollinations ( $L \times s/L$ ,  $M \times s/M$ ,  $S \times 1/S$ ). All flowers within a single inflorescence of *P. cordata* received the same pollination treatment and were used in pollinations. The average number of flowers pollinated per clone, with range, for each of the three pollination classes was: *P. cordata* var. *cordata*; (1) cross-pollination  $\bar{X}=302$ , range 45–1333. (2) most compatible self-pollination  $\bar{X}=677$ , range 30–2120. (3) least compatible self-pollination  $\bar{X}=115$ , range 43–223. *P. cordata* var. *lancifolia*; (1) cross-pollination  $\bar{X}=90$ , range 53–169. (2) most compatible self-pollination  $\bar{X}=100$ , range 21–180. (3) least compatible self-pollination  $\bar{X}=91$ , range 29–177. In *P. cordata* var. *cordata* the 36 clones were comprised of 12 per floral morph and in *P. cordata* var. *lancifolia* the 15 clones were made up of 5 per morph.

For each pollination treatment the number of flowers pollinated daily for each clone was recorded and the number of fruits was counted on mature infructescences. In *P. cordata* the anthesis period of individual flowers is 8–12 h; all pollinations were completed by 12:00 noon. Since flowers are uniovulate fruit set is equivalent to seed set. For each clone the total number of seeds was divided by the total number of flowers pollinated to give a compatibility value for each pollination treatment. Pollinations using short-level organs presented some difficulty because of their concealed and delicate nature. In order to accomplish pollinations the perianth tube was split using fine forceps to allow access to the anthers or stigma. Pollinations involving the S morph as an egg parent were particularly difficult to conduct.

To investigate the extent of intraclonal variation in the expression of self-incompatibility the four largest clones of each floral morph in *P. cordata* var. *cordata* were divided into three or four ramets by breaking the rhizome into segments and planting each in a separate pot. The pots were randomized on a glasshouse bench and the pollination treatments outlined above conducted on all ramets during 1982 and 1983.

### Inheritance of self-incompatibility levels

A preliminary experiment to examine whether offspring exhibit the level of self-incompatibility displayed by parents was conducted using four clones (L11, M3, M9, S9) of *P. cordata* var. *cordata*. Seed obtained by selfing the four clones was germinated and a small number of progeny raised to flowering and each was self-pollinated using pollen from the most compatible anther level. Of particular importance was to observe whether the level of self-incompatibility in segregating families differed among the floral morphs.

### Pollen size and incompatibility

Breakdown of heteromorphic incompatibility is often associated with a loss of pollen size differentiation in heterostylous taxa (Baker 1966; Ornduff 1972; Barrett 1979). To investigate whether a relationship exists between the level of self-incompatibility exhibited by a clone and pollen size the length, width, and volume of pollen grains from each anther level were obtained for all clones of *P. cordata* var. *cordata*. The equatorial and polar axes of 20 dry pollen grains per anther level for five flowers of each clone ( $n=100$  pollen grains per anther level per clone) were measured at  $\times 40$  power using a light microscope and bit pad accessory. Data on pollen size and level of incompatibility were analysed by regression analysis.

## Results

### Variation among floral morphs

The results of all controlled pollinations undertaken on clones of *Pontederia cordata* are presented in Table 1. The presence of a self-incompatibility system is confirmed in each of the floral morphs of both varieties. Percentage seed set of legitimate pollination was always significantly higher than in self-pollination. Analysis of variance demonstrates that there is no significant difference among the floral morphs in fertility following legitimate pollination (*P. cordata* var. *cordata*:  $F=1.00$  (2,75)  $P>0.05$ ; *P. cordata* var. *lancifolia*:  $F=0.143$  (2,12)  $P>0.05$ ). A high proportion of flowers that were cross-pollinated produced seeds (*P. cordata* var. *cordata*: 79.7%,  $n=10,849$  flowers; *P. cordata* var. *lancifolia* 86.7%,  $n=1,344$  flowers) indicating that the environmental conditions of the glasshouse and the pollination techniques employed were conducive to normal seed production.

**Table 1.** Percentage seed set of controlled self- and cross-pollinations of the floral morphs of *Pontederia cordata* vars. *cordata* and *lancifolia* under glasshouse conditions. Each pollination treatment involved 12 clones per morph (var. *cordata*) and 5 clones per morph (var. *lancifolia*).

Pollination <sup>a</sup> treatment	No. inflorescences	No. flowers pollinated	Percent flowers producing seed	Mean % seed set per clone $\pm$ S.D.
<i>Pontederia cordata</i> var. <i>cordata</i>				
Legitimate pollinations				
L $\times$ M or S	32	4,812	83.2	82.1 $\pm$ 8.7
M $\times$ m/L or S	24	3,173	76.9	76.0 $\pm$ 11.8
S $\times$ s/L or M	22	2,874	79.1	78.7 $\pm$ 6.6
Self pollinations				
L $\times$ s/L	12	1,579	4.6	3.8 $\pm$ 4.5
L $\times$ m/L	64	10,109	32.6	30.2 $\pm$ 25.7
M $\times$ s/M	12	1,379	8.6	7.5 $\pm$ 6.9
M $\times$ l/M	55	8,202	65.2	58.5 $\pm$ 17.6
S $\times$ l/S	12	1,192	2.0	2.0 $\pm$ 1.7
S $\times$ m/S	45	6,061	17.5	17.8 $\pm$ 10.1
<i>Pontederia cordata</i> var. <i>lancifolia</i>				
Legitimate pollinations				
L $\times$ M or S	5	508	84.4	86.8 $\pm$ 10.7
M $\times$ m/L or S	5	527	87.5	85.8 $\pm$ 10.1
S $\times$ s/L or M	5	309	88.3	89.0 $\pm$ 8.7
Self pollinations				
L $\times$ s/L	5	529	0.4	0.2 $\pm$ 0.5
L $\times$ m/L	5	579	14.9	13.6 $\pm$ 21.7
M $\times$ s/M	5	427	3.3	4.0 $\pm$ 2.5
M $\times$ l/M	5	477	47.0	46.2 $\pm$ 19.4
S $\times$ l/S	5	412	3.6	4.0 $\pm$ 4.9
S $\times$ m/S	5	440	18.2	16.0 $\pm$ 10.6

<sup>a</sup> Stylelength ( $\varphi$ )  $\times$  anther level/style length ( $\delta$ )

In both varieties of *P. cordata* the floral morphs exhibited striking differences in the level of self-compatibility following self-pollination. Furthermore, pollen from the two anther levels in each floral morph behaved differently. Self-pollination with one anther level was clearly more productive of seed than with the alternate level. In the L and S morphs, pollen from mid-level anthers was the most productive self-pollination, whereas in the M morph self-pollination using pollen from long-level anthers was considerably more productive than short-level anthers. The results indicate that although there are no differences in the fertility of the floral morphs following legitimate pollination, the expression of self-incompatibility varies considerably both among morphs and between anther levels within each morph.

The most striking feature of the data on self-pollination is the weak expression of self-incompatibility exhibited by the M morph when pollen from long-level anthers was used in pollinations. The average number of pollinated flowers producing seed in *P. cordata* vars. *cordata* and *lancifolia* was 65.2% and 47.0%, respectively for this treatment. These values are  $\times 2-3$  higher than the next most compatible self-pollination treatment in the L and S morphs of each variety (Table 1). In *P. cordata* var. *cordata* the L morph was, on average, more compatible than the S morph following self-pollination with mid-level pollen (L $\times$ m/L 32.6%, S $\times$ m/S 17.5%). These pollination treatments yielded similar results in *P. cordata* var. *lancifolia* although the limited sample of clones prevents any overall comparison between these two morphs.

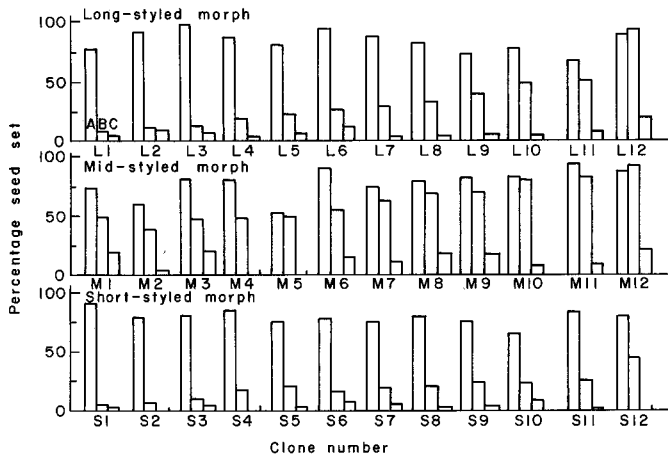
### Individual variation among clones

The expression of self-incompatibility varied greatly among individual clones within morphs. This variation is evident from inspection of Figs. 1 and 2 which present data for percentage seed set following the three pollination treatments for each clone of *P. cordata* vars. *cordata* and *lancifolia*, respectively. Examples of the spectrum of variation exhibited by clones of *P. cordata* var. *cordata* are: L $\times$ m/L, L1=4.0%, L12=93.7%; M $\times$ l/M, M1=33.3%, M12=86.6%; S $\times$ m/S, S8=4.0%, S5=40.0%. Similar patterns were evident among the smaller sample of clones in *P. cordata* var. *lancifolia* (Fig. 2).

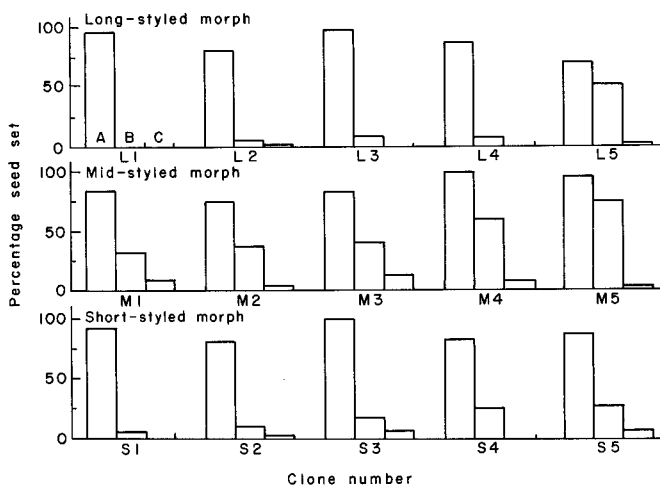
### Association between seasons in the expression of self-incompatibility

The differences among clones in the expression of self-incompatibility may result from genetic variation for this trait. To further investigate the basis of this variation the seed set data collected for the most compatible self-pollinations in 1981 and 1982 were compared

for each clone in *P. cordata* var. *cordata*. If a significant component of the variation is genetic in origin we might anticipate either similar values to be expressed from one growing season to the next, or, at the least, correlated changes among the clones. Figure 3 presents data for all clones irrespective of morph and for each morph separately. Significant positive associations are evident for all comparisons except those involving the S morph.



**Fig. 1.** Percentage seed set following three pollination treatments on 12 clones of each floral morph of *Pontederia cordata* var. *cordata* under glasshouse conditions. Treatment A is legitimate cross-pollination; treatment B is self-pollination with the most compatible anther level in each morph (L × m/L, M × l/M, S × m/S); and treatment C is self-pollination with the alternate anther level in each morph (L × s/L, M × s/M, S × l/S). The data are pooled over three years. Clones are ordered by increasing level of self-compatibility

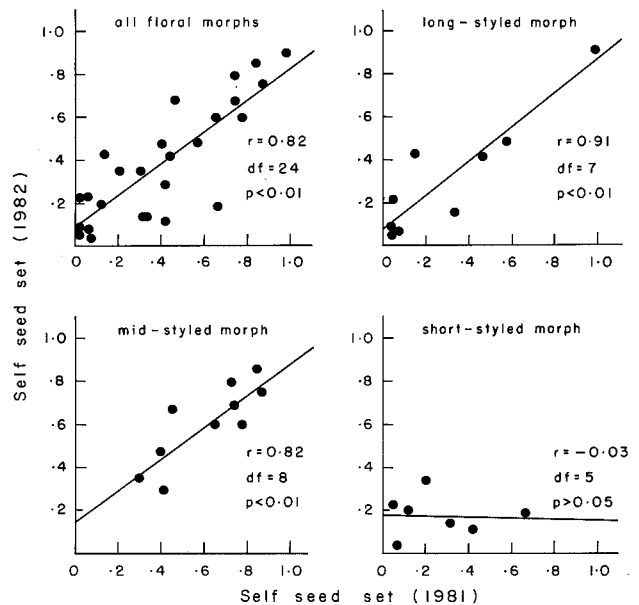


**Fig. 2.** Percentage seed set following three pollination treatments on 12 clones of each floral morph of *Pontederia cordata* var. *lancifolia* under glasshouse conditions. Treatments A, B, C are as in Fig. 1. Clones are ordered by increasing level of self-compatibility

The failure to detect an association between self-incompatibility levels in 1981 and 1982 in the S morph may result from an inflated level of environmental variation (technique error) associated with pollination procedures as well as the lower range of variation for this trait exhibited by the S morph. Evidence to support the suggestion of a high level of environmental variation influencing data for the S morph was obtained by comparison of the average coefficient of variation for self seed set among the floral morphs. This value was obtained by calculating the coefficient of variation for each clone based on repeated self-pollination of different inflorescences and averaging these values for each morph. The average C.V.s are 38.7%, 13.0% and 70.1% for the L, M, and S morphs, respectively.

*Genetic and environmental components of variation in expression of self-incompatibility*

The expression of self-incompatibility in individual clones of *P. cordata* is determined by an interaction of genetic and environmental factors. To determine whether the observed variation among clones of *P. cordata* var. *cordata* had a significant genetic component the seed set data for self-pollinations involving the most compatible anther level were analysed by ANOVA. Two procedures were adopted. Firstly a series of 12 one way ANOVAs were performed on the seed set data of inflorescences obtained from clones that had been divided into ramets. These analyses were used to deter-



**Fig. 3.** Comparison of self seed set data in 1981 and 1982 in *Pontederia cordata* var. *cordata* following self-pollination with the most compatible anther level in each floral morph. Pollinations performed under glasshouse conditions; each point represents values for an individual clone in the two years

**Table 2.** One-way analysis of variance (random effects model II) of percentage seed set values from individual clones of the floral morphs of *Pontederia cordata* var. *cordata*. Data is from the more self-compatible anther level of each morph. Values arc-sine  $\sqrt{P}$  transformed before analysis

Source of variation	D.F.	SS	MS	F	P	% variance
<b>Long-styled morph</b>						
Between clones	11	13,829.79	1,257.25	12.18	<0.001	68.3
Within clones	52	5,366.43	103.20			31.7
Total	63	19,196.22				
<b>Mid-styled morph</b>						
Between clones	11	6,122.39	556.58	18.43	<0.001	79.6
Within clones	43	1,298.31	30.19			20.4
Total	54	7,420.70				
<b>Short-styled morph</b>						
Between clones	7	1,500.30	214.4	1.87	>0.05	2.58
Within clones	35	4,010.98	114.6			97.42
Total	42	5,511.28				

**Table 3.** Percentage seed set of controlled self-pollinations of selfed progeny ( $F_1$ ) in four clones of *Pontederia cordata* var. *cordata*. Self-pollination involved the more self-compatible anther level of each floral morph

Parental clone	Progeny	Floral morph	No. flowers pollinated	% flowers producing seed	
L 11 (49.5) <sup>a</sup>	1	L	70	44.3	
	2	L	72	34.7	
	3	L	69	52.2	
				$\bar{X} = 43.7$	
M 9 (68.7)	1	M	77	54.5	
	2	M	16	62.5	
	3	M	92	72.8	
	4	M	77	55.8	
	5	M	48	66.7	
				$\bar{X} = 62.5$	
M 3 (45.3)	1	L	47	14.9	
	2	L	19	0	
	3	L	80	5.0	
	4	L	41	14.6	
					$\bar{X} = 8.6$
	5	M	52	69.2	
	6	M	90	65.6	
7	M	56	60.7		
				$\bar{X} = 65.2$	
S 9 (24.6)	1	M	92	76.1	
	2	S	79	36.7	
	3	S	70	40.0	
	4	S	30	0	
	5	S	67	19.4	
				$\bar{X} = 24.0$	

<sup>a</sup> Value in parentheses is the average percentage seed set of the parental clone following self-pollination with more compatible anther level

mine if significant differences among ramets of a single clone were evident. In none of the 12 clones was this the case. This finding permitted data from all clones, in which more than one inflorescence had been pollinated, to be analysed with the repeated measurement of percentage seed set per inflorescence used as the variate in the ANOVAs. Four of the 36 clones could not be used in the analyses since only a single inflorescence of each clone had been self-pollinated. These clones were all of the S morph.

Table 2 presents the results of the one-way ANOVAs (random effects model II) for the three floral morphs of *P. cordata* var. *cordata*. Estimates of the between and within clone variation in the expression of self-incompatibility are similar in the L and M morphs with most variation accounted for by individual differences among clones. In contrast, virtually all of the observed variation in self seed set in the S morph resides in the within clone component of variation. Once again this may stem from inflated environmental variation owing to technique error. The results provide further evidence that a significant component of the observed variation in the expression of self-incompatibility in *P. cordata* is of genetic origin.

#### *Inheritance of self-incompatibility levels*

The results involving the self seed set data of inbred progeny of *P. cordata* var. *cordata* clones are preliminary in nature and based on small samples. Nevertheless an inspection of offspring values for self-incompatibility presented in Table 3 indicates a clear pattern. Where segregation of style morphs occurs (M 3 and S 9) the level of self-incompatibility displayed by the off-

spring is strongly associated with style length. This can be seen by comparing the average values of the L and M offspring from the self of M3. Plants of the L morph averaged 8.6% seed set on selfing whereas M plants averaged 65.2%. The average values for self seed set in non-segregating families were close to the parental values although variation among individual clones was apparent and more detailed studies are necessary to elucidate the genetic factors controlling the variable expression of self-incompatibility.

#### Pollen size and incompatibility

As is typical of many tristylous species the pollen produced by the three anther levels of *P. cordata* can be clearly distinguished by size. The mean length, width and volume of pollen (in  $\mu\text{m}$ ) from long-, mid-, and short-level anthers is 65.6, 26.6 and 24,354.4 ( $\mu\text{m}^3$ ); 52.3, 21.5, and 12,946.8 ( $\mu\text{m}^3$ ); 34.6, 14.8, and 3,983.5 ( $\mu\text{m}^3$ ), respectively.

Nested ANOVA performed on pollen demonstrated that within each floral morph variation among clones accounted for approximately  $\times 2-3$  more of the variance than differences between flowers within a clone. This suggests that differences in pollen dimension among clones may have a genetic component.

Having established that pollen size varies between clones of a floral morph, regression analysis was used to determine whether a relationship existed between mean length, width, or volume of pollen of each clone and its strength of self-incompatibility. The analysis was performed on each floral morph separately and used data from the most self-compatible anther level. If some functional relationship exists between pollen size and incompatibility it may be predicted that in clones with weak self-incompatibility, the size of the compatible self pollen will tend towards the legitimate size class. For example, if a long-styled clone is highly self-compatible when self-pollinated with pollen from mid-level anthers, mid-level pollen grains may be larger than average tending towards the size class of legitimate pollen. These predictions were not supported by results from the regression analyses. In none of the nine analyses (3 morphs  $\times$  3 pollen grain measurements) was there a statistically significant relationship between pollen dimension and self-incompatibility level.

#### Discussion

The major findings of this study are that (1) the floral morphs of *Pontederia cordata* display different strengths of self-incompatibility, (2) the two anther levels within flowers of the floral morphs exhibit different behaviour in self-pollinations, (3) considerable genetically based variation in the expression of trimorphic incompatibility

is evident in natural populations of *P. cordata*. The first two observations are in accord with an earlier study of *P. cordata* by Ornduff (1966) and our own studies of *P. rotundifolia* (Barrett 1977 a) and *P. sagittata* (Glover and Barrett 1983).

The most striking feature of the data from the four taxa of *Pontederia* which have been studied experimentally is the high level of self-compatibility exhibited by the M morph (Fig. 4). With the exception of *P. rotundifolia*, where the L morph is moderately self-compatible, the M morph is  $\times 2-3$  more self-compatible than the L and S morphs. This behaviour is not restricted to self-pollination, but is also a feature of both intra-morph and inter-morph cross-pollination with pollen from long-level anthers of the M and S morphs, respectively (see data in Ornduff 1966; Barrett 1977 a; Glover and Barrett 1983). For some reason pollinations that involve the combination of large-sized pollen, with mid-length styles, overcome the trimorphic incompatibility system of *Pontederia* resulting in regular seed set. We now propose several hypotheses to explain the variation in expression of trimorphic incompatibility displayed by the floral morphs of *Pontederia*.

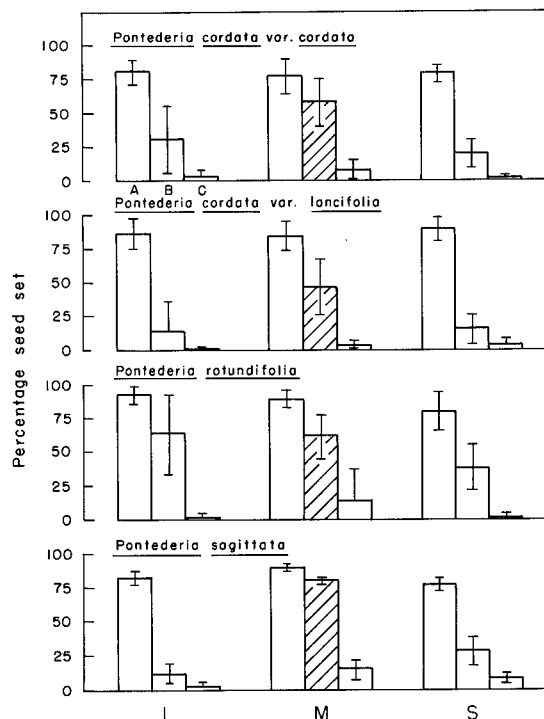


Fig. 4. Percentage seed set in four tristylous taxa of *Pontederia* following self- and cross-pollination. Treatments A, B, C are as in Fig. 1. Error bars represent the standard deviation around the mean value, the latter obtained by averaging data from individual clones. The hatched column highlights the weak self-incompatibility of the M morph. Data from *Pontederia rotundifolia* and *P. sagittata* from Barrett (1977 a) and Glover and Barrett (1983), respectively

The high level of self-compatibility in the M morph of *Pontederia* spp. could result from modifier genes linked to the dominant M allele at the locus governing mid-style length. Alternatively, weak self-incompatibility may be a pleiotropic effect of genes that directly control the expression of tristylous characters such as style length, anther height, or pollen size (Glover and Barrett 1983). Evidence to support the 'linked modifier gene hypothesis' would be difficult to obtain and would depend in part on observation of recombinant phenotypes with altered incompatibility behaviour. To date no genotype of the M morph that has been tested in each of the four taxa has exhibited high self-incompatibility, although occasional genotypes of the L morph can be highly self-compatible. Data from the pollination programme and from self-pollinations of segregating progenies favour the pleiotropy hypothesis to explain difference in incompatibility behaviour among the floral morphs. However, the patterns of expression within each of the floral morphs suggests that variation in trimorphic incompatibility also results from polygenes as well as environmental effects.

Although little is known of the genetical organization and developmental basis of tristylous the overall consistency among *Pontederia* species in the expression of trimorphic incompatibility in the M morph suggests a common causal explanation. In an effort to provide an explanation we propose an hypothesis that involves consideration of the developmental peculiarities of tristylous flowers. While the hypothesis is speculative in nature it does make predictions which are readily testable and in addition provides a unified explanation for several apparently unrelated characteristics of the tristylous genetic polymorphism in the Pontederiaceae.

Legitimate pollinations of the M morph involve pollen grains produced by mid-level anthers of the L and S morphs. In contrast to long- and short-level anthers the developmental patterns of these anthers differ in the two morphs. This arises because of differences in the insertion of stamens in tristylous members of the Pontederiaceae. Within flowers of each of the floral morphs the shorter anther set originates on the upper side of the perianth tube whereas the longer anther set develops on the lower side. The size of tepals on which the stamens are inserted also differs between the two anther sets. While two of the three anthers of the shortest set are associated with narrow tepals, the remaining anther is inserted on a broad tepal. The insertion patterns are reversed in the longest anther set, with two anthers associated with broad tepals and one anther inserted on a narrow tepal. Thus, while the long-level anthers of the M and S morphs and the short-level anthers of L and M morphs have identical insertion patterns, the positional arrangement of mid-level anthers differs between the L and S morphs. In the L

morph mid-level anthers are the longest set and are positioned on the lower portion of the perianth tube, whereas in the S morph they are the shorter set and are therefore inserted on the upper side of the perianth tube. These complex patterns were first noted in *P. cordata* by Price and Barrett (1982) and their developmental basis has been investigated in detail in the related *Eichhornia paniculata* by Richards and Barrett (1984).

The developmental heterogeneity of mid-level anthers in *P. cordata* is associated with differences in anther size and pollen production (Price and Barrett 1982). Mid-level anthers of the S morph are larger and produce approximately  $\times 2$  as many pollen grains than those of the L morph. A similar difference in mid-level pollen production is evident in *P. sagittata* (Glover and Barrett 1983). In contrast, pollen production within each of the two long-level and short-level anthers of the floral morphs is similar. The differences in the insertion, size and pollen production of mid-level anthers are associated with their different positional relationships and developmental patterns. We hypothesize that such effects may also influence the incompatibility phenotype of mid-level pollen. If pollen from mid-level anthers is biochemically heterogeneous, the M morph may require a broader physiological range of compatibility than the L and S morphs, to facilitate 'acceptance' of the two compatible mid-level pollens. This range may overlap with that involving illegitimate pollinations with pollen from long-level anthers. Why pollen from short-level anthers is prevented from producing seed in illegitimate pollinations of the M morph is unclear. This could result from inadequate storage reserves in the small pollen grains. Our studies of pollen tube growth in *P. cordata*, to be reported elsewhere, indicate that pollen tubes from small sized pollen grains cease growth approximately three mm into the styles of the L and M morph. In contrast, pollen tubes from medium and large-sized pollen grains grow for successively greater distances down styles in illegitimate pollinations, suggesting that part of the incompatibility mechanism is associated with pollen size effects (Anderson and Barrett, unpublished data).

A prediction from our hypothesis to account for the high-level of self-compatibility in the M morph of *Pontederia* is that the incompatibility behaviour of pollen from mid-level anthers of the L and S morphs in illegitimate pollinations should differ, whereas those involving long- and mid-level pollen should not. Examination of seed set data from controlled pollinations of *P. cordata* (Ornduff 1966) and *P. rotundifolia* (Barrett 1977a) provide some support for such an effect. In both species, mid-level pollen from the L and S morphs differs in behaviour in intermorph illegitimate pollinations. While both morphs express similar levels of seed set following self and intramorph pollinations with pollen from mid-level anthers, lower values result when mid-level pollen from the alternate morph is employed in intermorph illegitimate cross-

**Table 4.** Mean percentage seed set following self, intramorph, and intermorph illegitimate pollination with pollen from the mid-level anthers of the L and S morphs of *Pontederia cordata*<sup>a</sup> and *Pontederia rotundifolia*<sup>a</sup>

Pollination treatment	<i>Pontederia cordata</i>	<i>Pontederia rotundifolia</i>
Intramorph:		
L × m/L self	19.2	60.1
L × m/L cross	18.2	67.4
Intermorph:		
L × m/S	11.7	17.8
Intramorph:		
S × m/S self	12.8	35.1
S × m/S cross	11.4	45.9
Intermorph:		
S × m/L	2.7	28.1

<sup>a</sup> Data from Ornduff (1966)

<sup>b</sup> Data from Barrett (1977 a)

pollinations (Table 4). This pattern is not evident in intermorph illegitimate pollinations with pollen from long- or short-level anthers, or in illegitimate pollinations of the M morph. These data suggest that differences in incompatibility phenotype of mid-level pollen are present in illegitimate pollinations. We are currently extending these observations to examine in detail the incompatibility phenotypes of mid-level pollen, their biochemical characteristics and development. It would be of interest to examine the insertion patterns of stamens in the other tristylous families to determine whether a similar developmental model might apply. In *Lythrum salicaria*, Darwin (1877) and Stout (1923) reported that the weakest self-incompatibility was expressed in self-pollinations of the M morph with pollen from long-level anthers.

Considerable genetic variation in the expression of trimorphic incompatibility is evident in the Paugh Lake population of *P. cordata* var. *cordata*. Although a range of self-compatibility values are expressed, the mating system of *P. cordata* is probably largely outcrossed since pollen tubes from legitimate pollen grow more rapidly in styles than pollen tubes from illegitimate pollen (Anderson and Barrett, unpublished data). Studies of population structure, pollen flow patterns, and marker genes provide additional evidence that *P. cordata* is primarily an outcrossing species (Barrett et al. 1983; Price and Barrett 1984). However, if ecological conditions interfered with the maintenance of trimorphic incompatibility, self-compatible genotypes might readily be selected. Processes of this kind may have occurred in *P. parviflora*, a self-fertile monomorphic species, as well as in the related genus *Eichhornia* where tristily has broken down on a number of occasions to give rise to semi-homostylous selfing forms (Barrett 1985 b). Interestingly floral modifications most often involve the M morph. Why this morph appears more prone to modifications that promote increased selfing is an interesting problem. The answer may lie in the developmental constraints imposed by design of the polymorphism itself.

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