

R. W. Scribailo · S. C. H. Barrett

## Effects of prior self-pollination on outcrossed seed set in tristylous *Pontederia sagittata* (Pontederiaceae)

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**Abstract** The potential inhibitory effects of incompatible pollen on outcrossed seed set were investigated in mass-flowering, self-incompatible, tristylous *Pontederia sagittata*. Prior application of self pollen, followed after 2, 4, or 6 h by compatible pollen, was conducted on five genotypes of each of the three style morphs under uniform glasshouse conditions. The greatest reductions in seed set occurred in pollinations of the long-styled (L) morph at the 6 h time interval. Smaller reductions were also found for this treatment in the mid-styled (M) morph. No significant reductions in seed set were observed in the short-styled (S) morph or in the other morphs at shorter time intervals. Observations of pollen germination and pollen tube growth indicated that the lack of inhibitory effects in the S morph may occur because relatively few pollen grains adhered to stigmas in self-pollinations. In the L and M morphs, early germination of self pollen may cause physical clogging of the stigma and style, resulting in a reduced number of compatible pollen tubes in styles. Observations of the structural integrity of styles indicated that prior germination of self pollen resulted in more rapid onset of pistil senescence, particularly in the L morph. These influences may contribute to the morph-specific differences in seed set observed following prior self-pollination of outcrossed flowers. The negative effects of incompatible pollen are likely to be most evident where ecological factors cause delays in the delivery of outcross pollen to stigmas.

**Key words** Pollen-pistil interference · Prior self-pollination · Tristylous · Self-incompatibility · Styler senescence

### Introduction

The inhibitory effects of incompatible pollen on female function have often been invoked as a potential factor affecting plant reproductive success. This type of reproductive interaction and other forms of pollen-pistil interference have frequently been suggested as possible selective forces influencing the evolution of various plant reproductive traits (Bawa and Opler 1975; Zapata and Arroyo 1978; Snow 1982; Bertin 1985; Lloyd and Yates 1982; Lloyd and Webb 1986, 1992; Webb and Lloyd 1986; R. Bertin and C. Newman, unpublished data). Despite recent interest in pollen-pistil interference, there are few convincing demonstrations that the deposition of self pollen on stigmas leads to any significant negative effects on the female fertility of self-incompatible plants (although see Waser and Price 1991). Part of the difficulty, particularly in plant species with homomorphic incompatibility, lies in distinguishing whether observed reductions in seed set following mixed pollinations are attributable to mechanisms of interference, self-incompatibility phenomena, and/or the post-zygotic abortion of developing embryos (Crowe 1971; Seavey and Bawa 1986; Barrett 1988; Bertin and Sullivan 1988; Marshall and Ellstrand 1989; Marshall 1990; Weller and Ornduff 1991; Montalvo 1992; Walsh and Charlesworth 1992).

Heterostylous plants provide useful experimental systems for investigating various aspects of pollen-pistil interference (Shore and Barrett 1984; Barrett and Glover 1985; Nicholls 1987; Murray 1990; Kohn and Barrett 1992). Not only are the incompatibility relationships of plants greatly simplified because of the small number of mating groups (two in distyly, three in tristyly), but the presence of pollen heteromorphism allows measurement of the number of incompatible versus compatible pollen grains on stigmas and hence an eval-

R. W. Scribailo · S. C. H. Barrett  
Department of Botany,  
University of Toronto,  
Toronto, Ontario, Canada M5S 3B2,  
Fax no: (416) 978-5878

R.W. Scribailo (✉)  
Biological Sciences and Chemistry Section,  
Purdue University North Central,  
Westville, IN 46391, USA

uation of the likely intensity of such interactions. Studies of the pollen composition of open-pollinated stigmas in natural populations of heterostylous plants have demonstrated that floral heteromorphism promotes compatible pollen transfer (Ganders 1974; Barrett and Glover 1985; Piper and Charlesworth 1986; Lloyd and Webb 1992). Nevertheless, these studies and many others (reviewed in Ganders 1979) have also shown that stigmas of the floral morphs capture significant amounts of incompatible pollen. This is not unexpected, given the much higher probability that outcross pollen will be incompatible in heterostylous populations, compared to species with homomorphic incompatibility.

Observations of pollen loads raise the question of whether incompatible pollen may have deleterious effects on the ability of compatible pollen to achieve fertilization in heterostylous plant populations. The goal of the present study was therefore to determine whether any negative effects of the prior application of self pollen could be demonstrated in compatible pollinations of tristylous, self-incompatible *Pontederia sagittata*. This species is particularly appropriate for such a study for two reasons: (1) *P. sagittata* is a mass-flowering, clonal species (Scribailo and Barrett 1991a), hence opportunities for geitonogamous self-pollinations are high. Indeed, studies of the pollen loads of open-pollinated flowers in natural populations indicate that considerable amounts of incompatible pollen are deposited on stigmas by insect pollinators (Glover and Barrett 1983); (2) studies of pollen tube growth and seed set after self-pollination indicate that post-zygotic abortion of developing embryos occurs at very low levels (Scribailo and Barrett 1991b). Hence in this species these features are unlikely to complicate analyses of causes of reduced seed set. Failure to set seed in sequential incompatible and compatible pollinations is therefore more likely to result from pollen-pistil interference than other potential reproductive mechanisms.

Three specific questions were addressed in our study. (1) Does the prior application of self (incompatible) pollen to stigmas reduce the probability of seed set following subsequent cross-pollination? (2) Is such an effect influenced by the time interval between self- and cross-pollinations? (3) Do the three style morphs differ in their response to the pollination treatments because of intrinsic differences in style length? In addition, observations of pollen germination, pollen tube growth, and stylar function following the experimental pollinations were made in an attempt to interpret the mechanisms responsible for the patterns of seed set observed.

## Materials and methods

### Plant material

Plants were grown as emergent aquatics in submersed plastic pots in a single, flooded bench under glasshouse conditions at the University of Toronto. Plants were fertilized regularly and maintained at a temperature of 25–35°C during the experimental period. Five genotypes of each style morph were used in the experiments.

These represented genetic stocks originally collected as open-pollinated seed from a single population in Mexico (see Glover and Barrett 1983 and Scribailo and Barrett 1991a for further details of the culture, growth, and reproductive characteristics of the species).

### Pollination program

Experimental treatments consisted of three intervals (2, 4, and 6 h) between the application of incompatible and compatible pollen for each of the three style morphs (L, M, and S). Incompatible pollinations were conducted at the beginning of anthesis at 9:00 a.m. by contacting stigmas with a single anther of self pollen clasped in fine forceps, from the most incompatible stamen level for each morph (see Glover and Barrett 1983 and Scribailo and Barrett 1991b for data on the compatibility relationships of different pollen-pistil combinations in the species). These were short-level stamens in the L morph, short-level stamens in the M morph, and long-level stamens in the S morph. This was followed by pollinations with one anther of compatible pollen (legitimate pollen from a different floral morph) with either a 2, 4, or 6 h delay from the time of the initial self-pollination. Control pollinations consisted of flowers pollinated with compatible pollen at 0 (9:00 a.m.), 2 (11:00 a.m.), 4 (1:00 p.m.) or 6 h (3:00 p.m.) after the commencement of anthesis. For each experimental treatment (three style morphs × three time intervals, plus controls), all flowers on a single inflorescence per genotype were given the same pollination type.

All hand-pollinated inflorescences were bagged a week after pollination and mature seed was collected and counted 3 weeks later. Since flowers of *P. sagittata* are uniovulate, all seed set values refer to percentage seed set per inflorescence.

### Statistical analysis

Seed set data were analysed using a repeated measures univariate analysis of variance (ANOVA) in the GLM procedure of SAS (SAS Institute 1988). The analysis makes stringent assumptions about the variance-covariance structure of the data which were tested using Mauchly's test of sphericity (Potvin et al. 1990). The data did not violate the assumption of compound symmetry (Criteria = 0.888,  $P = 0.519$ ). Sources of variation used in the analysis included the between-subject effect of style morph (fixed effect), and the within-subject effects of time interval before compatible pollination (fixed effect), genotypes within morph (random effect), and the interaction of time interval and morph. Compatible pollinations (controls; see above) at each time interval were used to investigate differences in seed set resulting from the age of flowers. Relative reduction in seed set values were used in the ANOVA and were calculated as the percentage seed set for the treatment subtracted from the percentage seed set of the control. The number of pollinations conducted for each treatment was used to weight the analysis with values of relative seed set weighted by one half the sample size of the treatment plus one half the sample size of the control pollinations. Differences in relative seed set between main effects (style morph and time) were tested using specific contrasts.

### Pollen tube growth

In an effort to determine factors that may contribute to the observed decline in seed set after some pollination treatments (see below), observations of pollen tube growth using the following methods were undertaken. Experimental pollinations were performed as in the treatments and controls, but 6 h after the application of compatible pollen, pistils were excised from flowers and fixed overnight in 3:1 95% alcohol:acetic acid. Pistils were then cleared in 2 M NaOH at 55°C for 8 h, rinsed six times in water, and mounted in 0.1 M aniline blue (Polyscience) in tribasic potassium phosphate (pH 11.6) (Dumas and Knox 1983). Pollen and

pollen tubes were observed under a Zeiss Axioplan photomicroscope equipped with epifluorescence using a no. 2 ultraviolet filter system (excitor G-365, dichromatic beam splitter Ft-395, barrier filter Lp-420). Significant differences in pollen grain size (Glover and Barrett 1983) and pollen tube width (Scribailo and Barrett 1991b) among the pollen types from the three anther levels in *P. sagittata* allowed unambiguous distinction between compatible and incompatible pollen grains on the stigma surface and pollen tubes in the pistil.

## Results

### Floral characters

Flowers of *Pontederia sagittata* undergo anthesis within a single day. They open by 9:00 a.m., begin to wilt by late afternoon, and are completely senescent by evening. Complete flowering of an inflorescence takes 4–7 days with 15–35 flowers open each day. Inflorescence size varied among the style morphs with mean number of flowers (based on pooling inflorescences from all controls and treatments for each morph,  $n=40$ ). Mean flower number per inflorescence was 100.4 (SD 35.4) for the L morph, 115.1 (SD 43.1) for the M morph, and 88.5 (SD 25.4) for the S morph. Differences in means were marginally significant (at the  $P=0.05$  level) for comparisons between the L and M morphs and the L and S morphs. Differences between the M and S morphs were highly significant ( $P<0.005$ ).

### Effects of prior self-pollination on seed set

Data on the mean percentage seed set per style morph of all experimental treatments and controls are presented in Table 1. Control pollinations involving compatible pollen applied to stigmas at different time intervals after the commencement of flowering indicated a significant effect of flower age and style morph on percentage seed set (two-way ANOVA: interval  $F=5.55$ ,  $P<0.0024$ ; style morph  $F=31.35$ ,  $P<0.0001$ ; interaction  $F=0.76$ ,

NS). Percentage seed set in control pollinations declined significantly with a 6 h time delay in the application of compatible pollen, particularly in the L and M morphs (Table 1).

The overall effect of prior self-pollination on relative seed set was significant ( $F=4.05$ ,  $P<0.0001$ ), with the strength of the effects varying with style morph and the time of application of outcrossed pollen (Table 2). The L morph exhibited the largest decline in relative seed set, with the greatest effect evident with longest time interval between the application of the two pollen types (Fig. 1). A similar but weaker trend was evident in the percentage seed set data for the M and S morphs. Since there was no interaction between style morph and time interval in the analysis of relative seed set data, it was possible to test the main effects in the ANOVA. Both were statistically significant (Table 2). Relative seed set was significantly reduced in treatment flowers of the L and M morphs, but not in flowers of the S morph (L morph

**Table 2** Repeated-measures analysis of variance of relative seed set in *Pontederia sagittata*. The  $F$  test for the main effect of style morph and contrasts between morphs used the mean square of plant within morph as the denominator. All other sources of variation were tested over the mean square error (Neter et al. 1990)

	Source	df	Mean square	F
	Morph	2	517374.2	18.49***
	Plant (morph)	12	27974.4	1.23
	Time	2	110741.8	4.86*
	Morph*time	4	32399.2	1.42
Contrasts				
	Morph L vs S	1	937718.3	33.5***
	Morph L vs M	1	575605.5	20.6***
	Morph M vs S	1	68219.4	2.4
	Time 2 vs 4 h	1	127995.8	5.6*
	Time 2 vs 6 h	1	200616.0	8.8**
	Time 4 vs 6 h	1	10432.3	0.5

\*  $P<0.05$

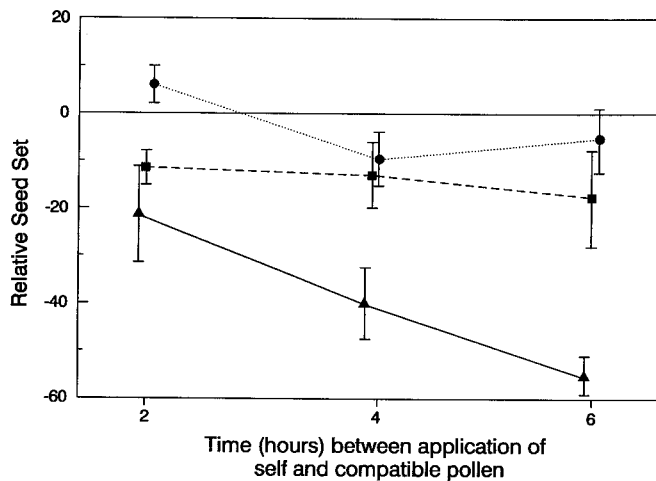
\*\*  $P<0.01$

\*\*\*  $P<0.001$

**Table 1** Mean percentage seed set ( $\pm$  SD) for control and treatment pollinations of the style morphs of *Pontederia sagittata*. Intervals indicate time since onset of anthesis in the case of control

Morph	Interval	Interval			
		0 h	2 h	4 h	6 h
L	Control	92.6 $\pm$ 3.61 (573)	90.6 $\pm$ 10.11 (371)	93.1 $\pm$ 5.60 (445)	82.8 $\pm$ 5. (520)
	Treatment		69.6 $\pm$ 21.7 (530)	53.0 $\pm$ 14.09 (536)	28.1 $\pm$ 11.92 (610)
M	Control	88.4 $\pm$ 8.22 (725)	94.4 $\pm$ 3.50 (614)	91.9 $\pm$ 5.54 (717)	80.3 $\pm$ 13.22 (475)
	Treatment		83.1 $\pm$ 10.94 (482)	79.0 $\pm$ 11.78 (569)	62.9 $\pm$ 11.60 (548)
S	Control	77.6 $\pm$ 10.35 (458)	69.5 $\pm$ 5.48 (454)	76.3 $\pm$ 14.24 (473)	66.5 $\pm$ 11.70 (492)
	Treatment		75.4 $\pm$ 6.18 (419)	66.8 $\pm$ 10.91 (488)	61.2 $\pm$ 8.13 (345)

pollinations and time since application of self pollen for treatment pollinations. The total numbers of pollinations for the five plants pollinated per treatment combination are in parentheses



**Fig. 1** Mean relative seed set ( $\pm$  standard error) in experimental pollinations of the three style morphs of *Pontederia sagittata* conducted at different times (2, 4, 6 h after application of self pollen). A value of 0 indicates no difference in seed set between the experimental treatment and the control (see Materials and methods for further details)

$t = -9.93$ ,  $P < 0.0001$ ; M morph  $t = -3.29$ ,  $P = 0.0031$ ; S morph  $t = -0.61$ ,  $P = 0.547$ ). Specific contrasts of relative seed set data from the experimental treatments are presented in Table 2. Reductions in relative seed set were significantly greater in the L morph than in the M and S morphs, whereas the M and S morphs did not differ significantly in their responses. Relative seed set values averaged across the style morphs for the 2 h time delay were significantly higher than values obtained for the 4 and 6 h treatments. There was no significant difference in the relative seed set of the 4 and 6 h treatments.

The lower seed set values of both control and treatment pollinations of the S morph compared to the L and M morphs (Table 1) may reflect technical difficulties associated with hand-pollination of this morph. Not only does the recessed stigma of the S morph reduce the accuracy of pollination, but its stigma is also significantly smaller than in the L and M morphs (Scribailo and Barrett 1991 a). Lower seed set of the S morph has been reported consistently in experimental hand-pollinations of *Pontederia* species, including *P. sagittata* (Barrett 1977, Glover and Barrett 1983; Barrett and Anderson 1985).

### Pollen tube growth

In *P. sagittata*, pollen tubes initially enter a hollow trilobed stylar canal that subsequently separates into three distinct canals at about the mid-point of the style. Only one of these canals leads to a fertile carpel containing the ovule. The other two canals end in vestigial carpels (Scribailo and Barrett 1991 a). The vast majority of pollen tubes enter the fertile carpel, although a small number of pollen tubes may terminate growth in the vestigial carpels (Figs. 3, 5, 7).

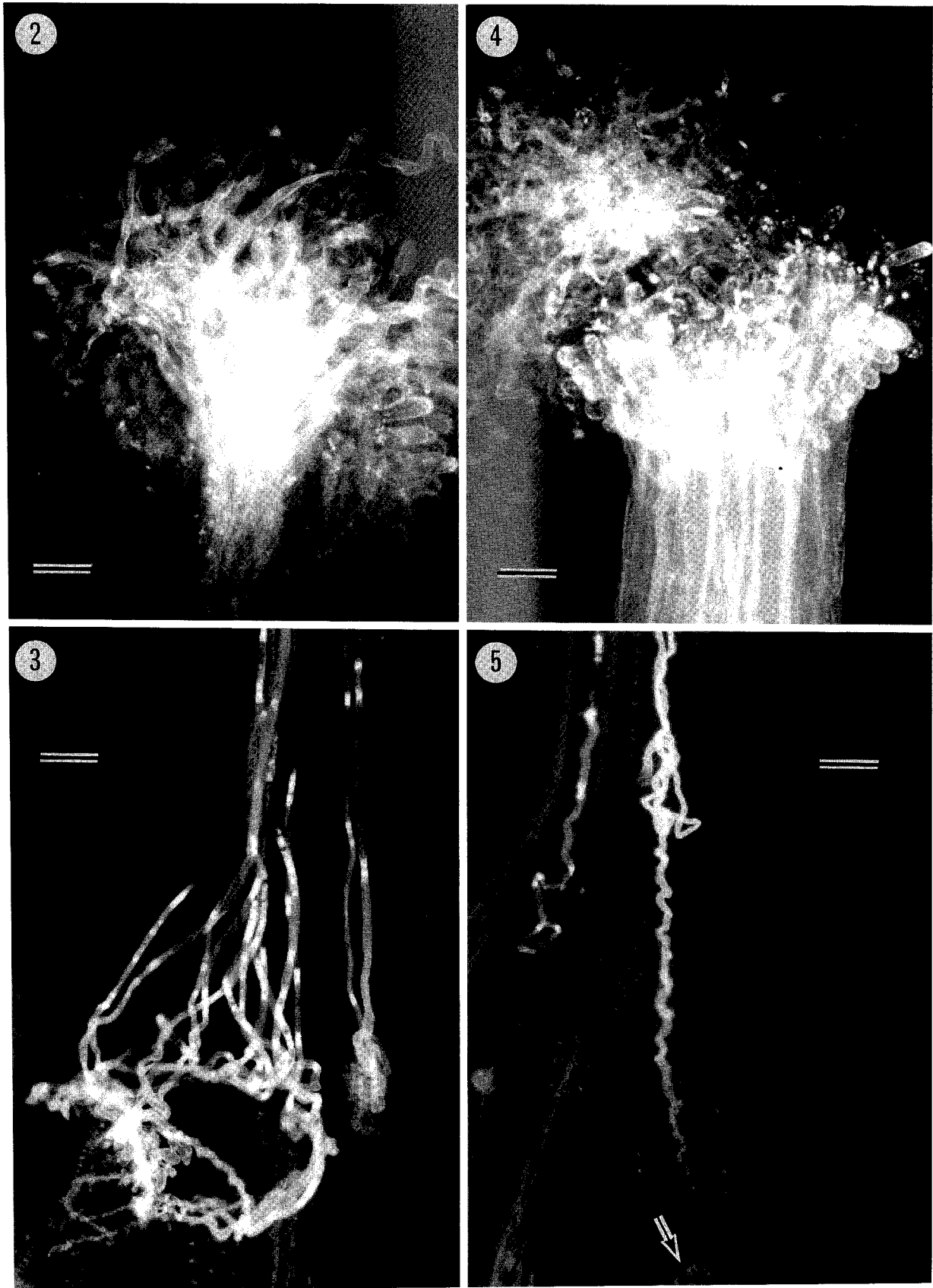
Observations of pollen tube growth in compatible control pollinations, over the range of intervals, indicated that some pollen tubes reach the ovule by 2 h after pollination. By 4 and 6 h after pollination, large numbers of pollen tubes were observed on the stigma (Fig. 2), in the style and ovary, and contacting the obturator of the ovule (Figs. 3, 7), although only a single pollen tube was ever observed entering the micropyle (Fig. 7).

For compatible pollination controls, no differences in behaviour of pollen tubes was observed for the M and S morphs at any of the intervals before pollination. In control pistils of the L morph pollinated at the 6 h interval (3:00 p.m.) and collected 6 h later, however, visibly fewer pollen tubes in the style were observed than in those examined from the 4 h (1:00 p.m.) interval.

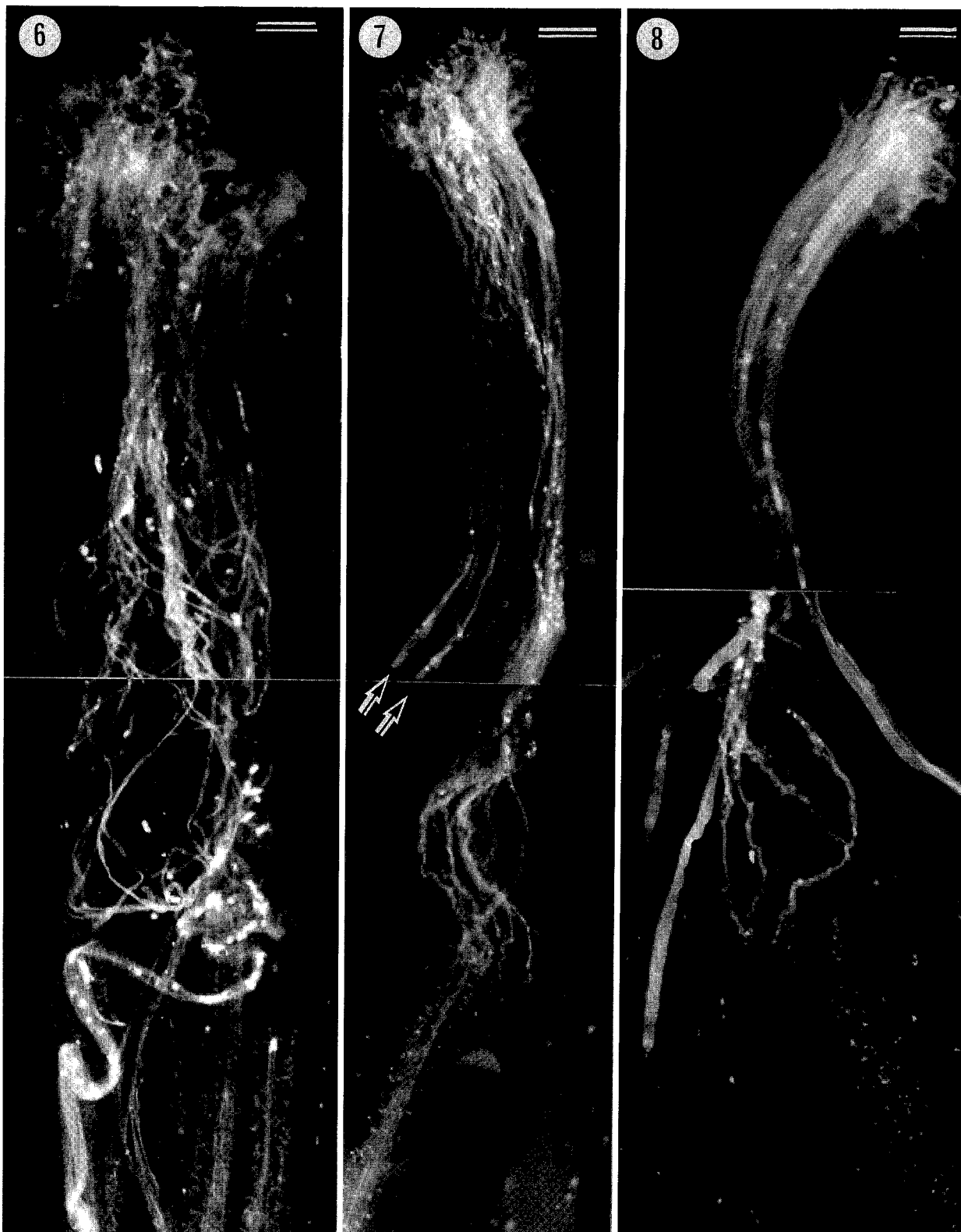
Observations of pollen tube growth in the 4 and 6 h experimental treatments, collected 6 h after application of compatible pollen, provided insights into possible mechanisms responsible for reductions in seed set in the L and M morphs. Many self pollen (short-level) grains were observed germinating on stigmas, with large numbers of pollen tubes entering into the style. The self pollen tubes then terminated growth in the upper third of the style in both morphs. The behaviour of compatible pollen in the experimental treatments varied among the pistils examined. A small number of pistils of the L and M morphs showed germination of compatible pollen on the stigma but not compatible pollen tubes entering into the stigma (Fig. 4). Inability of pollen tubes to enter into the style appeared to result from physical clogging of the stigma by germinating self pollen tubes. This effect was more pronounced at the 6 h interval between self- and compatible pollination, presumably because of an increase in the number of germinating self pollen grains.

The majority of pistils from the 6 h experimental treatments of the L and M morph showed compatible pollen tubes entering the stigma, by-passing self pollen tubes in the upper third of the style, and continuing down the style (Fig. 6). Many of the styles, however, had a reduced number of compatible pollen tubes present relative to the number observed in control pistils. In most of these pistils, particularly in the L morph, a rapid attrition in the number of pollen tubes was observed towards the base of the style, with either no pollen tubes

**Figs. 2–5** Pollen tube growth in the L morph of *Pontederia sagittata*. Bars 0.1 mm. **Fig. 2** Many compatible pollen tubes from a control pollination entering the stigma and style. **Fig. 3** Many pollen tubes entering the ovary from a compatible control pollination. The majority of pollen tubes have contacted the obturator of the ovule, which has been displaced to the left out of the micrograph. The two pollen tubes on the right have terminated growth in one of the vestigial carpels. **Figs. 4, 5** Six hour treatment pollinations. **4** A large majority of compatible pollen grains have germinated on the stigma but have not penetrated the stigma perhaps because of clogging by self pollen tubes. **5** A single pollen tube reaching the ovary and effecting fertilization. The micropyle is indicated by an arrow. The pollen tube on the left is in a vestigial carpel



Figs. 2-5



**Figs. 6-8** Pollen tube growth in the L and S morphs. Bars 0.1 mm. **Fig. 6** Six hour experimental pollinations of the L morph. A large number of incompatible pollen tubes have terminated growth in the upper third of the style. Two compatible pollen tubes have by-passed the incompatible pollen tubes. **Figs. 7, 8** Pollen tube growth in the S morph. **Fig. 7** Compatible control pollinations with many pollen tubes growing to the ovary and contacting the obturator but with only a single pollen tube effect-

ing fertilization. The two groups of pollen tubes on the left (*arrows*) are in the two styler canals leading to vestigial carpels. **Fig. 8** Six hour experimental pollinations showing compatible pollen tubes reaching the ovule. The incompatible pollen tubes are clearly visible because of their wider diameter. The ovule has been displaced to the left in preparation. The incompatible pollen tubes are by-passing the obturator of the ovule and continuing to grow down the face of the ovule

reaching the ovary or one or two pollen tubes reaching the ovary and apparent fertilization occurring (Fig. 4).

In the S morph, behaviour of compatible pollen tubes in experimental pollinations was identical to that observed in control pollinations (Figs. 7, 8). For the experimental pollinations, few self pollen grains were observed on stigmas. Only a small number of self pollen tubes were ever observed in the style and ovary, reflecting the small number of self pollen grains able to adhere to the stigma surface. Some self pollen tubes entered the ovary but in the majority of cases they by-passed the obturator of the ovule entirely, terminating growth on the funiculus or outer surface of the ovule (Fig. 8). In cases where contact with the obturator occurred, pollen tubes failed to grow through this structure and achieve fertilization. Lack of any inhibitory effects of prior self-pollination in this morph may be explained by the observation that both the number of self pollen grains on the stigma and self pollen tubes in the style were too few to cause interference with compatible pollen tube growth.

## Discussion

The major findings of this study are that prior self-pollination of stigmas in *P. sagittata* can reduce the seed set of flowers that are subsequently pollinated by compatible pollen. This effect, however, depends on both the time interval between the application of incompatible and compatible pollen and the particular style morph involved in pollinations. A significant reduction in seed set was observed in the L morph, particularly at the longest time interval between the two pollinations. Smaller but non-significant reductions in seed set were evident in the M morph, and prior self-pollination had no apparent effects on outcrossed seed set in the S morph. Analysis of the patterns of seed set in control and experimental treatments and observations of stylar behaviour, pollen germination, and pollen tube growth suggest several possible mechanisms that could account for the morph-specific patterns of seed set obtained.

### Potential mechanisms

The first potential mechanism of pollen-pistil interference that could account for reduced seed set is the physical clogging of stigmatic papillae and styles by early-germinating self pollen. This study confirmed earlier work on *P. sagittata* (Scribailo and Barrett 1991 b) that indicated large numbers of small, short-level pollen grains readily adhere, hydrate, and germinate on the larger, wetter stigmas of the L morph, in particular, but also on stigmas of the M morph which have similar characteristics. In contrast, large pollen grains from long-level anthers had difficulty adhering to and germinating on the significantly smaller and drier stigmas of the S morph. Since short-level anthers produce 5–6 times more pollen grains than long-level anthers

(Table 4 of Glover and Barrett 1983), stigmatic and stylar clogging is more likely to occur in self-pollinations of the L and M morphs with short-level pollen than in self-pollinations of the S morph using long-level pollen. While our observations of pollen tube growth in the S morph failed to reveal any significant difference between the experimental treatment and controls, in the L and M morphs a reduced number of compatible pollen tubes at the base of the style was evident in experimental flowers, particularly those involving longer time intervals between the two pollination types. This reduction could be associated with the large numbers of incompatible pollen tubes present in the upper parts of L and M styles.

Few studies have shown conclusively that self pollen reduces the number of cross pollen tubes in the style following mixed or sequential pollinations. In *Brassica oleracea*, the presence of incompatible pollen caused a two-thirds reduction in pollen tubes in the style at 0–6 h intervals, but this effect rapidly decreased and by 24 h was negligible. The self pollen alone produced virtually no pollen tubes in the style (Ockendon and Currah 1977). In pollinations using pairs of pollen donors in *Erythronium grandiflorum*, Cruzan (1990) found that pollen paired with self pollen exhibited greater rates of pollen tube attrition in the style than when paired with itself or with other outcrossed pollen. In distylous *Luculia gratissima*, Murray (1990) found that in both mixed and sequential pollinations involving compatible and incompatible pollen, incompatible pollen tubes had no effect on the rate of growth of compatible pollen tubes. The only interaction he observed was an increase in the percentage of abnormalities exhibited by incompatible pollen tubes in the presence of compatible pollen.

A second potential mechanism that could account for the morph-specific differences in outcrossed seed set observed in our study involved biochemical changes in styles of the morphs following the early application of incompatible pollen. Several studies of horticultural species have shown that pollination triggers ethylene production which brings about an onset of floral senescence (Labarca and Loewus 1973; Halevy 1986; Hoekstra and van Roekel 1986; Visser 1986; Reid 1989). In *Petunia hybrida* (Gilissen 1977, Sastri and Shivanna 1978) and *Lilium longiflorum* (Campbell and Linskens 1984), pollination with self pollen results in more rapid senescence of pistils than with outcrossed pollen. A similar phenomenon has been recorded in apple and pear (Visser and Marcucci 1983). Following from this, a hypothesis that may explain data from the experimental treatments of the L morph is that prior pollination with self pollen brings about an increased rate of pistil senescence, with the longer styles of the L morph losing their physiological integrity more rapidly than those of the M and S morphs. This hypothesis is supported by observation of the physical appearance of long styles which after 6 h appeared to be more wilted than the styles of the other two morphs. The observation of reductions in seed set in control pollinations of the L morph at the 6 h interval also suggests that the integrity of the longer,

more exposed styles of the L morph may be lost more rapidly than in the other morphs, irrespective of whether they are pollinated or not. It is possible that the protection afforded to styles of the M, and particularly the S morph, by the tubular perianth of *P. sagittata* flowers may result in a microclimate more favourable for maintaining stylar function. Tubular flowers are usually characterized by an intrafloral humidity gradient, with humidity increasing above ambient levels at the base of the flower (Corbet et al. 1979).

Another hypothesis that may explain the reductions in seed set of the L morph involves the possibility that the presence of self pollen grains on the stigma surface and self pollen tubes in the style delays germination and growth of compatible pollen tubes sufficiently that they cannot reach the ovule before floral senescence halts further growth. Observations of pollen tubes in the style indicated that, although compatible pollen tubes bypassed incompatible pollen tubes, numbers were greatly reduced relative to controls. The fact that in some L styles pollen tubes failed to reach the ovary supports the idea that stylar senescence may occur before pollen tubes have reached the ovule and achieved fertilization.

#### Ecological and evolutionary significance

While the mechanism(s) responsible for morph-specific differences in seed set following prior self-pollination in *P. sagittata* remain unknown, several lines of evidence from other heterostylous plants suggest that the L morph may be more prone to these types of effect than the other style morphs. In an experimental study involving distylous *Turnera ulmifolia*, Shore and Barrett (1984) found that prior self-pollination reduced outcrossed seed set in the L morph, but only with 1.5 and 3.5 h delays. They were unable to demonstrate any inhibitory effects of the experimental treatments on the S morph and suggested the difference may have resulted from contrasting sites of pollen tube inhibition in the style morphs (L morph-style vs S morph-stigma) and pollen production differences between the anther levels used in self pollinations. The narrower stylar transmitting tract in the L morph may be more susceptible to physical clogging by incompatible pollen tubes than the larger area afforded by the stigmatic surface.

Several field studies of tristylous species at the northern limits of their native ranges – *Pontederia cordata* in Ontario, Canada (Wolfe 1985), *Decodon verticillatus* in Ontario, Canada (C. Eckert and S. Barrett, unpublished data), and *Lythrum salicaria* in northern Sweden (J. Ågren, unpublished data) – have documented reduced seed set in the L morph compared with the M and S morphs. In these cases, lower seed set appears to be unrelated to differential pollinator visitation to the morphs and could perhaps be due to the timing and amount of self and outcross pollen delivery and slow rates of pollen tube growth under suboptimal conditions. It is noteworthy that in *P. cordata* reduced seed set

in the L morph was only apparent at the end of the season when temperatures were cool.

Despite the inhibitory effects of prior self-pollination on seed set in *P. sagittata*, it is possible that the effects obtained here under glasshouse conditions are uncommon in natural populations of this tropical species. In our study large amounts of incompatible pollen were applied to stigmas (e.g., a single anther from long-level or short-level stamens contains approximately 800 and 3500 pollen grains, respectively) whereas natural pollen loads usually contain much less incompatible pollen, even though it frequently outnumbers compatible pollen (see Table 9, Glover and Barrett 1983). Since flowers of *P. sagittata* are uniovulate and short-lived, the presence of even a small number of compatible pollen grains on stigmas may well suffice to bring about high levels of seed set. Indeed, pollinator activity and levels of seed set in tristylous *Pontederia* populations are usually high (e.g., Barrett 1977; Barrett and Glover 1985; Harder and Barrett 1992), suggesting that female reproductive success may only rarely be limited by the inhibitory effects of incompatible pollen. An attempt to demonstrate the negative effects of prior application of self pollen to open-pollinated flowers of *P. cordata* failed to demonstrate any reduction in seed set (Barrett and Glover 1985), presumably because sufficient numbers of compatible pollen grains were delivered to flowers by pollinators after self-pollination.

As pointed out by Shore and Barrett (1984), studies attempting to investigate the ecological and evolutionary significance of prior self-pollination should ideally include the range of compatible and incompatible pollination levels that populations commonly experience, notwithstanding the technical difficulties involved in designing such experiments. Even if realistic pollination levels can be achieved, however, the lack of data concerning the arrival, germination, and pollen tube growth schedules of different pollen types (Mulcahy et al. 1983; Galen et al. 1986; Snow 1986; Epperson and Clegg 1987; Thomson 1989; Barrett et al. 1992) under field conditions may still hamper attempts at a realistic evaluation of interactions between compatible and incompatible pollen. As this study indicates, the likelihood of negative influences of prior self-pollination will likely increase where ecological factors cause delays in the delivery of compatible pollen.

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