

## FERTILITY DIFFERENCES AMONG FLORAL MORPHS FOLLOWING SELFING IN TRISTYLOUS *EICHHORNIA PANICULATA* (PONTEDERIACEAE): INBREEDING DEPRESSION OR PARTIAL INCOMPATIBILITY?<sup>1</sup>

DOMENICA MANICACCI<sup>2</sup> AND SPENCER C. H. BARRETT<sup>3</sup>

Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

Reduction in seed set following self- vs. cross-pollination in flowering plants can result from abortion of selfed offspring owing to inbreeding depression and/or partial self-incompatibility. Previous studies on tristylous *Eichhornia paniculata* (Pontederiaceae) indicate that reduced seed set following self-pollination generally occurs in the short- (S), but not the long- (L) or mid-styled (M) morphs. To determine whether this pattern results from morph-specific differences in inbreeding depression owing to the sheltering of deleterious alleles at the *S* locus and/or partial self-incompatibility, we conducted controlled hand-pollinations of the floral morphs and measured seed set and levels of seed abortion. There were no significant differences in fertilization success and seed set following self-, illegitimate, and legitimate pollinations in the L and M morphs. In contrast, in the S morph self-, intramorph and intermorph illegitimate pollinations resulted in significant reduction in seed set in comparison with legitimate pollination. This indicates that the reduced seed set observed in self-pollination is the result of partial incompatibility rather than inbreeding depression. Significantly reduced fertilization success and low levels of ovule abortion in illegitimate pollinations of S plants also supported this conclusion. Reduced fertility in the S morph may have implications for the observed loss of this morph from natural populations and the evolutionary breakdown of tristily.

**Key words:** *Eichhornia paniculata*; floral morphs; inbreeding depression; partial incompatibility; Pontederiaceae; self-pollination; style morphs; tristily.

Experimental crossing studies of flowering plants commonly report differences in seed set between controlled self- and cross-pollinations. Where differences in seed set occur, it is almost always the case that the number of seeds produced by self-pollination is less than from cross-pollination (e.g., Darwin, 1876; Fryxell, 1957; Bawa, 1974; Zapata and Arroyo, 1978; Barrett and Helenurm, 1987; Lloyd and Schoen, 1992). There are two main causes for the reduced seed set following self-pollination. First, physiological self-incompatibility mechanisms can result in reduced fertilization of ovules, owing to prezygotic interactions between the male gametophyte and maternal tissues (de Nettancourt, 1977; Barrett, 1988; Williams, Clarke, and Knox, 1994). Second, early-acting inbreeding depression can lead to postzygotic reductions in seed set due to abortion of developing embryos homozygous for deleterious alleles (Seavey and Bawa, 1986; Johnston, 1992; Husband and Schemske, 1996). Distinguishing between these two phenomena is conceptually straightforward but may be difficult experimentally, particularly in species showing partial and/or late-acting self-

incompatibility (reviewed in Charlesworth, 1985; Seavey and Bawa, 1986; Barrett, 1988; Klekowski, 1988; Waser, 1993; Sage, Bertin, and Williams, 1994). In cases of partial incompatibility, self-rejection is often weakly expressed and manifested in a quantitative fashion (Cooper and Brink, 1940; Weller and Ornduff, 1977, 1991; Waser, 1993); as a result the patterns of seed set following selfing are similar to those expected from early-acting inbreeding effects (Wiens et al., 1987; Krebs and Hancock, 1991; Manasse and Pinney, 1991; Montalvo, 1992; Rigney et al., 1993; Seavey and Carter, 1994).

One way to distinguish between the effects of incompatibility vs. inbreeding depression on female fertility is to compare seed set among self-pollination, incompatible cross-pollination, and compatible cross-pollination. The use of incompatible cross-pollinations enables the separation of inbreeding effects from incompatibility, since these two phenomena are not confounded with one another as occurs in the traditional comparison of seed set from self- vs. cross-pollination. Unfortunately, in many homomorphic species with partial incompatibility the problem of detecting distinct mating groups can make it difficult to identify cross-incompatible individuals. In heterostylous plants, however, the limited number of mating groups (two in distyly, three in tristily) and the relative ease with which they can be distinguished morphologically provide opportunities to determine the factors responsible for seed-set variation. This approach has been used by Weller and Ornduff (1989, 1991) in distylous *Amsinckia grandiflora* in which both partial heteromorphic incompatibility and early-acting inbreeding depression occur.

In many heterostylous species, the strength of hetero-

<sup>1</sup> Manuscript received 12 January 1995; revision accepted 10 August 1995

The authors thank Tammy Sage for advice concerning observations of embryo development, Lawrence D. Harder for statistical advice, William W. Cole and Fanny Strumas for technical assistance, and Mitchell B. Cruzan, Lawrence D. Harder, Tammy L. Sage, and John D. Thompson for comments on the manuscript. Research was funded by a LaVoisier postdoctoral grant from the French ministry of foreign affairs to D. M. and a research grant from the Natural Sciences and Engineering Research Council of Canada to S.C.H.B.

<sup>2</sup> Current address: CEFÉ-CNRS, BP 5051, 34 033 Montpellier, France.

<sup>3</sup> Author for correspondence.

morphic incompatibility differs between the floral morphs (reviewed in Barrett and Cruzan, 1994). Variation is particularly evident in tristylous species, and has been studied in most detail in the Pontederiaceae (e.g., Barrett and Anderson, 1985; Scribailo and Barrett, 1991; Cruzan and Barrett, 1993). In tristylous *Eichhornia paniculata* (Spreng.) Solms-Laubach, experimental pollinations of plants originating from five populations in northeast Brazil all showed reduced seed set in self- vs. legitimate (between anthers and stigmas of equivalent height) pollinations of the short-styled (S) morph. In contrast, no significant differences in seed set were evident between these pollinations in the long- (L) and mid-styled (M) morphs (Barrett, 1985; Barrett, Morgan, and Husband, 1989; Kohn and Barrett, 1992). This pattern could arise from morph-specific differences in partial incompatibility; however, these authors also discussed an additional hypothesis involving inbreeding effects associated with the *S* locus governing the inheritance of short style length (genetic determination of tristylous involves two diallelic loci *S* and *M* with the *S* locus epistatic to *M*). Genotypes for the three floral morphs are thus *ss mm* for the L morph, *ss MM* and *ss Mm* for the M morph, and *Ss MM*, *Ss Mm*, and *Ss mm* for the S morph. In self-fertile species, homozygosity at the *S* locus can potentially occur (*SS* -) giving rise to three additional genotypes; see Barrett, Morgan, and Husband, 1989). Reduced seed set could arise either (1) directly as a result of selection against *SS* -genotypes or (2) from the exposure of deleterious genes sheltered by heterozygosity at the *S* locus since, owing to the outcrossed mating system of tristylous populations, most of the S plants are observed to be heterozygous at the *S* locus (Barrett, Kohn, and Cruzan, 1992).

In an effort to address these issues, we conducted a controlled crossing program under glasshouse conditions on two tristylous populations of *E. paniculata* originating from northeast Brazil. This program involved four pollination treatments: self-, intramorph, intermorph illegitimate, and legitimate pollinations. Comparisons of seed set among these treatments in conjunction with a study of embryo development enabled us to determine the factor(s) responsible for morph-specific differences in female fertility in *E. paniculata*.

## MATERIALS AND METHODS

*Eichhornia paniculata* is an aquatic annual native to the neotropics, primarily northeast Brazil and the islands of Jamaica and Cuba. Plants used in our experiments were obtained directly from open-pollinated families collected in 1987 from two populations B34 and B46 at Maranguape and Quixadá, respectively, Ceará state (see Barrett and Husband, 1990). Population size and morph frequency for long-, mid- and short-styled plants were  $N = 2500$ ,  $L = 0.40$ ,  $M = 0.34$ ,  $S = 0.26$  for B46, and  $N = 500$ ,  $L = 0.61$ ,  $M = 0.31$ ,  $S = 0.08$  for B34. Plants were grown in water-submersed pots and maintained in a heated (25°–30°C) glasshouse for the duration of the study (see Morgan and Barrett, 1989 for details on the cultivation of the species). Every day, plants produce up to 20 showy insect-pollinated flowers that last for 6 hr (from 8 a.m. to 2 p.m. under glasshouse conditions) and that are easily pollinated by hand.

**Experimental pollinations**—to determine whether seed-set reduction following self-pollination is due to inbreeding depression and/or partial incompatibility, four different types of hand-pollinations were used (Fig.

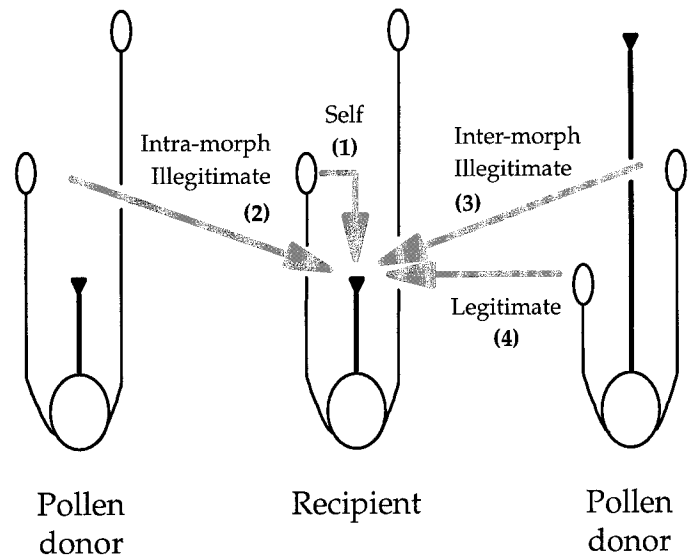


Fig. 1. Schematic illustration of the protocol used for controlled hand-pollinations of *Eichhornia paniculata*. Arrows indicate the four pollination treatments performed: (1) self-pollination, (2) within-morph illegitimate cross, (3) between-morph illegitimate cross, and (4) between-morph legitimate cross. In this figure, long- and short-styled plants are used as pollen donors on a short-styled recipient. Equivalent pollinations were performed on long-styled plants involving long- (treatment 2) and short-styled (treatments 3 and 4) pollen donors, and on mid-styled plants involving mid- (treatment 2) and short-styled (treatments 3 and 4) pollen donors.

1): (1) selfing was performed with pollen from the stamens closest to the stigma in flowers of L and S plants and from long-level stamens in M plants; (2) intramorph illegitimate crosses were performed using pollen from the same level as in treatment (1), but from different paternal plants of the same morph; (3) intermorph illegitimate crosses also involved pollen from the same stamen level but from paternal plants of a different morph; and (4) legitimate pollinations involved pollen from the same paternal plants as treatment (3) but from the stamen level equivalent to the level of the stigma in the recipient plant, i.e., legitimate pollen (Darwin, 1877).

Contrasts between these different pollination treatments provide information that enable us to discriminate between potential mechanisms influencing seed-set patterns in the floral morphs. For example, differences in seed set between treatments 1 and 2 would likely reflect inbreeding depression rather than incompatibility since both pollinations involve illegitimate pollen of the same stamen level. Differences in seed set between treatments 2 and 3 in the S morph could arise by morph-specific inbreeding depression. This is because intramorph illegitimate crosses would give rise to plants of genotype *SS* -, whereas all zygotes resulting from the corresponding intermorph illegitimate cross would be heterozygous at the *S* locus (*Ss* -). Finally, differences in seed set between pollination treatments 3 and 4 must result from the operation of trimorphic incompatibility rather than inbreeding effects since pollen used in both pollination treatments originates from the same paternal parent.

In each population, 12 plants of each of the three floral morphs were used as maternal recipients and six different plants per morph were used as pollen donors. Every day, each of the four lowest flowers in the inflorescence of each recipient plant received one of the treatments described above. The order of pollinations within an inflorescence was randomized. This procedure was repeated for six consecutive days. Within each population, all maternal plants per morph and treatment were pollinated by the same six pollen donors. On each day, the inter-

morph illegitimate and legitimate pollinations of each maternal plant involved the same pollen donor.

Both the long- and mid-level stamens in S plants, and the long-level stamen in M plants were removed prior to pollination. Pollen was evenly deposited on the whole stigmatic surface, resulting in an excess of pollen grains since flowers produce 50–120 ovules (Morgan and Barrett, 1989). In order to pollinate stigmas of S plants, the perianth tube was manually slit. Flowers from L and M plants were similarly slit so that each morph received similar manipulations. The position of all pollinated flowers with respect to branch number from the base of the inflorescence and flower position within a branch was recorded since it has previously been observed that the developmental position of flowers influences seed set in this species (S.C.H. Barrett, unpublished data). Fruits were collected after 11–13 d when ripe, and seeds were counted.

To assess whether there were differences in average seed mass per fruit as a result of inbreeding depression, seeds from each capsule obtained from the four pollination treatments in each population were weighed using a 0.0001 g precision electronic balance. The potential effects of inbreeding depression on germination were assessed in a glasshouse trial conducted at 25°–35°C. For all maternal plants and pollination types in each population, two replicates of 25 seeds were sown in 5.72 cm diameter pots filled with sieved sterilized soil. Pots were immersed in plastic trays filled with water and covered with clear plastic in order to maintain high humidity. Trays were placed on a glasshouse bench and rotated weekly in order to minimize microenvironmental effects on germination. Seedlings were counted and removed from the pots at weekly intervals until no new germination was observed for two consecutive weeks (after 7 and 6 wk for B34 and B46, respectively). For each population, the final proportion of germinated seeds per pot was analyzed.

**Embryo development**—to investigate whether variation in seed set observed in experimental pollinations was the result of postzygotic abortion, embryo development was studied following the four hand-pollination treatments. In each population, 12 plants per morph were examined, with two replicates of each hand-pollination treatment per plant. After 5 d, developing ovaries were fixed in 3:1 ethanol:acetic acid for 2 d and then transferred into 70% ethanol. The technique of Stelly et al. (1984) was modified for use in *E. paniculata* as follows. Ovaries were stained in Mayer's hemalum (Sass, 1958) for 48 h and then destained in 10% acetic acid for a period varying from 36 to 72 h depending on individual staining intensity. Ovaries were then rinsed in water, gradually dehydrated (at least 15 min in a graded ethanol series, 25–100%) followed by 24 h in 100% ethanol and cleared in methyl salicylate (see Scribailo and Barrett, 1991). Each ovary was opened and ovules and developing seeds were removed and placed in methyl salicylate on a microscope slide and observed under 100 × magnification using a Zeiss Axioplan compound microscope. Preliminary studies on 1–9 d old ovaries indicated that levels of embryo abortion were best assessed at 5 d, since after this time endosperm development obscured the developing embryo.

Seeds were classified into two categories: seeds with normally developed embryos and those with embryos that had aborted at different stages (see Results). Observations of ovaries at early stages (from 6 h to 2 d) revealed that syngamy occurs from 6 to 9 h after pollination. These observations also indicated that all unfertilized ovules had not expanded in size after 2 d. All expanded but shrunken seeds observed at the 5-d stage were therefore classified as aborted. The total number of seeds counted, i.e., normally developing plus aborted, was considered as an estimate of the number of fertilized ovules.

**Ovule number**—to determine whether ovule number per flower differed among the floral morphs, the number of ovules in each of two flowers was counted in all plants (12 plants per morph in each population) studied for embryo development. Ovules were removed from

flowers, placed on a dark glass slide and counted under 10 × magnification using a binocular microscope.

**Statistical analysis**—The pollination experiment involved a repeated-measures design with Morph as the between-plant fixed factor and Day and Treatment as within-plant factors. The data did not satisfy the sphericity assumption (Mauchly's criterion,  $P < 0.05$ ) required of a univariate repeated-measures ANOVA, so we adopted a multivariate approach. We intended to incorporate flower position as a covariate in the analyses of seed set and seed mass; however, multivariate ANOVA (PROC GLM; SAS, 1990) cannot accommodate within-plant covariates. We therefore used the residuals from the regressions of these variables against flower position as the dependent variables in the repeated-measures ANOVA. Flower position was not considered in the analyses of data on embryo development since all pollinations were applied to flowers from the bottom of the inflorescence and these did not differ greatly in position. We used pairwise comparisons to interpret interactions between Morph and Treatment, following Sidak's approach to control the experimentwise Type I error rate (Sidak, 1967).

## RESULTS

**Ovule number**—The number of ovules produced per flower did not differ significantly among floral morphs in either population [Mean (SE); B34: L = 110.3 (3.5), M = 104.0 (4.8), S = 103.7 (3.6),  $F_{(2,36)} = 0.49$ ,  $P = 0.60$ ; B46: L = 110.9 (3.9), M = 118.4 (4.3), S = 106.0 (4.4),  $F_{(2,35)} = 1.31$ ,  $P = 0.28$ ].

**Seed set from experimental pollinations**—Data on seed set following experimental pollinations of the three morphs from two populations of *E. paniculata* are presented in Fig. 2. In both populations, the pollination treatments differed significantly [B34:  $F_{(3,93)} = 6.2$ ,  $P = 0.002$ ; B46:  $F_{(3,78)} = 28.6$ ,  $P < 0.001$ ] and their effects differed among morphs [Morph × Treatment interaction: B34,  $F_{(6,93)} = 3.2$ ,  $P = 0.01$ ; B46,  $F_{(6,78)} = 13.4$ ,  $P < 0.001$ ]. In particular, pollination treatment did not significantly affect seed production by L and M plants, whereas in S plants of both populations the three types of illegitimate pollinations produced significantly fewer seeds than legitimate pollination (average decrease of 12.4% in B34 and 29.8% in B46; Table 1). In population B46, S plants set significantly fewer seeds following self-pollination compared to intramorph crosses, but not compared to intermorph illegitimate crosses (Table 1).

Seed set following illegitimate and legitimate intermorph pollinations were averaged among the six replicates for each S plant in both populations (Fig. 3). Illegitimate pollination significantly reduced seed set in four plants from population B34 (plants 5, 9, 11, and 12) and six plants from population B46 (plants 7 to 12).

**Seed mass and germination ability**—Seed mass did not differ among morphs in population B34 [ $F_{(2,31)} = 0.8$ ,  $P = 0.47$ ], but were slightly different in population B46 [ $F_{(2,31)} = 3.8$ ,  $P < 0.05$ ]. In both populations, Treatment and its interaction with Morph significantly affected seed mass [Treatment:  $F_{(3,93)} = 10.8$ ,  $P < 0.001$  and  $F_{(3,93)} = 9.8$ ,  $P < 0.001$ ; Morph × Treatment:  $F_{(6,93)} = 2.7$ ,  $P < 0.05$  and  $F_{(6,93)} = 3.4$ ,  $P < 0.01$ , for populations B34 and B46, respectively]. Pairwise contrasts among treatments for each morph separately showed significant differences

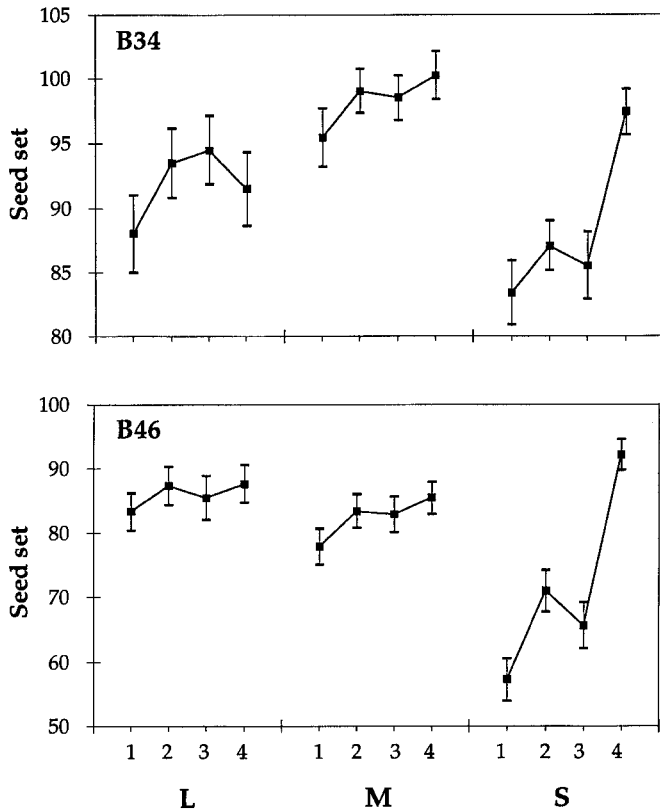


Fig. 2. Average seed set per fruit (developed seeds at fruit maturity) following four hand-pollination treatments (see Fig. 1) in the three floral morphs of tristylous *Eichhornia paniculata* from two Brazilian populations, B34 and B46. Vertical lines indicate 1 SE of the mean estimated from 12 plants per morph and six capsules per plant.

between treatment 1 and treatments 2–4 in L plants of population B34, between 1 and 3 in L plants of population B46, and between 1 and 2 in M plants of population B34. In contrast, in S plants of both populations self-pollinations did not significantly affect seed mass relative to outcross pollinations. Finally, in S plants of both populations, legitimate pollinations produced smaller seeds than illegitimate intermorph pollinations. This effect was probably associated with a trade-off between the size and number of seeds, since the latter pollination type produced fewer seeds than the former.

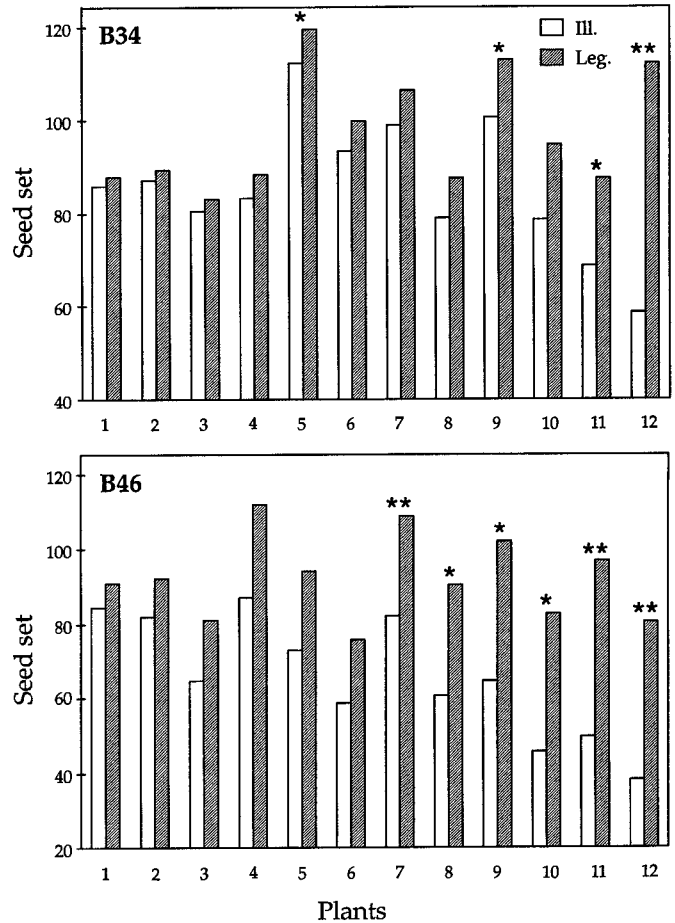


Fig. 3. Seed set per fruit (developed seeds at fruit maturity) following illegitimate (3) and legitimate (4) intermorph hand-pollinations in 12 plants of the short-styled morph of tristylous *Eichhornia paniculata* from two Brazilian populations, B34 and B46. For each maternal parent, the two treatments involved the same pollen donors. Observed variation in seed set results from differences in incompatibility among pollen types. Each bar is the average seed set from six hand-pollinated flowers. In the figure, plants from each population were ordered from the lowest to the highest relative decrease in seed set from illegitimate vs. legitimate crosses. Stars indicate the significant *t* values for the comparison of these treatments within each plant (\*  $P < 0.05$ , \*\*  $P < 0.01$ ).

TABLE 1. *F* values and their level of significance adjusted for multiple comparisons in a posteriori contrasts among hand-pollination treatments on the short-styled morph in tristylous *Eichhornia paniculata*. Four pollination treatments were performed: (1) selfing, (2) within-morph illegitimate cross, (3) between-morph illegitimate cross, and (4) between-morph legitimate cross (see Fig. 1). Fertilized ovules and developed and aborted seeds were counted 5 d after flowers were pollinated. Seed set was measured 11–13 d after pollination, at capsule maturity, and did not include small aborted seeds.

Pairwise contrasts	Population B34				Population B46			
	Seed set	Fertilized ovules	Aborted seeds	Developed seeds	Seed set	Fertilized ovules	Aborted seeds	Developed seeds
1 vs. 2	1.54	1.02	0.94	0.17	26.17**	0.14	0.09	0.07
1 vs. 3	0.53	4.82	0.15	3.63	8.57	0.33	1.39	0.10
1 vs. 4	16.89**	10.08*	5.02	17.39**	105.2***	19.87***	0.10	19.50***
2 vs. 3	1.43	2.65	0.38	3.14	4.45	0.15	1.13	0.02
2 vs. 4	18.67**	8.99*	0.77	14.63**	47.80**	14.33**	1.73	15.69**
3 vs. 4	19.11**	9.68*	2.17	14.57**	88.77***	17.47**	3.81	17.70**

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

The proportion of seeds germinating did not differ among morphs [B34:  $F_{(2,33)} = 1.5$ ,  $P = 0.25$ ; B46:  $F_{(2,32)} = 3.0$ ,  $P = 0.06$ ] or treatments [B34:  $F_{(3,99)} = 0.9$ ,  $P = 0.47$ ; B46:  $F_{(3,96)} = 1.3$ ,  $P = 0.28$ ], or with their interaction [B34:  $F_{(6,99)} = 0.9$ ,  $P = 0.52$ ; B46:  $F_{(6,96)} = 0.8$ ,  $P = 0.60$ ].

**Embryo development**—Five days after pollination, most seeds were normally developed and at the cotyledon stage (Fig. 4). A small proportion of aborted seeds were also observed. These were counted as one separate category although they differed in appearance (Figs. 5–7). Some of the aborted seeds had stained seed coats and embryos at the globular stage (Fig. 5), others had similar embryos but were smaller and did not retain stain (Fig. 6). Finally, the majority of aborted seeds were considerably smaller than the other two types and were characterized by a shrunken shape and an absence of stain (Fig. 7). In these seeds, embryos were not visible and had probably aborted early, i.e., between 1 and 2 d after pollination. The different types of aborted seeds were observed in each of the four pollination treatments and in all floral morphs.

The average numbers of fertilized ovules and seeds with developed embryos per fruit differed among pollination treatments in both populations (Fig. 8). In population B46, these treatment effects [number of fertilized ovules:  $F_{(3,90)} = 3.6$ ,  $P < 0.05$ ; number of developed seeds:  $F_{(3,90)} = 5.4$ ,  $P < 0.01$ ] varied among morphs [Morph  $\times$  Treatment interactions; number of fertilized ovules:  $F_{(6,90)} = 2.7$ ,  $P < 0.05$ ; number of developed seeds:  $F_{(6,90)} = 2.6$ ,  $P < 0.05$ ]. These interactions occurred because the three illegitimate pollinations produced significantly fewer fertilized ovules and developed seeds than legitimate crosses in the S morph (Table 1), while no difference among treatments was found in the L and M morphs (see Fig. 8). The treatment effects observed in population B34 [number of fertilized ovules:  $F_{(3,84)} = 4.7$ ,  $P < 0.05$ ; number of developed seeds:  $F_{(3,84)} = 6.5$ ,  $P < 0.01$ ] showed marginally significant interactions with morph [number of fertilized ovules:  $F_{(6,84)} = 1.7$ ,  $P = 0.12$ ; number of developed seeds:  $F_{(6,84)} = 1.8$ ,  $P = 0.10$ ]. However, because of similar observations in population B46, we considered these interactions as potentially indicative of a biological trend and therefore performed pairwise contrasts between treatments within each morph. As in population B46, the only significant contrasts revealed a lower number of fertilized ovules and developed seeds per fruit in the three illegitimate pollinations compared to the legitimate cross in the S morph. The difference in the number of fertilized ovules and developed seeds per fruit following illegitimate and legitimate pollinations in L and M morphs of both populations was either positive or negative and did not exceed absolute values of 4% and 7% for fertilized ovules and developed seeds, respectively. In S plants in contrast, we observed a reduction of 9.3% fertilized ovules and 11.7% developed seeds in population B34, and of 20.4% fertilized ovules and 22.3% developed seeds in B46 in illegitimate compared to legitimate pollinations (see Fig. 8).

In population B34, the number of aborted seeds per ovary did not differ significantly among morphs [ $F_{(2,84)} = 1.5$ ,  $P = 0.24$ ], treatments [ $F_{(3,84)} = 1.4$ ,  $P = 0.24$ ], or their interaction [ $F_{(6,84)} = 1.2$ ,  $P = 0.29$ ]. In population

B46 in contrast, those effects were significant [Morph:  $F_{(2,90)} = 4.4$ ,  $P < 0.05$ ; Treatment:  $F_{(3,90)} = 4.3$ ,  $P < 0.05$ ; Morph  $\times$  Treatment:  $F_{(6,90)} = 2.5$ ,  $P < 0.05$ ]. This interaction arose from increased seed abortion in self vs. legitimate pollination of M plants [ $F_{(1,30)} = 12.7$ ,  $P < 0.05$ ]; all other within-morph pairwise contrasts were nonsignificant.

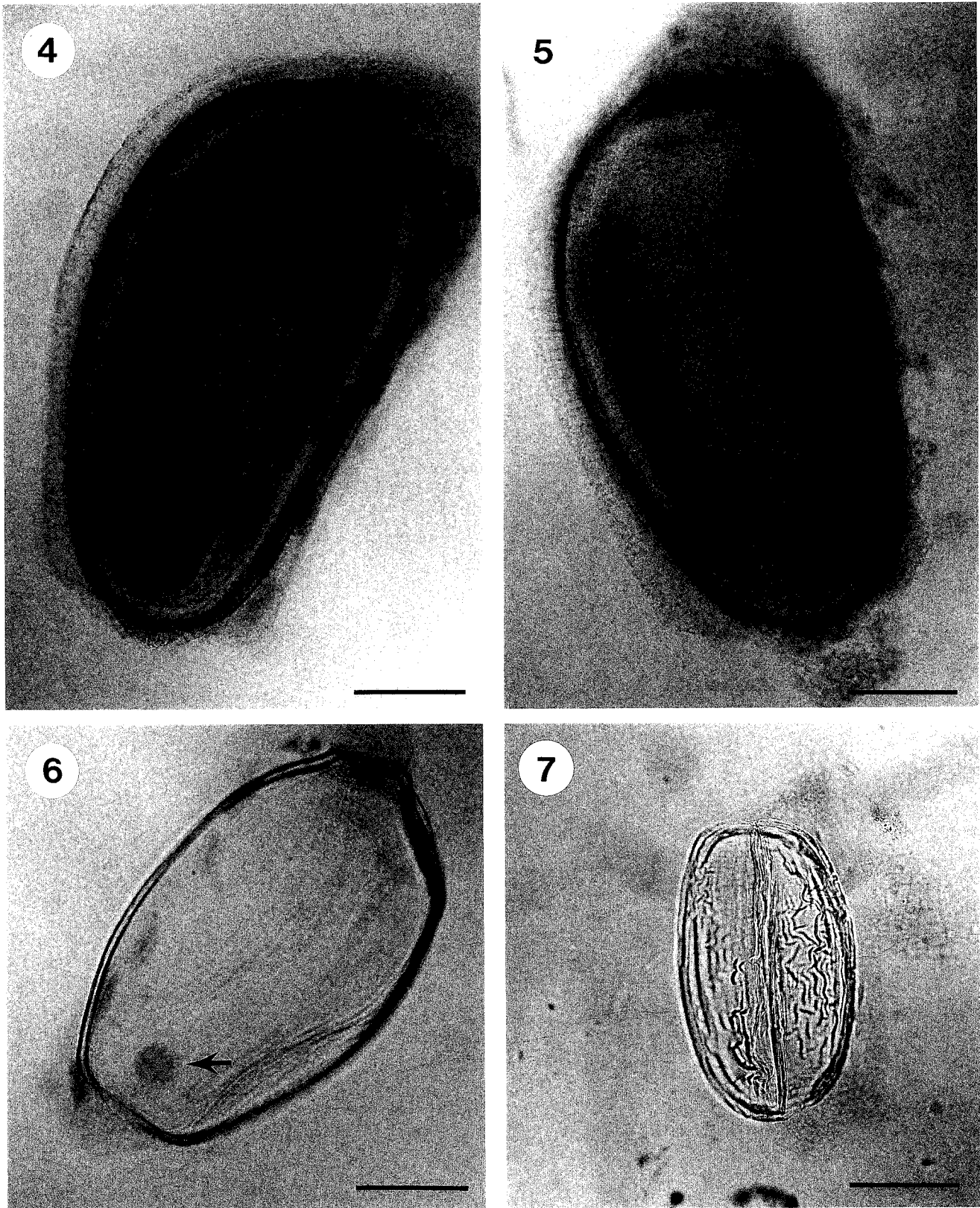
## DISCUSSION

The major finding of our experimental studies on *E. paniculata* is that differences in seed set following self- vs. legitimate pollinations of the S morph are largely the result of partial physiological self-incompatibility and not inbreeding depression. This conclusion was made possible in part because *E. paniculata* possesses a tristylous breeding system. Because of the presence of two pollen types within each flower, both illegitimate and legitimate cross-pollinations could be undertaken using a common pollen donor. When this was done, clear evidence for the presence of incompatibility was obtained in the S morph (Fig. 2), but not the L and M morphs.

While seed set among the four pollination treatments was not significantly different in the L and M morphs, this does not imply that these morphs do not possess physiological self-incompatibility. Previous studies in this species using marker genes have shown that competition between illegitimate and legitimate pollen leads to an excess of fertilizations by legitimate pollen (Cruzan and Barrett, 1993). This effect was demonstrated in all three morphs, indicating that each possesses a form of partial incompatibility. These findings emphasize that the traditional comparison of seed set between self- and cross-pollinations may not detect incompatibility in species where its expression is only weakly developed (and see Weller and Ornduff, 1977).

**Morph-specific differences in expression of partial incompatibility**—Cruzan and Barrett (1993) described the trimorphic incompatibility system in *E. paniculata* as cryptic because the species is highly self-fertile and competition experiments using marker genes were required for the detection of incompatibility. Elsewhere, Weller and Ornduff (1977), Casper, Sayigh, and Lee (1988), and Eckert and Barrett (1994) have also demonstrated cryptic incompatibility in other heterostylous species using mixed pollen loads and genetic markers. The results from the present study revealed partial incompatibility in the S morph using a single pollen source only. Partial incompatibility in *E. paniculata* is thus expressed when the S morph receives incompatible pollen alone, whereas in the L and M morphs it is manifested only through the disadvantage of illegitimate pollen in competition with legitimate pollen. This difference in functional response suggests that the mechanism(s) responsible for incompatibility in the S morph may be qualitatively different from those in the L and M morphs.

The suggestion that pollen–pistil interactions in the S morph of *E. paniculata* may differ from the L and M morphs has also been reached by Cruzan and Barrett (in press) in a recent marker-gene study of the species. In their study mixed pollinations of varying size and composition were applied to stigmas of the three morphs and



Figs. 4–7. Normally developed and aborted seeds, 5 d after pollination in *Eichhornia paniculata*. **4.** Normally developed seed with embryo at the cotyledon stage. **5.** Aborted seed with embryo at the globular stage and stained seed coat. **6.** Smaller aborted seed with embryo at the globular stage and poorly stained tissues. In Figs. 5, 6, arrows indicate aborted embryos. **7.** Aborted unstained seed resulting from earlier abortion than in Figs. 5, 6. In these seeds embryos were not visible. In *E. paniculata*, syngamy occurs at 6–9 h after pollination and seeds are mature after 11–13 d. The scale bars represent 0.2 mm, 100 × magnification.



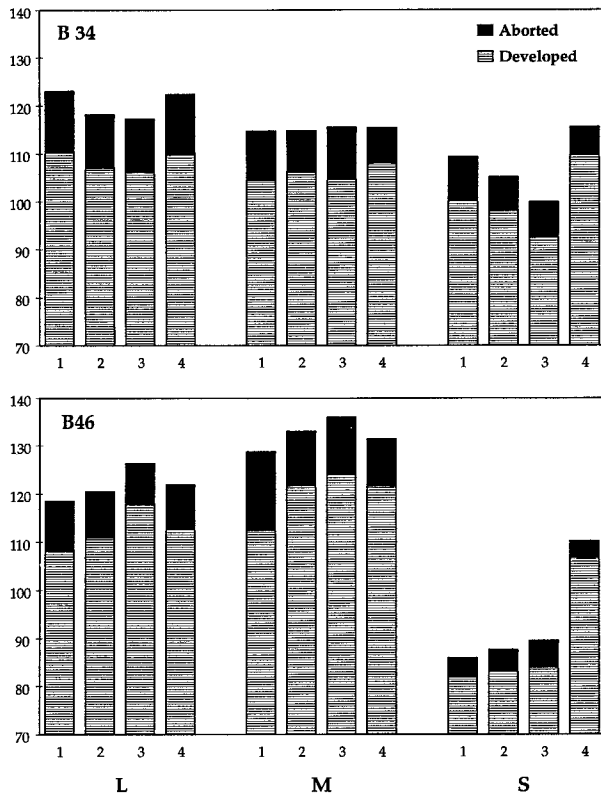


Fig. 8. Number of seeds with aborted and normally developed embryos per ovary following four hand-pollination treatments (see Fig. 1) in the three floral morphs of tristylous *Eichhornia paniculata* from two Brazilian populations, B34 and B46. Seed numbers were estimated 5 d after pollination. The total number of seeds counted in these two categories was an estimate of the number of fertilized ovules per flower. Each bar is an average of 12 plants and two flowers per plant.

the siring success of different pollen types was evaluated. In these experiments a different response in the S morph compared to the L and M morphs was also revealed. When the total number of pollen grains deposited on stigmas of S plants was lower than the number of ovules per flower, illegitimate pollen failed to achieve many fertilizations despite a surplus of available ovules. This effect was not observed in the L and M morphs. These authors propose that prezygotic pollen-tube attrition (see Cruzan, 1989) may be largely responsible for the patterns of siring observed in the S morph. In contrast, in the L and M morphs they suggest that differences in competition between legitimate and illegitimate pollen tubes largely govern siring success.

Why the expression of partial incompatibility in the S morph of *E. paniculata* appears to differ from the L and M morphs is unclear. Morph-specific differences have been commonly reported in other heterostylous species (Darwin, 1877; Charlesworth and Charlesworth, 1979; Richards, 1986; Barrett and Cruzan, 1994); and, in the Pontederiaceae, crossing studies of related tristylous *Pontederia* spp. have generally shown that incompatibility is more strongly expressed in the S morph than the L and M morphs (Ornduff, 1966; Barrett, 1977; Glover and Barrett, 1983; Barrett and Anderson, 1985). Lewis (1942) proposed that in distylous *Primula* stronger incompati-

bility in the S morph compared with the L morph resulted from selection to prevent self-fertilization since pollen tubes from large self pollen grains may traverse short styles more easily than the smaller self pollen grains in long styles. However, it seems unlikely that this explanation is of general validity in heterostylous plants since a recent survey of 91 distylous species found no evidence that the strength of incompatibility was consistently stronger in the S morph compared to the L morph as would be expected under this hypothesis (Barrett and Cruzan, 1994).

The morph-specific differences in expression of partial incompatibility that we have demonstrated in *E. paniculata*, and the possibility that these result from qualitatively different mechanisms of pollen-pistil interaction, are consistent with a recent model of the evolution of heterostyly by Lloyd and Webb (1992a, b). They propose that incompatibility may originate separately in the floral morphs after the evolution of the stamen-style polymorphism. If this scenario is true, it would seem reasonable that the mechanisms governing incompatibility may differ among the morphs, depending on their particular morphological and developmental features. This is also supported by the common finding that in many distylous species, the inhibition of illegitimate pollen tubes occurs on the stigmatic surface in the S morph but in the style in the L morph (reviewed in Dulberger, 1992) and by the fact that in related tristylous *Pontederia* spp. inhibition sites for incompatible pollen differ among the morphs (Anderson and Barrett, 1986; Scribailo and Barrett, 1991). These various morph-specific differences in pollen-pistil interactions may not in themselves be adaptive, but instead could arise as consequences of physiological and developmental constraints associated with the evolution of the heterostylous syndrome.

**Morph-specific inbreeding depression**—Our primary motivation for investigating inbreeding depression was the possibility that deleterious alleles linked to the S locus and made homozygous following selfing would result in reduced seed set in the S morph compared to legitimate crosses. The possibility of an association between genes controlling mating and loci determining genetic load would be of general significance for models of the evolution of self- and cross-fertilization (reviewed in Uyenoyama, Holsinger, and Waller, 1993). However, to our knowledge there are no convincing empirical examples of such associations. Several authors have discussed the issue of reduced viability of SS -genotypes in other heterostylous species (Mather and de Winton, 1941; Vuilleumier, 1967; Schou and Philipp, 1983; Shore and Barrett, 1985; Richards, 1986; Eckert and Barrett, 1993) and the issue of linkage of alleles to the S locus in heterostylous plants has been treated theoretically by Strobeck (1980).

Previous studies on *E. paniculata* suggested the possibility of reduced viability of SS -genotypes. First, such genotypes were not found among 22 short-styled maternal parents progeny-tested from a natural tristylous population (B5) in northeast Brazil (Barrett, Brown, and Shore, 1987). Second, four of 12 families obtained from selfing the S morph displayed significant deficiencies of the S morph when grown under glasshouse conditions

(Barrett, Morgan, and Husband, 1989). Despite these indications of potential selection against *SS* -genotypes, in the present study we found no evidence that inbreeding depression was any stronger in the S morph compared to the L and M morphs based on comparisons of seed set, seed mass, seed germination, and levels of embryo abortion. Therefore it seems unlikely, at least in the two populations that we investigated, that homozygosity at the S locus leads to deleterious effects on female fertility in the S morph. However, it is possible that viability selection against *SS* -genotypes could occur later in the life cycle and account for the patterns observed in earlier studies.

In order to detect inbreeding depression for seed set, it is necessary to remove any influence of incompatibility (see Table 3 in Charlesworth and Charlesworth, 1987). In heterostylous species with partial incompatibility, this can be achieved by comparing self- and illegitimate outcross pollinations. Statistical comparisons of these pollinations in *E. paniculata* failed to reveal any general effect of inbreeding on seed set. Only one significant contrast out of 12 among the three morphs was evident in our data. However, a consistent pattern was observed with selfed seed set always the lowest of the four pollination treatments in each morph and population (Fig. 2). In an earlier study of inbreeding depression in *E. paniculata*, no significant differences in seed set were detected between self- and outcross pollinations in each of 11 populations although the above trend was also evident (Toppings, 1989). Collectively, these studies on a total of 13 populations of *E. paniculata* therefore indicate that early-acting inbreeding depression at the seed set stage is at most weak in its effects and, in some populations, may be absent altogether. Data from *E. paniculata* are consistent with a recent survey of studies on inbreeding depression in seed plants by Husband and Schemske (1996). These authors found that in short-lived species, such as *E. paniculata*, most inbreeding depression was concentrated at later life cycle stages.

Our study of embryo development indicated relatively low levels of seed abortion with no major differences among morphs or pollination treatments (Fig. 8). While our technique may underestimate the true level of ovule abortion, particularly those occurring shortly after fertilization (Cooper, Brink, and Albrecht, 1937; Guth and Weller, 1986; Nakamura and Stanton, 1987), it seems unlikely that this possible bias would alter our conclusions concerning the roles of partial incompatibility and inbreeding depression on seed set. The fact that levels of abortion do not differ between self- and outcross pollinations is inconsistent with the occurrence of significant amounts of early-acting inbreeding depression.

**Evolutionary consequences**—The existence of a weak rather than a strong incompatibility system in *E. paniculata* may be associated with ecology and life history of the species (Cruzan and Barrett, 1993). The ephemeral aquatic habitats occupied by this facultatively annual species are associated with wide fluctuations in population size and pollinator service. When pollinators are abundant and the pollen loads deposited on stigmas are high, the competitive advantages of legitimate pollen can lead to most progeny resulting from outcrossing. However, with limited pollinator service and small pollen loads re-

productive assurance is guaranteed since all pollen types are capable of producing seeds. Ecological variation of this type undoubtedly plays a role in causing the wide diversity of mating patterns found in the species (reviewed in Barrett, Kohn, and Cruzan, 1992).

Surveys of floral morph representation in natural populations of *Eichhornia paniculata* in northeast Brazil have revealed a striking pattern. The S morph is often at a lower frequency than the L and M morphs, and, among dimorphic populations the S morph is almost always missing (Barrett, Morgan, and Husband, 1989; Husband and Barrett, 1993). While stochastic forces undoubtedly play a major role in causing these patterns, particularly for dimorphic populations, selection involving morph-specific fitness differences may also be involved (reviewed in Barrett, 1993). Under a pollination regime involving reduced levels of legitimate pollen deposition, the fertility of the S morph would be influenced more strongly than the L and M morphs. Theoretical models indicate that, with reduced fertility and weak disassortative mating, the S morph can be driven down in frequency and lost from populations (see Fig. 8 in Barrett, Morgan, and Husband, 1989). Such conditions may prevail where pollinator service is limited owing to local environmental conditions or where specialized pollinators capable of mediating legitimate pollination among the morphs are absent. Reduced female fertility of the S morph may therefore contribute to the loss of this morph from trimorphic populations, potentially leading to the evolutionary breakdown of tristylly in the species.

#### LITERATURE CITED

- ANDERSON, J. M., AND S. C. H. BARRETT. 1986. Pollen tube growth in tristylous *Pontederia cordata* (Pontederiaceae). *Canadian Journal of Botany* 64: 2602–2607.
- BARRETT, S. C. H. 1977. The breeding system of *Pontederia rotundifolia* L., a tristylous species. *New Phytologist* 78: 209–220.
- . 1985. Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Biological Journal of the Linnean Society* 25: 41–60.
- . 1988. The evolution, maintenance, and loss of self-incompatibility systems. In J. and L. Lovett Doust [eds.], *Plant reproductive ecology: patterns and strategies*, 98–124. Oxford University Press, Oxford.
- . 1993. The evolutionary biology of tristylly. In D. Futuyma and J. Antonovics [eds.], *Oxford surveys in evolutionary Biology*, vol. 9, 283–326. Oxford University Press, Oxford.
- , AND J. M. ANDERSON. 1985. Variation in expression of trimorphic incompatibility in *Pontederia cordata* (Pontederiaceae). *Theoretical and Applied Genetics* 70: 355–362.
- , A. H. D. BROWN, AND J. S. SHORE. 1987. Disassortative mating in tristylous *Eichhornia paniculata* (Pontederiaceae). *Heredity* 58: 49–55.
- , AND M. B. CRUZAN. 1994. Incompatibility in heterostylous plants. In E. G. Williams, A. E. Clarke, and R. B. Knox [eds.], *Genetic control of self-incompatibility and reproductive development in flowering plants*, *Advances in cellular and molecular biology of plants*, vol. 2, 189–219. Kluwer, Dordrecht.
- , AND K. HELENURM. 1987. The reproductive biology of boreal forest herbs. 1. Breeding systems and pollination. *Canadian Journal of Botany* 65: 2036–2046.
- , AND B. C. HUSBAND. 1990. Variation in outcrossing rates in *Eichhornia paniculata*: the role of demographic and reproductive factors. *Plant Species Biology* 5: 41–55.
- , J. R. KOHN, AND M. B. CRUZAN. 1992. Experimental studies of mating-system evolution: the marriage of marker genes and flo-



- ral biology. In R. Wyatt [ed.], Ecology and evolution of plant reproduction: new approaches. 192–230. Chapman and Hall, New York, NY.
- , M. T. MORGAN, AND B. C. HUSBAND. 1989. The dissolution of a complex genetic polymorphism: the evolution of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae). *Evolution* 43: 1398–1416.
- BAWA, K. S. 1974. Breeding systems of tree species of a lowland tropical community. *Evolution* 28: 85–92.
- CASPER, B. B., L. S. SAYIGH, AND S. S. LEE. 1988. Demonstration of cryptic incompatibility in distylous *Amsinckia douglasiana*. *Evolution* 42: 248–253.
- CHARLESWORTH, D. 1985. Distribution of dioecy and self-incompatibility in angiosperms. In J. J. Greenwood and M. Slatkin (eds.), *Evolution—essays in honour of John Maynard Smith*, 237–268. Cambridge University Press, Cambridge.
- , AND B. CHARLESWORTH. 1979. A model for the evolution of heterostyly. *American Naturalist* 114: 499–513.
- , AND ———. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics* 18: 237–268.
- COOPER, D. C., AND R. A. BRINK. 1940. Partial self-incompatibility and the collapse of fertile ovules as factors affecting seed formation in alfalfa. *Journal of Agricultural Research* 60: 453–472.
- COOPER, D. C., R. A. BRINK, AND H. R. ALBRECHT. 1937. Embryo mortality in relation to seed mortality in alfalfa, *Medicago sativa*. *American Journal of Botany* 24: 203–213.
- CRUZAN, M. B. 1989. Pollen tube attrition in *Erythronium grandiflorum* (Liliaceae). *American Journal of Botany* 76: 562–570.
- , AND S. C. H. BARRETT. 1993. Contribution of cryptic incompatibility to the mating system of *Eichhornia paniculata* (Pontederiaceae). *Evolution* 47: 925–934.
- , AND ———. In press. Post-pollination mechanisms influencing mating patterns and fecundity: an example from *Eichhornia paniculata*. *American Naturalist*.
- DARWIN, C. 1876. The effects of cross and self fertilization in the vegetable kingdom. Murray, London.
- . 1877. The different forms of flowers on plants of the same species. Murray, London.
- DE NETTANCOURT, D. 1977. Incompatibility in angiosperms. Springer-Verlag, Berlin.
- DULBERGER, R. 1992. Floral polymorphisms and their functional significance in the heterostylous syndrome. In S. C. H. Barrett [ed.], *Evolution and function of heterostyly. Monographs on Theoretical and Applied Genetics* 15: 41–84.
- ECKERT, C. G., AND S. C. H. BARRETT. 1993. The inheritance of tristylous in *Decodon verticillatus* (Lythraceae). *Heredity* 71: 473–480.
- , AND ———. 1994. Post-pollination mechanisms and the maintenance of outcrossing in self-compatible, tristylous, *Decodon verticillatus* (Lythraceae). *Heredity* 72: 396–411.
- FRYXELL, P. A. 1957. Mode of reproduction in higher plants. *Botanical Review* 23: 135–233.
- GLOVER, D. E., AND S. C. H. BARRETT. 1983. Trimorphic incompatibility in Mexican populations of *Pontederia sagittata* Presl. (Pontederiaceae). *New Phytologist* 95: 439–455.
- GUTH, C. J., AND S. G. WELLER. 1986. Pollination, fertilization and ovule abortion in *Oxalis magnifica*. *American Journal of Botany* 73: 246–253.
- HUSBAND, B. C., AND S. C. H. BARRETT. 1993. Multiple origins of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae): inferences from style morph and isozyme variation. *Journal of Evolutionary Biology* 6: 591–608.
- , AND D. W. SCHEMSKE. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.
- JOHNSTON, M. O. 1992. Effects of cross and self-fertilization and progeny fitness in *Lobelia cardinalis* and *L. siphilitica*. *Evolution* 46: 688–702.
- KLEKOWSKI, E. J. JR. 1988. Mutation, developmental selection and plant evolution. Columbia University Press, New York, NY.
- KOHN, J. R., AND S. C. H. BARRETT. 1992. Experimental studies on the functional significance of heterostyly. *Evolution* 46: 43–55.
- KREBS, S. L., AND J. F. HANCOCK. 1991. Embryonic genetic load in the highbush blueberry, *Vaccinium corymbosum* (Ericaceae). *American Journal of Botany* 78: 1427–1437.
- LEWIS, D. 1942. The physiology of incompatibility in plants. I. The effect of temperature. *Proceedings of the Royal Society London Series B* 131: 13–26.
- LLOYD, D. G., AND D. J. SCHOEN. 1992. Self- and cross-fertilization in plants. I. Functional dimensions. *International Journal of Plant Science* 153: 358–369.
- , AND C. J. WEBB. 1992a. The evolution of heterostyly. In S. C. H. Barrett [ed.], *Evolution and function of heterostyly. Monographs on Theoretical and Applied Genetics* 15: 151–178.
- , AND ———. 1992b. The selection of heterostyly. In S. C. H. Barrett [ed.], *Evolution and function of heterostyly. Monographs on Theoretical and Applied Genetics* 15: 179–207.
- MANASSE, R. S., AND K. PINNEY. 1991. Limits to reproductive success in a partially self-incompatible herb: Fecundity depression at serial life-cycle stages. *Evolution* 45: 712–720.
- MATHER, K., AND D. DE WINTON. 1941. Adaptation and counter-adaptation of the breeding system in *Primula*. *Annals of Botany* 5: 297–311.
- MONTALVO, A. M. 1992. Relative success of self and outcross pollen comparing mixed- and single-donor pollinations in *Aquilegia caerulea*. *Evolution* 46: 1181–1198.
- MORGAN, M. T., AND S. C. H. BARRETT. 1989. Reproductive correlates of mating system variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). *Journal of Evolutionary Biology* 2: 183–203.
- NAKAMURA, R. R., AND M. L. STANTON. 1987. Cryptic seed abortion and the estimation of ovule fertilization. *Canadian Journal of Botany* 65: 2463–2465.
- ORNDUFF, R. 1966. The breeding system of *Pontederia cordata*. *Bulletin of the Torrey Botanical Club*. 93: 407–416.
- RICHARDS, A. J. 1986. Plant breeding systems. Allen and Unwin, London.
- RIGNEY, L. P., J. D. THOMSON, M. B. CRUZAN, AND J. BRUNET. 1993. Differential success of pollen donors in a self-compatible lily. *Evolution* 47: 915–924.
- SAGE, T. L., R. I. BERTIN AND E. G. WILLIAMS. 1994. Ovarian and other late-acting self-incompatibility systems. In E. G. Williams, A. E. Clarke, and R. B. Knox [eds.], *Genetic control of self-incompatibility and reproductive development in flowering plants*, Advances in cellular and molecular biology of plants, vol. 2, 116–140. Kluwer, Dordrecht.
- SAS. 1990. SAS/STAT user's guide, Release 6.03 edition. SAS Institute, Cary, NC.
- SASS, J. E. 1958. Botanical microtechnique. Iowa State University, Ames, IA.
- SCHOU, O., AND M. PHILIPP. 1983. An unusual heteromorphic incompatibility system. III. On the genetic control of distyly and self-incompatibility in *Anchusa officinalis* L. (Boraginaceae). *Theoretical and Applied Genetics* 68: 139–144.
- SCRIBAILO, R. W., AND S. C. H. BARRETT. 1991. Pollen–pistil interactions in tristylous *Pontederia sagittata* (Pontederiaceae). II. Patterns of pollen tube growth. *American Journal of Botany* 78: 1662–1682.
- SEAVEY, S. R. AND K. S. BAWA. 1986. Late-acting self-incompatibility in angiosperms. *Botanical Review* 52: 195–219.
- , AND S. K. CARTER. 1994. Self-sterility in *Epilobium obcordatum* (Onagraceae). *American Journal of Botany* 81: 331–338.
- SHORE, J. S., AND S. C. H. BARRETT. 1985. The genetics of distyly and homostyly in *Turnera ulmifolia* L. (Turneraceae). *Heredity* 55: 167–174.
- SIDAK, Z. 1967. Rectangular confidence regions for the means of multivariate normal distributions. *Journal of the American Statistical Association* 62: 626–633.
- STELLY, D. M., S. J. PELOQUIN, R. G. PALMER, AND C. F. CRANE. 1984. Mayer's hemalum-methyl salicylate: a stain-clearing technique for observation within whole ovules. *Stain Technology* 59: 155–161.
- STROBECK, C. 1980. Heterozygosity of a neutral locus linked to a self-incompatibility locus or a balanced lethal. *Evolution* 34: 779–788.
- TOPPINGS, P. 1989. The significance of inbreeding depression to the evolution of self-fertilization in *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Msc. thesis, University of Toronto, Toronto, Ontario.

- UYENOYAMA, M. K., K. E. HOLSINGER, AND D. M. WALLER. 1993. Ecological and genetic factors directing the evolution of self-fertilization. In D. Futuyma and J. Antonovics [eds.], *Oxford Surveys in Evolutionary Biology*, 9: 327–381.
- VUILLEUMIER, B. S. 1967. The origin and evolutionary development of heterostyly in angiosperms. *Evolution* 21: 210–226.
- WASER, N. M. 1993. Population structure, optimal outbreeding, and assortative mating. In Thornhill, N. W. [ed.], *The natural history of inbreeding and outbreeding: theoretical and empirical perspectives*, 173–199. University of Chicago Press, Chicago, IL.
- WELLER, S. G., AND R. ORNDUFF. 1977. Cryptic self-incompatibility in *Amsinckia grandiflora*. *Evolution* 31: 47–51.
- , AND ———. 1989. Incompatibility in *Amsinckia grandiflora* (Boraginaceae): distribution of callose plugs and pollen tubes following inter- and intramorph crosses. *American Journal of Botany* 76: 277–282.
- , AND ———. 1991. Pollen tube growth and inbreeding depression in *Amsinckia grandiflora* (Boraginaceae). *American Journal of Botany* 78: 801–804.
- WIENS, D., C. L. CALVIN, C. A. WILSON, C. I. DAVERN, D. FRANK, AND S. R. SEAVEY. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 71: 501–509.
- WILLIAMS, E. G., A. E., CLARKE, AND R. B. KNOX [eds.]. 1994. Genetic control of self-incompatibility and reproductive development in flowering plants, *Advances in cellular and molecular biology of plants*, vol 2. Kluwer Academic, Dordrecht.
- ZAPATA, R. T., AND M. T. K. ARROYO. 1978. Plant reproductive ecology of a secondary deciduous tropical forest in Venezuela. *Biotropica* 10: 221–230.