

STAMEN ELONGATION, POLLEN SIZE, AND SIRING ABILITY IN TRISTYLOUS *EICHHORNIA PANICULATA* (PONTEDERIACEAE)¹

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Selfing variants in tristylous *Eichhornia paniculata* (Pontederiaceae) possess an elongated, short-level stamen adjacent to mid-level stigmas, which causes autonomous selfing. The variants commonly spread in dimorphic, but not trimorphic populations in northeast Brazil. We investigated the effect of stamen elongation on pollen size and siring ability. Competition experiments using controlled hand-pollination and allozyme markers were used to compare different pollen types. Pollen from the elongated stamen was significantly larger in size than pollen from unmodified short-level stamens. In mixed pollinations of mid-level stigmas, pollen from the elongated stamen sired significantly more seed than pollen from unmodified short-level stamens. Despite these differences, the size and compatibility of pollen from the elongated stamen were more similar to short- than mid-level pollen, indicating that alterations to stamen level were not associated with major changes in pollen characteristics. The results suggest that the advantage of selfing variants in dimorphic populations is mainly due to efficient pollen transfer to mid-level stigmas rather than increased postpollination siring success of pollen from modified stamens. In addition, the absence of major changes in pollen size and compatibility associated with stamen elongation support other lines of evidence indicating that selfing variants are not the result of recombination in the putative heterostyly supergene.

The relative positions of male and female reproductive organs within angiosperm flowers are important determinants of plant fitness since they strongly influence pollen dispersal and patterns of mating (Breese, 1959; Thomson and Stratton, 1985; Webb and Lloyd, 1986; Barrett and Shore, 1987; Campbell 1989; Harder and Barrett, 1995). Heterostylous plants provide model systems for studies on the evolutionary ecology of floral traits since they exhibit more intrapopulation variation in the placement of sex organs than is found in monomorphic species (Harder and Barrett, 1993). Populations of heterostylous plants are usually characterized by three associated sets of traits: the reciprocal positioning of stigmas and anthers in two (distyly) or three (tristyly) floral morphs (reciprocal herkogamy), a sporophytically controlled self- and intramorph incompatibility system that favors intermorph mating, and a suite of ancillary morphological polymorphisms, particularly involving pollen size and stigmatic papillae length (Ganders, 1979; Dulberger, 1992; Lloyd and Webb, 1992a; Barrett and Cruzan, 1994).

While the modes of inheritance of both distyly and tristyly are well known (reviewed in Lewis and Jones, 1992), controversy still exists concerning the genetic architecture of traits making up the heterostylous syndrome, particularly in tristylous species. Two basic models have been proposed to account for the recurrent as-

sociations that are found between morphological and physiological traits. The supergene model proposes that several closely linked genes each controlling different morphological or physiological traits govern the expression of the polymorphism. Studies in several unrelated distylous taxa of self-compatible recombinant phenotypes with anthers and stigmas at the same position within a flower (homostyles) provide good evidence for the supergene model (Ernst, 1955; Dowrick, 1956; Shore and Barrett, 1985), although the precise number and order of genes is still poorly understood (but see Lewis and Jones, 1992). In tristylous species, homostylous variants commonly occur, but do not appear to be recombinant in origin and there is no general consensus concerning whether supergenes govern the expression of the tristylous syndrome (Charlesworth, 1979; Ganders, 1979; Barrett, 1993; Eckert and Barrett, 1994).

A second model to account for the close association between morphological and physiological traits invokes pleiotropy as a mechanism. According to this view incompatibility in heterostylous plants may be a pleiotropic effect of genes governing styles and stamen growth. This assumption was used by Charlesworth (1979) in theoretical models to account for the evolution of tristyly. The suggestion that incompatibility is a direct physiological outcome of differences between the floral morphs in development and morphology was first proposed by Mather and De Winton (1941). More recently, Dulberger (1975, 1992) has extended these ideas by suggesting that morph-specific recognition substances involved in the incompatibility mechanism may be synthesized during cell wall extension in the morphs. Several observations support the idea that incompatibility in heterostylous plants may be directly influenced by the expression of morphological traits. For example in *Primula sinensis* two modifier genes (Primrose Queen and Fertile Double) are known that alter stigma and anther height, respectively. The two

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genes cause concurrent modifications to the incompatibility reaction of stigmas and pollen (Mather and De Winton, 1941). In populations of tristylous *Oxalis alpina* lacking the mid-styled morph, the positions of mid-level stamens in the long- and short-styled morphs are modified in opposite directions, taking up positions close to the remaining anther level within flowers of each of the two morphs. These changes in position have also been accompanied by alterations to the behavior of pollen, making them compatible on stigmas of the alternate morph (reviewed in Weller, 1992). Both examples indicate that modification to pollen characteristics can accompany alterations to the positions of stamens in heterostylous plants. Such effects could have important consequences for the male fitness of variants with modified stamen positions as a result of changes in the dispersal and siring success of pollen.

Eichhornia paniculata Solms-Laubach (Pontederiaceae) exhibits considerable floral variation associated with the evolutionary breakdown of its tristylous breeding system. Populations vary from those in northeast Brazil that contain all three floral morphs and are largely outcrossing to those in Jamaica that are composed exclusively of self-pollinating variants of the mid-styled morph (Barrett, 1985; Glover and Barrett, 1986; Barrett and Husband, 1990). Selfing variants in *E. paniculata* possess different numbers (1-3) of modified short-level stamens with anthers adjacent to mid-level stigmas (Seburn, Dickinson and Barrett, 1990; Barrett and Harder, 1992). The transition from outcrossing to predominant selfing in *E. paniculata* commences with the spread of selfing variants in dimorphic populations from northeast Brazil in which the short-styled morph is absent (Barrett, Morgan, and Husband, 1989). These variants usually possess a single elongated short-level stamen with the anther at the mid-level. Genetic and developmental studies of these variants indicate that the allele(s) controlling stamen modification is recessive (Fenster and Barrett, 1994) and that elongation occurs ≈ 24 hr before anthesis, largely through cell elongation (Richards and Barrett, 1992).

In an effort to understand why selfing variants commonly spread in dimorphic but not trimorphic populations, Kohn and Barrett (1994) employed experimental manipulations of population morph structure to measure the male and female fertility of selfing and outcrossing morphs using genetic markers. An important result of their studies was the finding that while modified (selfing) and unmodified (outcrossing) mid-styled plants had similar reproductive success in trimorphic populations, in populations missing the short-styled morph the selfing variants sired nearly twice as many seeds as unmodified mid-styled plants. Such context-dependent mating may help explain why selfing variants are rare in trimorphic populations but common in dimorphic and monomorphic populations.

The siring success of selfing variants in experimental nontrimorphic populations of *E. paniculata* could be explained by two contrasting reproductive mechanisms. Modification to anther position may result in improved outcross pollen dispersal to mid-level stigmas of both modified and unmodified plants as a result of more efficient pollen transfer by pollinators. A second mechanism could involve alterations to the compatibility of pollen as

a result of stamen elongation in the mid-styled variants. Although controlled self- and cross-pollinations of *E. paniculata* result in similar levels of fruit and seed set, experiments using genetic markers have demonstrated that the species possesses a cryptic trimorphic incompatibility system (Cruzan and Barrett, 1993). Legitimate pollen is favored over illegitimate pollen through faster pollen tube growth when the two occur together in mixtures on the stigma. If pollen produced by modified stamens behaves like mid-level pollen as a result of changes in pollen tube growth, its siring success on mid-level stigmas may be improved. Of course, both of these mechanisms could be involved in the observed fitness advantage that selfing variants experience in dimorphic populations of *E. paniculata*.

To investigate these issues we examined the effects of stamen modification on the size and siring ability of pollen. We used selfing variants of the mid-styled morph of *E. paniculata* originating from several populations in northeast Brazil, including material from the population (B46) used in the Kohn and Barrett (1994) study discussed above. Two major questions were addressed: (1) Do changes in pollen size and number accompany elongation of short-level stamens in selfing variants of the mid-styled morph? (2) Is modification to stamen position in the variants also associated with changes in pollen compatibility, as assessed in competition experiments with pollen from unmodified stamens? The results of our study are used to assess the potential reproductive mechanisms contributing to the spread of selfing variants in *E. paniculata*, as well as to more general issues concerned with the developmental genetics of the tristylous polymorphism.

MATERIALS AND METHODS

Eichhornia paniculata is an annual, emergent aquatic, native to the Neotropics, primarily northeast Brazil, Jamaica, and Cuba. Plants used in our experiments were derived from open-pollinated families collected from six populations in northeast Brazil between 1987 and 1989. Information concerning the locality, size, and floral morph representation of each population is presented in Table 1. Plants were grown in water-submersed pots and maintained in a heated glasshouse (25–35 C) for the duration of the study. The culture of *E. paniculata* (Morgan and Barrett, 1989) and the mating system and genetic structure of populations (Barrett and Husband, 1990) are described elsewhere.

The arrangement of floral organs in the long-, mid- and short-styled morphs (hereafter L, M, and S morphs) of *E. paniculata* are illustrated in Fig. 1. In each morph, flowers possess two stamen levels that occupy positions not taken up by the stigma and are referred to as long-, mid-, or short-level stamens (hereafter l, m, and s stamen levels). Each stamen level is composed of three stamens. In selfing variants of the M morph (hereafter M') the filament of one of the outer short-level stamens is elongated so the anther is at a position adjacent to mid-level stigmas. This modification to anther position results in autonomous self-pollination of flowers and gives rise to increased levels of self-fertilization of this morph in both natural and experimental populations (Barrett, Morgan

TABLE 1. The locality, population size, morph structure, and frequency of modified mid-styled plants (M') in populations of *Eichhornia paniculata* from northeast Brazil from which plants used in this study originated.

Population	Locality	Year sampled	Population size	Morph structure	Frequency of M'
B46	Quixadá, Ceará	1987	500	L, M, M', S	0.05
B56	Patos, Paraíba	1989	640	L, M, M', S	0.01
B63	Garanhuns, Pernambuco	1989	5	M'	1.00
B69	Murici, Alagoas	1989	200	M, M'	0.80
B72	Ibateguara, Alagoas	1989	5	L, M, M'	0.63
B115	Capela, Alagoas	1989	1,550	L, M, M'	0.12

and Husband, 1989; Kohn and Barrett, 1994). Which of the two outer stamens of the s level in the M' morph becomes modified is determined by the position of the flower within each branch of the inflorescence (see Seburn, Dickinson, and Barrett, 1990; Richards and Barrett, 1992, fig. 39). The modified stamen is always on the side of the flower away from the inflorescence branch and is inserted on an outer tepal whorl. The central stamen of the s level does not elongate in these variants. Following the terminology developed by Troll (1937) for vegetative shoots we refer to the nonelongated outer stamen as acroscopic in position, the elongated outer stamen as basiscopic, and the remaining stamen as central. For convenience these are coded as 1, 3, and 2, respectively (see Fig. 1). In natural populations, inflorescences of M' plants sometimes contain some unmodified flowers. In our studies these flowers occurred rarely and were not used in experiments. While other selfing variants of *E. paniculata* with two and three stamens elongated to the mid-level length are reported (see Barrett, 1985), the single modified stamen condition discussed above is most common in nature and our studies therefore focused exclusively on this phenotype.

Pollen size—In common with most tristylous plants, *E. paniculata* displays size trimorphism of pollen produced by the three anther levels. To determine whether modification to anther position in plants of the M' variant was generally associated with changes in pollen size, pollen was collected separately from each of the three s stamens in plants originating from the six populations of *E. paniculata* listed in Table 1. Pollen was collected on glass microscope slides and the polar axes of ten dry pollen grains per anther type from each of two flowers per plant were measured under $\times 1000$ magnification using an ocular micrometer (10 μm per ocular unit) and a Zeiss Axioptan compound microscope. Pollen was sampled from five plants in populations B56 and B72, six plants in populations B63, B69 and B115, and nine plants in population B46. In population B46, further comparisons were undertaken to investigate how pollen size of the three s stamens of M' plants compared with pollen from s stamens of the L and M morphs and with pollen from m stamens of the L and S morphs. In the first comparison, pollen from stamens s2 and s3 were compared in L, M, and M' plants, and in the second comparison, pollen from stamen s3 in M' plants was compared to pollen from stamen m3 in L and S plants. These comparisons involved 10 L, 11 M, 11 S, and nine M' plants. For each plant and stamen type, data obtained for the two flowers were averaged and subjected to ANOVA using PROC GLM (SAS 1990). Factors involved in the first compar-

ison were Population, Plant nested within Population, and Stamen Type. The second comparison involved Floral Morph, Plant nested within Morph, and Stamen Type.

Pollen number—The number of pollen grains was estimated for the three stamens used in the pollen competition experiments described below. These were stamens s2 and s3 in the M' morph and stamen m3 in the S morph. Plants from population B46 were used and flowers were sampled at the same time as the competition experiments were being conducted. Estimates were made to determine whether the tests performed on siring ability of the different pollen types would need to take into account differences in pollen production among the stamen types. Two samples of four mature buds per plant were collected the evening before blooming and stored dry in microcentrifuge tubes. Pollen number was later estimated using a haematocytometer under $400\times$ magnification following the method of Lloyd (1965). The data on pollen number per stamen were analyzed by a mixed-model ANOVA using PROC GLM in SAS (1990). The comparison between different stamens of M' plants involved Stamen Type and Plant effects and their interaction, and the comparison between stamens occurring at the mid position in the M' and S morphs involved Morph and Plant nested within Morph effects.

In the ANOVAs performed on both pollen size and number, the effect of Plant and its interaction with other factors was considered as random and *F*-tests followed Sokal and Rohlf (1981, pp. 387–393).

Pollen competition—To investigate whether stamen modification in the M' morph is associated with changes in pollen compatibility, pollen competition experiments were undertaken using controlled hand-pollinations and allozyme markers. Plants from population B46 were used in the experiments. Three alternate homozygous genotypes at the AAT-3 locus were available in plants from this population, allowing unambiguous determination of seed paternity (see Cruzan and Barrett, 1993, for further details). Pollen from the elongated stamen competed against pollen from an unelongated short-level stamen from M' plants (experiment 1), and against pollen from a mid-level stamen from S plants (experiment 2). In the first experiment, mixtures of pollen from the elongated (s3) and central (s2) short-level stamens in the M' morph competed against each other. This represents a comparison between two stamens from the same stamen level (s level) and morph (M' morph) but involving anthers in different positions within the flower (s vs. m position). Nine plants of the M' morph were used and these were paired in 16 combinations depending on their genotype

stamen

level

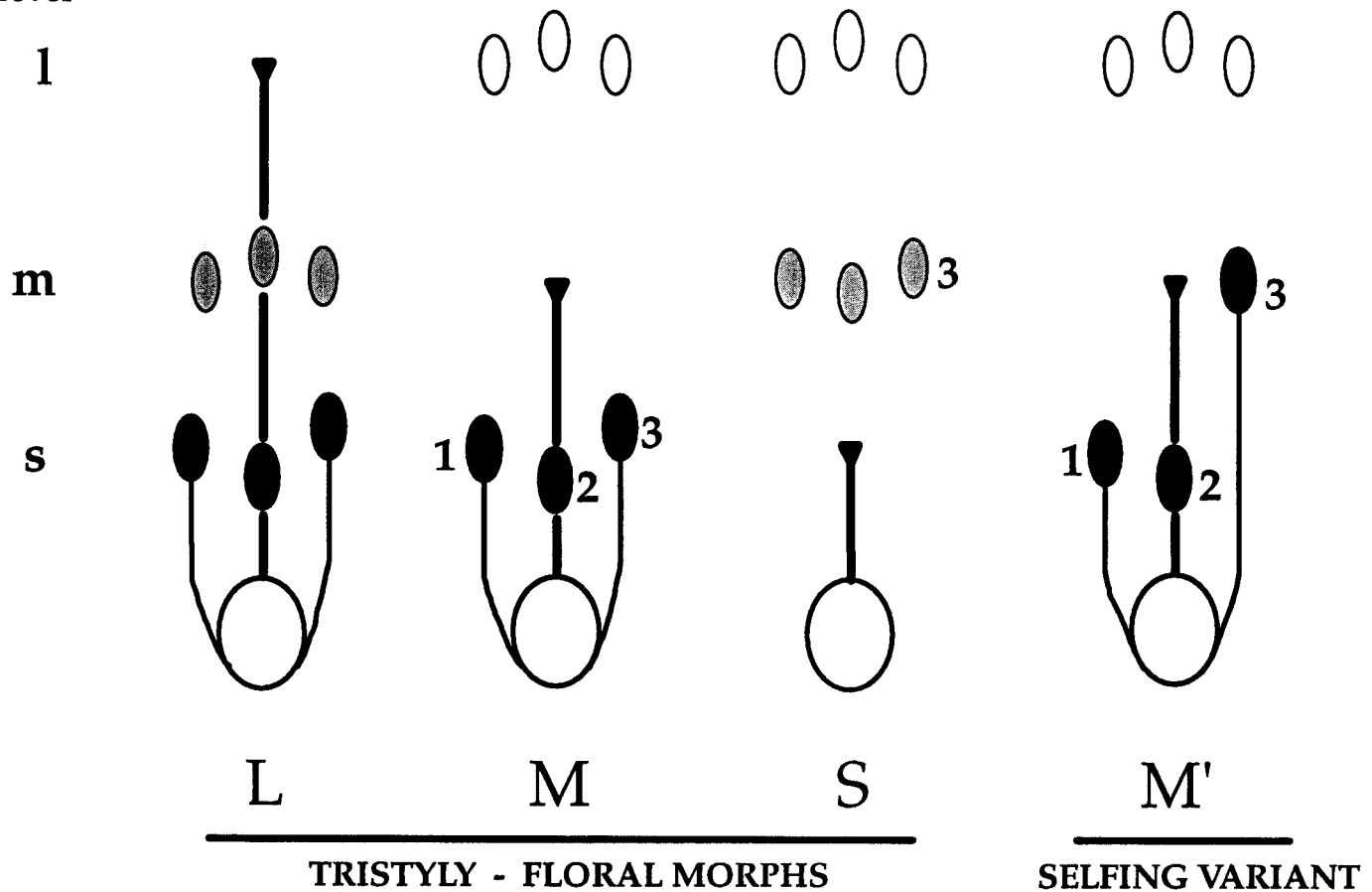


Fig. 1. Schematic illustration of stigma and anther positions in floral morphs of *Eichhornia paniculata*. The floral morphs in a tristylous population are referred to as long-, mid-, and short-styled (L, M, and S, respectively). The selfing variant (M') has a single short-level stamen elongated with the anther adjacent to the mid-level stigma. The long-, mid-, and short-level stamens (l, m, and s, respectively) are represented by different shadings (white, gray, and black, respectively). Within each level, stamens represented from left to right are coded 1, 2, and 3, respectively.

at the *AAT-3* locus. Each pair was used to produce a reciprocal pollen mixture giving a total of 32 pollen mixtures. In the second experiment, the siring ability of pollen from the elongated stamen of the M' morph was compared with pollen from the basiscopic mid-level stamen (m3) in the S morph. These two anthers are in the same position within the flower (m position) but originate from different stamen levels (s vs. m level) and floral morphs (M' vs. S morph). Nine M' plants and 11 S plants were paired depending on their genotypes at the *AAT-3* locus, leading to 32 pollen mixtures. One replicate was lost, giving a total of 31 pollen mixtures. Self-pollen was not used in pollen mixtures of either experiment.

Each experiment was conducted over a 4-d period, with eight pollen mixtures being applied each day. All mixtures were composed of pollen from six stamens (one from each of six flowers) from each of the two donors. Stamens were collected prior to dehiscence and placed in a porcelain spot plate. Shortly after dehiscence the pollen was mixed thoroughly using fine nylon monofilament and then transferred to stigmas. Each pollen mixture was applied to ten flowers, one on each of five M and five S

recipient plants, a total of 11 plants of the M and S morphs being used as recipients in the experiments. Stigmas were covered evenly with the pollen mixture and the identity of the treatment recorded. Fruits were collected 10 d later when they were ripe and five seeds per fruit were assayed at the *AAT-3* locus using electrophoretic techniques outlined in detail by Kohn and Barrett (1992) and Cruzan and Barrett (1993). Data were pooled for the five fruits obtained for each pollen mixture and each recipient morph in experiment 2 (32 sets of 25 seeds for each recipient morph) and, in experiment 1, data for the two reciprocal pollen mixtures from each pollen donor pair were also pooled (16 sets of 50 seeds for each recipient morph). Data were treated as counts of the number of seeds sired by each pollen donor. Replicated goodness-of-fit *G*-tests (Sokal and Rohlf, 1981, p. 721) were performed to assess the siring success of pollen from the two stamen types being compared in each experiment. The null hypothesis used for siring ratios depended on whether the pollen production of the stamens that were being compared differed significantly.

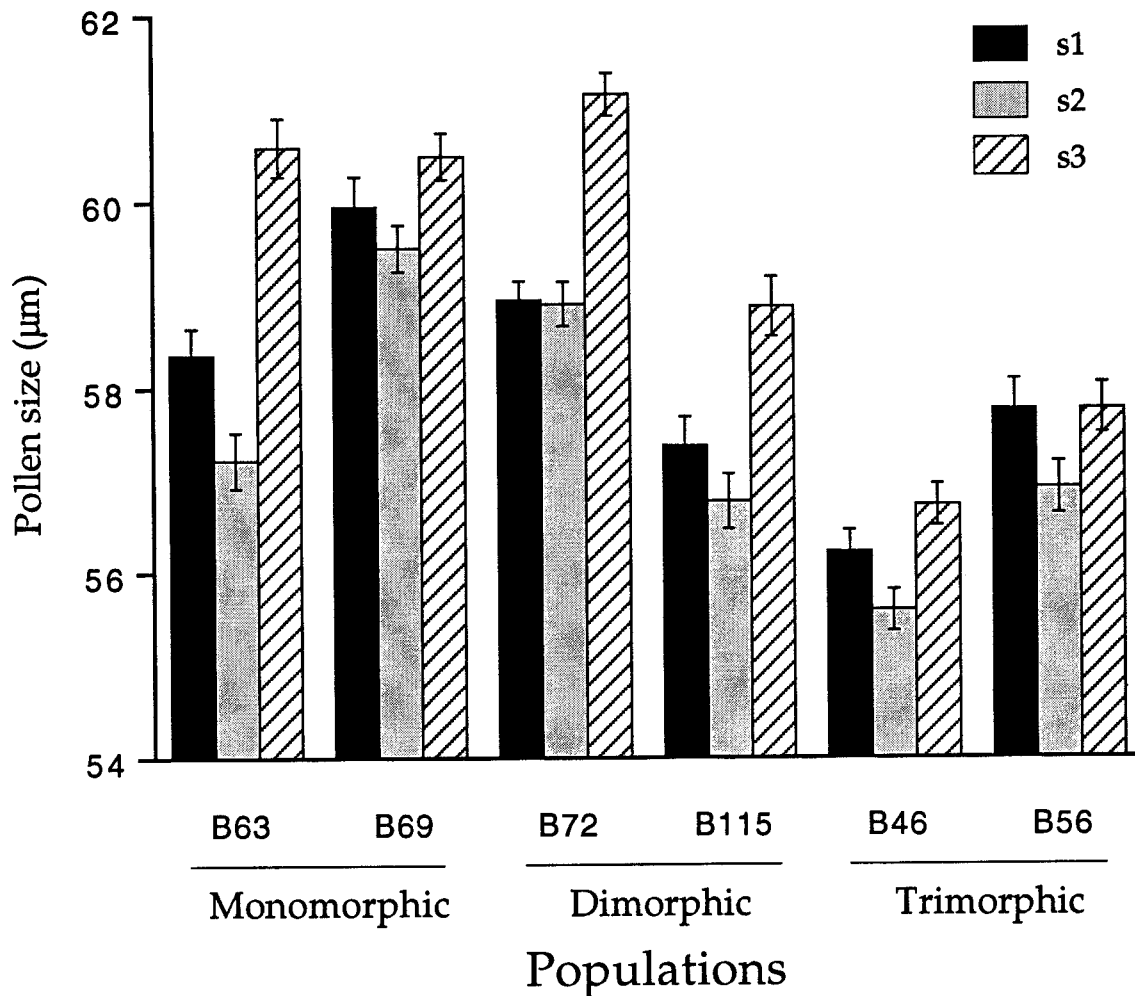


Fig. 2. Pollen size for the three short-level stamens in modified mid-styled plants (M') from six populations of *Eichhornia paniculata* from northeast Brazil. The elongated stamen is coded s3, the central stamen s2, and the unelongated outer stamen s1. Samples were based on five M' plants in B56 and B72, nine M' plants in B46, and six M' plants in other populations. Vertical lines represent ± 1 SE.

RESULTS

Pollen size—Measurements of the mean size of pollen from the three short-level stamens in the M' morph showed significant differences between stamen types when data were pooled from the six populations from northeast Brazil (Fig. 2; $F_{2,62} = 51.1$, $P < 0.001$). Pollen from the elongated stamen (s3) was significantly larger (mean in micrometers = $59.1 \mu\text{m}$, $\text{SE} = 0.13$) than pollen from the unelongated outer stamen (s1, mean = $58.0 \mu\text{m}$, $\text{SE} = 0.12$) and pollen from the central stamen (s2) was significantly smaller (mean = $57.4 \mu\text{m}$, $\text{SE} = 0.12$) than pollen from the other two stamen types. There was significant variation in mean pollen size among the six populations ($F_{5,31} = 2.8$, $P < 0.05$). The interaction between population and stamen type was significant ($F_{10,62} = 3.5$, $P < 0.001$), indicating that the general pattern evident in the pooled data was not consistent among all populations. When each population was analyzed separately it was found that in three of six populations (B63, B72, B115) the elongated stamen contained significantly larger pollen than the other two stamens. In population B46, pollen

from the elongated stamen was only significantly larger than pollen from the central stamen (Tukey-Kramer test 1, Table 2). In the remaining two populations (B56, B69), there were no significant differences in pollen size among the three stamen types. Although the three pollen types were not always significantly different in size within each population, pollen from the elongated stamen was consistently larger in size than pollen from the central stamen (see Fig. 2).

In population B46, further measurements compared pollen from s-level stamens in the M' morph with s- and m-level stamens in the L, M, and S morphs (Table 2). Comparisons of pollen size between short-level stamens s2 and s3 (see Fig. 1) in the L, M, and M' morphs revealed significant Morph ($F_{2,27} = 6.9$, $P < 0.01$) and Stamen ($F_{1,27} = 12.8$, $P < 0.01$) effects. Differences among the stamen types also varied between the morphs (Morph \times Stamen: $F_{2,27} = 4.9$, $P < 0.05$). Of particular importance was the finding that pollen from all three short-level stamens of the M' morph was significantly larger than pollen from the homologous s-level stamens in the

TABLE 2. Pollen size (mean and SE of axis length) and sample size (number of plants) for different stamens in M', M, L, and S plants from population B46 of *Eichhornia paniculata*. The three Tukey-Kramer tests were performed on different groups of stamens, i.e., 1) short-level stamens in the M' morph, 2) central s2 and basis-copic s3 stamens in L, M, and M' morphs, and 3) basis-copic stamens at the mid-level in flowers of the M', L, and S morphs. The means associated with different letters are significantly different ($P < 0.05$).

Morph	Stamen	Number of plants	Pollen size (μm)	Tukey-Kramer		
				1	2	3
M'	s1	9	56.2 \pm 0.23	ab		
M'	s2	9	55.6 \pm 0.23	b	b	
M'	s3	9	56.7 \pm 0.22	a	a	c
M	s2	11	53.6 \pm 0.16		c	
M	s3	11	54.2 \pm 0.17		c	
L	s2	10	53.4 \pm 0.17		c	
L	s3	10	53.3 \pm 0.18		c	
L	m3	10	64.6 \pm 0.24			b
S	m3	11	68.0 \pm 0.19			a

TABLE 3. Replicated goodness-of-fit G tests (GP : pooled G and GH : G for heterogeneity; Sokal and Rohlf, 1981) for pollen competition experiments using plants from population B46 of *Eichhornia paniculata*. The first experiment involves pollen from the central and elongated short-level stamens in the M' morph. The second involves pollen from the elongated stamen in the M' morph and pollen from the homologous mid-level stamen in the S morph. Both pollen competition trials were carried out on M and S recipient plants.

Recipient morph	M		S	
Experiment 1				
	df	G	df	G
Pooled G	1	4.6*	1	0.2
G for heterogeneity	15	21.5	15	19.7
Experiment 2				
	df	G	df	G
Pooled G	1	742.3***	1	15.9***
G for heterogeneity	30	40.1	30	67.7***

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

L and M morphs, the pollen of which did not differ in size between the two morphs (Tukey-Kramer test 2, Table 2). There were significant differences in the size of pollen from stamens positioned at the mid-level in the L, M', and S morphs (Morph: $F_{2,27} = 62.4$, $P < 0.001$). While pollen grains of the elongated stamen of the M' morph are larger in size than pollen from short-level stamens in the L and M morphs, they were still considerably smaller than pollen from mid-level stamens of the L and S morphs (Tukey-Kramer test 3, Table 2). Pollen from mid-level stamens in the L and S morphs differed significantly from each other, with pollen from the S morph larger in size (Tukey-Kramer test 3, Table 2).

Pollen number—The number of pollen grains produced by elongated (s3) and central (s2) stamens in the M' morph were not significantly different [Mean (SE); s3 = 3209 (118); s2 = 3064 (202); $F_{1,8} = 0.36$, $P = 0.57$]. Hence in the competition experiment between these two pollen types described below a null hypothesis of 1:1 for the expected siring ratio was used. In the second comparison involving stamens s3 of the M' morph

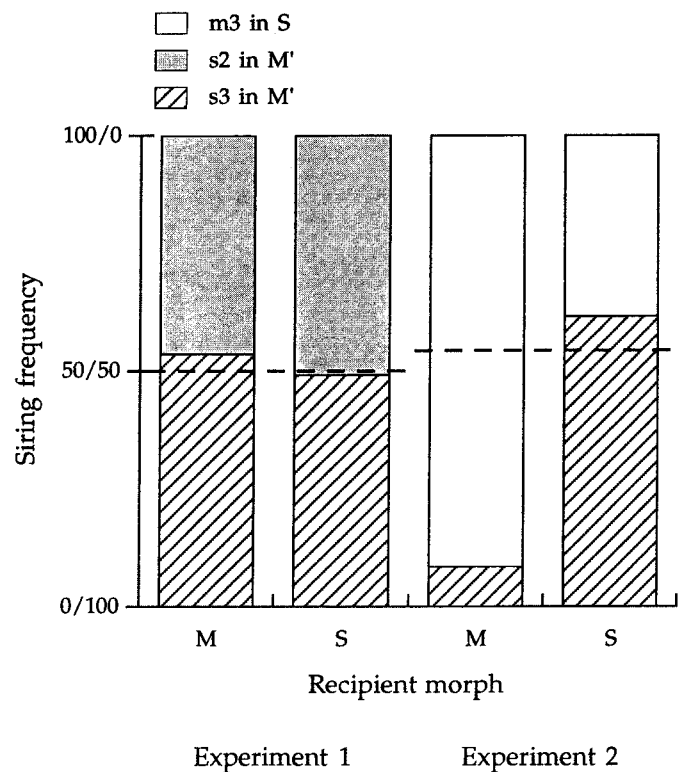


Fig. 3. Percentage of seeds sired by each pollen type following hand-pollination of M and S plants with mixtures of pollen from different stamen types in population B46 of *Eichhornia paniculata*. Experiment 1: competition between pollen from the central (s2) and elongated (s3) stamens from the short level in modified mid-styled (M') plants. Experiment 2: competition between pollen from the elongated stamen (s3) in the M' morph and the homologous mid-level stamen (m3) in the S morph. Expected siring percentage based on pollen counts from stamens involved in pollen mixtures are shown as dotted lines.

and m3 of the S morph a significant difference in the number of pollen grains per stamen was found with less pollen produced by stamen m3 [$m3 = 2716$ (105); $F_{1,18} = 8.04$, $P < 0.05$]. A siring ratio of 54.5:45.5 for the s3:m3 stamens was therefore used in the competition experiment.

Pollen competition—Figure 3 illustrates the results of the pollen competition experiments. Experiment 1 involved pollen from s3 (elongated) and s2 stamens of the M' morph when applied to stigmas of the M and S morphs. Pollen from the elongated stamen sired 53.8% (vs. a 50% expected frequency) of the progeny produced by M plants when competing against pollen from the central stamen. This small advantage to s3 pollen was reflected by a significant G pooled statistic (Table 3) and did not vary among pollen donor pairs (nonsignificant G heterogeneity in Table 3). The siring ratio of the same pollen mixtures on S morph recipients did not differ significantly from 1:1 or show significant heterogeneity (Table 3). Experiment 2 involved pollen mixtures from stamen s3 of the M' morph and stamen m3 of the S morph when applied to stigmas of the M and S morphs. On M stigmas, pollen from m3 stamens strongly outcompeted pollen from s3 stamens with no significant heterogeneity

among mixtures (Fig. 3; Table 3). Although pollen from the elongated s3 stamens of the M' morph made up 54.5% of the pollen mixture, only 8.5% of the seeds produced in pollinations were sired by this pollen type. On recipients of the S morph, pollen from the elongated stamens of the M' morph sired 61.6% of the seeds vs. an expected 54.5% when competing against pollen from stamens m3 in S plants. Although significant heterogeneity was evident among the pollen mixtures (Table 3), the heterogeneity G value was low compared to the pooled G value ($F_{1,30} = 7.0$, $P < 0.025$).

DISCUSSION

Morphological and physiological characteristics of pollen grains differ among stamen levels in most heterostylous species that have been studied (Dulberger, 1992; Barrett and Cruzan, 1994). In tristylous *Eichhornia paniculata*, pollen grains from short-level stamens tend to be smaller in size than pollen from long- and mid-level stamens (Barrett, 1985). Furthermore, in competition experiments with other pollen types, pollen from short-level stamens has a siring advantage on short-level stigmas but a disadvantage on mid- and long-level stigmas (Cruzan and Barrett, 1993). In the present study it was shown that subtle differences in the size and compatibility of pollen may also occur within a stamen level where genetic alterations to the position of individual anthers have occurred. In selfing mid-styled (M') variants from Brazilian populations, pollen from short-level stamens elongated to the mid-position was slightly larger in size than pollen from unmodified short-level stamens. In addition, pollen from the modified stamen sired a small excess of seeds on mid-styled recipients when competing with pollen from unmodified short-level stamens. Pollen from the elongated stamen was nevertheless found to be markedly different from typical mid-level pollen, being smaller and exhibiting both a strong siring disadvantage on mid-level stigmas and a siring advantage on short-level stigmas relative to typical mid-level pollen. These results indicate that although the anther of the modified stamen occurs at the mid-level position within tristylous flowers of *E. paniculata*, the basic properties of its pollen are those of short-level anthers.

Kohn and Barrett (1994) have shown that in experimental populations of *E. paniculata* lacking the S morph, M' plants have an outcrossed siring advantage over unmodified M plants. Although we cannot directly refute the hypothesis that postpollination mechanisms account for this difference, several aspects of our results cast doubt on this as a general explanation. While short-level pollen from M' plants is significantly larger than short-level pollen from M plants, the difference in size is relatively small in comparison with the large difference between short- and mid-level pollen from unmodified plants. This size difference could result in a small siring advantage of M' over M plants; however, it would seem unlikely to be of sufficient magnitude to account for the large mating asymmetry (1.5–2.2 M' vs. M) that was observed by Kohn and Barrett (1994). These considerations suggest that Kohn and Barrett's results are more likely to be explained by preferential pollen transfer from elongated short-level stamens with anthers at the mid-position

to stigmas of mid-styled plants than by postpollination differences in siring success. Elsewhere, several field studies of heterostylous populations have demonstrated preferential transfer of pollen between anthers and stigmas of equivalent height (Ganders, 1974; Barrett and Glover, 1985; Piper and Charlesworth, 1986). These studies provide support for Darwin's hypothesis (Darwin, 1877) that the reciprocal arrangement of reproductive organs in heterostylous plants is a floral mechanism promoting insect-mediated cross-pollination (Barrett, 1990; Lloyd and Webb, 1992b).

Modifications to pollen size and compatibility in the elongated stamen of M' plants are relevant to questions about the genetic and developmental mechanisms controlling the expression of heterostylous traits. The phenotype of the selfing variant is unlikely to have resulted from crossing-over in a putative heterostyly supergene since it is difficult to imagine how the traits that it possesses could have arisen from recombination. Pollen size in all three short-level stamens of M' plants is significantly larger than the homologous stamens of M plants but is markedly smaller than mid-level pollen of unmodified plants. Similarly, the siring ability of pollen from the elongated stamen of M' plants differs from both short- and mid-level pollen in the unmodified morphs. Moreover, crossing-over would seem unlikely to have caused elongation of just a single stamen of the three short-level stamens. These patterns are not readily interpreted under the supergene model, although they do not by themselves constitute evidence against the existence of a supergene. It seems more reasonable, therefore, to interpret the variants as resulting from the action of modifier gene(s). Indeed, genetic studies of selfing variants from contrasting parts of the geographical range have demonstrated that stamen modification is under the control of recessive allele(s), and different loci are responsible for stamen elongation in northern and southern parts of the Brazilian range (Fenster and Barrett, 1994).

One of the most striking results that we obtained was the finding that modifications to pollen size in M' plants from population B46 were not restricted to the elongated stamen. The other two short-level stamens produced pollen that was significantly larger than pollen from short-level stamens of unmodified plants. This suggests that the modifier gene(s) responsible for stamen elongation in selfing variants also has pleiotropic effects on pollen size. Why only a single stamen becomes elongated whereas all short-level pollen is affected seems anomalous at first. Our observations of occasional aberrant flowers produced by selfing variants, however, may help explain this apparent dissociation between pollen size and stamen elongation within short-level stamens of M' plants (see below).

A characteristic feature of selfing variants in natural populations of *E. paniculata* is the high degree of stamen instability expressed by certain genotypes (Barrett, 1985; Seburn, Dickinson, and Barrett, 1990; Barrett and Harder, 1992). One manifestation of this is the production of both modified and unmodified flowers within the same inflorescence. Also, while most modified flowers possess a single elongated stamen (s3), occasional flowers are observed with two (s3, s1) and sometimes three short-level stamens with anthers at the mid-position. Selfing variants

from population B46 produced a low frequency of unmodified flowers and, very rarely, flowers with two elongated stamens. The absence of continuous variation in the length of short-level stamens in conjunction with the instability of discrete stamen phenotypes within plants suggests that the control of stamen elongation in M' plants is governed by a threshold response (and see Richards and Barrett, 1992).

We propose that all short-level stamens in M' plants are affected by a quantitative signal that varies in intensity among the three stamens. This variation may result from both a position effect among short-level stamens (see Barlow and Carr, 1984) and differences in timing of stamen development (Richards and Barrett, 1984). Indeed, in *E. paniculata* both lateral short-level stamens are associated with outer tepals, belonging to the outer stamen whorl, and initiate their development earlier than the central short-level stamen, which is associated with an inner tepal belonging to the inner stamen whorl (Richards and Barrett, 1984, fig. 1). As a result of these differences in position and timing of origin, the outer stamens may experience different developmental conditions than the central stamen, e.g., different hormonal concentration(s) and/or a longer duration of sensitivity to hormonal effect(s). We propose that whether or not a particular stamen elongates depends on the intensity of the signal it receives, and this is likely determined by both genetic factors and position and/or timing effects in development. The control of pollen size in M' plants, on the other hand, appears to be unaffected by such a threshold and, as a result, the three stamens respond similarly to the signal produced by the modifier gene(s). The subtle differences in pollen size that are evident among the three short-level stamens may also result from position and/or timing effects in development. These differences are, however, small in comparison with the overall increase in pollen size in M' plants.

Although the developmental and physiological mechanisms responsible for stamen elongation have received little attention, it is known that elongation occurs shortly before the opening of the flower due to cell elongation induced by an appropriate balance of growth hormones (gibberellin or auxin) and/or ethylene concentration (Koning, 1983; Koning and Raab, 1987). In *E. paniculata*, stamen elongation in M' plants occurs 24 hr before anthesis (Richards and Barrett, 1992) through differential cell elongation. It would thus be of interest to examine whether differences in stamen elongation occur in response to spatial variation in hormonal concentration within the flower. The correlation between pollen size and stamen length observed both among stamen levels and within the short-level of M' plants suggests a pleiotropic effect, which may be mediated by hormones.

Pollen from the elongated stamens sired a small but significant excess of progeny when competing against pollen from the central short-level stamen on stigmas of M plants. As observed in other heterostylous species (see Dulberger, 1992 for a review), in *E. paniculata* there is a correlation between pollen size and compatibility among the three stamen levels (Cruzan and Barrett, 1993). The difference in siring ability observed between pollen from the elongated and central s-level stamens of M' plants matches the difference in pollen size with the

larger pollen exhibiting higher siring success. This result therefore parallels the pattern observed among stamen levels but occurs at a finer scale, being expressed within a stamen level. These findings support the hypothesis that pollen size is associated in some way, either directly or indirectly, with the compatibility mechanism (Mather and De Winton, 1941; Scribailo and Barrett, 1991; Dulberger, 1992). Further work comparing the siring success of pollen from homologous short-level stamens of M and M' plants would be of particular interest in this regard because of the differences in pollen size exhibited by the two types of plants. In addition, comparisons of pollen size and siring ability between modified and unmodified flowers of M' plants would also be valuable, since it may enable a more refined analysis of environmental effects on pollen characteristics.

The frequency of selfing variants of the M morph differs among trimorphic, dimorphic, and monomorphic populations of *E. paniculata* in northeast Brazil (Husband and Barrett, 1993). Variants are rare in trimorphic, common in dimorphic, and usually fixed in monomorphic populations. We compared pollen size in short-level stamens of M' plants from each of these population morph structures. Pollen was significantly smaller in size in trimorphic (Mean in micrometers; B46:56.2; B56:57.5) compared to dimorphic (B115:57.7; B72:60.1) and monomorphic (B63:58.7; B69:60.0) populations (a posteriori contrast between trimorphic and nontrimorphic populations, $t = 2.8$, $P < 0.01$). This pattern is consistent with pollen size data obtained from an independent sample of *E. paniculata* populations of contrasting morph structure (Barrett 1988, fig. 5). Virtually all nontrimorphic populations of *E. paniculata* lack the S morph (Barrett, Morgan, and Husband, 1989; Husband and Barrett, 1993). In populations in which the S morph is missing, there may be no selection to maintain the pollen size and compatibility characteristics of short-level pollen. As a result, there is likely to be selection to increase its compatibility on mid-level stigmas. The general increase in pollen size observed in nontrimorphic compared with trimorphic populations may be explained by this process if we assume, as argued above, that pollen size and compatibility are functionally correlated.

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