J. evol. Biol. 2: 183–203 (1989)

1010–061X/89/03183–21 $ 1.50 + 0.20/0

Reproductive correlates of mating system variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae)

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Key words: Sex allocation; heterostyly; *Eichhornia*; mating system variation; maternal regulation.

Abstract

Comparative studies of related plant species indicate that evolutionary shifts in mating systems are accompanied by changes in reproductive attributes such as flower size, floral morphology, and pollen/ovule ratio. Recent theoretical work suggests that patterns of investment in reproduction should also change with the mating system. In a glasshouse study, we investigated the extent to which mating system differences among populations of *Eichhornia paniculata* (Pontederiaceae) were correlated with changes in allocation to male and female function, floral display, and the regulation of investment in reproduction through fruit and ovule abortion.

Significant differences in the amount of biomass allocated to reproductive structures were evident among six populations of *E. paniculata*. As predicted by sex allocation theory, the proportion of dry weight allocated to male function decreased with the outcrossing rate of populations. Six of the eight attributes used to characterize floral display also differed significantly among populations. However, with the exception of two attributes describing the number of flowers produced by inflorescences, these were not correlated with outcrossing rate. Levels of fruit and ovule abortion were determined in two populations with contrasting mating systems under different nutrient and pollination treatments. Virtually all fruits initiated by plants from a self-fertilizing population were matured, while the amount of fruit abortion in an outcrossing population increased with flower production. Ovule abortion was low in both populations. Our results demonstrate that the evolution of self-fertilization in *E. paniculata* is associated with changes in investment to reproduction that normally distinguish selfing and outcrossing species.

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Introduction

Evolutionary shifts in plant mating systems are often accompanied by manifold changes in the reproductive biology, life history, and population genetic structure of plant species (Ornduff, 1969; Jain, 1976; Hamrick et al., 1979; Bawa, 1980a; Wyatt, 1983). Since the shift from cross-fertilization to self-fertilization is the most common pathway of mating system change in flowering plants (Stebbins, 1970), it has been a particular focus of both theoretical and empirical studies. Analytic models and qualitative arguments concerned with the evolution of selfing predict changes in floral morphology, allocation of resources to sexual function, and population genetic structure (Allard et al., 1968; Ornduff, 1969; Lloyd, 1979a,b, 1984; Wells, 1979; Charlesworth and Charlesworth, 1981, 1987; Lande and Schemske, 1985).

Since the evolution of selfing is often associated with rapid character divergence, reproductive isolation, and speciation (Baker, 1961; Grant, 1971; Barrett, 1989), intraspecific studies of the consequences of mating system change for reproductive traits have been limited (e. g. Lloyd, 1965; Moore and Lewis, 1965; Rick et al., 1978; Schoen, 1982). Instead, most empirical work has involved survey data (Cruden, 1977; Lemen, 1980; Lovett Doust and Cavers, 1982; Sutherland and Delph, 1984; Cruden and Lyon, 1985; Sutherland, 1986) or population studies of closely related taxa (e. g. Arroyo, 1973; Solbrig and Rollins, 1977; Garnock-Jones, 1981; Thomas and Murray, 1981; Wyatt, 1983; Ritland and Jain, 1984; Layton and Ganders, 1984). While survey data and interspecific comparisons provide valuable information on the characters that distinguish outcrossing and selfing species, population studies at the intraspecific level are more likely to provide evidence of functional relationships since factors responsible for reproductive change are less confounded with taxonomic differences.

Eichhornia paniculata (Pontederiaceae) provides suitable experimental material to investigate the reproductive consequences of mating system variation. Populations of this short-lived perennial or annual emergent aquatic display a wide range of mating systems associated with the breakdown of tristyly to semi-homostyly (Barrett, 1988). Populations in northeast Brazil are primarily trimorphic and outcrossing whereas those on the island of Jamaica are predominantly monomorphic and composed of self-fertilizing, semi-homostylous variants of the mid-styled morph. These two conditions describe the extremes of mating system variation in the species. In Brazil, dimorphic and monomorphic populations exhibit intermediate selfing rates and may represent stages in the breakdown process.

Comparisons of the floral biology of Brazilian and Jamaican populations of *E. paniculata* have revealed differences in flower number per inflorescence, flower size, nectar guide intensity, degree of pollen heteromorphism, pollen production, and ovule and seed number per flower (Barrett, 1985a,b). Jamaican populations usually exhibit smaller inflorescences containing smaller, less showy flowers with lower reproductive potential than trimorphic Brazilian populations. Similar but less dramatic alterations in floral and reproductive characters are evident in dimorphic and monomorphic populations from Brazil. Differences in the size and showiness of

flowers from trimorphic, dimorphic, and monomorphic populations of *E. paniculata* are illustrated in Fig. 1.

In this study we focus on three additional features of the reproductive biology of *E. paniculata* that might be expected to change with shifts in mating system. First, the theory of sex allocation predicts decreased allocation to male function with greater levels of selfing (Charlesworth and Charlesworth, 1981, 1987; Charnov, 1982; Lloyd, 1983). Accordingly, we expect a lower allocation to male function associated with the evolution of selfing in *E. paniculata*. This pattern has been demonstrated in *Gilia achilleifolia* (Schoen, 1982) and *Oryza perennis* (Charnov, 1987). Second, pollination biologists have suggested that floral display influences the pattern of mating, and is subject to selective forces imposed by pollinators (Fäegri and van der Pijl, 1979; Stephenson, 1979; Willson, 1979; Thomson, 1980; Beach, 1981; Bawa, 1983). Autogamy is usually associated with a decreased reliance on pollinators for reproduction, and might therefore be expected to result in a relaxation of the selection pressures that maintain inflorescence traits contributing to floral display. In *E. paniculata*, inflorescences of selfing populations are smaller than outcrossing populations, but it is not known if other aspects of floral display (e. g. flowering synchrony, flowering duration) have become modified in association with changes in mating system. Finally, Lloyd (1980) has argued that maternal expenditure is determined by a temporal series of controls on the number of potential fruit in which investment is made, and that assured reproduction in self-fertilizing species might result in the regulation of maternal investment earlier in the reproductive period. Using a selfing and an outcrossing population of *E. paniculata*, we address Lloyd's hypothesis in the context of changes in the mating system by documenting patterns of flower production and fruit and ovule abortion under experimental conditions.

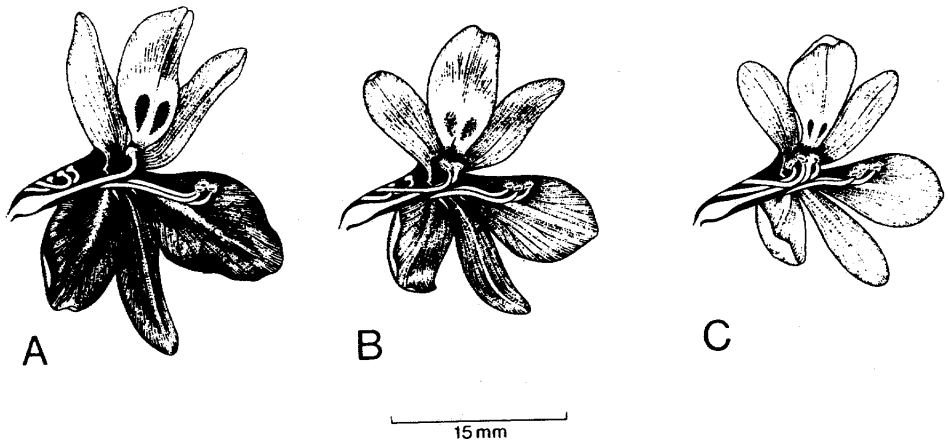

Fig. 1. Differences in the size and showiness of flowers from (A) trimorphic (Brazilian), (B) dimorphic (Jamaican), and (C) monomorphic (Jamaican) populations of *Eichhornia paniculata*.

Materials and methods

The three experiments described in this study used individuals of *Eichhornia paniculata*, grown under uniform glasshouse conditions during the summer and fall of 1986. Details of growing conditions and glasshouse culture are given in Barrett (1985b). Each reproductive shoot (module) of *E. paniculata* consists of an inflorescence, two bracts (one with a cordate lamina, short petiole, and ensheathing leaf base, the other greatly reduced), and an elongate internode (Richards and Barrett, 1984). Inflorescences produce between 40 and 125 flowers over a period of seven to 18 days. Flowers open between 7:00 am and 9:00 am, and the anthesis period of individual flowers is six to eight hours. Fruits mature in 10 to 12 days, and produce a total of 80 to 150 seeds in three locules.

Plants were grown from seed collected in six populations of *E. paniculata*, four from northeast Brazil and two from Jamaica. B5 and B11 were trimorphic, B9 and J15 were dimorphic, and B8 and J13 were monomorphic. Details of the locality, habitat and size of populations can be found in Glover and Barrett (1986). The populations were chosen to represent the range of mating system variation exhibited by the species. Previous electrophoretic studies of the populations determined their selfing rates and levels of genetic variation (Glover and Barrett, 1986, 1987). The absence of variation at 21 isozyme loci in population J13 precluded measurement of its selfing rate. The semi-homostylous floral morphology, capacity for autonomous self-pollination, and homozygosity at isozyme loci in plants from this population suggest a high level of self-fertilization under field conditions. Because of the similarity in floral morphology between plants from J13 and mid-styled plants from J15, an outcrossing rate ($t = 0.10$) similar to the latter population was used for J13 in statistical analyses. To facilitate comparisons among populations, only the mid-styled morph, or semi-homostylous derivative of it, were used in experiments 1 and 2. Experiment 3 compared populations B11 and J13 and employed seed selected at random from maternal plants in the populations.

Statistical analyses involved ANOVA and the calculation of correlation coefficients. ANOVA was performed on either raw or transformed data, depending on which most closely met the assumptions of normality and homoscedasticity of variances. Even with transformations, some violation of these assumptions occurred; these are noted in the appropriate tables. In experiments 1 and 2, populations were treated as random effects. All other treatments were considered fixed effects. Correlation coefficients were calculated using population mean values, and the significance of correlations were determined using a randomization test (Edgington, 1980). Details of analyses used in each experiment are described below.

Experiment 1: Allocation of resources to reproduction

The allocation of dry matter to reproductive and vegetative function was estimated in six populations of *E. paniculata*. Reproductive structures were defined to include all flowers and fruits, the smaller subtending bract, and the inflorescence

branches and rachis (following Thompson and Stewart, 1981), while the larger subtending bract ('leaf') and elongated internode was classified as vegetative structures. The entire above-ground module of five plants per population was harvested two to three days after inflorescences had started flowering, and the number of flowers and buds on the inflorescence were counted. The dry weight of the inflorescence rachis and branches, with flowers removed, and of the subtending vegetative structures were determined. Mean flower weight for each population was estimated using the dry weight of two pairs of randomly selected flowers from each of five genotypes. The mean number of seeds per capsule was estimated on a different inflorescence by counting all seeds in twelve randomly chosen cross-fertilized capsules from ten genotypes in each population. Mean seed weight was calculated in a similar manner using four samples of 50 seeds per genotype. Estimates of mean seed number and seed weight were used to calculate mean fruit mass. Total allocation to reproduction at flowering was estimated by multiplying mean flower weight by the number of flowers on the inflorescence, and adding the weight of the inflorescence branches and rachis. Total allocation to reproduction at fruit maturation was estimated in a similar way, with mean fruit mass substituted for mean flower weight. Relative allocation was calculated as the ratio of reproductive to vegatative allocation.

Biomass allocated to male, female, and attractive structures, at anthesis, were determined by selecting four fully expanded flowers at random from five plants in each of the six populations. Flowers from each plant were placed in pairs and separated into androecium, gynoecium, and perianth. As in other studies of sex allocation (e. g. Schoen, 1982), allocation to male function was calculated as a percent of total allocation, including flower and fruit mass. Because the perianth may have either male or female function, relative allocation to male function was calculated in three ways: assigning the perianth an entirely male function, assigning the perianth an entirely female function, and partitioning the perianth equally between male and female function. More recent theoretical models (Charlesworth and Charlesworth, 1987) consider allocation to male, female, and attractive structures relative to total floral biomass at flowering, and we calculated these measures also.

All plant material was dried at 70° C for 72 hours, and weighed on a Sartorius microbalance to the nearest 0.01 mg. ANOVA was used to detect significant between-population differences for all samples in the experiment. Correlation coefficients were calculated between the attributes described above and the selfing rates for each population.

Experiment 2: Inflorescence display

To contrast the patterns of flowering in selfing and outcrossing populations of *E. paniculata*, ten plants from each of six populations were grown on a glasshouse bench during the summer of 1986. All flowers on the first two inflorescences initiated after August 15 were censused daily. The following attributes were measured: total

number of flowers produced, inflorescence duration, mean number of flowers produced per day, and the mean, variance, skewness, and kurtosis of the distribution of number of flowers in anthesis each day. To determine whether fruit production influenced flowering patterns at the inflorescence level, two pollination treatments were applied to all plants in the experiment. In the first treatment, all flowers were hand pollinated, while in the second no flowers were pollinated. Treatments were randomized over two successive inflorescences, with five plants from each population receiving the first and then second treatments, and five plants receiving treatments applied in the reverse order. To prevent autonomous self-pollination in selfing populations, unpollinated stigmas were removed with forceps. Statistics derived from the distribution of flowers in anthesis on each inflorescence were then used as entries in a two-way ANOVA with population and pollination treatment as main effects. Correlation coefficients were calculated between the means of these attributes and the selfing rates of each population.

Experiment 3: Regulation of maternal investment

To investigate whether there are differences in the regulation of maternal investment in populations of *E. paniculata* with contrasting mating systems, plants from a highly outcrossing population (B11) and a predominantly selfing population (J13) were compared. To investigate the influence of resource levels on maternal regulation, all plants in the experiment were assigned to one of two nutrient treatments. The 'high' treatment involved fertilizing plants weekly for the duration of the experiment (14 consecutive inflorescences), while plants in the 'low' treatment were fertilized during the production of the first three inflorescences only. An additional manipulation of resource levels within each population and nutrient treatment was applied by varying the number of flowers pollinated. In the 'heavy' pollination treatment, all flowers were hand pollinated, while in the 'light' pollination treatment approximately one-third of flowers, chosen randomly within inflorescences, were pollinated. To prevent autonomous self-pollination in the selfing population, unpollinated stigmas were removed with forceps. Thus the design consisted of a three-way factorial experiment, with two levels of each factor and five plants assigned to each treatment combination.

Plants were grown from seed and measurements of four attributes were made until each plant had produced 14 inflorescences. The attributes were the date on which each inflorescence began flowering, the number of flowers produced, the number of flowers hand-pollinated, and the number of fruits produced. Values for the last three attributes were summed over all fourteen inflorescences, and cumulative values were used to calculate percent fruit set (fruits per pollination). An additional three attributes associated with ovule abortion were measured on the third, fifth, eighth, and fourteenth inflorescences. Flowers in six pre-determined positions were pollinated and capsules harvested four, eight, or ten days later. Capsules were stained with Mayer's hemalum and cleared in methyl salicylate following a modified version of the technique described by Stelly et al. (1984). All

ovules in one randomly chosen locule were scored as either 'seeds' (ovules plump, embryo present and developing), 'aborted' (embryo present but under-developed, ovules collapsed), or 'unfertilized' (no evidence of embryo development). The technique allows recognition of fertilized ovules after they have reached the globular stage. Embryo abortion occurring at very early stages of development might have been mistakenly classified as unfertilized, resulting in a conservative estimate of the level of ovule abortion. The six capsules were used to estimate the number of seeds, unfertilized ovules, and aborted ovules per capsule for each inflorescence. These values were summed over the four inflorescences, and percent fertilization and ovule abortion (seeds per fertilized ovule) were calculated from the cumulative samples from the four inflorescences. All measures were analyzed using a three-way ANOVA.

Results

Experiment 1: Allocation of resources to reproduction

Populations differed significantly in vegetative module mass, flower number and mass, and seed number and mass (Table 1). Vegetative structures ranged between 0.47 and 0.67 g, while allocation to reproductive structures was 1–2 times the allocation to vegetative function. ANOVA (results not shown) indicated no significant differences between populations for total allocation to reproduction at time of flowering or fruiting. Corresponding measures of relative allocation to reproductive and vegetative structures were not significantly different between populations, nor were they significantly correlated with the selfing rate of populations.

There were significant differences among populations in dry weights of androecium, gynoecium, and perianth (Table 2), although only allocation to androecium was significantly correlated with selfing rate (Table 3). Allocation to individual flowers (either at flowering or including seed number and mass), and relative allocation at the time of flowering to androecium, gynoecium, and perianth were not significantly correlated with selfing rate (Table 3). When seed number and mass are included as components of female function, relative allocation to male function is significantly correlated with selfing rate, regardless of how the perianth is partitioned into male or female function.

Experiment 2: Inflorescence display

Summary statistics of inflorescence display are presented in Table 4. The distributions of the number of flowers in anthesis for each population and pollination treatment are shown in Fig. 2. ANOVA results (Table 4) indicate significant differences between populations for all attributes except kurtosis of the flower production distribution. Correlations between these measures and estimates of

Table 1. Allocation of dry weight to vegetative and reproductive function in six populations of *Eichhornia paniculata*. Values in the upper portion of the table are the population means and standard errors, ANOVA results are reported in the lower portion.

Population	Outcrossing rate[a]	Vegetative module ($g \times 10^{-2}$)	Inflorescence infrastructure ($g \times 10^{-2}$)	Flower number	Flower mass ($g \times 10^{-5}$)	Seed number per flower	Seed mass per flower ($g \times 10^{-2}$)
J13	0.10	47 (3.3)	1.5 (0.21)	45.8 (4.7)	321 (9.2)	66.4 (1.36)	15 (0.1)
J15	0.12	49 (2.4)	2.4 (0.52)	65.8 (6.1)	419 (14.9)	76.1 (1.69)	15 (0.2)
B9	0.36	47 (5.9)	2.1 (0.30)	69.1 (5.3)	437 (12.1)	76.5 (2.28)	12 (0.3)
B8	0.47	51 (3.7)	1.2 (0.30)	57.0 (4.0)	444 (14.4)	68.2 (2.20)	17 (0.6)
B5	0.97	67 (1.2)	1.5 (0.46)	76.5 (5.8)	446 (12.2)	64.7 (1.98)	14 (0.2)
B11	1.01	50 (8.1)	1.8 (0.43)	113.0 (6.3)	434 (9.1)	68.2 (2.18)	13 (0.2)
$MS_{pop}(df)$		0.056 (5)*	1814130 (5)	3185 (5)***	22912 (5)***	3058 (5)***	22.9***
$MS_{error}(df)$		0.022 (24)	1508550 (24)	177 (54)	1748 (54)	468 (714)	0.967

[a] After Glover and Barrett, 1986.
*: $P < 0.05$;***: $P < 0.001$.

Table 2. Allocation of dry weight to androecia, gynoecia, and perianth in six populations of *Eichhornia paniculata* with contrasting mating systems. Values in the upper portion of the table represent population means and standard errors in $g \times 10^{-5}$, ANOVA results are presented below.

Population	Androecium	Gynoecium	Perianth
J13	41.0 (3.48)	52.6 (3.25)	228 (7.0)
J15	57.1 (4.58)	72.9 (5.19)	289 (16.4)
B9	57.9 (5.94)	61.8 (5.52)	317 (11.5)
B8	56.4 (3.49)	67.4 (3.85)	320 (10.8)
B5	69.0 (4.34)	76.7 (5.21)	303 (13.0)
B11	64.9 (4.96)	61.2 (2.78)	308 (10.9)
MS_{pop}(df)	915.7 (5)***	645.6 (5)***	11726 (5)***
MS_{error}(df)	115.9 (54)	126.3 (54)	1424 (54)

***: $P < 0.001$

Table 3. Correlation between selfing rate and attributes describing allocation to male, female, and attractive structures in six populations of *Eichhornia paniculata* with contrasting mating systems.

Parameter	r	Probability
Total Allocation		
androecium (a)	0.826	0.026
gynoecium (g)	0.278	0.571
perianth (p)	0.517	0.313
seed number (sn)	−0.508	0.278
seed mass (sm)	−0.327	0.514
flower (a + g + p)	0.585	0.175
flower and fruit (a + g + p + sn*sm)	−0.366	0.489
Allocation at flowering (following Charlesworth and Charlesworth, 1987)		
a/(a + g + p)	0.636	0.065
g/(a + g + p)	0.560	0.393
p/(a + g + p)	0.160	0.625
Allocation at fruiting (following Schoen, 1982)		
a/(g + p + sn*sm)	0.919	0.022
(a + 0.5*p)/(g + 0.5*p + sn*sm)	0.865	0.033
(a + p)/(g + sn*sm)	0.837	0.043

selfing rate in the six populations are presented in Table 5. The total number of flowers produced per inflorescence and the mean number of flowers produced per day show strong negative correlations with selfing rate. No other attributes are significantly correlated with selfing rate.

For all attributes except the mean number of flowers per day and kurtosis of flower distribution, there was a statistically significant effect of pollination treat-

Table 4. Parameters describing inflorescence display in six populations of *Eichhornia paniculata* under two pollination regimes. Values represent means of 10 observations per cell. ANOVA results presented at the bottom of the table.

Population	Pollination treatment	Inflorescence duration (days)	Total # of flowers	Mean # flowers/day	Mean of flower distribution	Variance of flower distribution	Skewness of flower distribution	Kurtosis of flower distribution
J13	Poll	7.9 (0.79)	45 (4.7)	5.9 (0.30)	4.0 (0.33)	3.9 (0.59)	0.22 (0.063)	−0.85 (0.098)
	Unpoll	10.1 (0.50)	51 (4.4)	5.1 (0.31)	4.7 (0.18)	6.5 (0.66)	0.36 (0.064)	−0.69 (0.070)
J15	Poll	10.7 (0.55)	65 (6.0)	6.1 (0.45)	5.0 (0.20)	6.9 (0.71)	0.41 (0.072)	−0.70 (0.165)
	Unpoll	12.4 (0.45)	73 (4.9)	6.1 (0.58)	5.5 (0.18)	8.8 (0.68)	0.52 (0.034)	−0.46 (0.095)
B9	Poll	10.1 (0.86)	69 (5.2)	6.9 (0.39)	5.0 (0.27)	5.9 (0.94)	0.21 (0.065)	−0.72 (0.127)
	Unpoll	10.7 (0.65)	71 (5.8)	6.7 (0.36)	5.0 (0.27)	6.8 (0.75)	0.30 (0.064)	−0.77 (0.064)
B8	Poll	8.2 (0.59)	57 (4.0)	7.2 (0.68)	4.2 (0.17)	3.9 (0.50)	0.20 (0.100)	−0.70 (0.151)
	Unpoll	9.2 (0.35)	60 (4.2)	6.6 (0.34)	4.7 (0.15)	4.5 (0.27)	0.15 (0.055)	−0.74 (0.070)
B5	Poll	8.9 (0.46)	76 (5.8)	8.7 (0.74)	4.5 (0.19)	5.0 (0.47)	0.25 (0.042)	−0.89 (0.055)
	Unpoll	10.6 (0.52)	82 (6.4)	7.8 (0.43)	4.9 (0.24)	6.4 (0.66)	0.38 (0.039)	−0.69 (0.035)
B11	Poll	12.3 (0.57)	113 (6.2)	9.2 (0.43)	5.6 (0.19)	8.3 (0.80)	0.29 (0.046)	−0.79 (0.058)
	Unpoll	13.3 (0.63)	123 (6.4)	9.4 (0.62)	6.1 (0.24)	9.8 (0.98)	0.39 (0.047)	−0.66 (0.056)
$MS_{pop'n}$	5 df	49.39***	11532.0***	39.64***	6.28***	65.12***	0.187***	0.115
$MS_{poll'n}$	1 df	56.03**	1165.0**	4.93	6.12**	66.27**	0.222*	0.317
$MS_{pop \times poll}$	5 df	1.77	40.9	0.98	0.28	2.47	0.025	0.078
MS_{error}	108 df	3.54	297.6	2.45	0.52	4.86	0.036	0.091

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

Table 5. Correlations between attributes describing inflorescence floral display and estimates of selfing rate in six populations of *Eichhornia paniculata* under two pollination regimes.

Parameter	Pollinated		Unpollinated	
	r	Probability	r	Probability
Inflorescence duration	−0.380	0.433	−0.298	0.550
Total number of flowers	−0.786	0.025	−0.768	0.036
Mean number flowers/day	−0.989	0.001	−0.924	0.003
Mean of flower distribution	−0.432	0.367	−0.370	0.496
Variance of flower distribution	−0.365	0.465	−0.204	0.674
Skewness of flower distribution	0.202	0.811	0.078	0.883
Kurtosis of flower distribution	0.416	0.381	0.275	0.672

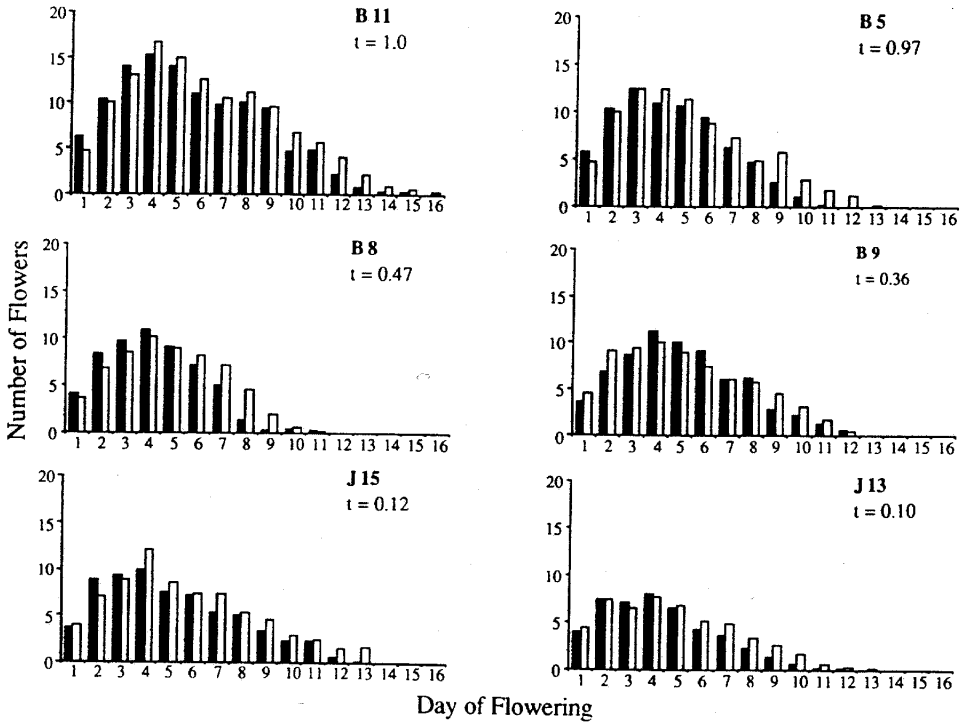

Fig. 2. Inflorescence floral display in six populations of *Eichhornia paniculata*. Each bar represents the number of flowers produced on each day of flowering. Solid bars: flowers pollinated; open bars: flowers unpollinated.

Table 6. Means and standard errors of parameters describing regulation of maternal investment in two populations of *Eichhornia paniculata* with contrasting mating systems under two nutrient and pollination treatments.

Population	Nutrient level	Pollination treatment	Days until infl. 14	Total number of flowers	Fruit set (%)	Number of ovules per flower	Ovules fertilized (%)	Ovules aborted (%)
J13	High	Heavy	110 (5.9)	409 (38.6)	91 (1.3)	109 (3.39)	94 (1.1)	4.3 (1.5)
		Light	108 (2.5)	496 (54.9)	100 (4.2)	112 (3.31)	96 (1.2)	4.5 (2.1)
	Low	Heavy	144 (3.1)	296 (20.6)	91 (2.6)	100 (3.10)	97 (0.6)	3.9 (1.5)
		Light	143 (9.3)	342 (19.5)	98 (2.9)	100 (3.03)	97 (0.9)	7.3 (4.4)
B11	High	Heavy	113 (5.3)	1186 (80.4)	58 (3.5)	141 (8.08)	87 (5.8)	8.0 (1.7)
		Light	116 (3.2)	1439 (42.8)	82 (2.5)	151 (10.10)	91 (2.4)	5.6 (1.9)
	Low	Heavy	251 (11.7)	619 (90.6)	75 (5.1)	115 (3.78)	96 (1.1)	5.2 (1.2)
		Light	208 (23.7)	806 (18.5)	91 (3.8)	109 (6.53)	91 (2.1)	6.2 (2.0)

Table 7. ANOVA of parameters describing regulation of maternal investment in two populations of *Eichhornia paniculata* with contrasting mating systems under two nutrient and pollination treatments. Values are mean squares.

Source	df	Days until infl. 14[a]	Total number of flowers[b,c]	Fruit set (%)	Number of ovules[a]	Ovules fertilized[c,d] (%)	Ovules aborted[d] (%)
Population	1	20.51**	1394.0***	0.472***	24.70***	1.890*	0.158
Nutrient	1	102.94***	424.9***	0.006	22.31***	1.030	0.0002
pop*nutr	1	10.43*	98.68***	0.144***	3.0*	0.193	0.141
Pollination	1	0.663	72.70***	0.316***	0.002	0.00058	0.035
pop*pol	1	0.219	9.87	0.001	0.093	0.027	0.159
nutr*pol	1	1.29	0.20	0.056***	1.28	0.703	0.261
pop*nutr*pol	1	1.14	0.74	0.005	0.33	0.368	0.0002
error	32	0.887	150.95	0.006	21.59	0.306	0.254

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

[a] $1/x$ transformed. Values $\times 10^{-6}$.

[b] square-root transformed.

[c] heterogenous variances.

[d] Values $\times 10^{-4}$.

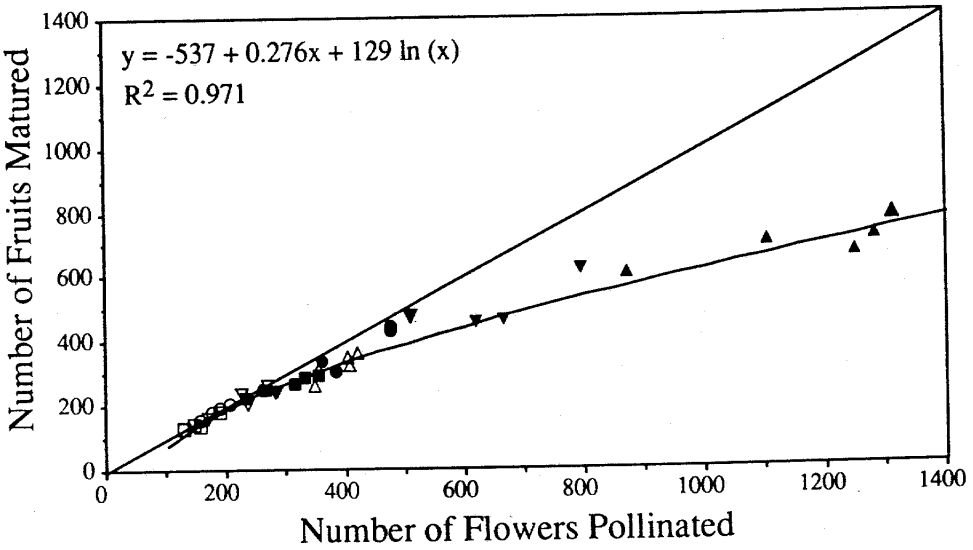

Fig. 3. Relationship between flower production and fruit maturation in 40 plants of *Eichhornia paniculata* from two populations with contrasting mating systems grown under glasshouse conditions. The line was fitted using least-squares regression. Both linear and logarithmic coefficients are significantly different from zero ($P < 0.001$). Filled symbols: population B11; hollow symbols: population J13. Squares: low nutrient, high pollination; circles; low nutrient, low pollination; triangles: high nutrient, low pollination; inverted triangles: high nutrient, high pollination.

than capsules in the low nutrient treatment (Table 6). This is reflected in the ANOVA as significant population, nutrient treatment, and interaction terms (Table 7). While in all treatment combinations a mean of 94% of ovules were fertilized (Table 6), in the outcrossing population fewer were fertilized than in the selfing population (Table 7, $P < 0.05$). Levels of ovule abortion were low (0.056 ± 0.008, mean $\pm$ SE) and did not differ between treatment combinations. As noted above, the technique used for ovule classification might not detect embryo abortion occurring at very early stages in development. If all ovules not reaching maturity are assumed to have been aborted, then the outcrossing population shows significantly higher levels of ovule abortion than the selfing population (0.142 ± 0.019 vs. 0.089 ± 0.015, mean $\pm$ SE).

Discussion

Variation in the mating system among populations of *Eichhornia paniculata* is associated with differences in patterns of investment to reproduction. The allocation of biomass to male function decreased with increased levels of self-fertilization. Inflorescence size and mean number of flowers produced per day were negatively

correlated with selfing rate, and significant differences between populations were found for most other attributes describing floral display. In addition, flower production and fruit maturation differed markedly between populations with contrasting mating systems when they were subjected to various experimental treatments.

The finding that relative allocation of dry matter to male function is negatively correlated with selfing rate in *E. paniculata* is in accord with two previous studies of the relationship between mating system and reproductive investment (Schoen, 1982; Charnov, 1987). Collectively the three studies support models of sex allocation which predict that lower levels of outcrossing should be associated with reduced investment in male function.

Sex allocation studies to date have assumed that limiting resources at the time of flowering are the same as those that limit fruit maturation, and investment in attractive structures can be accurately partitioned into male and female function (Bell, 1985). Charlesworth and Charlesworth (1987) have recently developed a model that addresses both of these assumptions by calculating the allocation to male, female, and attractive structures at the time of flowering. The model explicitly incorporates attributes describing the relationship between allocation to attractive structures and male and female fitness, but requires knowledge of several additional attributes including estimates of inbreeding depression. While our study has not attempted to quantify these parameters, the results presented in the Charlesworths' paper suggest that greater selfing rates will be associated with decreased allocation to perianth and androecium and increased allocation to gynoecium at the time of flowering. We observed trends consistent with these expectations, although only the correlation between allocation to male function and selfing rate approached statistical significance. For some values of the parameters in the Charlesworths' model, only small changes in allocation patterns are predicted over the entire range of selfing levels. Detection of significant correlations may require sampling more populations than were employed in our study.

Results presented by Charlesworth and Charlesworth (1987) suggest that attractive structures should usually represent substantially less than 50% of the total allocation to angiosperm flowers. However, our results and those of others (references in Charlesworth and Charlesworth, 1987; Lloyd, 1987) indicate that a high proportion (69% to 75% in our study) of floral biomass can be invested in attractive structures. This discrepancy may be because biomass does not accurately reflect the cost of different floral parts (Goldman and Willson, 1986; Charlesworth and Charlesworth, 1987). Use of biomass as a unit of currency could result in proportionately greater allocation to perianth compared to other measures such as energy content (Smith and Evenson, 1978; Schemske, 1978; but see Lovett Doust and Harper, 1980). Calculations with our data including a constant of proportionality, to accommodate the assumption that biomass overestimates perianth allocation, increases the correlation between selfing rate and allocation to androecium and gynoecium (M. T. Morgan, unpubl. data).

Difficulties in establishing the precise nature of the relationship between allocation to attractive structures and fitness gain through male or female function might

also account for the high proportion of biomass allocated to these structures. Charlesworth and Charlesworth (1987) assumed parameter values that corresponded to a decreasing gains curve. However, an increasing gains curve, such as when the cost of attractive structures must be outlaid before reproduction, is also possible (Lloyd, 1984). Our calculations indicate that employing this assumption results in expected allocation to attractive structures similar to that observed in our study. These difficulties reflect the problem of assessing the actual metabolic cost and functional role of different floral structures.

Populations of *E. paniculata* with contrasting mating systems also differ in their patterns of flower, fruit, and ovule maturation. Lloyd (1980) suggested that reproductive assurance in self-fertilizing populations should lead to earlier regulation of maternal investment than in outcrossing populations. In experiment 3, flower number and fruit abortion per inflorescence were greater in plants from the outcrossing population, whereas plants in the selfing population produced fewer flowers but matured virtually all fruits that were initiated. In addition, in experiment 2, unpollinated inflorescences produced more flowers than those in which all flowers were pollinated. These results are consistent with Lloyd's hypothesis, suggesting that 'excess' flower production may function as a mechanism for regulating investment in response to successful pollination (Aker, 1982).

Excess flower production may allow plants to compensate for fluctuating resource levels during fruit maturation (Stephenson, 1981; Udovic, 1981; Udovic and Aker, 1981; Sutherland, 1986). However, in *E. paniculata* fruit abortion in the outcrossing population was greatest in the treatments where nutrients were most abundant, and it seems unlikely that resource availability would fluctuate dramatically during the 10 to 12 days required for fruit maturation. Moreover, this explanation does not account for the high levels of fruit maturation in the self-fertilizing population.

Fruit initiation and maturation patterns observed in experiment 3 and illustrated in Figure 3 are consistent with sexual selection involving either male-male competition or female mate choice through selective fruit and seed maturation. Excess flower production might influence male fitness by increasing pollen representation in the outcrossed pollen pool (Bawa, 1980b; Queller, 1983; Bawa and Webb, 1984; Sutherland and Delph, 1984; Sutherland, 1987). If excess flower production benefits male function, we expect attributes characterizing floral display to be correlated with selfing rate because selection will act in outcrossing populations, but not selfing populations, to enhance pollinator attraction (Thomson, 1980, 1985; Waser, 1983; Bawa, 1983; Brown et al., 1986; Zimmerman, 1987). Flower production per inflorescence was negatively correlated with selfing rate, although other attributes associated with the phenology of inflorescences were not. The lack of correlations may result from developmental constraints associated with the complex inflorescence design in *E. paniculata* (see Richards and Barrett, 1984). The scorpioid cyme may restrict opportunities for selection to alter the phenological pattern of flowering, and only modifications involving allometric changes in the size of inflorescences and hence the duration of flowering appear to have occurred.

Willson and Burley (1983) have argued that sexual selection mediated by female choice could also occur through selective fruit and seed maturation. In our study,

fruit and ovule abortion were highest in the outcrossing population, with the greatest control over progeny number exercised through fruit rather than ovule abortion. Because levels of ovule abortion were relatively low, there may be little scope for selective maturation of embryos within fruits of *E. paniculata*. Fruit abortion will be more effective than seed abortion at eliminating inferior progeny when the diversity of progeny within fruits is less than between fruits. Reduced diversity might arise when the number of paternal parents represented within fruits is small as a result of correlated matings (Schoen and Clegg, 1984).

Our study has examined intraspecific variation in reproductive traits and has sought to determine correlations between these traits and the selfing rate of populations from which plants were obtained. This approach relies on the accuracy of outcrossing estimates and assumes that the outcrossing rate is, in part, under genetic control. The close association between population structure, style and stamen condition and mating system (Glover and Barrett, 1986; Barrett et al., 1987) supports the view that the control of outcrossing rate in *E. paniculata* has a significant heritable component. Nevertheless, the relatively small number of populations examined and the likelihood that outcrossing rates fluctuate in association with local demographic and environmental conditions may have restricted opportunities for detecting significant correlations in our study. A larger sample of populations and year to year estimates of outcrossing rate would assist in defining more precisely the quantitative relationships between patterns of sex allocation and mating system.

Among the six populations of *E. paniculata* that were examined, four originated from Brazil and two were from Jamaica. For several comparisons values for Brazilian populations were significantly different from those for Jamaican populations regardless of the mating system. Brazilian populations are likely to be more recently derived from tristylous ancestors and may have experienced a shorter history of inbreeding in comparison with those from Jamaica. If this is true, selection may have had less opportunity to alter floral traits and allocation patterns to the extent that is evident among Jamaican populations (Barrett, 1985b). Although outcrossing rates for the dimorphic and monomorphic Brazilian populations indicated significant levels of self-fertilization, this effect largely results from minor genetic alterations in stamen length in the mid-styled morph (Glover and Barrett, 1986). Early stages in the evolution of self-fertilization in outcrossing species provide opportunities to identify the proximate causes of self-fertilization and to separate these effects from subsequent changes to phenotype that occur following the establishment of selfing. The evidence from *E. paniculata* suggests that modifier genes affecting floral form initially exert their influence on mating system independent of allocation patterns. Alterations in the reproductive economy of plants appear to be a secondary consequence of the adoption of the selfing habit, rather than a cause.

Acknowledgements

We thank Petra Donnelly and Peter Toppings for technical assistance, Elizabeth Campolin for drawing the illustrations, and the Natural Sciences and Engineering Research Council of Canada for research support.

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Received 11 April 1988;
accepted 8 November 1988.
Corresponding Editor: G. Bell