

ON THE DARWINIAN HYPOTHESIS OF THE ADAPTIVE SIGNIFICANCE OF TRISTYLY

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Abstract.—Darwin proposed that the function of the stamen-style polymorphism in heterostylous plants is to increase the probability of legitimate (compatible) pollinations among the floral morphs. Conspicuous pollen trimorphism in tristylous *Pontederia cordata* enables a test of the hypothesis. Comparison of the composition of pollen loads in naturally pollinated stigmas of intact and emasculated flowers were made at a population in Paugh Lake, Ontario, which was visited primarily by bumblebees. The magnitude of legitimate pollination was analyzed by ANOVA. In intact flowers, significant legitimate pollination was detected in the long-styled morph only. Following emasculation legitimate pollination was evident in the long- and short-styled morphs, with the mid-styled morph just short of displaying significant legitimate pollination. Similar results were obtained by chi-square analysis.

It has been suggested that heterostyly may reduce mutual interference between maternal and paternal reproductive function. Two aspects of pollen-stigma interference were investigated in *P. cordata*. The potential importance of stigmatic or stylar clogging by incompatible pollen was examined by controlled field pollinations and measurements of seed set. The results indicate that prior application of large amounts of incompatible pollen has no significant effect on the seed set of open-pollinated inflorescences. Comparison of legitimate pollen capture in intact and emasculated flowers provided no evidence that the presence of stamens within flowers of the floral morphs interferes with the receipt of legitimate pollen. Pollen-stigma interference remains to be demonstrated in heterostylous plants.

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The most widely accepted explanation of the functional significance of heterostyly was originally formulated by Darwin (1877), who hypothesized that the reciprocal placement of stamens and styles in the floral morphs is a mechanical device to promote insect-mediated cross-pollination among morphs with anthers and stigmas at equivalent levels (legitimate pollination). According to Darwin, pollen from different stamen levels would adhere to various parts of an insect's body corresponding to the position where compatible stigmas would contact the insect. This results in phenotypic disassortative pollination between flowers of different floral morphs.

The hypothesis that the stamen-style polymorphism promotes legitimate pollination was never tested directly by Darwin. However, in recent years, several workers, following Levin (1968), have investigated the magnitude of legitimate pollination in natural populations of heterostylous plants by using the marked size heteromorphism of pollen produced by

different stamen levels in the floral morphs. By examination of stigmatic pollen loads, the number of legitimate (compatible) and illegitimate (incompatible) pollen grains deposited by pollinating insects can be measured.

The great majority of pollen flow studies have involved distylous species and have provided only limited support for the Darwinian hypothesis or, because of the experimental design, have been inconclusive (Ganders, 1979). To evaluate the efficacy of floral heteromorphism in promoting legitimate pollination, the intrafloral illegitimate component of the pollen load should be removed by emasculation (Ganders, 1974). This is because pollen transfer between anthers and stigmas within a flower is necessarily unaffected by the heterostylous polymorphism. Unfortunately, few studies have employed this approach.

Here we present the results of an investigation of pollination patterns in a population of *Pontederia cordata* L. (Pontederiaceae) at Paugh Lake, Ontario,

specifically designed to test Darwin's hypothesis that tristily promotes legitimate pollination among the floral morphs. This objective was undertaken by a comparison of the number of legitimate and illegitimate pollen grains deposited on naturally pollinated stigmas of intact and emasculated flowers. In addition, by comparison of pollen loads of intact and emasculated flowers, and by performing controlled field pollinations and measurements of seed set, we evaluate the importance of an additional hypothesis, concerned with aspects of pollen-stigma interference (Lloyd and Yates, 1982; Webb and Lloyd, 1986), formulated to explain the adaptive significance of heterostyly.

MATERIALS AND METHODS

The test of Darwin's hypothesis was conducted at a population of *P. cordata* bordering Paugh Lake, near Barry's Bay, Ontario, (N45°35', W77°45') between August 6–24, 1982. This corresponds to the middle of the seven-week flowering period, although peak flowering occurs about one week earlier. Further information on the floral biology, flowering phenology, and details of temporal variation in pollen flow at the site during the 1979 season, can be found in Price and Barrett (1982, 1984) and Barrett et al. (1983). At peak flowering, the stand consists of approximately 3,000 reproductive shoots and extends from the lake-shore outwards along a gently sloping incline for 10–15 m, and laterally for 200 m. Water depth throughout the area where plants occur ranges from 0–1.5 m. During 1982, the floral morph frequencies in the population were L:0.324, M:0.276, and S:0.400, $N = 377$.

Examination of Pollen Loads

Stigmatic pollen loads were collected from naturally pollinated stigmas of *P. cordata* following procedures detailed in Price and Barrett (1984). For the emasculated treatment, the anthers from all open flowers on two or three inflorescences per morph were removed prior to

flower opening (0730–1100 hr, depending on night temperature). The degree of contamination resulting from this procedure was estimated by collecting and preserving stigmas from 40 flowers of each floral morph immediately after emasculation. Both emasculated and intact inflorescences were marked with tape, and, just before the flowers closed, 40 stigmas were removed and preserved in FAA separately by morph and treatment. This was repeated for nine days for a total of 360 flowers per treatment per morph. Different groups of 2–3 inflorescences per treatment were used on each day.

Preserved stigmas were acetolyzed in groups of 40 corresponding to the daily treatments (for method see Ganders, 1974; Price and Barrett, 1984), and the resultant solution of pollen grains was suspended in lactophenol-glycerin with cotton blue, subsampled, and scored by size. Standards were obtained by acetolyzing undehisced anthers from each stamen level of five individuals per floral morph. The length of 100 pollen grains of each level was measured under a compound microscope equipped with an ocular micrometer. Fresh, dry pollen from glasshouse-grown plants transplanted from the Paugh Lake population was also measured for comparative purposes. The number and proportion of the stigmatic pollen load composed of pollen grains originating from long-, mid-, and short-level anthers was then determined for intact and emasculated flowers of each floral morph.

Statistical analysis of pollen load data employed a four-way fixed effects model analysis of variance, conducted using the GLM procedure of SAS (SAS Inst., 1982), on log-transformed data of the number of pollen grains deposited on stigmas of the floral morphs. Main effects were: 1) pollination treatment: intact flowers versus emasculated flowers; 2) floral morph: long-, mid-, and short-styled morphs (hereafter L, M, S, respectively); 3) pollen type: large-, medium-, and small-sized pollen (hereafter l, m, s, respectively); 4) day of treatment (1–9). Following this

analysis, separate two-way ANOVAs for intact and emasculated treatments were performed with floral morph and pollen type as main effects. Residuals from these analyses, containing the floral morph \times pollen type interaction + error, reflect the relative amounts of each pollen type deposited on stigmas of the floral morphs. Mean residuals (\bar{R}) were then tested for significant departure from 0 by single sample one tailed t tests. Darwin's hypothesis predicts significant (\bar{R}) values for the legitimate floral morph-pollen type combinations (e.g., L/l, M/m, S/s).

Pollinator Visitation

If pollinators of *P. cordata* distinguish between intact and emasculated inflorescences, they may not visit them with the same frequency and, thus, may influence pollen grain deposition. To determine whether differences in visitation were evident, observations were conducted (Aug. 18–19) of the foraging behavior of bees on pairs of inflorescences of each floral morph. These were trimmed to the same flower number and were chosen for similarity in size, height above the water, and exposure. One was emasculated prior to flower opening and the other left intact. The three pairs were each observed for 10 minute time intervals for a total of 140 minutes during the two day period. The number of bees visiting each inflorescence as well as the number of flowers probed at each visit was recorded. Data were compared using G tests of independence.

Pollen-Stigma Interference

To evaluate whether the presence of stamens in a flower interferes with the capture of compatible pollen, comparison of the legitimate pollen loads of stigmas from intact and emasculated flowers were made. Mean pollen loads were compared by t tests.

To investigate the potential importance of pollen-clogging to seed set in *P. cordata*, a field experiment involving hand pollinations and measurements of

seed set was undertaken in a population at Lake Opinicon, Ontario, during July 1982. The experiment compared the seed set of inflorescences that received one of two treatments: 1) open-pollination; and 2) self-pollination by hand followed by open-pollination. The experiment commenced July 28 and involved a cohort of 60 inflorescences (10 inflorescences per treatment per morph) which commenced flowering on July 28–29. All flowers on an inflorescence received the same treatment.

In self-pollinations the contents of a single anther were applied with fine forceps to stigmas, and an attempt was made to cover the entire stigmatic surface with pollen. The pollen of *P. cordata* is yellow and visible on the white stigmas. A single anther contains several thousand pollen grains. Hand pollinations were completed each day before insect activity had commenced.

After 21 days, all inflorescences were bagged to facilitate the collection and counting of seeds approximately six weeks later. Comparisons of percentage seed set were made, following arcsine transformation, with pollination treatment and morph as main effects.

RESULTS

Pollinator Visits

The major visitors to flowers of *P. cordata* at Paugh Lake are the bumblebees *Bombus vagans* F. Smith, *B. terricola* Kirby, and *B. ternarius* Say. Pooled G values, for goodness-of-fit tests of independence, between intact and emasculated inflorescences for both the number of visits to inflorescences and the number of flowers visited were not significant. This indicates that bumblebees do not discriminate between intact and emasculated flowers of the floral morphs ($G = 1.17$, $d.f. = 1$; and $G = 1.53$, $d.f. = 1$; respectively). In both cases, there was no significant heterogeneity among the floral morphs ($G = 1.15$, $d.f. = 2$, and $G = 0.67$, $d.f. = 2$, respectively).

TABLE 1. Pollen deposition on naturally pollinated stigmas of intact and emasculated flowers of *Pontederia cordata* at Paugh Lake, Ontario. Each value is a mean, based on eight hemacytometer counts from 40 combined stigmas, repeated for nine days. Standard deviations are in parentheses. Legitimate pollen loads are underlined.

Pollen type	Floral morph		
	L	M	S
A. Intact flowers			
l	<u>70.6</u> (45.8)	37.0 (24.5)	21.1 (36.1)
m	65.6 (26.6)	<u>62.5</u> (33.8)	42.1 (18.9)
s	145.1 (51.0)	607.7 (291.1)	<u>104.8</u> (76.8)
Total	281.3 (101.8)	707.2 (310.0)	167.8 (119.3)
B. Emasculated flowers			
l	<u>62.5</u> (55.7)	26.0 (21.3)	10.3 (9.0)
m	46.5 (16.4)	<u>74.9</u> (58.4)	31.0 (15.3)
s	123.6 (84.4)	164.2 (100.2)	<u>138.7</u> (72.7)
Total	232.6 (141.0)	265.0 (131.1)	180.0 (71.8)

Stigmatic Pollen Loads

Data on pollen size trimorphism in *P. cordata* during the 1982 flowering season at Paugh Lake are in accord with values previously published (Price and Barrett, 1982, 1984). Pollen from corresponding anther levels of acetolyzed samples is smaller than from newly opened flowers; for l pollen, mean diameter (μm) is 43.82 ± 2.43 versus 65.65 ± 3.22 ; for m pollen, 36.93 ± 1.78 versus 53.95 ± 3.60 ; and for s pollen 25.59 ± 1.43 versus 34.52 ± 2.57 . Nevertheless, the relative size classes are preserved, demonstrating that the origins of pollen can be identified unambiguously following acetolysis.

Contamination of stigmas as a result of the emasculation procedure was detected. However, the magnitude of contamination was minor in comparison with the observed total pollen loads. The mean number of pollen grains found on unvisited emasculated stigmas was 4.14% of the total stigmatic pollen load of intact flowers.

During the nine-day study period, stigmas of intact flowers of the M morph received the largest total pollen loads, while flowers of the L and S morphs received considerably smaller pollen loads

(Table 1). The floral morphs differed less markedly in the number of legitimate pollen grains deposited on stigmas. Mean pollen loads of stigmas for both intact and emasculated flowers of the three morphs were composed primarily (>50%) of s pollen (Table 1). The predominance of s pollen on stigmas of *P. cordata* is a general feature of the pollination biology of the species and results from the fact that pollen originating from short-level anthers is produced in far greater numbers than is pollen from the other two anther levels (l pollen 13.6%, m pollen 22.0%, s pollen 64.4%; see also Price and Barrett, 1984).

Results of the four-way ANOVA of pollen load data are presented in Table 2. All main effects except day were highly significant. Lack of significance for the effect of day indicates that the overall patterns of pollination were relatively similar during the study period. The significant floral morph effect indicates that stigmas of the three morphs received different total numbers of pollen grains, whereas the significant pollen type effect largely reflects differences in pollen production among anther levels and unequal morph frequencies in the population.

TABLE 2. Four-way ANOVA of the stigmatic pollen load of intact and emasculated flowers of *Pontederia cordata* at Paugh Lake, Ontario. Pollination treatment = intact and emasculated; Floral morph = long-, mid-, and short-styled flowers; Pollen type = large-, mid-, and small-sized pollen; Day = day of treatment, 1-9. Data were log-transformed prior to ANOVA.

Source	d.f.	SS	F
Pollination treatment	1	5.33	11.18**
Floral morph	2	25.01	26.18***
Pollen type	2	99.74	104.41***
Day	8	5.39	1.41
Pollination treatment × floral morph	2	1.74	1.82
Pollination treatment × pollen type	2	0.20	0.21
Floral morph × pollen type	4	17.00	8.90***
Pollination treatment × floral morph × pollen type	4	5.20	2.73*
Error	136	64.96	

* = $P < 0.05$.
 ** = $P < 0.001$.
 *** = $P < 0.0001$.

Since these two main effects are not directly involved in determining the magnitude of legitimate pollination, and in fact can obscure its detection, they were removed in further analyses. The effect of pollination treatment was also highly significant, demonstrating that the total pollen loads of intact and emasculated flowers differed, with intact flowers capturing more pollen grains. Within morphs, the difference provides an estimate of self-pollination and intra-inflorescence geitonogamous pollination. The M morph experienced the highest degree of self and geitonogamous pollination (64.3% of the total pollen load), whereas values for the L and S morphs were considerably less, 14.4% and 13.0%, respectively.

Of the interaction terms in the four-way ANOVA the most important to the Darwinian hypothesis is the floral morph × pollen type interaction. This was highly significant, indicating that different amounts of each of the pollen types were captured by the floral morphs. To examine this effect further separate two-way ANOVAs of the pollen load data for intact and emasculated flowers were undertaken. The mean residuals from these analyses are presented in Table 3 and indicate the direction and degree of association between all floral morph-pollen

type combinations. Of major interest for the evaluation of the Darwinian hypothesis are the results for emasculated flowers. Significant legitimate pollination is revealed for the combinations involving l pollen on stigmas of the L morph and s pollen on stigmas of the S morph. Pollen from m anthers is not significantly associated with stigmas of the M morph, although the mean residual is positive and therefore in the predicted direction. No significant positive associations occur for the six illegitimate combinations of emasculated flowers, a result fully concordant with Darwin's predictions.

Pollen-Stigma Interference

Effects of Emasculation.—There was no significant difference between intact and emasculated flowers of each floral morph in the number of legitimate pollen grains captured. (L:intact 70.6, emasculated 62.5, $t = 0.77$ NS; M:intact 62.5, emasculated 74.9, $t = 0.32$ NS; S:intact 104.6, emasculated 138.7, $t = 1.14$ NS). Thus there is no evidence that the presence of stamens in flowers of *P. cordata* obstructs the receipt of outcrossed, legitimate pollen.

Pollen Clogging.—Application of large amounts of incompatible pollen to stigmas prior to open pollination had no significant effect on the seed set of inflores-

TABLE 3. Mean residuals (\bar{R}) for log-transformed stigmatic pollen load data after controlling for the effects of pollen type and floral morph. Single sample, one-tailed t tests examine the null hypothesis that $\bar{R} = 0$. A. Intact inflorescences. B. Emasculated inflorescences.

Floral morph	Pollen type								
	l			m			s		
	\bar{R}	SD	t	\bar{R}	SD	t	\bar{R}	SD	t
A. Intact									
L	0.523	0.70	2.26*	-0.031	0.46	-0.20	-0.501	0.36	-4.23
M	-0.293	0.99	-1.69	-0.333	0.44	-2.29	0.626	0.52	3.62***
S	-0.239	0.51	-0.73	0.364	0.57	1.92*	-0.125	0.60	-0.62
B. Emasculated									
L	0.631	0.92	2.08*	-0.192	0.59	-0.97	-0.440	0.67	-1.98
M	-0.103	0.78	-0.40	0.109	1.06	0.31	0.005	0.53	-0.03
S	-0.528	1.07	-1.48	0.082	0.61	0.41	0.445	0.60	2.21*

* = $P < 0.05$.
 *** = $P < 0.001$.

cences under field conditions (Table 4). The level of open pollinated seed set at Lake Opinicon averaged 86.4%, with no significant differences in fecundity among the floral morphs.

DISCUSSION

Studies of pollen flow in tristylous Lythraceae and Oxalidaceae are complicated by considerable overlap in the size of pollen originating from the three anther levels. The marked pollen trimorphism displayed by *Pontederia* species enables the magnitude of legitimate pollination to be measured unambiguously. Accordingly, they provide suitable experimental systems for testing Darwin's hypothesis of the adaptive significance of floral trimorphism.

Our previous work on pollen flow in *Pontederia cordata* (Price and Barrett, 1984) and *P. sagittata* (Glover and Barrett, 1983) involved samples of stigmas from intact flowers from four populations of each species. In both studies, considerable variation in the levels of legitimate pollination was observed among populations. Despite this variation, the L morph consistently exhibited significant legitimate pollination whereas the M and S morphs more commonly experienced random or assortative pollination. The present investigation involved several differences in experimental

design and data analysis and represents a more detailed investigation of pollination patterns within a single population. We therefore begin our discussion by briefly examining the rationale for the use of new procedures.

Since our previous work on *Pontederia* involved intact flowers, stigmatic pollen loads almost certainly contained a significant self and geitonogamous pollination component (Price and Barrett, 1984). The presence of self pollen can obscure the illegitimate component of outcrossed pollinations and complicate attempts to test the Darwinian hypothesis (Ganders, 1979 fig. 5). How tristylous might influence geitonogamy is not clear, but it seems

TABLE 4. Seed set of the floral morphs of *Pontederia cordata* at Lake Opinicon, Ontario following open pollination (OP) and self-pollination (SP) prior to open pollination. Values are the mean, standard deviation and sample size (inflorescences/flowers). Two-way ANOVA used eight randomly chosen inflorescences per treatment.

Treatment	L	M	S
OP	85.5 ± 18.2 (10/1,925)	86.4 ± 6.4 (9/2,218)	87.3 ± 10.6 (10/2,476)
SP	83.5 ± 8.3 (8/1,915)	85.4 ± 8.9 (9/1,991)	74.2 ± 13.1 (9/1,541)

Two-way ANOVA: Floral morph $F = 2.0222_{42}$ ns., Pollination treatment $F = 1.7821_{42}$ ns., Floral morph × Pollination treatment $F = 0.5932_{42}$ ns.

reasonable to assume that factors such as the number of inflorescences per plant, the number of open flowers per inflorescence, and the foraging behavior of bees play a more significant role. These factors are unrelated to the polymorphism itself and are common to non-heterostylous species. Since the removal of anthers from all flowers of a clone is impractical in *P. cordata*, we chose the inflorescence as our unit for emasculation. As a result, our measures of the magnitude of outcrossed illegitimate pollination are conservative, since they contain a component due to inter-inflorescence geitonogamous pollination. However, at Paugh Lake this may not be large, since clone sizes are relatively small with an average of 3.6 inflorescences produced per clone during the seven week flowering season (Price and Barrett, 1982).

Previous pollen flow studies of heterostylous plants have compared, by chi-square analysis, observed pollen loads with random expectations based on population pollen production (see Ganders [1974] for details). Instead, in this study, we determined statistical associations between the three pollen sizes and style lengths by analysis of variance. This method makes no assumption about random pollination and does not require population estimates of floral morph frequency or pollen production. However, since data were available for these parameters, we also analyzed pollen load data from Paugh Lake using goodness-of-fit tests in order to compare the two methods. The results from intact and emasculated flowers were qualitatively similar to those obtained by ANOVA. Only intact flowers of the L morph displayed significant levels of legitimate pollination, but all three morphs showed this pattern in emasculated flowers (*Intact* L: $\chi^2 = 33.62$, $P < 0.001$, excess legitimate; M: $\chi^2 = 71.9$, $P < 0.001$, excess illegitimate; S: $\chi^2 = 0.316$, NS. *Emasculated* L: $\chi^2 = 35.14$, $P < 0.001$, excess legitimate; M: $\chi^2 = 5.99$, $P < 0.05$, excess legitimate; S $\chi^2 = 12.55$, $P < 0.01$, excess legitimate). The results for intact flowers

were similar to an earlier study conducted during the 1979 season at Paugh Lake (Price and Barrett, 1984). Elsewhere work on *Lythrum salicaria* (Mulcahy and Caporello, 1970) and *L. junceum* (Ornduff, 1975) has also demonstrated legitimate pollination of the L morph.

Several patterns have emerged from the twenty or so pollen flow studies conducted primarily on intact flowers of distylous plants (reviewed in Ganders, 1979; more recent reports include Ornduff, 1980a, 1980b; Weller, 1980; Philip and Schou, 1981; Lewis, 1982; Schou, 1983). In all cases, marked asymmetries in the amount and type of pollen deposited on stigmas of the floral morphs occur. The L morph usually receives a greater number of total pollen grains than the S morph, but the S morph is more likely to experience legitimate pollination. In most studies large amounts of illegitimate pollen have been recorded on stigmas, particularly of the L morph. Although fewer studies have been performed on tristylous species, it would appear that different patterns of pollen flow occur with the likelihood of legitimate pollination in the L and S morphs reversed in comparison with distylous populations. The contrasting patterns may result from differences in floral morphology between *Pontederia* and *Lythrum* and the distylous species that have been studied. Virtually all distylous species examined possess relatively unspecialized actinomorphic flowers, many of which are vertically orientated. In contrast, *Pontederia* and *Lythrum* flowers are zygomorphic with horizontal orientation and sternotribic pollination. Studies of the interaction between floral architecture and pollinator behavior are required to determine whether these differences influence the magnitude of legitimate pollination in distylous and tristylous species.

Since much of the asymmetry in pollination between the floral morphs of heterostylous populations resides in differences in the illegitimate pollen load, it is also of importance to determine what

factors account for the observed variation. Most workers have explained the disparity as the effect of contrasting pollinator positioning with respect to reproductive organs in the floral morphs (Levin, 1968; Ganders, 1974; Price and Barrett, 1984; Schou, 1983). However, differences in illegitimate pollen deposition on stigmas of the floral morphs may simply result from the distance separating a given stigma type from the stamen set that produces the largest number of pollen grains overall. For example, in tristylous plants, short-level anthers are considerably more productive of pollen, and hence mid-level stigmas should be most susceptible to illegitimate pollination. This is what is observed in *P. cordata*, where $\frac{2}{3}$ of all pollen is produced by short-level anthers and the largest illegitimate pollen loads are captured by mid-level stigmas (Price and Barrett, 1984; Glover and Barrett, 1983). Similarly, in distylous plants, short-level stamens produce the largest amounts of pollen, and the L morph is most prone to illegitimate pollination. According to this interpretation, differences in the magnitude of illegitimate pollination follow directly from the floral morphology and pollen production characteristics of the morphs.

Darwin (1877) proposed that floral heteromorphism, by lowering the likelihood of illegitimate pollination, might reduce pollen wastage and thus conserve resources. Ganders (1974) argued that, in a species with diallelic incompatibility, an increased level of legitimate pollination over that obtained from random pollination would result in higher fecundity. He postulated that, under conditions in which ovules were pollen limited, any heritable trait that increases compatible pollination would spread, leading to the establishment of the stamen-style heteromorphism. While this process is entirely plausible as an historical model to account for the evolution of heterostyly, it seems unlikely to be involved in the *maintenance* of the polymorphism in contemporary populations.

Sufficient numbers of legitimate pollen grains to achieve maximum potential seed set are usually deposited on stigmas of heterostylous plants with random pollination (Lewis, 1982). *Pontederia cordata* flowers are uniovulate, and stigmatic pollen loads are usually composed of many compatible pollen grains. As a result, seed set is rarely pollen limited (Barrett and Glover, unpubl.). As long as pollinator service is reliable and the close developmental association with self-incompatibility is maintained, tristily in *P. cordata* appears to be relatively insensitive to fluctuations in the levels of legitimate and illegitimate pollination. While floral trimorphism may have little direct influence on regulating levels of seed set in *P. cordata*, the polymorphism may still have evolved in the Pontederiaceae because it increased fecundity.

Lloyd and Yates (1982) propose that the stamen-style polymorphism, that characterizes heterostylous species, is a floral mechanism to reduce mutual interference between maternal and paternal reproductive functions. They propose that the spatial separation of anthers and stigmas increases the maternal fitness of morphs by leaving more space on the stigma for compatible pollen or by reducing adverse effects of incompatible pollen. Our experiments to investigate the potential importance of two components of pollen-stigma interference failed to reveal any significant effects. The presence of stamens within flowers of the floral morphs had no effect on the capture of legitimate pollen. Application of incompatible pollen to stigmas of the morphs prior to open-pollination did not result in any marked difference in seed set in comparison with controls. A detailed study of pollen clogging in the floral morphs of distylous *Turnera ulmifolia* also failed to demonstrate any large scale effects of incompatible pollen on seed set (Shore and Barrett, 1984). Taken together these studies cast doubt on the overall significance of pollen clogging as a factor influencing the maintenance of floral heteromorphism. Whether stigmatic and

pollen polymorphisms play a role as "anti-clogging" devices in heterostylous species, as Yeo (1975) has suggested, requires further study.

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