

CONTRIBUTION OF CRYPTIC INCOMPATIBILITY TO THE MATING SYSTEM OF *EICHHORNIA PANICULATA* (PONTEDERIACEAE)

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Abstract.—Tristyloous populations of the annual aquatic *Eichhornia paniculata* have high levels of outcrossing and intermorph mating despite being fully self- and intramorph compatible. Experimental studies of pollen germination, pollen-tube growth, and pollinations with mixtures of genetically marked pollen were used to determine whether postpollination processes contribute to the observed mating patterns. Differences in pollen germination were small and did not contribute to differences in pollen siring ability. The fraction of pollen tubes first entering the ovary, however, was greater for legitimate outcross pollen than for either of the other two pollen types (self or outcross illegitimate pollen) in all recipient morphs. Moreover, legitimate pollen had higher siring success when in competition with illegitimate pollen types (self or outcross) in each recipient style morph. The ranking of pollen performance for different pollen-style combinations was the same for both the pollen-tube growth and marker-gene experiments indicating that differences in pollen-tube growth rate are the principal cause of differences in pollen siring ability. Cryptic incompatibility in *E. paniculata* may represent a weak heteromorphic incompatibility system because the observed patterns of pollen-tube growth parallel pollen-tube growth and seed-set patterns that occur in related species with strong trimorphic incompatibility. The ability to produce mostly outcrossed progeny when pollinators are abundant, but to reliably produce seed under a variety of environmental and demographic conditions may be favored in *E. paniculata* because of its colonizing life history and occurrence in ephemeral habitats. Cryptic incompatibility may be more likely to occur in species subject to wide fluctuations in population size and levels of pollinator service.

Key words.—Cryptic incompatibility, *Eichhornia paniculata*, genetic markers, pollen-tube growth, Pontederiaceae, siring success, tristily.

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Outcrossing in hermaphroditic plants can be controlled by specific incompatibility reactions through the inhibition of self pollen (Nettancourt 1977; Lewis 1979), postzygotic failure of selfed embryos caused by inbreeding depression, or the expression of postzygotic self-incompatibility (Seavey and Bawa 1986; Waser and Price 1991; Montalvo 1992). Many self-incompatible species produce little or no seed after self-pollination because of nearly complete inhibition of self pollen. The resulting avoidance of selfing is a benefit to plants because of reduced inbreeding depression (Lande and Schemske 1985; Uyenoyama 1986; Charlesworth and Charlesworth 1987; Olmstead 1989); however, it also precludes seed production when pollen vectors are unreliable and may represent a greater liability for some species. The rigid mating system imposed by strong self-incompatibility would be particularly disadvantageous for species with annual life cycles that occupy ephemeral habits, because they rely on seed production to maintain populations

and colonize new sites. Such species are generally self-compatible and often have floral mechanisms (e.g., autogamy, cleistogamy) that assure high seed set (Baker 1965; Lloyd 1980).

Weak (or cryptic) incompatibility permits a flexible mating strategy that leads to the production of outcrossed progeny during pollinator abundance and the production of selfed seed during pollinator scarcity (Bowman 1987; Becerra and Lloyd 1992). Bateman (1956) first described "cryptic" self-incompatibility as the production of primarily outcrossed progeny by a self-compatible species after pollination with a mixture of self and outcross pollen. He originally ascribed the preferential production of outcrossed progeny in *Cheiranthus* to differential pollen-tube growth in the style, but selective abortion of selfed seeds caused by inbreeding depression also increases the frequency of outcrossing in self-compatible species (Moran and Brown 1980; Levin 1984; Cheliak et al. 1985; Charlesworth 1988). Cryptic incompatibility is difficult to detect without genetic markers, and only a few studies have demonstrated its action (Bateman 1956; Weller and Ornduff 1977, 1989; Bowman 1987; Casper et al. 1988). Consequently, the prevalence of

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cryptic self-incompatibility in species that appear to be self-compatible, as judged by substantial seed set after self-pollination, is unknown (Walsh and Charlesworth 1992).

Here we investigate the mechanisms responsible for the high rates of outcrossing and intermorph mating in tristylous populations of *Eichhornia paniculata* (Spreng.) Solms-Laubach (Pontederiaceae). Plants of *E. paniculata* can be one of three floral morphs (long-, mid-, or short-styled, hereafter L, M, and S morphs, respectively). Each morph is fully self-, intra-, and intermorph compatible (Barrett 1985; Kohn and Barrett 1992). In spite of high self- and intramorph fertility, trimorphic populations typically outcross (Glover and Barrett 1986; Barrett and Husband 1990) with most outcrossed progeny resulting from intermorph matings (Barrett et al. 1987; Morgan and Barrett 1990; Kohn and Barrett 1992). The reciprocal placement of stigmas and anthers in different morphs of heterostylous species has been hypothesized to promote pollen transfer between morphs (Darwin 1877; Ganders 1979; Lloyd and Webb 1992). Indeed, experimental manipulation of morph structure in garden populations of *E. paniculata* demonstrated that reciprocal morphologies contribute to the high levels of outcrossing and intermorph mating (Kohn and Barrett 1992). However, stigmas of heterostylous plants typically capture abundant self and outcross pollen from all anther levels (Ganders 1979; Barrett and Glover 1985), suggesting that postpollination discrimination among pollen types may also produce the observed mating patterns. Here we determine the potential of cryptic incompatibility to contribute to the high rates of outcrossing and intermorph mating found in *E. paniculata* by examining pollen germination, pollen-tube growth, and the siring ability of different pollen types when in competition.

MATERIALS AND METHODS

Eichhornia paniculata is an aquatic annual that inhabits temporary ponds and ditches throughout the seasonally arid caatinga region of northeastern Brazil (Barrett 1985). The showy, bee-pollinated flowers, last for a single day and contain 50–120 ovules (Morgan and Barrett 1989). Flowers of each morph bear reproductive organs at three different levels; the stigma occupies one level, and anthers occupy the other two levels (long-, mid-, and short-level anthers, hereafter l, m, and s, respectively). Pollinations in heterostylous plants are defined as legitimate

(pollen from the same anther level as the stigma) or illegitimate (pollen from either of the other two anther levels). Legitimate pollinations involve only intermorph crosses, whereas illegitimate pollinations can be either self, intramorph, or intermorph. Plants were grown under glasshouse conditions (temperature ranged from 22° to 30°C) under which flowers opened between 8:00 and 9:00 A.M. each day and wilted 6–8 h later. Tristylous plants of *E. paniculata* do not normally produce seed unless crossed. All pollinations were conducted between 9:00 and 11:00 A.M.

The experiments described below involved plants that were derived from a trimorphic population (B46: Barrett and Husband 1990) in Ceará state, northeastern Brazil. Seed progeny from open-pollinated maternal parents were intercrossed to produce a second generation from which we selected the experimental plants. Only one individual was selected from each maternal family.

Pollen Germination

We measured pollen germination following all possible anther level-style morph combinations for self, intra-, and intermorph crosses. Eight flowers on a single inflorescence of one plant of each morph were pollinated on each day with each flower receiving one of the eight treatments indicated in figure 1. Pollinations were carried out over 9 days, and three different plants (one of each morph) were used each day for a total of nine plants per morph. Flowers were pollinated by evenly coating stigmas with a single newly dehisced anther. Forty-five minutes after pollination, stigmas were fixed in 70% EtOH in 1.5 mL microcentrifuge tubes.

Pollen germination was recognized by the absence of cytoplasm and/or the presence of a pollen tube projecting from the grain. Pollen-grain walls and cytoplasm were stained by adding a drop of aniline blue (0.1% by weight in water) and basic fuchsin (0.05% by weight in water) to the microcentrifuge tubes containing stigmas. Stained stigmas were placed on a microscope slide with a drop of water and the tissue evenly spread by gently pressing on the cover slip. We counted germinated and ungerminated grains under a compound microscope at 100×. The examination of the contents of a subsample of microcentrifuge tubes after stigmas had been removed indicated that very few if any grains were dislodged from stigmas by this procedure. The

proportion of germinated grains was analyzed separately for each morph by analysis of variance with recipient identity as a random factor, anther level, donor morph, and pollination type (self or outcross) as fixed factors, and donor identity nested within donor morph using the GLM procedure of SAS (SAS Institute 1985). The untransformed data approximated a normal distribution.

Pollen-Tube Growth

We measured pollen-tube growth by counting the number of pollen tubes at the base of the style after the fastest tubes had reached the ovary. We chose this measure of pollen-tube growth because the number of pollen tubes typically reaching the ovary after growth has ceased is greater than the number of fertilized ovules. Our approach assumes that ovule fertilization occurs in the sequence of pollen-tube arrival to the ovary; pollen types that grow to the ovary more quickly will procure the majority of fertilizations. By measuring the growth of only the fastest pollen tubes, we obtained a measure of pollen-tube growth that more accurately reflects the fertilization ability of different pollen types when in competition with each other.

To measure pollen-tube growth for different anther level-style morph combinations we used the same design that was used to assess pollen germination. Three hours after pollination we excised styles at the top of the ovary and fixed them in 70% EtOH. Preliminary observations indicated that the fastest pollen tubes entered the ovary after this time, but the majority of tubes were still growing in the style. Styles were stored in alcohol for at least 24 h before clearing in 8 N NaOH at 60°C for 1 h. Cleared styles were rinsed with water three times and then stained with 0.1% aniline blue in 0.5 M tris-HCl buffer (pH 7) for at least 24 h. Stained styles were then placed on a microscope slide, and the lower 2 mm of the style were excised. This section was macerated in a drop of water and a cover slip was applied. Pollen tubes were counted at 200× using an epi-illumination fluorescence microscope (365 nm excitation, 420 nm barrier filters) by scanning the entire preparation underneath the cover slip. The total number of pollen grains on the stigma was also counted after squashing the top portion of the style under a cover slip on the same slide. The clearing process removed ungerminated pollen, thus, only germinated grains remained on the stigma.

Cross	Donor	i	m	s	Donor	i	m	s	Donor	i	m	s
Inter-morph	M	●	*	*	L	●	*	*	L	●	*	*
Inter-morph	S	●	*	*	S	*	●	*	M	*	*	●
Intra-morph	L	*	*	*	M	*	*	*	S	*	*	*
Self	L	*	*	*	M	*	*	*	S	*	*	*
		L			M			S				
		Recipient										

FIG 1. Experimental design used to examine pollen germination and pollen-tube growth in *Eichhornia paniculata*. Solid circles indicate legitimate pollinations (pollen from the same anther level as the stigma) and asterisks indicate illegitimate pollinations (anther level and style length not the same).

The data were analyzed to determine whether pollen from different sources differed in their germination and growth in each morph (table 1). The number of germinated grains present on the stigma, the number of pollen tubes reaching the base of the style after 3 h, and the proportion of tubes reaching the base of the style (tubes divided by the number of grains present) were dependent variables for ANOVAs (GLM procedure, SAS Institute 1985). Independent variables included were the same as for pollen germination, with the number of grains present on the stigma included as a covariate in the analyses of pollen tubes at the base of the style.

Siring Success

The competitive ability of pollen from different sources was assessed as siring success using electrophoretic markers. Mixtures of two pollen types were used to compare self with intermorph pollen, self with intramorph pollen, and intramorph with intermorph pollen. For these comparisons we used both anther levels of the recipient morph and paired each level with one of three possible donor anther levels; legitimate, illegitimate of the same level, or illegitimate of the alternate level (see table 2). Each of the 12 comparisons per morph was replicated five times over 5 days for a total of 60 pollinations per morph. Each day two plants of each morph were pollinated with 6 of the 12 pollen mixtures indicated in table 2, with each plant receiving the mixtures that involved one of the two recipient anther levels for that morph. Pollen donors and recipients were homozygous for one of three alternate alleles at the *AAT-3* locus, allowing unambiguous determination of seed paternity.

Pollen was applied to the stigma sequentially, with the order of application of pollen types reversed on alternate days. On the average, each pollen type should sire 50% of the seeds in each pollen comparison, because switching the order

TABLE 1. ANOVAs of the proportion of pollen germination after 45 min, the total number of germinated grains after 3 h, and the number and proportion of pollen tubes reaching the base of the style after 3 h for the three style morphs of *Eichhornia paniculata*. *F*-values are given for each test. The experimentwise error rate ($\alpha' = 0.02$) was adjusted for three analyses.

Source	df	Pollen germination		Pollen tubes at style base	
		45 min	3 h	Number	Proportion
A. Long-styled morph					
Anther level	2	0.56	16.60***	18.67***	15.25***
Recipient	7	6.73***	5.39***	1.19	1.42
Donor morph	2	0.05	1.18	1.57	1.88
Donor(morph)	24	1.14	2.08*	1.06	0.90
Self versus outcross	1	0.04	1.70	0.00	0.19
Number of grains	1	—	—	0.16	1.50
B. Mid-styled morph					
Anther level	2	3.67*	11.36***	8.78***	9.07***
Recipient	7	5.18***	2.27*	0.84	1.53
Donor morph	2	1.34	0.38	1.13	1.04
Donor(morph)	24	2.62**	0.65	0.78	0.86
Self versus outcross	1	13.28***	0.05	0.00	0.06
Number of grains	1	—	—	7.19**	0.00
C. Short-styled morph					
Anther level	2	1.27	7.95***	1.29	2.76†
Recipient	7	4.19***	1.09	0.71	1.79
Donor morph	2	0.23	0.69	2.53	2.20
Donor(morph)	24	1.68†	0.84	1.64†	0.73
Self versus outcross	1	0.00	0.06	1.69	0.49
Number of grains	1	—	—	18.86***	1.77

† $P < 0.10$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

of application for each replicate should equalize any advantage of the pollen type applied first. Treatments were indicated by marking flowers at their base with a unique color combination of acrylic paint. Fruits were collected upon ripening, 10 days after pollination. Ten seeds from each fruit were assayed using starch-gel electrophoresis after grinding in sodium phosphate buffer (pH 7.5; with 1 mg/mL diethyldithiocarbamate, 0.5 mg/mL EDTA, 1.25 mg/mL bovine serum albumin, and a trace of Tween 80). Gels were stained using previously described methods (Kohn and Barrett 1992).

Data were treated as counts of the number of seeds fertilized by each pollen type for the five pollinations made for each comparison. Tests for departure from an expectation of 50% for each pollen comparison were made using replicated goodness of fit *G*-tests. Least-squares ANOVAs were used to control for the effects of pollen-application order and differences among recipients while testing for differences among pollen mixtures in each morph. Using the method outlined by Rao (1973, pp. 427–431), counts were transformed by calculating the inverse sine of the square root of the ratio $(y + \frac{3}{8}) / (n + \frac{3}{4})$, where y is the number of seeds fertilized by one pollen

type and n is the number of seeds scored. We calculated χ^2 values for each test, using type III sums of squares (from the GLM procedure; SAS Institute 1985) as the numerator and an estimate of the binomial variance $[1/(4n + 2)]$; Rao 1973] as the denominator. The 12 pollen comparisons made for each morph were entered as a single variable and the effect of legitimate versus illegitimate, self versus outcross, and intra- versus intermorph pollen sources were tested using orthogonal contrasts.

RESULTS

Pollen Germination

The number of pollen grains that germinated after 45 min for different pollen-style combinations varied from 23 to 62%. More pollen germinated on stigmas of the L morph ($F = 19.58$, $P < 0.001$, $df = 2, 24$) than on stigmas of either the M or S morphs for all three pollen types (fig. 2a). Although pollen from s anthers tended to germinate better than pollen from other anther levels this difference was not significant ($F = 1.86$, $P > 0.15$, $df = 2, 152$; pooled across style morphs with the independent variables indicated in table 1). Analyses within style morphs indicated little

TABLE 2. Relative frequency of fertilization by different classes of donors after paired pollinations of the style morphs of *Eichhornia paniculata*. Each of five recipients per cross (except as indicated) received self or intramorph pollen plus pollen from another source. Asterisks adjacent to the fertilization frequencies indicate significant departures from a random expectation (0.5; replicated goodness-of-fit test). Tests for heterogeneity among recipients are in parentheses.

Self versus intramorph			Self versus intermorph			Intramorph versus intermorph		
Self anther	Intra-morph anther	Proportion intramorph	Self anther	Inter-morph/anther	Proportion intermorph	Intra-morph anther	Inter-morph/anther	Proportion intermorph
A. Long-styled morph								
m	m	0.54 (20.34***)	m	S/m	0.70** (6.84)	m	S/m	0.68* (14.58**)
s	s	0.78*** (17.72**)	s	M/s	0.20*** (4.83)†	s	M/s	0.37 (8.49*)‡
m	s	0.04*** (4.83)	m	S/l	0.54 (27.21***)	m	S/l	0.62 (19.53***)
s	m	0.94*** (8.24)	s	M/l	0.98*** (1.78)	s	M/l	0.78*** (43.10***)
B. Mid-styled morph								
l	l	0.38 (14.78**)	l	S/l	0.52 (16.23**)	l	S/l	0.35* (6.50)
s	s	0.78*** (5.78)‡	s	L/s	0.72** (12.05*)	s	L/s	0.54 (9.04)
l	s	0.24*** (10.49*)	l	S/m	0.57 (6.25)	l	S/m	0.86*** (3.43)
s	l	0.80*** (8.90)	s	L/m	0.80*** (8.90)	s	L/m	0.60 (20.47***)
C. Short-styled morph								
l	l	0.48 (17.04**)	l	M/l	0.62 (22.98***)	l	M/l	0.50 (12.42*)
m	m	0.64* (13.25*)	m	L/m	0.39 (6.19)	m	L/m	0.39 (31.44***)
l	m	0.82*** (11.78*)	l	M/s	0.92*** (6.04)	l	M/s	0.72** (25.47***)
m	l	0.17*** (4.80)	m	L/s	0.84*** (9.88*)	m	L/s	0.76*** (9.93*)

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

† Sample size was three recipients.

‡ Sample size was four recipients.

difference in germination of the three pollen types on stigmas of the L and S morphs (table 1); however, a smaller fraction of l pollen germinated on stigmas of the M morph than m or s pollen (fig. 2a). The M morph was the only morph for which more self pollen germinated than outcross pollen (0.35 and 0.27 for self and outcross pollen, respectively). Pollen germination also varied among individual recipients in all three style morphs and among individual donors in the M morph (table 1). The morph of the donor did not contribute to differences in germination (table 1).

Pollen-Tube Growth

The number of germinated pollen grains present on stigmas after 3 h differed depending on both recipient morph and donor anther level (fig. 2b). More grains germinated on stigmas of the L

morph (mean = 441.1) than on stigmas of the M morph (mean = 227.7) or the S morph (mean = 177.1); ($F = 38.43$, $P < 0.001$, $df = 2, 23$). More germinated grains from s anthers were present on stigmas of all three morphs, followed by pollen from m and l anthers (fig. 2b; table 1). On all recipient stigmas the ranking of the number of germinated grains from each anther level was the same and the recipient morph by anther level interaction was small ($F = 0.69$, $P > 0.60$, $df = 4, 145$; fig. 2b).

Pollen-tube growth to the ovary after 3 h, expressed as the number of tubes at the base of the style and the proportion of grains with tubes at the base of the style, both depended on the combination of the recipient morph and donor anther level (fig. 2c). The ranking of pollen-tube growth differed for each recipient morph; there were more

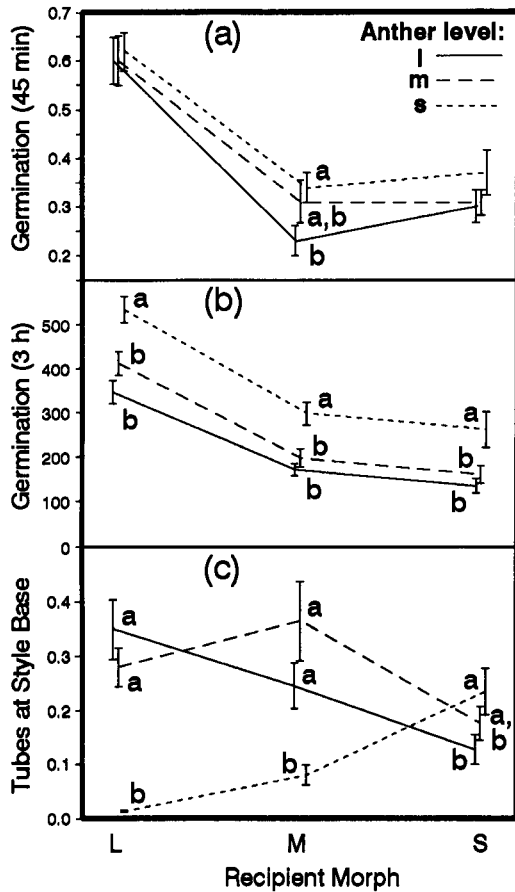


FIG 2. The proportion of pollen germinated after 45 min (a), total number of grains germinated after 3 h (b), and the proportion of pollen tubes reaching the style base after 3 h (c) following controlled pollinations with pollen from differing anther level and recipient style morph combinations in *Eichhornia paniculata*. Means having the same lowercase letter do not differ for comparisons within a recipient morph (Tukey's multiple-range test). Vertical bars represent two standard errors of the mean.

pollen tubes at the base of styles after pollination with legitimate pollen than either of the illegitimate pollen types for all morphs. As a result, there was a strong recipient morph by anther level interaction for the proportion of pollen tubes reaching the ovary ($F = 11.34, P < 0.001, df = 4, 144$; pooled across style morphs with independent variables as indicated in table 1). Analyses for each recipient morph revealed large differences in pollen-tube growth for pollen from different anther levels for the L and M morphs, and a smaller difference in the S morph (fig. 2c, table 1). There were only minor differences in

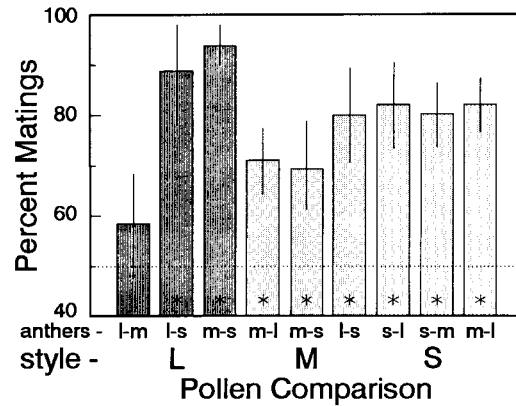


FIG 3. Siring success following controlled pollinations with pollen mixtures in *Eichhornia paniculata*. Bars indicate the frequency of matings obtained by the first pollen type listed from pollinations using pairs of pollen types for each recipient morph. Vertical bars represent two standard errors of the mean, and asterisks indicate means that differ ($P < 0.05$ from G -tests) from the random expectation of 50% (dotted line).

pollen-tube growth caused by differences among recipients or donors. Self pollen did not differ from outcross pollen of the same anther level in either the number or proportion of tubes reaching the ovary for each of the recipient morphs (table 1).

Siring Success

When two pollen types from different donors were placed on stigmas of the three style morphs, the anther level and recipient morph largely determined the siring success of each pollen type. These patterns were complicated by the order of pollen application and differences among recipients (tables 2, 3). Once application-order effects were held constant, however, the differences in pollen-fertilization success resulted primarily from source-anther levels (i.e., legitimate versus illegitimate; table 3). Legitimate pollen consistently outcompeted illegitimate pollen in each morph (fig. 3). Competition between illegitimate pollen types resulted in pollen from the anther level closest to the stigma (i.e., the m anther level) procuring most of the fertilizations in the L and S morphs, and pollen from l anthers outcompeting pollen from the s anthers in the M morph (fig. 3). No differences in pollen competitive ability were detected when intramorph pollen was compared to intermorph pollen, or when self-pollen was compared to intramorph pollen (table 3).

TABLE 3. Least-squares ANOVAs of the probability of fertilization by different classes of pollen donors after paired pollinations of the three style morphs of *Eichhornia paniculata*. Type III sums of squares (Proc GLM; SAS Institute 1985) and the binomial error variance were used to calculate χ^2 values for the effects for recipient identity, order of pollination, and the pollen mixture used (from table 2). Contrasts were made for legitimate versus illegitimate, intra- versus intermorph, and intramorph versus self pollen sources. The experimentwise error rate ($\alpha' = 0.02$) was adjusted for three analyses.

Source	df	Style morph		
		Long	Mid	Short
Recipient	9	52.08***	16.89	34.31***
Order	1	16.72***	32.68***	27.76***
Pollen mixture	11	158.34***	70.35***	96.81***
Contrasts:				
Legitimate versus illegitimate	1	26.08***	17.25***	49.27***
Intramorph versus intermorph	1	1.42	0.00	0.58
Intramorph versus self	1	0.87	0.09	0.39

** $P < 0.01$, *** $P < 0.001$.

DISCUSSION

The observed patterns of pollen-tube growth and competitive ability indicate that postpollination processes contribute to levels of outcrossing and intermorph mating in populations of *Eichhornia paniculata*. In all three style morphs, legitimate pollen grew faster and outcompeted pollen from other anther levels. The observed differences in pollen siring ability most likely reflect differences in pollen-tube growth, because (1) stigma loads were sufficiently large to ensure competition for access to ovules, (2) the ranking of pollen in terms of competitive ability in each morph are the same as the ranking for pollen-tube growth, and (3) ovule abortion occurs infrequently in *E. paniculata* (5%–10%), minimizing the potential contribution of postfertilization processes to variation in siring ability (Morgan and Barrett 1989). Under field conditions, pollen competition would result in fewer self and intramorph matings and an excess of intermorph matings when mixtures of all three pollen types are deposited in amounts in excess of the number of ovules present. The patterns of preferential production of progeny sired by legitimate pollen are also maintained when mixtures of all three pollen types are used (Cruzan and Barrett, unpubl. data).

The differences in pollen-tube growth and siring ability observed in *E. paniculata* indicate a weak (or cryptic) incompatibility system. An outcross pollen advantage after pollination with pollen mixtures in a normally self-compatible species is "cryptic" in the sense that it is difficult to detect without the use of genetic markers (see Walsh and Charlesworth 1992). Cryptic incompatibility has been reported in both nonheter-

ostylous (*Cheiranthus cheiri*, Bateman 1956; *Clarkia unguiculata*, Bowman 1987; also see Ockendon and Currah 1978) and heterostylous species (*Amsinckia grandiflora*, Weller and Ornduff 1977, 1989, 1991; *A. douglasiana*, Casper et al. 1988). Furthermore, observed differences in pollen-tube growth could result in the preferential production of outcrossed progeny when mixtures of pollen are applied (e.g., Aizen et al. 1990; Manasse and Pinney 1991; Snow and Spira 1991). However, extrapolation from single-donor pollinations to mixed pollinations is not always possible (Cruzan 1990), thus, conclusions about the presence of cryptic incompatibility cannot be drawn from observations of pollen-tube growth alone. Differential pollen-tube growth was invoked by Bateman as the mechanism for the preferential production of outcrossed seed in *Cheiranthus*, but the possible contribution of selective ovule abortion was not excluded in his study or in several more recent reports (e.g., Bowman 1987). Other investigations of the preferential production of outcrossed seed have implicated prezygotic factors by considering variation in ovule abortion (Casper et al. 1988), or by directly observing pollen tubes (Weller and Ornduff 1989; but see Weller and Ornduff 1991). Our study indicates that preferential production of progeny from legitimate crosses in *E. paniculata* results from cryptic incompatibility because it is primarily due to differential pollen-tube growth rather than differences in embryo abortion.

Several of our results are in accord with an earlier investigation of *E. paniculata* that examined pollen siring ability in mixtures (Glover and Barrett 1986). That study also suggested dis-

crimination against self pollen in competition with outcross pollen; however, discrimination against illegitimate outcross pollen was not detected, leading to speculation that inbreeding effects might account for the observed prepotency of outcross over self pollen (see Barrett 1988; Casper et al. 1988). Mulcahy and Mulcahy (1983) and Waser et al. (1987) have suggested that pollen-tube growth may be affected by the genetic relatedness between the pollen and stylar tissue resulting in discrimination against matings between related individuals. However, our experiments clearly demonstrate that discrimination among pollen types in *E. paniculata* is not a manifestation of inbreeding, because illegitimate outcross pollen also had reduced siring ability when in competition with legitimate pollen.

Although Glover and Barrett used plants from a different population in northeastern Brazil (B5), both populations exhibit high levels of outcrossing and intermorph mating (Barrett et al. 1987; Kohn and Barrett 1992). The previous study may have failed to detect discrimination against illegitimate outcross pollen for several reasons: (1) comparisons involved the pairs of anther levels closest to each other in competitive ability (i.e., l and m on L, m and l on M, and s and m on S); (2) no statistical control existed for either the application sequence of pollen or variation among recipients; and (3) sample sizes were small. In the earlier study, pollen was placed on opposite sides of stigmas to reduce the influence of pollen precedence, but this technique can also lead to an advantage for the first pollen type applied (Graham and Barrett 1990, unpubl. data). If we had used only the same pollen comparisons as those of Glover and Barrett (1986) and tested them in the same manner (see table 2), then we may have come to similar conclusions.

Differential pollen-tube growth determined more of the variation in pollen competitive ability than pollen germination; differences in germination were small and had little influence on the observed patterns of siring ability. Pollen from s anthers had higher initial and total germination than pollen from either l or m anthers on stigmas of each style morph. Differences in pollen germination may be a consequence of the variation in pollen size among anther levels; pollen from s anthers is smallest, followed by m, and then l pollen (Barrett 1985). Because pollen hydration must precede germination (Johri and Vasil 1961), the higher surface-to-volume ratio of the smaller

s grains may allow them to hydrate and germinate more quickly than other pollen types. Smaller size may also have allowed more s grains to adhere to stigmas, resulting in a greater total number of germinated grains for pollinations with pollen from this anther level. In spite of the advantage that pollen from s anthers had during germination, s pollen grew slowest and procured the fewest fertilizations when competing with pollen from the other two anther levels in the L and M morphs.

Cryptic incompatibility in *E. paniculata* may have the same physiological basis as the trimorphic incompatibility system that occurs in related tristylous taxa. Differences in pollen-tube growth among various pollen-style combinations in *E. paniculata* follow the same patterns of pollen-tube growth and seed set as tristylous *Pontederia* species with stronger inhibition of illegitimate pollen types (Barrett and Anderson 1985; Anderson and Barrett 1986; Scribailo and Barrett 1992). Differences in pollen-tube growth are based strictly on the pollen-style combination rather than the relatedness of the pollen to the recipient. Moreover, in these tristylous taxa, a gradient in pollen performance on each style morph is correlated with the similarity in grain size between legitimate and illegitimate pollen types. The resemblance between the patterns of pollen-tube growth in *E. paniculata* and pollen-pistil interactions in related species with strong heteromorphic incompatibility indicates that cryptic incompatibility in *E. paniculata* represents a weak form of heteromorphic incompatibility. Self-compatibility has traditionally been considered to be a derived condition in angiosperm families (Stebbins 1974; Lewis 1979), but the susceptibility of incompatibility systems to evolutionary modification makes it difficult to determine whether cryptic incompatibility in *E. paniculata* represents a weakened or incipient form of trimorphic incompatibility.

The presence of weak rather than strong heteromorphic incompatibility in *E. paniculata* may be associated with its population biology. Most populations in northeastern Brazil occupy ephemeral aquatic habitats and exhibit an annual life history. This makes reliable production of seed critical for the maintenance of populations and the colonization of new sites (Husband and Barrett 1992). In contrast, species of *Pontederia* have stronger incompatibility systems, are longer lived, and tend to occur in more permanent wet-

lands. Because all pollen types can sire seeds in *E. paniculata*, plants are assured of producing seed when pollen loads are small because of scarce pollinators. However, when more pollen grains are deposited on stigmas, they still have the ability to produce largely outcrossed progeny. Incompatibility systems that favor outcross matings when pollinators are abundant, while allowing for the production of seed when pollinators are infrequent, have also been found in other species with annual life cycles (Bowman 1987; Casper et al. 1988). Flexible mating systems of this type then provide the "best of both worlds," because the preference for outcross matings does not sacrifice reliable production of seed (Becerra and Lloyd 1992). Given the advantages that accrue from such a system, it is likely that cryptic incompatibility is more widespread than the few documented cases would suggest, and may help explain instances of high outcrossing rates in many apparently self-compatible taxa (Barrett and Eckert 1991).

Although weak incompatibility allows the mating system of *E. paniculata* to be more resilient to varying pollen environments than one based solely on legitimate pollen transfer, the inability to prevent fertilizations by self and illegitimate outcross pollen may have consequences for the maintenance of tristily. Experimental studies have demonstrated that, in addition to differential pollen-tube growth, the size as well as the composition of the pollen load can determine the level of outcrossing and intermorph mating (Cruzan and Barrett unpubl. data). When few grains are deposited on stigmas, the levels of selfing and intramorph mating increase. Such effects contribute to the loss of morphs from populations, because reduced levels of intermorph mating make the loss of alleles at the loci controlling tristily more likely (Barrett et al. 1989). Weak trimorphic incompatibility in *E. paniculata* combined with wide fluctuations in population size and pollinator service may have contributed to the diversity of selfing rates and morph structures found in populations in northeastern Brazil.

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