

STIGMATIC POLLEN LOADS IN POPULATIONS OF PONTEDERIA CORDATA FROM THE SOUTHERN U.S.¹

DEBORAH E. GLOVER AND SPENCER C. H. BARRETT

Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1, Canada

ABSTRACT

Stigmatic pollen loads were examined in four tristylous populations of *Pontederia cordata* from the southern U.S. to evaluate Darwin's hypothesis that floral trimorphism promotes legitimate pollination. In each population, morph frequency, pollen production, the composition of stigmatic pollen loads and seed production were estimated. Goodness-of-fit tests and a measure of the efficiency of legitimate pollination were employed to evaluate pollen load patterns. Results from the four populations demonstrate that the long-styled (L) morph usually experiences legitimate pollination, whereas the mid-styled (M) and short-styled (S) morphs often display random pollination. However, at Taylor Road (Louisiana) all three floral morphs exhibited statistically significant levels of legitimate pollination. The size of legitimate pollen loads suggests that in populations of *P. cordata* tristily may have only a minor influence on the fecundity of morphs. Two hypotheses are proposed to explain the maintenance of floral trimorphism in contemporary populations of *Pontederia* spp. The first assumes the polymorphism is selectively neutral; the second suggests that it contributes towards the male component of fitness by increasing the distance, and hence the number of genets, that pollen can be transported to by pollinators. In clonal species, such as *Pontederia cordata*, where geitonogamous pollinations are promoted by large floral displays, floral mechanisms that increase pollen carryover may have selective value.

HETEROSTYLOUS PLANT populations contain two (distyly) or three (tristyly) floral morphs which differ principally in style length, anther ht, pollen size and incompatibility behavior. Darwin (1877) proposed that the reciprocal arrangement of reproductive organs which characterizes heterostylous plants functions to promote insect-mediated pollen transfer between anthers and stigmas of equivalent height (legitimate pollination). This hypothesis can be tested in many heterostylous species because of the presence of conspicuous pollen size heteromorphism. The composition of stigmatic pollen loads can be examined and the magnitude of legitimate pollination determined (see Ganders, 1979, for review).

Pollen load studies have largely involved distylous species. Among tristylous taxa considerable overlap in pollen size complicates the measurement of legitimate pollination in most genera (e.g., *Lythrum* and *Oxalis*). However, in *Pontederia* the three pollen types can be identified unambiguously by size and as a result

the levels of legitimate and illegitimate pollination can be estimated. Recent studies of *Pontederia cordata* populations from eastern North America (Price and Barrett, 1984; Barrett and Glover, 1985) indicate that the composition of pollen loads differs from most distylous taxa (see Discussion). Here we extend the geographical scope of this work by examining the extent of legitimate pollination in populations from Louisiana and Mississippi. We summarize the general features of stigmatic pollen load data in the species, compare them with the closely related *P. sagittata* Presl. (Glover and Barrett, 1983), and speculate on the selective forces that maintain floral trimorphism in natural populations.

MATERIALS AND METHODS—We conducted studies in four populations of *P. cordata* in May 1982: 1) *Phoenix* population—borders Route 39 7 km north of Phoenix, St. Bernards Parish, Louisiana, 2) *New Orleans* population—along Route 90 east of New Orleans 6 km past the junction of Route 47, Orleans Parish, Louisiana, 3) *Taylor Road* population along Route 90 just west of Paradis, St. Charles Parish, Louisiana, 4) *Jordan River* population off Route 40, south of Kiln, Hancock County, MS. The Louisiana populations all occur in drainage ditches whereas the Mississippi population oc-

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TABLE 1. Reproductive parameters in four populations of *Pontederia cordata* from the southern U.S. Flower and fruit data based on 15–20 inflorescences per floral morph; values are means \pm one S.D.

Population	Floral morph	Floral morph frequency	No. open flowers per inflorescence	Seed set per inflorescence
Phoenix	L*	0.30	18.4 \pm 9.8	59.4 \pm 14.0
	M	0.47	19.4 \pm 10.5	56.1 \pm 11.1
	S	0.23	18.4 \pm 9.8	58.7 \pm 15.8
			$F = 0.18$ ns	$F = 0.05$ ns
New Orleans	L	0.30	15.5 \pm 8.9	63.9 \pm 13.9
	M	0.37	23.5 \pm 9.6	68.0 \pm 19.8
	S	0.33	13.0 \pm 7.4	78.8 \pm 11.4
			$F = 7.4^{**}$	$F = 2.10$ ns
Taylor Road	L	0.34	32.8 \pm 12.3	84.5 \pm 7.6
	M	0.32	29.9 \pm 11.3	83.9 \pm 5.2
	S	0.34	28.8 \pm 8.4	85.3 \pm 7.0
			$F = 0.78$ ns	$F = 0.37$ ns
Jordan River	L	0.38	20.4 \pm 6.9	71.6 \pm 14.4
	M	0.40	19.1 \pm 7.9	44.0 \pm 15.5
	S	0.22	19.2 \pm 8.9	81.9 \pm 8.7
			$F = 0.17$ ns	$F = 8.60^{**}$

* L, M, S: long-, mid-, and short-styled morph, respectively.

** $P < 0.001$.

cupies a sandy riverbank. At Taylor Road the population was large, dense and contained approximately 3,000 inflorescences; the remaining populations contained between 500–700 inflorescences. Extensive clonal growth precluded estimates of the number of genets in each population. The relative frequency of inflorescences of the floral morphs, and measurements of pollen size and pollen production were made in each population. Methods employed were those detailed in earlier work (Glover and Barrett, 1983; Price and Barrett, 1984). Sample sizes are given in tables. The seed set of floral morphs was compared in each population by sampling infructescences (13–20 per morph) and counting fruits. Since *P. cordata* produces one functional ovule per flower, fruit set is equivalent to seed set.

To quantify the legitimate (compatible) and illegitimate (incompatible) component of stigmatic pollen loads, 40 stigmas were sampled at random from each floral morph within populations in early afternoon just prior to floral senescence. Stigmas were removed with fine forceps and preserved in FAA (Formaldehyde, acetic acid and 80% absolute ethanol, 1:1:8 v/v/v) and later acetolysed in groups of 40 following methods detailed in Ganders (1974) and Price and Barrett (1984). The resulting pollen pellet was suspended in lactophenol-glycerin with cotton blue, sub-sampled and the number of pollen grains originating from each of the three anther levels scored. Raw values of the number of pollen grains of each pollen type (large size = 1, mid size = m, small size =

s) deposited on stigmas of the floral morphs were expressed per flower for further calculations.

Random pollination, with respect to the floral morphs, occurs if pollen from each anther level is deposited on stigmas in proportions equivalent to its production in the population. With random pollination, the expected number of pollen grains of each type deposited on stigmas, when anther levels produce different numbers of pollen grains and the floral morphs are unevenly represented, can be calculated as follows:

$$N_{ij} = \frac{T_j \sum_{j=1}^3 P_{ij} m_j}{\sum_{i=1}^3 \sum_{j=1}^3 P_{ij} m_j} \text{ for } P_{i \neq j}$$

where N_{ij} = the expected number of i pollen grains on stigmas of floral morph j with $i = 1, 2,$ or 3 corresponding to pollen from long-, mid-, and short-level anthers, respectively; and $j = 1, 2,$ or 3 corresponding to the L, M, and S morphs, respectively; T_j = the total number of pollen grains observed on stigmas of floral morph j ; P_{ij} = the number of pollen grains of type i produced by floral morph j , and m_j = the frequency of floral morph j in the population. Deviations from random pollination, detected by goodness-of-fit tests, were employed to establish whether significant legitimate or illegitimate pollination occurred in each population.

TABLE 2. Estimates of the mean number of pollen grains per flower produced by the three anther levels in four populations of *Pontederia cordata*. Values are means of six hemacytometer counts with coefficient of variation in parentheses

Population	Organ level	Floral morph		
		L	M	S
Phoenix	l*	—	4,281 (0.10)	2,725 (0.31)
	m	4,633 (0.21)	—	7,944 (0.34)
	s	20,873 (0.13)	19,644 (0.13)	—
New Orleans	l	—	3,066 (0.36)	2,725 (0.31)
	m	3,123 (0.22)	—	6,577 (0.23)
	s	22,236 (0.06)	20,562 (0.12)	—
Taylor Road	l	—	4,118 (0.17)	5,451 (0.24)
	m	3,188 (0.32)	—	9,437 (0.22)
	s	18,162 (0.15)	18,346 (0.22)	—
Jordan River	l	—	1,968 (0.17)	1,466 (0.13)
	m	1,851 (0.33)	—	4,736 (0.16)
	s	19,674 (0.06)	15,633 (0.13)	—

*l, m, s: long-, mid-, and short-level organs, respectively.

RESULTS—Reproductive parameters—The three floral morphs were represented in each of the four populations examined (Table 1). In three populations there were no significant differences among the floral morphs in the number of open flowers per inflorescence and the number of seeds per infructescence (Table 1). At the New Orleans population the estimate of open flower number per inflorescence in the M morph was significantly higher than in the other morphs and at Jordan River the M morph produced significantly fewer seeds per infructescence than the L and S morphs. There are marked differences in pollen size and pollen production among the three anther levels with short-level anthers producing by far the largest number of pollen grains and long-level anthers the least (Table 2). Mid-level anthers of the S morph produce on average twice as many pollen grains as mid-level anthers of the L morph (see Price and Barrett, 1982; Barrett et al., 1983).

Pollen loads—Total pollen loads per stigma varied considerably both among populations and among floral morphs within populations (Table 3). The total number of pollen grains deposited on stigmas ranged from 83.3–393.3. In three of the four populations the M morph received the largest total pollen load and the S morph the smallest. The largest loads for all morphs were at the Taylor Road population. Legitimate pollen loads were also highest in this population with floral morphs receiving similar numbers of legitimate pollen grains. In the remaining populations the number of legitimate pollen grains on stigmas differed markedly among morphs (Table 3).

Pollination regime—Data from each population on pollen production and floral morph frequency were used to calculate the expected

pollen loads presented in Table 3. Goodness-of-fit tests indicate whether significant departure from random expectation occurs. If the observed number of legitimate pollen grains is significantly greater than random expectation, the pollination regime is classified as legitimate. Conversely, if fewer legitimate pollen grains than would be expected with random pollination are found on stigmas, pollination is classed as illegitimate. In each of the four populations, the L morph experienced a significant level of legitimate pollination, whereas the M and S morphs were legitimately pollinated in three and one population, respectively (Table 3). Only at Taylor Road was the pollination regime legitimate for all three floral morphs.

Ganders (1974) developed an efficiency measure (E) to assess the magnitude of legitimate pollination in the floral morphs of heterostylous species. The measure is given by the formula $E = o - r/d - r$ where o = the observed frequency of one of the pollen types, r = the expected frequency of the pollen type with random pollination, and d is the expected frequency with legitimate pollination. Values of E may range from 0–100% (random pollination—complete legitimate pollination) and are calculated for the four populations of *P. cordata* examined in this study, as well as for each of four previously studied populations of *P. cordata* (Price and Barrett, 1984) and *P. sagittata* (Glover and Barrett, 1983). Several generalizations can be made from the values which are presented in Table 4. The L morph of *Pontederia* is most likely to experience the highest degree of legitimate pollination. In only one of the 12 populations sampled was pollination not legitimate. The remaining two morphs vary

TABLE 3. Mean pollen grain deposition ($N = 40$ stigmas per floral morph) on stigmas in four populations of *Pontederia* cordata from the southern U.S. Expected values based on random pollination are calculated as explained in text. G values indicate departure of observed pollen grain deposition (Obs.) from random expectation (Exp.)

Population	Morph		Pollen type			G^a (df = 1)	Pollination regime ^b
			l	m	s		
Phoenix	L	Obs.	38.8	7.7	37.7	66.26**	L
		Exp.	9.2	12.6	63.1		
	M	Obs.	38.8	70.0	214.4	10.46**	L
		Exp.	35.5	48.4	239.0		
	S	Obs.	7.7	12.6	94.4	3.17 ns	R
		Exp.	12.5	17.2	86.5		
New Orleans	L	Obs.	40.0	6.6	84.4	42.16**	L
		Exp.	13.1	20.9	96.9		
	M	Obs.	22.2	62.2	194.4	7.79*	L
		Exp.	27.6	44.4	205.7		
	S	Obs.	13.3	44.1	28.3	55.67**	I
		Exp.	8.3	13.3	61.6		
Taylor Road	L	Obs.	117.7	57.7	158.8	74.19**	L
		Exp.	53.4	70.2	207.0		
	M	Obs.	33.3	108.8	251.1	9.84**	L
		Exp.	62.8	82.5	243.0		
	S	Obs.	8.8	34.4	122.2	10.62**	L
		Exp.	26.4	34.6	102.4		
Jordan River	L	Obs.	61.1	38.8	186.6	60.71**	L
		Exp.	20.0	34.3	231.0		
	M	Obs.	3.3	20.0	200.0	2.12 ns	R
		Exp.	15.6	26.7	180.0		
	S	Obs.	3.3	34.4	117.7	2.95 ns	R
		Exp.	10.8	18.6	125.5		

^a G value compares legitimate pollen load with illegitimate pollen load.

^b Pollination regime: L = legitimate, R = random, I = illegitimate.

* $P < 0.05$.

** $P < 0.005$.

in behavior. In eight of the twelve populations, the M morph was legitimately pollinated although individual values were generally low and its overall mean efficiency (scoring illegitimate pollination as 0) was only 7.3%. The S morph displayed the most variable pollen loads, with five of the twelve populations exhibiting legitimate pollination, but individual values ranging from 21.3–73.1 with an overall mean efficiency of 15.1%. It should be noted that the pollen load data in Table 4 are for intact (unmanipulated) flowers of *Pontederia*. Measurements of the efficiency of heterostyly in promoting legitimate pollination are higher for emasculated flowers as discussed in detail by Ganders (1974) and Barrett and Glover (1985).

DISCUSSION—Darwin's hypothesis that heterostyly promotes legitimate pollination should ideally be tested by comparisons of stigmatic pollen loads and seed set among populations which exhibit diallelic incompatibility and pollen heteromorphism but differ by the presence or absence of the stamen-style polymorphism (Ornduff, 1986). In such comparisons it is predicted that legitimate pollen loads, seed set and

possibly seed quality should be higher in heterostylous populations than in homomorphic populations. Unfortunately, no species has been found that possesses the appropriate floral variation and as a result the problem of the evolution and adaptive significance of floral heteromorphism remains difficult to investigate experimentally.

Instead most studies of the pollination biology of heterostylous plants have evaluated the Darwinian hypothesis by estimating the relative magnitudes of legitimate and illegitimate pollination in natural populations (Ganders, 1979). Here the approach is to observe whether significantly more legitimate pollen grains are deposited on stigmas than would be predicted with random pollination. 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 total number of pollen grains than the S morph, but the latter 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, and the common finding of random

TABLE 4. Efficiency of legitimate pollination in the floral morphs of *Pontederia*. See text for calculation of efficiency. Data from this study, Glover and Barrett (1983) and Price and Barrett (1984)

Population		Efficiency of legitimate pollination (%)			
		L	M	S	
<i>Pontederia cordata</i> (N. America)	Phoenix, LA	39.3	8.2	26.9	} This study
	New Orleans, LA	22.2	7.1	*	
	Taylor Road, LA	22.6	8.8	32.4	
	Jordan River, MS	15.0	*	*	
	Ft. McCoy, FL	24.3	15.4	27.1	} Price and Barrett (1984)
	Elizabeth City, NC	36.6	33.7	*	
	Osborn, SC	33.8	*	*	
	Paugh Lake SW, Ont.	*	*	21.3	
<i>Pontederia sagittata</i> (Vera Cruz, Mexico)	Buena Vista	6.6	*	73.1	} Glover and Barrett (1983)
	Laguna	63.0	4.6	*	
	Paso San Juan	35.9	*	*	
	Minatitlan	16.0	10.2	*	
	Mean	26.3	7.3	15.1	

* Pollination illegitimate.

and even assortative pollination has prompted workers to question the validity of Darwin's hypothesis.

Our studies of tristylous populations of *P. cordata* also demonstrate asymmetries in pollen deposition patterns among the floral morphs. The likelihood of legitimate pollination in the L and S morphs is, however, reversed in comparison with distylous species, and in addition, the overall levels of legitimate pollination in populations tend to be higher. Why this contrast occurs is not clear but may involve differences between the species studied in floral architecture and pollinator positioning (Price and Barrett, 1984; Barrett and Glover, 1985). The composition of pollen loads may also be influenced by interactions between pollen grains and stigmatic papillae.

Differences among the three pollen types in their adhesive properties to the stigmatic papillae of floral morphs could influence pollen loads in *Pontederia* populations. Variations in topographical complementarity between different pollen and stigma types, as occur in several distylous taxa (Dulberger, 1975a, b), could affect the composition of pollen loads in as yet undetermined ways. Such effects might act to increase the retention of legitimate pollen following the initial deposition of random pollen loads. It is of interest to note that in distylous *Linum* and several taxa of the Plumbaginaceae adherence of both pollen types is evident on stigmas of the S morph, whereas only compatible pollen tends to adhere to stigmas of the L morph (Dulberger, 1975a, b). Unfortunately, virtually nothing is known about this stage in the pollination process of *Pontederia* spp., so it is difficult to evaluate whether stigmatic poly-

morphisms participate in regulating the composition of pollen loads. Controlled pollinations, with pollen mixtures of known composition, followed by observations of stigmatic capture should help answer these questions.

Pontederia cordata flowers are uniovulate and large numbers of compatible pollen grains are deposited on naturally pollinated stigmas, even with random pollination (Table 3). This raises the question: what selective factors maintain floral trimorphism in contemporary populations of *Pontederia*? We offer two hypotheses to account for the maintenance of floral trimorphism. The first views the stamens polymorphism as selectively neutral under contemporary conditions whereas the second focuses on its functional significance. It is possible that outbreeding advantage maintains the trimorphic incompatibility system and because of a close developmental association between the incompatibility system and the floral polymorphisms the latter are also maintained. Under this view the components of the heterostylous system are functionally integrated (see Dulberger, 1975b) and loss of the self-incompatibility system is likely to lead to dissolution of floral trimorphism. This breakdown process has occurred in the related *Eichhornia*, although self-compatible tristylous is maintained if population densities and outcrossing rates remain high (Barrett, 1985). This hypothesis requires a demonstration of inbreeding depression in inbred progeny of *Pontederia* as well as information on the developmental genetics of the polymorphism.

A second explanation focuses on the adaptive role of floral trimorphism per se and is

simply an extension of Darwin's pollen wastage hypothesis (Darwin, 1877). In *Pontederia* several attributes of the plant enhance illegitimate pollination; these include the clonal habit and large number of flowers per inflorescence. Despite these influences considerable amounts of legitimate pollen are deposited on stigmas indicating significant pollen carryover (Price and Barrett, 1984). It is possible that floral trimorphism may serve to reduce pollen wastage by increasing the levels of pollen carryover over that which could be achieved with floral monomorphism. In this way, the polymorphism may influence the male component of fitness by increasing the distance that pollen can be transported by pollinators. Such an effect might be of importance in species with extensive clonal growth, since near neighbor inflorescences are usually the same genet and increased pollen dispersal distances are more likely to result in a greater number of genets encountered. It is of interest that tristily is frequently associated with the clonal habit (e.g., *Decodon*, *Oxalis*, *Pontederia*, *Eichhornia*). Unfortunately, there are few available data to evaluate this suggestion, although Waser and Price (1984) have shown that high floral variation in the non-heterostylous *Ipomopsis aggregata* increases the carryover distance of dyes that mimic pollen. Similar conclusions on the effects of floral variation on pollen carryover distance have also been obtained by Lertzman (1981) on the basis of theoretical studies. Waser and Price (1983) suggest that floral polymorphisms, such as heterostyly, may have evolved partly as a means of enhancing pollen carryover. The presence of pollen size heteromorphism in heterostylous species should enable experimental study of pollen carryover and evaluation of these ideas.

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