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Trimorphic incompatibility in *Eichhornia azurea* (Pontederiaceae)

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Abstract *Eichhornia azurea* (Pontederiaceae) is a mat-forming, clonal aquatic that inhabits lakes, marshes and river systems in many parts of the Neotropics. The species is tristylous with long-, mid-, and short-styled morphs commonly represented in natural populations. To investigate whether *E. azurea* possesses a trimorphic incompatibility system typical of tristylous species, we conducted a controlled pollination experiment on 15 clones representing the three style morphs from a natural population near Rosario, Argentina. Comparisons of fruit and seed set following self-, illegitimate, and legitimate pollinations clearly demonstrated the presence of trimorphic incompatibility in *E. azurea*. Self- and illegitimate pollinations produced significantly less fruit and seed than legitimate pollinations in all three style morphs. Pollen from the two anther levels within a flower exhibited contrasting compatibility relations in self-pollinations. In common with several other tristylous species in Pontederiaceae, the expression of self-incompatibility was weakest in the mid-styled morph and strongest in the short-styled morph. We discuss the ecological and evolutionary significance of the partial expression of trimorphic incompatibility in *E. azurea*.

Key words Tristyly · Trimorphic incompatibility · Pollen trimorphism · Partial incompatibility · Clonal aquatic plant

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Introduction

In flowering plants, the principle mechanism that prevents self-fertilization and its harmful effects on progeny fitness is physiological self-incompatibility. Several classes of incompatibility systems are distinguished depending on their genetic control, sites of self-recognition and rejection, and whether or not mating types are morphologically identical (Lewis 1949; Nettancourt 1977; Gibbs 1986; Williams et al. 1994). In species with heteromorphic incompatibility, cross-compatible mating types are distinguished by their floral morphology (Darwin 1877; Ganders 1979; Barrett 1992). Populations are usually composed of two (distyly) or three (tristyly) self- and intramorph incompatible classes of plants that differ reciprocally with respect to style length, anther height and a suite of ancillary polymorphisms of pollen and stigmas. In most heterostylous species, pollinations between anthers and stigmas of equivalent height (legitimate pollinations) are compatible whereas all other cross- and self-pollinations are incompatible. However, not all species that possess the morphological components of heterostyly exhibit heteromorphic incompatibility (reviewed in Barrett and Cruzan 1994), and experimental pollination studies are required to determine the compatibility status of heterostylous plants.

The Pontederiaceae is a small monocotyledonous family of from six to nine genera and 35–40 species of mostly New World freshwater aquatics (Barrett and Graham 1998). Tristyly occurs in two genera, *Eichhornia* with three of nine species and *Pontederia* with four of six species tristylous, respectively. Experimental studies indicate that expression of the tristylous syndrome differs between the two genera. *E. crassipes* (Mulcahy 1975; Barrett 1977a) and *E. paniculata* (Barrett 1985) are highly self-fertile with weakly developed pollen-size trimorphism, whereas tristily in *P. cordata* (Ornduff 1966; Price and Barrett 1982; Barrett and Anderson 1985), *P. rotundifolia* (Barrett 1977b) and *P. sagittata* (Glover and Barrett 1983; Scribailo and Barrett 1991a,b) is associated with a well-developed trimorphic

incompatibility system and conspicuous size trimorphism of pollen. An earlier study of *E. azurea* (Barrett 1978), the remaining tristylous species of *Eichhornia*, revealed strong pollen-size trimorphism (and see Lallana and Marta 1981; Kirkwood 1996), a feature normally associated with the presence of trimorphic incompatibility. Unfortunately, in the Barrett (1978) study only a single clone was available for experiments and therefore a detailed examination of the compatibility status of *E. azurea* was not possible. The goal of the present study was to investigate this issue further by conducting controlled self- and cross-pollinations of the three style morphs to establish whether *E. azurea* possesses a trimorphic incompatibility system.

Here we address two specific questions concerning the reproductive system of *E. azurea*. (1) Do self- and illegitimate intramorph cross-pollinations produce less seed than legitimate cross-pollinations? If so the species must possess trimorphic incompatibility. (2) Do the style morph differ in their responses to the various pollination treatments, particularly self- and illegitimate cross-pollinations? This question is relevant for comparisons with other tristylous species in Pontederiaceae where data often indicate morph-specific differences in the expression of trimorphic incompatibility (reviewed in Barrett and Anderson 1985). Following the presentation of our results we discuss the relevance of our findings to issues concerning with the evolution and function of tristylous reproductive systems.

Materials and Methods

Study organism

Eichhornia azurea is a bee-pollinated, mat-forming, clonal aquatic that occurs in lakes, marshes and rivers throughout the Neotropics. It is particularly common in lowland South America in aquatic habitats associated with the Amazon (Brazil) and Paraná (Argentina) river systems and in the Pantanal wetlands. All work described here was conducted on plants originating from populations grow-

ing on swampy islands associated with the River Paraná opposite the city of Rosario, Santa Fe Province, Argentina. A survey of two populations conducted during the flowering period (January-May) verified that populations were tristylous. The frequencies of style morphs were: Laguna La Chilena; long-styled morph (hereafter L-morph) = 0.221, mid-styled morph (hereafter M-morph) = 0.543, short-styled morph (hereafter S-morph) = 0.236, $N=140$; and Laguna Charigüé; L-morph = 0.280, M-morph = 0.267; S-morph = 0.453, $N=150$.

Experimental study

We sampled 15 clones (five per style morph) from the Paraná River populations in April 1992 and returned them to a swimming pool at Rosario (water depth 0.60 m) where they were grown for subsequent experimental studies. Plants flowered prolifically during the course of the experiment, enabling us to conduct controlled hand-pollinations on individual inflorescences. We applied a given pollination treatment to ten emasculated flowers on an inflorescence, with two flowers left unpollinated to serve as controls. No seeds were produced by these unfertilized flowers, demonstrating that *E. azurea* does not have the capacity for autonomous self-fertilization. Six pollination treatments were conducted on each clone using a single inflorescence for each treatment. These were: self-pollination with each anther level within a flower; illegitimate intramorph cross-pollinations with each anther level in the S-morph and with the mid- and long-level anthers in the L- and M-morphs, respectively; and legitimate cross-pollinations with each morph. Reduced flowering of some clones, inflorescence damage and experimental error reduced sample sizes for some treatments. Following hand pollinations we tagged inflorescences and surrounded them by mesh bags. Infructescences of *E. azurea* exhibit geniculation of the floral axis and mature their seeds under water. At maturity (4–6 weeks after pollination) we retrieved submersed infructescences and counted the number of fruits and seeds produced by each flower. We analysed data on seeds per fruit (square-root transformed) by ANOVA to examine the effects of the pollination treatments and style morph on seed set (Sokal and Rohlf 1995).

Results

Fruit and seed set in self- and illegitimate pollinations were significantly lower than in legitimate pollinations. This demonstrates that *E. azurea* possesses a trimorphic

Table 1 Fruit and seed set following controlled self, illegitimate and legitimate pollinations of the three style morphs of tristylous *Eichhornia azurea*

Morph	Treatment	Anther level	<i>N</i>	Percent fruit set (SE)	Seed set/per pollination (SE)	Seed set/per fruit (SE)	
Long-styled	Self	<i>m</i>	44	81.8 (5.8)	31.73 (4.4)	38.78 (4.6)	
		<i>s</i>	48	10.4 (4.5)	0.77 (0.7)	7.4 (5.9)	
	Cross	Illegitimate	<i>m</i>	19	10.5 (7.2)	0.74 (0.5)	7.0 (1.0)
		Legitimate	<i>l</i>	46	100 (0.0)	77.59 (4.1)	77.59 (4.1)
Mid-styled	Self	<i>l</i>	78	100 (0.0)	60.27 (6.5)	60.27 (6.5)	
		<i>s</i>	40	50 (8.0)	14.38 (3.0)	28.75 (3.8)	
	Cross	Illegitimate	<i>l</i>	12	92 (8.3)	55.75 (10.5)	60.82 (10.2)
		Legitimate	<i>m</i>	43	100 (0.0)	103.51 (5.2)	103.51 (5.2)
Short-styled	Self	<i>l</i>	58	0 (0.0)	0 (0.0)	0 (0.0)	
		<i>m</i>	63	50.8 (6.3)	6.9 (1.4)	13.59 (2.2)	
	Cross	Illegitimate	<i>l</i>	39	15.4 (5.9)	3.56 (2.0)	23.17 (10.5)
		Legitimate	<i>m</i>	30	13.3 (6.3)	3.47 (2.1)	26.00 (11.8)
	Legitimate	<i>s</i>	65	76.9 (5.3)	115.36 (11.1)	149.96 (10.1)	

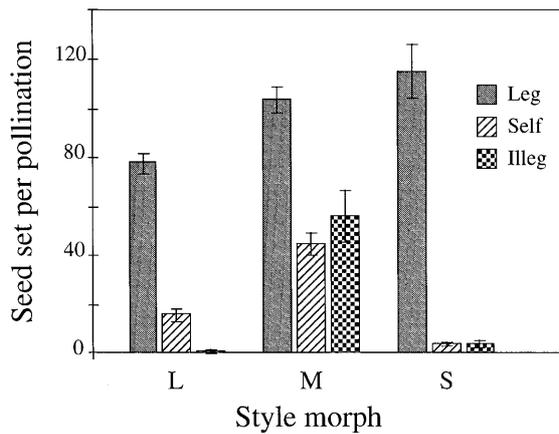


Fig. 1 Seed set per pollination following controlled self, legitimate and illegitimate pollinations of the three style morphs of tristylous *Eichhornia azurea*. Data were pooled within each of the pollination classes. See Table 1 for individual means. Bars are standard errors

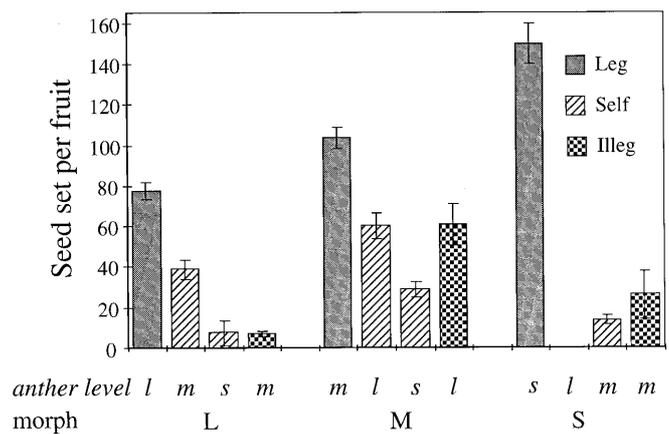


Fig. 2 Seed set per fruit following controlled self, illegitimate and legitimate pollinations in the three style morphs of tristylous *Eichhornia azurea*. Data are presented to illustrate variation in compatibility of pollen from different anther levels (*l*, *m*, *s* = long-, mid-, and short-level anthers, respectively). Bars are standard errors

Table 2 ANOVA for seed set per fruit (on transformed data, see text) following controlled pollinations on *Eichhornia azurea* (Pontederiaceae). Style morph and pollination type were considered fixed effects, plant was considered a random effect

Source	df	Sum of squares	F Ratio	P>F
Style morph	2	73.522	7.59	0.0006
Pollination type	2	1494.075	154.25	<0.0001
Morph × pollination type	4	165.032	8.52	<0.0001
Plant (morph)	13	787.676	12.51	<0.0001

incompatibility system (Table 1, Fig. 1). Average percent fruit and seed set per fruit for the three classes of pollination were: percent fruit set; self=51.7, illegitimate=23.0, legitimate=90.3 and seeds per fruit; self=21.6, illegitimate=9.3, legitimate=100.8. An ANOVA of seed set per fruit for all pollinations in the crossing programme indicated significant effects of style-morph, pollination type (self, illegitimate and legitimate) and their interaction (Table 2). For most pollen-pistil combinations the seed set of self- and illegitimate crosses was similar, as expected for a species with trimorphic incompatibility. The only clear exception to this pattern involved self- and illegitimate crosses of the L-morph with mid-level pollen. These pollinations resulted in strikingly different mean values of seed set (self=31.73; intramorph=0.74, see Table 1) contributing towards the significant morph × pollination type interaction in the ANOVA (Table 2).

The strength of the incompatibility system varied significantly among the three style morphs in *E. azurea* (Table 1, Fig. 1). The M-morph exhibited the weakest incompatibility system with high levels of seed produced from self- and illegitimate pollinations, especially with pollen from long-level anthers. In contrast, incompatibility was strongest in the S-morph, with self- and illegitimate pollinations producing only a small fraction of the seed that was obtained from legitimate pollinations. In-

compatibility was also generally strong in the L-morph although, as discussed above, self-pollinations with pollen from mid-level anthers produced moderate levels of seed.

A diagnostic feature of trimorphic incompatibility is the differentiation in behaviour of pollen produced by the two anther levels within a flower. This is clearly evident from comparisons of data for seed per fruit following self-pollinations with alternate anther levels within flowers of each style morph (Fig. 2). Pollen from one anther level always produced significantly more seeds than pollen from the other. In the L- and M-morphs, the most compatible pollen originated from mid- and long-level anthers, respectively (L-morph: mid-level anthers=38.78, short-level anthers=7.40, $t=3.39$, $P=0.0016$; M-morph: long-level anthers=60.27, short-level anthers=28.75, $t=2.31$, $P=0.0239$). In the S-morph, pollen from mid-level anthers resulted in 13.59 seeds per fruit whereas pollen from long-levels was strongly incompatible, producing no seeds.

Discussion

The major finding of this study is that the clonal aquatic *E. azurea* possesses a trimorphic incompatibility system. Self- and illegitimate pollinations resulted in significantly less seed per fruit than cross-pollinations with pollen from anthers of an equivalent level as the stigma. We begin the discussion by providing evidence that these results are indeed indicative of the operation of an incompatibility system in *E. azurea*, and then discuss the ecological and evolutionary significance of the variable expression of incompatibility that characterizes this species.

Lower seed set from controlled self-pollinations than from cross-pollinations has traditionally been taken as

evidence for the presence of self-incompatibility in a species. However, this result can also arise from the abortion of embryos due to early-acting inbreeding depression (Charlesworth 1985; Seavey and Bawa 1986; Charlesworth and Charlesworth 1987; Husband and Schemske 1996). In recent years, authors have been more cautious in interpreting the results of controlled pollination studies and have attempted to distinguish the proximate mechanisms governing the fertility of selfs and crosses (e.g. Manicacci and Barrett 1996; Sage et al. 1999). However, there are two reasons why we can reject early-acting inbreeding depression as contributing significantly to the patterns of seed set we obtained in our pollination experiment. First, low seed set was not only obtained from most self-pollinations but also occurred in cross-pollinations when pollen originated from illegitimate anther levels. Second, the amount of seed produced by self-pollinations differed dramatically depending on which anther level within a flower was used. Neither of these results would be predicted by the inbreeding depression hypothesis, but both are entirely consistent with the operation of a trimorphic incompatibility system in *E. azurea*.

An earlier pollination study involving a single clone of the L-morph of horticultural origin provided preliminary evidence of self-incompatibility in *E. azurea* (Barrett 1978). Self-pollinations with pollen from mid-level anthers produced an average of 14.3 seeds per fruit, whereas those using pollen from short-level anthers resulted in only 4.3 seeds. These values could not be compared with the productivity of cross-pollinations because only a single clone was available. Nevertheless the seed set data and observed differences in pollen-tube growth in this study were consistent with the presence of self-incompatibility. Later work on the origin and evolution of tristylous reproductive systems in the family (Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1998; Graham et al. 1998) assumed that *E. azurea* possessed trimorphic incompatibility. The results of this investigation fully support this supposition.

Experimental studies of related *E. crassipes* and *E. paniculata* indicate that both are highly self-fertile with relatively little difference in the seed set obtained from self- and cross-pollinations (Barrett 1977a, 1985). In these species, self-compatibility is accompanied by weak size-trimorphism of pollen. Our results for *E. azurea* contrast with these earlier findings. Trimorphic incompatibility is well developed and is accompanied by a clear size-trimorphism of pollen (Barrett 1978; Lallana and Marta 1981; and for our experimental material Kirkwood 1996). The morph-specific differences in the strength of incompatibility revealed in *E. azurea* resemble those in *Pontederia* species (reviewed in Barrett and Anderson 1985) with incompatibility only partially expressed in the M-morph and strongest in the S-morph. In addition, the most compatible self-pollinations in the L- and S-morphs involve pollen from mid-level anthers, and pollen from long-level anthers is most compatible in self-pollinations of the M-morph. These differences in

compatibility between anther levels in self-pollinations were also revealed in earlier studies of *Pontederia* spp.

In tristylous members of the Pontederiaceae, the contrasting levels of seed set that result from different pollen-pistil combinations occur because trimorphic incompatibility is only partially expressed in the family. In tristylous species from other families, only legitimate pollinations usually produce significant amounts of seed [e.g. *Lythrum junceum*, Dulberger 1970; *Oxalis* spp. Weller 1980; although see work on *Decodon verticillatus* by Eckert and Barrett (1994) for an exception]. However, in Pontederiaceae the strength of trimorphic incompatibility varies considerably both among style morphs and closely related species. In *E. paniculata*, despite strong self-fertility, a cryptic incompatibility system (sensu Bateman 1956) involving differential pollen-tube growth (Cruzan and Barrett 1993) favors fertilizations by legitimate pollen rather than self or illegitimate pollen in mixed pollinations. Thus, in this species trimorphic incompatibility is very weakly expressed. In contrast, in *E. azurea* the incompatibility system, while variable in expression is much stronger, with some pollen-pistil combinations resulting in virtually no seed set (e.g. pollinations of the L- and S-morphs with pollen from short- and long-level anthers, respectively), while others (e.g. pollinations of the L- and M-morphs with pollen from mid- and long-level anthers, respectively) are moderately compatible. It would be interesting to obtain information on the competitive relations of illegitimate and legitimate pollen in mixed pollinations, particularly in the L- and M-morphs. It is possible that despite the moderate self-compatibility of these morphs, in populations with a mixture of style morphs most seed is sired by legitimate pollen because of its superior competitive ability.

The general association between the strength of incompatibility and pollen-size trimorphism in Pontederiaceae has led to suggestions that there may be a functional link between them (Anderson and Barrett 1986; Scribailo and Barrett 1991b). However, whether size per se can explain patterns of seed set is unclear. Dulberger (1992) suggested that if the association between pollen-size heteromorphism and the expression of incompatibility in heterostylous plants has a functional basis, neither the storage product nor the pollen grain size itself is likely to be directly involved. She proposed that a protein or growth factor produced during differential growth of sporogenous tissue or pollen mother cells may be critical to governing inhibition sites. To date such 'incompatibility proteins' have not been identified in heterostylous species (although see Athanasiou and Shore 1997). Interestingly, in tristylous *Narcissus triandrus*, a species with a self-incompatibility system distinct from all other tristylous species, there are no differences in pollen size among the three anther levels, indicating that pollen-size differences are not a prerequisite for successful pollen tube growth in styles of contrasting length (Sage et al. 1999). However, no species with trimorphic incompatibility, irrespective of its strength, has

been found that does not possess some pollen-size trimorphism, and hence this association still requires a satisfactory functional explanation.

The adaptive significance of variation in the strength of trimorphic incompatibility in Pontederiaceae remains uncertain. It is possible that partial self-incompatibility in *E. azurea* can at times assure reproduction either when populations are composed of a single style morph, as can occur in this species (Barrett 1978 and unpublished observations), or when pollinators are limiting. The widespread occurrence of a selfing homostylous race of *E. azurea* in Costa Rica (Barrett 1978 and unpublished observations) is consistent with the view that under some ecological conditions the ability to produce seed through self-fertilization may be beneficial. If this interpretation is correct, the M-morph of *E. azurea* would appear to have a selective advantage, since this form has by far the weakest incompatibility system of the three morphs. Of course this advantage would be important only in seasonal habitats that demand frequent sexual recruitment, and in most aquatic habitats clonal propagation probably enables single clones to persist over long periods regardless of their style form.

Many workers studying the evolution of heterostyly have assumed that incompatibility evolves before the morphological components of heterostyly and where self-compatibility occurs it has resulted from a relaxation and eventual loss of the incompatibility system (reviewed in Ganders 1979, but see Lloyd and Webb 1992). However, recent reconstructions of the phylogenetic history of the tristylous syndrome in Pontederiaceae cast doubt on this sequence of events (Graham and Barrett 1995; Kohn et al. 1996; Barrett and Graham 1998; Graham et al. 1998). Instead, this work suggests that morphological tristylous originated first in the family, with trimorphic incompatibility subsequently evolving in some species but not others. If this latter sequence of events is true, then the variable expression of trimorphic incompatibility in members of the family should not necessarily be interpreted as the result of a loss in strength of a formerly strong incompatibility system. Rather, the present variable expression may be an adaptive compromise between the ecological benefits of partial incompatibility in habitats where assured reproduction by seed is advantageous and developmental constraints imposed by the morphological architecture of the polymorphism itself.

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References

- Anderson JM, Barrett SCH (1986) Pollen tube growth in tristylous *Pontederia cordata* (Pontederiaceae). *Can J Bot* 64:2602–2607
- Athanasiou A, Shore JS (1997) Morph-specific proteins in pollen and styles of distylous *Turnera* (Turneraceae). *Genetics* 146:669–679
- Barrett SCH (1977a) Tristyly in *Eichhornia crassipes* (Mart.) Solms (water hyacinth). *Biotropica* 9:230–238
- Barrett SCH (1977b) The breeding system of *Pontederia rotundifolia* L., a tristylous species. *New Phytol* 78:209–220
- Barrett SCH (1978) Floral biology of *Eichhornia azurea* (Swartz) Kunth (Pontederiaceae). *Aquat Bot* 5:217–228
- Barrett SCH (1985) Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). *Biol J Linn Soc.* 25:41–60
- Barrett SCH (ed) (1992) *Evolution and function of heterostyly*. Springer, Berlin Heidelberg New York
- Barrett SCH, Anderson JM (1985) Variation in expression of trimorphic incompatibility in *Pontederia cordata* L. (Pontederiaceae). *Theor Appl Genet* 70:355–363
- Barrett SCH, Cruzan MB (1994) Incompatibility in heterostylous plants. In: Williams EG, Clarke AE, Knox RB (eds) *Genetic control of self-incompatibility and reproductive development in flowering plants*. Kluwer, Dordrecht, pp 189–219
- Barrett SCH, Graham SW (1998) Adaptive radiation in the aquatic plant family Pontederiaceae: insights from phylogenetic analysis. In: Givnish TJ, Sytsma KJ (eds) *Molecular evolution and adaptive radiation*. Cambridge University Press, Cambridge, pp 225–258
- Bateman AJ (1956) Cryptic self-incompatibility in the wallflower: *Cheiranthus cheiri* L. *Heredity* 10:257–261
- Charlesworth D (1985) Distribution of dioecy and self-incompatibility in angiosperms. In: Greenwood PJ, Slatkin M (eds) *Evolution: essays in honour of John Maynard Smith*. Cambridge University Press, Cambridge, pp 237–268
- Charlesworth D, Charlesworth B (1987) Inbreeding depression and its evolutionary consequences. *Annu Rev Ecol Syst* 18:237–268
- Cruzan MB, Barrett SCH (1993) Contribution of cryptic incompatibility to the mating system of *Eichhornia paniculata* (Pontederiaceae). *Evolution* 47:925–934
- Darwin C (1877) *The different forms of flowers on plants of the same species*. Murray, London
- Dulberger R (1970) Tristyly in *Lythrum junceum*. *New Phytol* 69:751–759
- Dulberger R (1992) Floral polymorphisms and their functional significance in the heterostylous syndrome. In: Barrett SCH (ed) *Evolution and function of heterostyly*. Springer, Berlin Heidelberg New York pp 41–84
- Eckert CG, Barrett SCH (1994) Tristyly, self-compatibility and floral variation in *Decodon verticillatus* (Lythraceae). *Biol J Linn Soc* 53:1–30
- Ganders FR (1979) The biology of heterostyly. *NZ J Bot* 17:607–635
- Gibbs PE (1986) Do homomorphic and heteromorphic self-incompatibility systems have the same sporophytic mechanism? *Plant Syst Evol* 154:285–323
- Glover DE, Barrett SCH (1983) Trimorphic incompatibility in Mexican populations of *Pontederia sagittata* Presl. (Pontederiaceae). *New Phytol* 95:439–455
- Graham SW, Barrett SCH (1995) Phylogenetic systematics of Pontederiales: implications for breeding-system evolution. In: Rudall PJ, Cribb PJ, Cutler DF, Humphries CJ (eds) *Monocotyledons: systematics and evolution*. Royal Botanic Garden Kew, pp 415–441
- Graham SW, Kohn JR, Morton BR, Eckenwalder JE (1998) Phylogenetic congruence and discordance among one morphological and three molecular data sets from Pontederiaceae. *Syst Biol* 47:545–567
- Husband BC, Schemske DW (1996) Evolution of the magnitude and timing of inbreeding depression. *Evolution* 50:54–70
- Kirkwood J (1996) A study of post-pollination events in the pistils of *Eichhornia azurea* (Swartz) Kunth (Pontederiaceae) a tristylous species. BSc honours thesis, The University of St. Andrews, Scotland
- Kohn JR, Graham SW, Morton B, Doyle JJ, Barrett SCH (1996) Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. *Evolution* 50:1454–1469

- Lallana VH, Marta MC (1981) Biología floral de *Eichhornia azurea* (Swartz) Kunth (Pontederiaceae). Rev Asoc Cienc Nat Litoral 12:128–135
- Lewis D (1949) Incompatibility in angiosperms. Biol Rev 24:472–496
- Lloyd DG, Webb CJ (1992) The evolution of heterostyly. In: Barrett SCH (ed) Evolution and function of heterostyly. Springer, Berlin Heidelberg New York pp 151–178
- Manicacci D, Barrett SCH (1996) Fertility differences among floral morphs following selfing in tristylous *Eichhornia paniculata* (Pontederiaceae): inbreeding depression or partial incompatibility? Am J Bot 83:594–603
- Mulcahy DL (1975) The reproductive biology of *Eichhornia crassipes* (Pontederiaceae). Bull Torrey Bot Club 102:18–21
- Nettancourt D de (1977) Incompatibility in angiosperms. Springer, Berlin Heidelberg New York
- Ornduff R (1966) The breeding system of *Pontederia cordata*. Bull Torrey Bot Club 93:407–416
- Price SD, Barrett SCH (1982) Tristyly in *Pontederia cordata*. Can J Bot 60:897–905
- Sage TL, Strumas F, Cole WW, Barrett SCH (1999) Differential ovule development following self- and cross-pollination: the basis of self-sterility in *Narcissus triandrus* (Amaryllidaceae). Am J Bot 86:855–870
- Scribailo RW, Barrett SCH (1991a) Pollen-pistil interactions in tristylous *Pontederia sagittata* (Pontederiaceae). I. Floral heteromorphism and structural features of the pollen tube pathway. Am J Bot 78:1643–1661
- Scribailo RW, Barrett SCH (1991b) Pollen-pistil interactions in tristylous *Pontederia sagittata* (Pontederiaceae). II. Patterns of pollen tube growth. Am J Bot 78:1662–1682
- Seavey SR, Bawa KS (1986) Late-acting self-incompatibility in angiosperms. Bot Rev 52:195–219
- Sokal, RR, FJ Rohlf (1995) Biometry. WH Freeman, New York
- Weller SG (1980) The incompatibility relationships of tristylous species of *Oxalis* section *Ionoxalis* of southern Mexico. Can J Bot 58:1908–1911
- Williams EG, Clarke AE, Knox RB (1994) Genetic control of self-incompatibility and reproductive development in flowering plants. Kluwer, Dordrecht