

# Variation in Outcrossing Rates in *Eichhornia paniculata*: Temporal Changes in Populations of Contrasting Style Morph Structure

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**Abstract** Surveys of mating-system parameters in populations of the annual, self-compatible, tristylous, emergent aquatic, *Eichhornia paniculata* (Pontederiaceae) from N.E. Brazil and Jamaica have indicated that the species exhibits a wide range of outcrossing rates. To investigate whether temporal variation in outcrossing rate was also a feature of populations, open-pollinated families were sampled from five populations of contrasting style morph structure from N.E. Brazil over three consecutive years (1987–1989). Multilocus estimates of outcrossing rate ( $t$ ) were obtained from assays of isozyme polymorphisms using starch gel electrophoresis. There was significant variation both among populations and between years in the frequency of outcrossing. Outcrossing in three tristylous populations was high ( $t > 0.80$ ), with relatively small fluctuations occurring over the three-year sampling period. In contrast, in a dimorphic and monomorphic population considerable self-fertilization occurred and the frequency of outcrossing declined significantly from 1987 to 1989 in both populations. In the dimorphic population, increased selfing was associated with a marked reduction in population size and an increase in the frequency of selfing variants of the mid-styled morph. The significance of temporal variation in outcrossing frequency in plant populations is discussed in relation to its effect on population genetic structure and recent models of mating-system evolution.

**Key words:** *Eichhornia paniculata*, mating-system variation, selfing, tristily.

Over the past two decades the availability of electrophoretic marker loci has resulted in considerable progress in the quantification of mating-system parameters in both experimental and natural plant populations (Clegg, 1980; Ritland, 1983; Brown, 1990). Measurements of the frequency of outcrossing ( $t$ ) and selfing ( $s$ ) are now commonly reported in studies of the reproductive ecology and genetics of plant populations (reviewed in Schemske and Lande, 1985; Barrett and Eckert, 1990). Isozyme markers have enabled the description of patterns of variation in outcrossing rate among species and, more recently, investigations of the proximate ecological and genetic mechanisms governing the mating system of populations at both the individual and flower levels (Ritland and Ganders, 1985; Morgan and Barrett, 1990; Schoen and Brown, 1991; Schoen and Lloyd, 1992; Barrett et al., 1994). This data can provide valuable information for the development and testing of models of mating-system evolution, particularly those that place emphasis on the role of ecological factors in the selection of various levels of self- and

cross-fertilization (Lloyd 1979, 1980, 1992; Holsinger, 1991, 1992).

Most measurements of the frequency of outcrossing reported in the literature are population-level estimates obtained from bulk samples of open-pollinated families sampled at one time during a single season. These point estimates almost certainly subsume considerable heterogeneity manifested at both spatial (variation between individuals, inflorescences and flowers) and temporal scales (variation within and among years), particularly in self-compatible species with mixed mating systems. There have been relatively few studies that have measured year-to-year variation in outcrossing rate within populations and the results obtained to date have been mixed. For example, among animal-pollinated taxa only small amounts of between-year variation were evident in populations of *Papaver dubium* (Humphreys and Gale, 1974), *Limnanthes douglasii* (Kesseli and Jain, 1985), *Clarkia tembloriensis* (Holtsford and Ellstrand, 1991) and *Mimulus* spp. (Dole and Ritland, 1993), whereas in *Lupinus* spp. outcrossing rates fluctuated widely, probably as a result of yearly variation in the availability of insect pollinators (Harding, 1970; Horovitz and Harding, 1972; Harding et al., 1974; Harding and Barnes, 1977). Determining the temporal patterns of variation in outcrossing frequency among years is of particular interest to the problem of the evolutionary maintenance of mixed mating in flowering plants (Lande and Schemske, 1985; Waller, 1986;

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Schemske and Lande, 1987; Holsinger, 1988, 1991, 1992; Barrett and Eckert, 1990). If outcrossing rates in populations with intermediate values are of similar magnitude over many years it might provide some evidence in support of the mixed mating system as an evolutionary stable strategy.

*Eichhornia paniculata* (Pontederiaceae) is a self-compatible, insect-pollinated, tristylous, annual which is native to seasonal pools and ditches in N.E. Brazil and the Caribbean islands of Cuba and Jamaica. It exhibits the widest range of population-level estimates of outcrossing frequency reported to date in the flowering plants ( $t=0.002-0.96$ ,  $n=54$  populations; Barrett and Husband, 1990; Barrett et al., 1992). Studies of both natural and experimental populations have demonstrated that this variation is correlated with differences in the style-morph structure of populations (Barrett, 1985; Glover and Barrett, 1986; Barrett and Husband, 1990; Kohn and Barrett, 1992, 1994). Trimorphic populations exhibit moderate to high levels of outcrossing, whereas dimorphic and monomorphic populations usually experience considerable self-fertilization. High levels of selfing in non-trimorphic populations of *E. paniculata* are associated with the spread and fixation of self-pollinating variants of the mid-styled morph.

The data on outcrossing frequencies in populations of *E. paniculata* have been based on geographical surveys conducted throughout most of the species range. In this study we consider the temporal rather

than spatial context to mating-system variation, by examining year-to-year differences in outcrossing rate within populations of contrasting style morph structure. This approach is of interest for at least two reasons. First, populations of *E. paniculata* can vary dramatically in size and density from year to year as a result of seasonal fluctuations in the availability of moisture. Changes in population size are associated with variation in style morph frequencies and in some cases the loss of morphs from populations (Husband and Barrett, 1992a). Second, it has been proposed that the evolution of self-fertilization in *E. paniculata* is associated with the spread of self-pollinating variants in non-trimorphic populations. Hence, estimates of outcrossing in at least some of these populations might be expected to vary more than in trimorphic populations, because of changes in the relative frequency of selfing and outcrossing morphs. To investigate these issues we measured year-to-year variation in the outcrossing rates of a sample of populations of *E. paniculata* from N.E. Brazil with contrasting style morph structure using electrophoretic techniques.

## Methods

### 1. Sampling

Open-pollinated families were sampled from a total of

**Table 1.** The localities, sample sizes for electrophoretic loci, families and individuals, and the outcrossing rate (SE) in 1987, 1988, and 1989 for the five populations of *Eichhornia paniculata* with contrasting style morph structure sampled in N.E. Brazil.

Population	Year	Location	Number of loci screened	Number of families	Number of individuals	Outcrossing rate (SE)
<b>Trimorphic</b>						
B34	1987	Maranguape, Ceará	8	20	175	0.88 (0.019)
	1988		8	18	92	0.86 (0.102)
	1989		7	59	610	0.78 (0.033)
B58	1987	Belo Jardim, Pernambuco	3	78	1950	0.93 (0.030)
	1988		2	28	165	0.77 (0.130)
	1989		9	23	167	0.91 (0.032)
B85	1987	Limoeiro, Pernambuco	8	23	126	0.80 (0.060)
	1988		6	20	115	0.87 (0.045)
	1989		7	7	39	0.76 (0.073)
<b>Non-Trimorphic</b>						
B69	1987	Garanhuns, Pernambuco	1	9	60	0.70 (0.077)
	1988		4	25	179	0.29 (0.068)
	1989		5	36	383	0.38 (0.090)
B72	1987	Itabaiana, Sergipe	2	16	128	0.47 (0.068)
	1988		2	5	40	0.24 (0.249)
	1989		2	7	56	0.06 (0.035)

five populations of *Eichhornia paniculata* occurring in different regions of N.E. Brazil. Sampling was undertaken in May and June of 1987, 1988 and 1989. In large populations families were sampled at random, whereas in very small populations all seed-bearing plants were sampled. The geographical location and style morph structure (trimorphic, dimorphic and monomorphic) of the five populations as well as the number of families and progeny sampled per population are given in Table 1.

At each sampling interval two demographic parameters, population size ( $N$ ) and the density of plants in the population ( $d$ ), were estimated in each population to assess whether variation in outcrossing rate was associated with changes in these parameters. In addition, the frequencies of the three style morphs (long-, mid-, and short-styled morphs, hereafter L, M, and S morphs, respectively) were recorded each year in each population as well as the frequency of self-pollinating mid-styled variants (MM). For further details of the sampling methods adopted in this study, see Barrett et al. (1989) and Barrett and Husband (1990).

## 2. Electrophoresis

The frequency of outcrossing for each year was estimated using polymorphisms at loci encoding for isozymes, by starch gel electrophoresis. Flower buds (3–5) from progeny were crushed in DL-Dithiothreitol extraction buffer, adsorbed onto filter paper wicks and inserted into 11% starch gels. Populations were screened for polymorphic isozyme loci, the number of loci screened in each population is given in Table 1. Running conditions, gel buffer systems and allozyme variation at polymorphic loci are described in Glover and Barrett (1987) and Husband and Barrett (1991), where further details of electrophoretic procedures can be found.

## 3. Statistical Analyses

Multilocus outcrossing rates ( $t$ ) were estimated for each sampling interval in each population by the method of Ritland and Jain (1981) and Ritland (1990a). Population estimates were based on all polymorphic loci and standard errors for each estimate were calculated by bootstrapping across progeny arrays. Genotypes of maternal parents were inferred from progeny arrays during the estimation of  $t$ , using the method of Brown and Allard (1970). To determine whether estimates of  $t$  varied significantly among sampling intervals within each population, heterogeneity tests were performed using the test statistic,

$$\sum_{i=1}^n \left( \frac{t_i - \bar{t}}{\sigma_t} \right)^2,$$

where  $t_i$  is the outcrossing rate in year  $i$ ,  $\bar{t}$  is the arithmetic mean over  $n$  years, and  $\sigma_t$  is the standard error of  $t$  pooled across  $n$  estimates. The statistic is distributed as a  $\chi^2$  with  $n-1$  degrees of freedom.

Student's  $t$ -tests were then used to test for significant differences in outcrossing rate between years for populations that showed significant heterogeneity.

## Results

Estimates of outcrossing in the five populations were consistent with previous studies (see Barrett and Husband, 1990) in revealing two distinct patterns associated with the demography and style morph structure of populations. Trimorphic populations tended to be larger with higher plant densities and exhibited high rates of outcrossing, whereas non-trimorphic populations experienced moderate to high levels of self-fertilization and were often smaller in size with plants that were more sparsely distributed (Table 2, Fig. 1).

*Trimorphic populations*—In the three trimorphic populations that were sampled outcrossing rates were generally high (Mean  $t$ —B34=0.85, B58=0.87, B85=0.81) with relatively minor fluctuations observed from year to year, despite large changes in population size and the frequency of style morphs within each population (Table 2). For example, in population B34 population size declined from approximately 2500 individuals in 1987 and 1988 to only 120 plants in 1989. Yet outcrossing rate only changed by a relatively small amount over this time period (1987,  $t=0.88$ ; 1989,  $t=0.78$ ). Similarly, in population B85 the decline in frequency of the S morph from 1987 to 1988 and the loss of this mating type from the population in 1989, were not associated with any striking change in the outcrossing rate. Statistical analysis of variation in outcrossing within each of the three trimorphic populations indicated no significant heterogeneity among years ( $\chi^2$  with  $df=2$ , B34=2.13 N.S.; B58=3.71 N.S.; B85=1.76 N.S.).

*Non-trimorphic populations*—Mating patterns in the two non-trimorphic populations were markedly different from those observed in the three trimorphic populations (Table 1, Fig. 1). Both populations exhibited high levels of self-fertilization and there was significant heterogeneity among years in outcrossing rate within both populations ( $\chi^2$  with  $df=2$ , B69=15.13,  $P<0.005$ ; B72=6.13,  $P<0.05$ ). In the dimorphic population (B72), the frequency of outcrossing decreased significantly from 1987 to 1989 ( $t=5.36$ ,  $P<0.001$ ). This change in the mating system was associated with a marked decrease in the size of the population from 53 to 8 individuals, an increase in the frequency of selfing variants of the M morph, and a decline in the frequency of the L morph (Table 2). In the monomorphic population (B69) outcrossing decreased significantly from 1987 to 1989 ( $t=2.7$ ,  $P<0.01$ ), but this change in mating pattern was not associated with fluctuations in population size or changes in the frequency of selfing

**Table 2.** The frequencies of style morphs (L, M, S), selfing variants (MM) and the size ( $N$ ) and density ( $d$ ) at each sampling interval in the five populations of *Eichhornia paniculata* investigated in this study. Density values range from 1 (low density) to 4 (high density).

Population	Year	L	M	S	MM	$N$	$d$
<b>Trimorphic</b>							
B34	1987	0.40	0.34	0.27	0.00	2500	4
	1988	0.34	0.35	0.31	0.00	2500	3
	1989	0.51	0.32	0.17	0.00	120	3
B58	1987	0.32	0.47	0.21	0.10	1400	4
	1988	0.36	0.34	0.30	0.01	275	3
	1989	0.46	0.29	0.25	0.07	256	3
B85	1987	0.48	0.37	0.15	0.00	85	2
	1988	0.46	0.52	0.02	0.00	87	2
	1989	0.29	0.71	0.00	0.00	15	1
<b>Non-Trimorphic</b>							
B69	1987	0.00	1.00	0.00	1.00	82	1
	1988	0.00	1.00	0.00	0.32	100	3
	1989	0.00	1.00	0.00	0.80	200	2
B72	1987	0.23	0.77	0.00	0.55	53	2
	1988	0.38	0.63	0.00	0.63	8	1
	1989	0.13	0.88	0.00	0.88	8	1

variants of the M morph (Table 2).

## Discussion

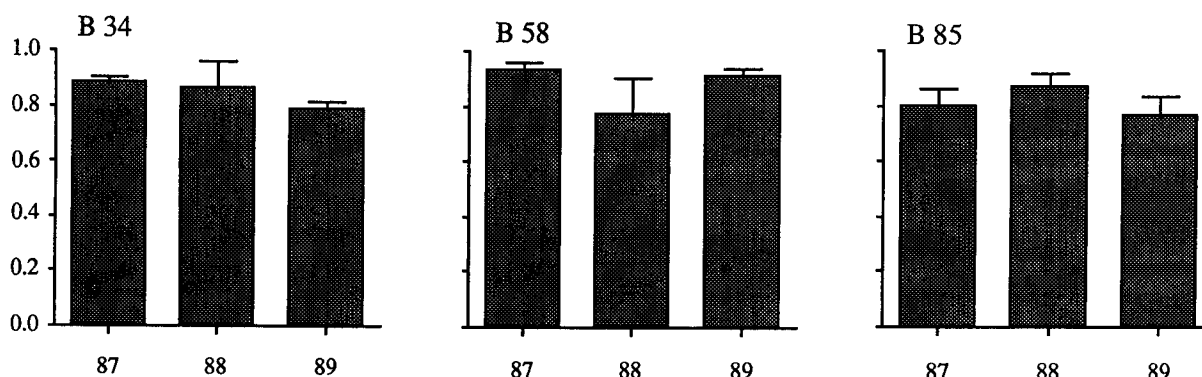
The mating system is not a static property of plant populations but is subject to environmental and genetic influences operating at both ecological and evolutionary time scales. A major challenge for workers interested in understanding the evolution of plant mating systems is to determine the extent of variation in mating patterns within and between populations and to elucidate the relative importance of ecological and genetic factors governing this variation. This study has highlighted how outcrossing rates can vary greatly in both space and time within a single species. The causes of this variation are complex and involve interactions between demographic factors and genetic modifications to the mating system associated with the evolutionary breakdown of the species' tristylous breeding system.

In the three trimorphic populations that were sampled, outcrossing rates generally exceeded 0.80 and there were only relatively small fluctuations from year to year, despite large changes in population size and, in population B85, periods of low density. It has often been suggested that reductions in population size or plant density are likely to be associated with decreased

pollinator activity resulting in increased levels of self-fertilization (Lloyd, 1965, 1980; Vasek and Harding, 1976; Wyatt, 1986; Burdon et al., 1988; Barrett et al., 1989). However, our results suggest that the mating system of tristylous populations may be quite resilient to environmental perturbations likely to influence pollen dispersal. The maintenance of high outcrossing in tristylous populations of *E. paniculata* may be associated with the species cryptic trimorphic-incompatibility system.

Although plants in tristylous populations are thoroughly self-compatible, setting abundant seed upon self-pollination (Barrett, 1985; Barrett et al., 1989; Kohn and Barrett, 1992), competition experiments between genetically-marked illegitimate and legitimate pollen have demonstrated that legitimate pollen has a substantial advantage in siring ovules (Glover and Barrett, 1986; Cruzan and Barrett, 1993). Hence as long as a sufficient supply of outcrossed pollen is delivered to stigmas by pollinators, post-pollination mechanisms of discrimination between self and outcross pollen will buffer the mating system against variable pollen environments. Similar mechanisms involving post-pollination discrimination against self pollen have also been demonstrated in self-compatible, tristylous *Decodon verticillatus* (Lythraceae) and probably account for the relatively narrow range of outcrossing rates displayed in populations of contrasting size and density in this species (Eckert and Barrett, 1994a).

### Trimorphic



### Non-trimorphic

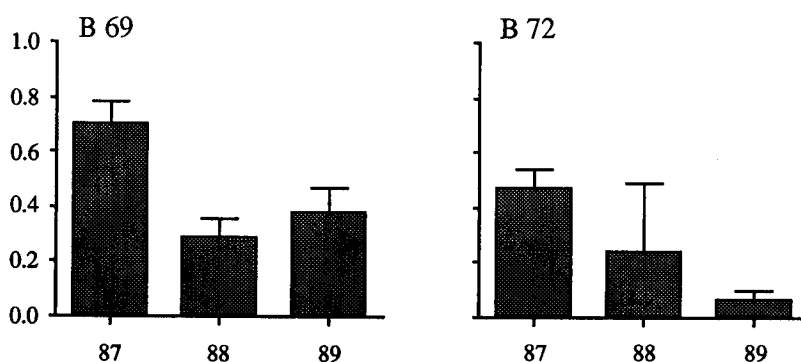


Fig. 1. Outcrossing rates and their standard errors estimated over three consecutive years (1987, 1988, 1989) in five populations of *Eichhornia paniculata* with contrasting style morph structures from N.E. Brazil. Populations B34, B58 and B85 are trimorphic (L,M, S), Population B72 is dimorphic (L, M) and population B69 is monomorphic (M) for style morph.

The two non-trimorphic populations of *E. paniculata* investigated in this study experienced higher levels of self-fertilization and much greater year-to-year variation in mating patterns than trimorphic populations. The differences in temporal variation of outcrossing rate between the two classes of populations are well illustrated by comparisons of their coefficient's of variation in  $t$ . Values for trimorphic populations B34, B58, and B85, were 6.30, 10.02, and 6.87, respectively, compared with 47.19 and 80.07 for non-trimorphic populations B69 and B72. Samples from more non-trimorphic populations would be required to determine whether these patterns are generally found. High self-fertilization in the non-trimorphic populations is undoubtedly associated with the presence of moderate to high frequencies of self-pollinating variants of the M morph within each population (Table 3). Previous studies involving morph-specific estimates of the selfing rate have clearly demonstrated that these variants display a higher degree of self-fertilization than

unmodified floral morphs, particularly in non-trimorphic populations (Barrett et al., 1989; Kohn and Barrett, 1994). In the dimorphic population (B72) an increase in the frequency of selfing variants over the three-year period was accompanied by a decrease in the outcrossing rate, a pattern expected if selection were favoring increased self-fertilization. However, in population B69, changes in the frequency of selfing variants were not associated in any obvious manner with alterations to mating pattern. Between 1987 and 1988 selfing rates increased by 41%, yet the frequency of selfing variants decreased by 48%, suggesting that other factors were influencing mating. Population sizes were similar in the two years and the density of plants in the population increased.

Observations of pollinator activity were not undertaken during the sampling of populations investigated in this study. Clearly these would have been useful in attempting to interpret mating patterns, particularly those observed in non-trimorphic populations. In a

study of pollinator visitation to 16 populations of *E. paniculata* in N.E. Brazil, Husband and Barrett (1992b) found that pollinator densities were not correlated with the size, density or morph ratio of populations. However, insect visitation rates were more variable among small populations, a pattern consistent with the idea that selfing variants are favored by reproductive assurance in small populations with unreliable pollinator service. Elsewhere, attempts to relate levels of pollinator activity to selection for selfing have largely been unsuccessful (Rick et al., 1978; Wyatt, 1986). Difficulties in determining the functional relationships between the pollination biology of populations and their mating patterns highlight the need for experimental studies in this area (reviewed in Harder and Barrett, 1994). Ideally, studies that measure the response of outcrossing rate to experimental manipulations of the size and density of populations are required to determine the importance of pollinator activity to mating patterns.

What are the implications of temporal variation in outcrossing to population genetic structure and mating-system evolution? There has been no detailed theoretical analysis on this problem and it is unclear whether any novel selective forces on the mating system would be generated by year-to-year variation in mating parameters. Nevertheless, it is clear that a single estimate of outcrossing rate for a population with mixed mating is unlikely to adequately reflect its inbreeding history, leading to the possibility of biased predictions in evolutionary models. For example, models that predict the frequency of heterozygotes in a population from the current outcrossing rate assume that the mating system is constant through time. However, Brown and Albrecht (1980) showed that single generation estimates of outcrossing result in underestimates of the levels of heterozygosity expected in populations based on the mating system alone. They concluded that the bias resulting from a lack of multigeneration estimates of outcrossing may, in part, account for the common observation of a discrepancy between observed and expected levels of heterozygosity in predominantly selfing species (Brown, 1979).

Recently, Ritland (1990b) has developed methods for estimating the magnitude of inbreeding depression in field populations of partially self-fertilizing species by measuring multigenerational changes in the inbreeding coefficient of parents and progeny. His equilibrium  $F$  approach also assumes that rates of selfing are constant between years. Simulations conducted by Ritland (1990b) indicate that temporal variation in selfing will cause, on average, an underestimate of inbreeding depression relative to the true value. In an application of the equilibrium method to populations of *Mimulus* spp., Ritland (1990b) used information on spatial variation in mating patterns to investigate the potential bias. By assuming that variation in selfing rates among popula-

tions was representative of variation among years within a population, he obtained an upper limit for the bias in estimates of fitness for individual populations. While this approach seems reasonable for species with a relatively narrow range of population-level outcrossing rates (and see Dole and Ritland, 1993; Eckert and Barrett, 1994b), it is probably inappropriate for *E. paniculata* because of the extreme range of mating patterns displayed by populations of different morph structure.

Fluctuations in outcrossing are also of relevance to quantitative models of the evolution of inbreeding depression (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987, 1990; Charlesworth et al., 1990). If genetic load builds up slowly in a population through deleterious mutations, but is purged rapidly by brief episodes of selfing, then information on the temporal variance in outcrossing will lead to more accurate predictions of inbreeding depression at equilibrium. This raises the question of what measure provides the best integrated estimate of outcrossing for a population. Brown and Albrecht (1980) suggested measurements of outcrossing rate for a few generations, however, at least for models of inbreeding depression, the harmonic mean of  $t$  may be more biologically relevant, since extreme episodes of selfing are likely to be more important than the average value of  $t$  over generations. Of course as discussed above, accounting for variation in  $t$  can also be achieved by using the observed inbreeding coefficient  $F$ , rather than  $t$ , since this parameter will reflect the recent mating history of the population.

Temporal variation in outcrossing rate has some relevance to the debate over whether mixed mating is an evolutionary stable strategy (Lande and Schemske, 1985; Waller, 1986; Schemske and Lande, 1987; Barrett and Eckert, 1990; Holsinger, 1988 1991, 1992). If the causes of fluctuations in outcrossing in self-compatible species are largely environmental in origin, then the evolution of predominant selfing or outcrossing as stable end points of mating-system evolution may be quite protracted. In such cases outcrossing estimates over several generations are unlikely to provide much insight into mating-system evolution, since the time scale involved is likely to be too brief for the detection of a selection response. Even in species such as *E. paniculata*, where the evolution of predominant selfing is well documented and there is good evidence for a strong genetic control to mating patterns, it is unclear whether mixed mating may be stable under certain ecological conditions in N.E. Brazil.

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