

Variation in Outcrossing Rates in *Eichhornia paniculata*: The Role of Demographic and Reproductive Factors*

SPENCER C. H. BARRETT and BRIAN C. HUSBAND

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

Abstract A survey of 32 populations of the self-compatible, tristylous, aquatic, *Eichhornia paniculata* (Pontederiaceae), from N. E. Brazil and Jamaica, was undertaken to examine the role of demographic and reproductive factors on variation in outcrossing rate (t), and to investigate the association between t and population genetic structure. Multi-locus outcrossing rates, estimated using isozyme techniques, varied widely ($\hat{t}=0.002-0.960$) among populations and were uniformly distributed across the entire range of t . Sixty percent of variation in \hat{t} was explained by style morph diversity (E) and the frequency of selfing variants within populations (M). Population size (N) and plant density (d) also accounted for a significant portion of variation in \hat{t} , particularly in Jamaica, where variation in style morph diversity was low.

Outcrossing rates were significantly correlated with the proportion of loci that were polymorphic (P), the average number of alleles per polymorphic loci (N_a), the average observed heterozygosity, (H_o), and the inbreeding coefficient (\hat{f}). A strong regional effect was evident in the association of \hat{t} with P and N_a . This largely results from the confounding effect of genetic bottleneck(s) and high levels of self-fertilization associated with the colonization of Jamaica. Comparisons of \hat{f} with the equilibrium inbreeding coefficient, (F_{eq}), indicated that outcrossing populations had a significant deficiency of heterozygotes, while selfing populations had an excess. This demonstrates that levels of heterozygosity cannot be accounted for by the mating system alone. Outcrossing rate variation in *E. paniculata* is controlled by a complex interplay of local demographic and genetic factors. These influences operate within contrasting regional contexts as a result of the different evolutionary histories of populations in N. E. Brazil and Jamaica.

Key words: *Eichhornia paniculata*, outcrossing rate, tristily, mating system, Pontederiaceae.

Mating systems have long been recognized as primary determinants of the amounts and organization of genetic diversity within and among plant populations (Darlington and Mather, 1940; Stebbins, 1957; Grant, 1958; Baker, 1959; Allard et al., 1968; Jain, 1976). Theoretical arguments concerning the loss of heterozygosity upon inbreeding, as well as data on the distribution of quantitative variation in open-pollinated progeny arrays, gave rise to the early generalization that predominantly selfing populations possess lower genetic diversity and a greater amount of population differentiation than populations with a high degree of outcrossing. The great diversity of reproductive systems in plants (reviewed by Fryxell, 1957; Richards, 1986) provides a rich

source of material for comparative studies on the genetic consequences of variable mating patterns. The occurrence of this variation also enables investigation of the ecological and genetic factors that underlie the evolution of plant mating systems.

During the past two decades the development of electrophoretic techniques has allowed the simultaneous measurement of many population genetic parameters including outcrossing rates, inbreeding coefficients, allelic diversity and heterozygosity. This has enabled workers to examine the quantitative relationships between the mating system and population genetic structure (Brown, 1979; Clegg, 1980; Brown and Weir, 1983; Ritland, 1983). Extensive surveys involving large numbers of species (Hamrick et al., 1979; Loveless and Hamrick, 1984) and comparisons of closely related taxa with contrasting mating systems (e.g., Ellstrand and Levin, 1980; Layton and

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Ganders, 1984) have provided empirical support for earlier predictions that the levels and organization of genetic variation differ between inbreeding and outbreeding species.

A variety of ecological and historical factors also play important roles in influencing the patterns of genetic variation in plant populations, and considerable heterogeneity in genetic structure has been revealed both within and among plant species with similar mating systems (Loveless and Hamrick, 1984). Variation in the demography and life history characteristics of populations complicates attempts to isolate the mating system as a primary force regulating genetic diversity. This problem is particularly evident in interspecific comparisons because of the confounding influences of evolutionary history and adaptive divergence. An approach that may reduce these effects involves investigation of intraspecific variation in mating systems and population genetic structure. Unfortunately, the occurrence of species that display wide variation in outcrossing rates in nature appears to be relatively uncommon, presumably because large mating system shifts are often accompanied by ecological differentiation, reproductive isolation and speciation (Baker, 1961; Barrett, 1989). Despite this difficulty, several species have been identified in which populations exhibit a broad range of outcrossing rates (reviewed in Schemske and Lande, 1985; Barrett and Eckert, 1990) and studies of these groups have provided important insights into the genetic consequences of mating system variation (e.g., Rick et al., 1977; Schoen, 1982; Holtsford and Ellstrand, 1989).

Eichhornia paniculata (Pontederiaceae) is a tristylous, self-compatible annual or short-lived perennial, aquatic of seasonal pools and ditches of N. E. Brazil (Fig. 1a) and the Caribbean islands of Cuba and Jamaica (Barrett, 1985a). Studies of the floral biology and mating system of populations have revealed a great deal of variation associated with the evolutionary breakdown of tristily to semi-homostyly (Barrett, 1985b; Glover and Barrett, 1986; Morgan and Barrett, 1989). Modifications in floral traits that increase selfing rates commonly occur in populations that are dimorphic or monomorphic for style length but are less frequent in

tristylous populations (Fig. 1b). While the majority of populations in N. E. Brazil are tristylous and predominantly outcrossing, in Jamaica, populations exhibit a high degree of self-fertilization (Barrett et al., 1989; Husband and Barrett, 1991).

An electrophoretic survey of enzyme polymorphism in 11 populations of *E. paniculata* from N. E. Brazil (six pops.) and Jamaica (five pops.) indicated that the breakdown of tristily is associated with reductions in the proportion of loci that are polymorphic and in the allelic diversity and heterozygosity of populations (Glover and Barrett, 1987). Populations in Jamaica were genetically depauperate at enzyme loci in comparison with those from N. E. Brazil. The low levels of enzyme polymorphism in Jamaica are likely the result of both genetic bottleneck(s) associated with island colonization and the high levels of self-fertilization that occur within populations. In the above study, the relationships between outcrossing rate and population genetic structure were complicated by the different evolutionary histories of populations in N. E. Brazil and Jamaica, and the small number of populations sampled. In this paper, we examine a larger sample of populations from the two regions in an effort to further investigate the causes and consequences of mating system variation in *E. paniculata*.

The major objectives of our study were to: 1) document the range of variation in outcrossing rates exhibited by natural populations of *E. paniculata* 2) determine the relative importance of demographic and reproductive factors on outcrossing rates, and 3) examine the consequences of variable mating patterns in *E. paniculata* on population genetic structure. After presenting our results, we discuss the evolutionary significance of variation in outcrossing rates in *E. paniculata* for models of mating system evolution and examine whether the demographic characteristics of populations play a significant role in promoting evolutionary changes in mating patterns.

Methods

1. Sampling

A total of 32 populations of *E. paniculata* were

Fig. 1a. *Eichhornia paniculata* growing at the edge of a seasonal pool in Pernambuco, N. E. Brazil.

The yellow-flowered floating aquatic is *Hydrocleis* sp. (Limnocharitaceae). **1b.** Range of morphological variation of flowers from three *Eichhornia paniculata* populations with contrasting mating systems. Top left-trimorphic population, N. E. Brazil; top right-monomorphic population, N. E. Brazil; bottom center-monomorphic population, Jamaica. Note the differences among populations in the size, color and intensity of nectar guides. All flowers are mid-styled.



sampled from N. E. Brazil (21) and Jamaica (11) during May–June 1982 and 1987 (N. E. Brazil) and December–January 1983 and 1987 (Jamaica). At each population, during peak flowering, data on the following attributes were collected: frequency of long-, mid-, and short-styled plants (hereafter L, M, S), frequency of M plants that exhibited genetic modifications to short-level anthers (see Seburn et al., 1990) promoting self-pollination (hereafter termed modified M plants), population size (N) and plant density (d). Estimates of population size involve only reproductive individuals and plant density was assessed qualitatively using a five point scale (1 low density–5 high density). For details of the sampling protocols for these attributes see Barrett et al. (1989). Data on style morph frequency in each population was later summarized using a normalized index of diversity (E) following Simpson (1949). The localities of populations and details of all demographic and genetic variables measured are listed in the Appendix.

At each population, seed families were collected from randomly selected plants, or from all mature individuals in small populations. Each family comprised seed from several capsules (5–10), reducing any bias in outcrossing estimates due to correlated matings (see Morgan and Barrett, 1990). Seeds were germinated as families on moist soil and after 2–3 weeks between 7 and 25 progeny per family were transplanted individually into separate pots. The number and size of each family varied among populations depending on population size and germination levels. Where possible, the number of seedlings transplanted from each population was divided equally among maternal parents. Germination rates were high, with no evidence of differences in germination or seedling survival between progeny derived from controlled self- and cross-fertilization grown under glasshouse conditions (P. Toppings and S. C. H. Barrett, unpublished data).

2. Electrophoresis

Population genetic structure and outcrossing rates were estimated using polymorphisms at loci encoding for isozymes, assayed with horizontal starch gel electrophoresis. Flower buds (3–5) from progeny were crushed in a DL-Dithiothreitol extraction buffer (Glover and Barrett, 1987), adsorbed onto filter paper wicks and inserted into 11% starch gels. Populations were screened for between 19 and 27 loci. Running conditions, gel buffer systems and isozymes scored are described in Glover and Barrett

(1987) and Husband and Barrett (1991), where further details of electrophoretic procedures can be found.

3. Statistical Analyses

Standard population genetic parameters were estimated for each population. These included P , the proportion of loci that are polymorphic (frequency of the most common allele < 0.95), N_a , the average number of alleles per polymorphic locus, and H_o , the observed frequency of heterozygotes, averaged over all loci. Wright's fixation index, f , a measure of the degree to which observed heterozygosity deviates from that expected under random mating, was calculated for each polymorphic locus. The population estimates of f comprised an average over loci, weighted by allele frequency and sample size (Weir and Cockerham, 1984). Inbreeding, or the number of heterozygotes observed relative to random mating, was also estimated from the multi-locus outcrossing estimates (\hat{t}_{ml}) using the relationship:

$$F_{eq} = (1 - \hat{t}_{ml}) / (1 + \hat{t}_{ml}), \quad (1)$$

where F_{eq} is the level of inbreeding expected in a population with a given outcrossing rate, at equilibrium (Hedrick, 1985).

Single- and multiple-locus outcrossing rates (\hat{t}_{sl} and \hat{t}_{ml} , respectively) were estimated for each population by the method of Ritland and Jain (1981). 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 estimation of t , using the method of Brown and Allard (1970). The goodness of fit of observed genotype frequencies to that expected by the mixed mating model was tested by Chi-Square significance tests. Single- and multi-locus measures of outcrossing rate were compared for each population using a Student's t -test for paired comparisons.

Inbreeding can result from both self-fertilization and outcrossing when pollen and ovule parents are more related than randomly chosen individuals. The latter form of inbreeding has been termed biparental inbreeding (Uyenoyama, 1986). Estimates of single- and multi-locus outcrossing rates were combined to investigate the extent of biparental inbreeding within populations of *E. paniculata*. The frequency of inbred progeny derived from out-

crossing, expressed as a proportion of all outcrossed progeny, or the genotypic correlation (Waller and Knight, 1989), is described as:

$$(t_{ml} - t_{sl}) / (t_{ml}) \quad (2)$$

where t_{ml} is a measure of all outcrossed progeny and t_{sl} comprises those progeny recognized as being from outcrosses, excluding biparental inbreeding and selfing.

To explain variation in outcrossing rate estimates in *E. paniculata*, we examined three ecological variables, region, population size (N), density (d), and two reproductive variables, style morph diversity (E), and the frequency of modified M plants (M). Since region is a nominal variable, we compared the average \hat{t} among regions using a Kruskal-Wallis test. Populations were identified as occurring in one of three different geographic regions: 1-Jamaica, 2-Ceará, Brazil or 3-Pernambuco-Paraíba-Alagoas, Brazil (hereafter termed Pernambuco). The associations among all other variables were examined using Spearman's Rank correlation coefficients (Conover, 1980). To assess the association between \hat{t} and each variable, in the absence of correlated effects from other variables, partial correlation coefficients, where all other variables are held constant, were calculated using Spearman's Rank correlation coefficients ($df = n - 2 - m$, where m is the number held constant). A multiple regression of the same independent variables on \hat{t}_{ml} was conducted using the ranks of each variable. This procedure was applied to all populations as well as for Brazilian and Jamaican populations separately. The relationship of \hat{t} and population genetic structure was also examined through the association between \hat{t} and N_o , H_o , P and \hat{f} . Each relationship was assessed using the Spearman's Rank correlation coefficient.

Results

1. Distribution of Outcrossing Rates

Populations of *Eichhornia paniculata* exhibit continuous variation in multi-locus outcrossing rate (range of $\hat{t} = 0.002$ to 0.960) with the distribution of \hat{t} uniform throughout the 0–1 range (Figure 2). There was no significant difference in the number of populations that occurred within each of the five 0.20 classes of \hat{t} based on a Chi-Square goodness of fit test ($\chi^2 = 2.816$, $P = 0.5891$). Outcrossing rates in Brazil range from 0.255 to 0.960, whereas those in Jamaica ranged from 0.002 to 0.680. There were

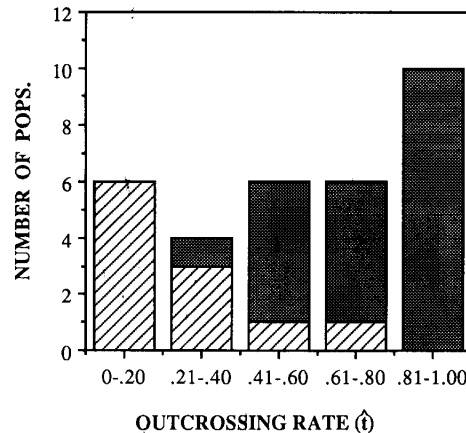


Fig. 2. The frequency distribution of outcrossing rates (\hat{t}) for 32 populations of *Eichhornia paniculata*. Dark, stippled bars represent populations from N. E. Brazil; hatched bars represent populations from Jamaica.

significant differences among the three regions (Jamaica; Ceará, Brazil; Pernambuco, Brazil) in mean outcrossing rates ($H = 17.03$, $P = 0.001$). The mean for Jamaican populations ($\bar{x} = 0.213$, $sd = 0.20$, $N = 11$) was significantly lower than the mean for either of the two Brazilian regions, which did not differ (Ceará $\bar{x} = 0.734$, $sd = 0.7$, $N = 7$; Pernambuco $\bar{x} = 0.722$, $sd = 0.23$, $N = 14$).

2. Demographic and Reproductive Correlates

Table 1 illustrates the results of the correlation analysis for the four variables that were measured in each population. There is a high degree of association between \hat{t} and both the diversity of style morphs (E) and frequency of modified M plants within *E. paniculata* populations. Since both variables are also highly correlated ($\rho = -0.923$), only E is used

Table 1. Spearman's rank correlation coefficients among outcrossing rate (\hat{t}) and four population-level variables: population size (N), plant density (d), morph diversity (E), and the frequency of self-pollinating variants of the mid-styled morph (M) for 32 populations of *Eichhornia paniculata*.

	\hat{t}	N	d	E	M
N	0.288	1.000			
d	-0.017	0.433*	1.000		
E	0.734*	0.203	0.242	1.000	
M	-0.674*	-0.241	-0.047	-0.923*	1.000

in subsequent analyses. There was no association between outcrossing rate and population size or plant density for the total sample of populations.

Partial correlations and multiple regression indicated that population size, plant density and style morph diversity each show a significant association with outcrossing rate. With all other variables held constant, E accounted for a significant portion (59.5%) of the variation in \hat{t} (partial correlation, $\rho = 0.772$). Outcrossing rate increases as the number of style morphs and their evenness within populations increases (Fig. 3a). The reverse relationship was found between \hat{t} and the frequency of modified M plants (Fig. 3b).

Approximately 32 percent of the variation in outcrossing rate can be explained by population size and density combined (partial correlations $\rho = 0.376$ and -0.426 respectively), when data from both Brazil and Jamaica are pooled (Table 2). Within each region, population size exhibited a non-linear

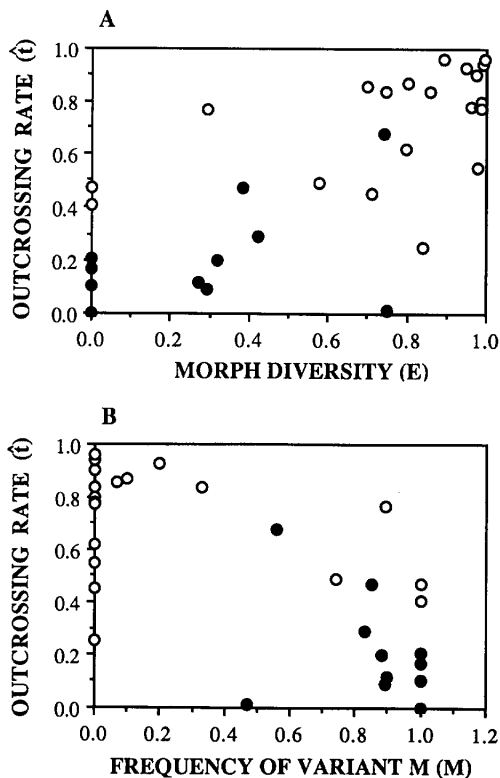


Fig. 3. The association between outcrossing rate (\hat{t}) and a) style morph diversity (E) and b) the frequency of self-pollinating, mid-styled variants (M) in 32 populations of *Eichhornia paniculata*. Open circles represent populations from N. E. Brazil, solid circles from Jamaica.

Table 2. Results from multiple regressions of morph diversity, population size and plant density on outcrossing rate for 32 populations of *Eichhornia paniculata*. Partial F values and partial correlation coefficients are presented for the analysis of populations in Jamaica and N. E. Brazil pooled as well as separately. Each multiple regression explained a significant portion of the variation in t ($P < 0.05$).

Variable	F	P	R_{ijk}
Total sample (N=32)			
Style morph diversity	41.19	0.0001	0.772**
Population size	4.62	0.04	0.376*
Plant density	6.19	0.019	-0.426*
Jamaica (N=11)			
Style morph diversity	3.01	0.126	0.548
Population size	11.28	0.012	0.786*
Plant density	5.79	0.047	-0.673*
Brazil (N=21)			
Style morph diversity	11.18	0.0039	0.639**
Population size	1.55	0.2296	0.289
Plant density	3.98	0.0622	-0.436

relationship with \hat{t} (Fig. 4). In general, values of \hat{t} were more variable in small populations than in large. The weak relationship that was obtained between population size and outcrossing rate is strongly influenced by a single Brazilian population (B63).

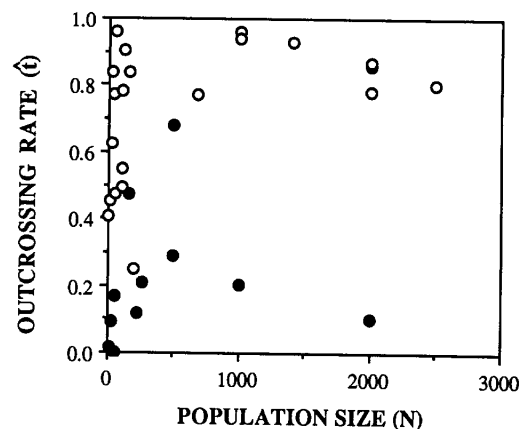


Fig. 4. The association between outcrossing rate (\hat{t}) and population size (N) in 32 populations of *Eichhornia paniculata*. Open circles represent populations from N. E. Brazil, solid circles are from Jamaica. Population B63 is omitted from the figure (see Results).

This population was monomorphic for modified variants of the M morph and, in the year sampled (1987), consisted of approximately 10,000 plants. This was the only example of a large population with a low outcrossing rate and, in subsequent years, the population contained less than 100 plants. When this population is removed from the analysis, the proportion of variation in \hat{t} explained by population size is considerably higher ($\rho=0.403$, $P<0.05$). Density exhibits a strong inverse relationship with outcrossing rate but is highly variable at any given level (Table 2).

While there are no significant differences between Jamaica and Brazil in population size or plant density (N , $F=1.1$, $P<0.319$; d , $F=0.08$, $P>0.78$), their relative effects on outcrossing rate depend on region. In Jamaica, population size and plant density were the only variables with significant partial correlations with outcrossing rate. In contrast, in Brazil neither variable had a significant influence on \hat{t} , or was as important as style morph diversity or the frequency of modified M plants. While the association between population size and plant density, as measured by Spearman's rank correlation (Table 1), is moderately positive ($\rho=0.433$), their effects on \hat{t} are in opposite directions (Table 2).

A mechanism that could explain the lower levels of outcrossing observed in both small and dense

populations is increased inbreeding through biparental matings to related individuals. The mean genotypic correlation among parents of outcrossed progeny is 0.136 (range, $=-0.140-0.517$; $sd=0.112$), for 28 populations in which this parameter was estimated. Holding the number of polymorphic loci constant, population size and density were not significantly correlated with the genotypic correlation among outcrossed progeny (Table 3, N , $\rho=-0.277$; d , $\rho=0.160$). Thus, these data do not support the hypothesis that population size and plant density influence \hat{t} through their effects on biparental inbreeding. Because there are strong regional differences in genotypic correlations ($F=4.9$, $P<0.016$), we also examined the same regression for Brazilian and Jamaican populations, separately. Within each region, the number of loci used to estimate t_{ml} and t_{sl} explains a significant (marginal so in Jamaica) portion of the variation, while population size and density explain almost none.

3. Outcrossing Rates and Population Genetic Structure

All four population genetic parameters that were investigated (P , N_a , H_o , \hat{f}) were significantly correlated with outcrossing rate when populations from both regions were combined (Table 4). As expected, the proportion of polymorphic loci and average heterozygosity of populations decline with increased self-fertilization (Fig. 5a, b). When these relationships are examined separately for each region, only the associations involving heterozygosity and inbreeding coefficient, \hat{f} , remain significantly correlated with outcrossing rate. All genetic variables were significantly correlated with one another, with the exception of inbreeding coefficient

Table 3. Results from multiple regressions of population size, density and the number of polymorphic loci used in each \hat{t} estimate, on $(\hat{t}_{ml}-\hat{t}_{sl})/\hat{t}_{ml}$, a measure of biparental inbreeding in 28 populations of *Eichhornia paniculata*. Separate regression analyses were also conducted on populations in Brazil and Jamaica.

Variable	F	P	$R_{i,jk}$
Total sample (N=28)			
Number of loci	0.20	0.969	0.007
Population size	1.98	0.170	-0.277
Plant density	0.64	0.434	0.160
Jamaica (N=9)			
Number of loci	3.75	0.111	0.655
Population size	0.15	0.718	-0.168
Plant density	0.06	0.812	-0.112
Brazil (N=19)			
Number of loci	5.67	0.031	0.524*
Population size	1.67	0.216	-0.316
Plant density	0.49	0.494	-0.178

Table 4. Spearman's rank correlation (ρ) between the multi-locus outcrossing rate (\hat{t}_{ml}), and four population genetic parameters, proportion of loci polymorphic (P), average number of alleles per polymorphic loci (N_a), average observed heterozygosity (H_o) and inbreeding coefficient (\hat{f}).

	Overall	Jamaica	Brazil
P	0.702**	0.562	0.235
N_a	0.648**	-0.196	0.477*
H_o	0.818**	0.811**	0.507*
\hat{f}	-0.741**	-0.876**	-0.486*

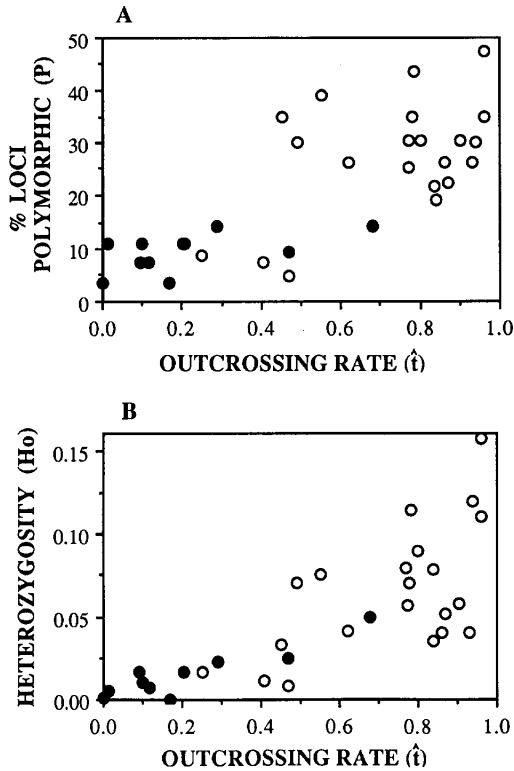


Fig. 5. The association between outcrossing rate (\hat{t}) and a) the proportion of loci that are polymorphic (P) and b) the average observed heterozygosity (H_o) in 32 populations of *Eichhornia paniculata*. Open circles represent populations from N.E. Brazil, solid circles from Jamaica.

and the number of alleles per polymorphic locus (Table 4). Variables representing a change in allele frequency (P , N_a) were not independent from changes in genotypic frequency (H_o , \hat{f}).

To determine if levels of inbreeding within each population are solely determined by the mating system, the inbreeding coefficient, \hat{f} , estimated from heterozygosity, was compared to F_{eq} , derived from the outcrossing rate. Regardless of whether Jamaican and Brazilian populations were combined or separated, \hat{f} was significantly correlated with F_{eq} (Table 4, combined, $\rho = 0.741$, $P < 0.01$). In a paired t-test, the mean inbreeding coefficient, \hat{f} , was not significantly different than levels of inbreeding at equilibrium (student's $t = 1.85$, $P < 0.074$). Within Jamaica, the inbreeding coefficient in 7 of 11 populations indicated an excess of heterozygotes compared to F_{eq} (\bar{x} difference = -0.055 ; student's $t = -1.095$, $P < 0.299$), while in Brazil, 16 of 20 populations

showed a significant deficiency (\bar{x} difference = 0.132 ; student's $t = 3.233$, $P < 0.004$).

To account for the variation in \hat{f} , not explained by \hat{t}_{mi} , we conducted a multiple regression, including N , d and \hat{t}_{mi} as independent variables. Unfortunately, this is not completely satisfactory since outcrossing rate and population size are not independent. When Jamaican and Brazilian populations are combined, population size had a significant, non-linear relationship with \hat{f} ($\rho = -0.455$, $P < 0.01$); however, only \hat{t}_{mi} explained a significant portion of the variation in inbreeding (t_{mi} , $P < 0.0001$; N , $P < 0.15$; d , $P < 0.75$) in the multiple regression. This result is directly related to the degree of independence between outcrossing rate and population size. In Jamaica, where much of the variation in outcrossing rate is accounted for by population size and plant density, only \hat{t}_{mi} explains a significant portion ($P < 0.027$) of the variation in \hat{f} . In contrast, in Brazil population size is more important ($P < 0.04$) and outcrossing rate less so ($P < 0.07$). Because of the interdependence between population size and outcrossing rate, the importance of population size in explaining variation in the levels of inbreeding is likely to be underestimated.

Discussion

Populations of *Eichhornia paniculata* exhibit among the largest range of outcrossing rates measured to date in flowering plants (see Barrett and Eckert, 1990). Tristyloous populations in N.E. Brazil are often predominantly outcrossing, whereas on the island of Jamaica those that are monomorphic for semi-homostyloous variants of the mid-styled morph can be highly autogamous. These extremes are linked by populations with various morph structures and levels of self- and cross-fertilization. Despite conspicuous differences in the floral morphology and allocation patterns in selfing and outcrossing populations (Barrett, 1985a; Morgan and Barrett, 1989), genotypes from throughout the range of outcrossing rates are fully interfertile, demonstrating that wide variation in mating system can be maintained within a single biological species.

1. Significance for Mating System Evolution

The distribution of outcrossing rates in *E. paniculata* is near uniform across the range of \hat{t} values that are possible. This pattern is relevant to Lande and Schemske's (1985) models of the evolution of self-fertilization and inbreeding depression.

These authors propose that predominant selfing and outcrossing should be alternative stable states of the mating system in most plant populations. Their models predict a bimodal distribution of outcrossing rates, owing to selection for the maintenance of outcrossing in historically large populations with substantial inbreeding depression, and selection for selfing when increased inbreeding, through bottlenecks or pollinator failure, reduces the level of inbreeding depression below 0.5 (Lande and Schemske, 1985; Schemske and Lande, 1985). At first glance the wide distribution of \hat{t} values in *E. paniculata* would not appear to support their prediction that populations should be primarily selfing or outcrossing. Indeed, Schemske and Lande (1985) drew attention to several other taxa (e.g., *Lupinus*, *Clarkia*, *Gilia* and *Collinsia*) with wide variation in outcrossing rates as cases that did not support their models. However, a number of considerations need to be taken into account when evaluating the significance of the *E. paniculata* data for testing the Lande and Schemske models.

First, the 32 populations of *E. paniculata* used for estimating outcrossing rates do not represent a random sample. Jamaican populations are over-represented in the sample in comparison with their species-wide frequency, because the sampling of populations in Jamaica was more intensive than in N. E. Brazil. Also, sampling in N. E. Brazil involved an attempt to include a representative sample of large and small populations from the two major areas in which the species occurs. One way of evaluating whether this sampling scheme is likely to bias our interpretation is to examine the distribution of style morph diversity (E) values that have been sampled for all populations of *E. paniculata*. Since this parameter is positively correlated with outcrossing rate (Table 1) it may provide a rough indicator of the true extent of mating system variation in the species.

We have sampled a total of 110 populations of *E. paniculata* from N. E. Brazil and Jamaica with most populations encountered being sampled for style morph frequency (see Barrett et al., 1989). The distribution of E values among the five 0.20 classes is 0–0.20, 25; 0.21–0.40, 8; 0.41–0.60, 4; 0.61–0.80, 21; 0.81–1.00, 52. This distribution is significantly different from the uniform distribution of outcrossing rates in Figure 2 ($\chi^2=25.8$, $P<0.0001$). A similar pattern is observed if the distribution of E values is compared between the sample of populations studied for outcrossing rates and the total sam-

ple of *E. paniculata* populations surveyed in N. E. Brazil. In both of these comparisons, populations with high E values, and to a lesser extent those with the lowest E values, are more frequent than those in the remaining classes. If all Jamaican populations, which are over-represented here, are excluded the distribution becomes unimodal and skewed to the left. This indicates that trimorphic populations are under-represented in our sample of outcrossing rates.

These comparisons raise the issue of what distribution of \hat{t} values should be expected in outcrossing species in which selfing populations have evolved. In those that are wide ranging, such as *E. paniculata*, and in which the evolution of selfing is restricted to small, isolated regions, a random sample of populations is unlikely to yield a bimodal distribution of outcrossing rates, even though the selective forces described in the Lande and Schemske models could be operating. Geographical patterns of mating system variation need to be considered if outcrossing rate distributions are to be used in testing the predictions of the Lande and Schemske models at the intraspecific level.

A more difficult but related problem concerns the strong regional differentiation in mating systems that is evident in our data. While the distribution of \hat{t} values in Figure 2 is uniform in a statistical sense, this pattern arises from combining two quite different distributions each of which alone is unimodal and skewed to the right (Jamaica) and left (N. E. Brazil). Highly selfing populations of *E. paniculata* have not been found in N. E. Brazil, whereas predominantly outcrossing populations do not occur in Jamaica. The biological interpretation of these patterns are important for understanding the evolution of mating systems in *E. paniculata*.

The absence of highly outcrossing populations of *E. paniculata* in Jamaica may largely result from founder effects and the associated stochastic loss of genetic variation during island colonization (Glover and Barrett, 1987). Not only is trimorphic population structure prevented in Jamaica, because of the absence of the S morph, but selfing variants may have been favored in island colonization and establishment. A paucity of specialized long-tongued pollinators that visit *E. paniculata* in Jamaica, and the ephemeral nature of habitats that the species occupies, likely favors the maintenance of autogamy (see Barrett et al., 1989). Under these ecological conditions there may be restricted opportunities for selection to increase outcrossing rates.

Moreover, since Jamaican populations are genetically depauperate (Glover and Barrett, 1987; and see Appendix), heritable variation in traits influencing the mating system may be low. In order for there to be selection for increased outcrossing, offspring from outcrosses would need to show significant fitness advantages to counteract the transmission bias possessed by selfing M variants (Barrett et al., 1989). Studies of the relative fitness of selfed and outcrossed progeny in seven predominantly selfing Jamaican populations of *E. paniculata* have failed to detect evidence of inbreeding depression or heterosis (Toppings and Barrett, unpublished data). Thus, the evolution of a predominantly outcrossed mating system in Jamaican populations of *E. paniculata* may be prevented by a combination of ecological factors (pollinator scarcity, ephemeral habitats) and because of limited genetic variation owing to genetic bottlenecks associated with island colonization.

The apparent absence of predominantly selfing populations of *E. paniculata* in N. E. Brazil is more difficult to explain. Unlike Jamaica, where monomorphic populations usually exhibit high levels of self-fertilization, these types of population structures in N. E. Brazil are more commonly associated with intermediate outcrossing rates. For example, \hat{t} values of 0.47 and 0.41 were recorded for populations B8 and B63, both of which were fixed for variant M plants. One explanation for these intermediate outcrossing rates is that nonequilibrium conditions prevail in nontrimorphic populations of *E. paniculata* in N. E. Brazil. Selection intensities may not have been strong enough, or exerted for long enough time periods to drive local populations to predominant selfing. Alternatively, intermediate outcrossing rates in N. E. Brazil may be maintained because of local selection pressures associated with the more abundant and specialized pollinators that visit populations (S. C. H. Barrett, unpubl. data) and which, in general, tend to characterize continental as opposed to island faunas.

Interestingly, in N. E. Brazil phenotypes of mid-styled variants differ from those in Jamaica in two important ways that are likely to influence mating patterns. Flowers in monomorphic Brazilian population (see Figure 1a) are considerably larger than in Jamaican populations and are, in fact, comparable in size to many trimorphic populations (Morgan and Barrett, 1989 and unpubl. data). Large flowers may serve to attract pollinators thereby maintaining moderate levels of outcross-

ing. In addition, there is considerable genetically based developmental instability in the position of modified short-level stamens of M plants in Brazilian populations (Seburn et al., 1990). The variation results in the occurrence of many flowers that cannot automatically self-pollinate. With effective pollinator service these flowers have the potential to be outcrossed.

Whether Brazilian monomorphic populations display stable mixed mating systems is unclear, but our failure to locate small-flowered highly autogamous populations in N. E. Brazil suggests that constraints on the evolution of predominant selfing may exist in this region. These constraints may not involve strong inbreeding depression since comparisons of the relative fitness of selfed and outcrossed progeny in Brazilian populations with mixed mating systems have found only minor differences in progeny fitness for a small number of traits (Toppings and Barrett, unpublished data). Furthermore, constraints, if they exist, are unlikely to be caused by developmental limits to morphological evolution since the occurrence of autogamous populations of *E. paniculata* in Jamaica demonstrates that, under the appropriate conditions, the evolutionary transition from outcrossing to predominant selfing can occur.

Much of the discussion surrounding the Lande and Schemske models (e.g., Waller, 1986; Schemske and Lande, 1987) concerns the relative importance of genetic and ecological factors determining variation in outcrossing rates as well as the extent to which populations with a known outcrossing rate are at evolutionary equilibrium. Schemske and Lande (1985) suggested that the causes of variation in outcrossing rate in taxa with wide ranges were the result of either local ecological factors (*Lupinus*, *Collinsia*) or genetically based differences in floral traits (*Gilia*, *Clarkia*). They argued that where outcrossing rates varied greatly, owing to local environmental conditions, selection intensities on the mating system would be reduced. These considerations highlight the importance of determining for species with variable outcrossing rates the relative roles of genetic and ecological factors in maintaining variation.

2. Demographic and Reproductive Correlates

In our study, we investigated the association between variation in outcrossing rates and four population-level attributes. Two of these attributes (*E*, *M*) involved genetically-based reproductive traits that

have a direct bearing on the mating system. The other variables (N and d) were demographic in nature and likely to reflect the local ecological conditions in which populations occur. Our results clearly indicate that attempts to explain variation in outcrossing rates as the result of either ecological or genetic factors will likely oversimplify a more complex situation. In *E. paniculata*, not only do both sorts of factors interact to influence the mating system, but these interactions vary with the geographic locale and evolutionary history of populations.

The correlation analyses demonstrated that outcrossing rates were strongly associated with both demographic and reproductive factors. Further, partial correlations indicated that population size, plant density and style morph diversity all exert a significant influence on variation in outcrossing levels. The role of genetically-based reproductive characters in determining levels of outcrossing has been demonstrated in several other studies (e.g., Rick et al., 1978; Ennos, 1981; Schoen, 1982; Shore and Barrett, 1990). In *E. paniculata* the relative frequencies of self-pollinating M variants within populations directly influences population-level estimates of \hat{t} as does the degree of stamen modification displayed by individual phenotypes. These modifications are recessive in nature and under relatively simple genetic control (Barrett, unpublished data). The origin of these variants may be associated with periods of inbreeding caused by small population size (Barrett, 1989).

Morph diversity (E) likely contributes to mating system variation in two ways that are also related to demographic factors. First, populations with low diversity measures are often small in size and contain the highest frequency of modified M variants. Moreover, populations with a single morph are usually fixed for the variant M phenotype whereas populations with all three morphs rarely contain modified M plants. This suggests that in small populations with unreliable pollinator service, the variants experience a fertility advantage. Comparisons of fruit set in populations with modified and unmodified floral phenotypes have demonstrated significant fertility advantages to the selfing morph (Barrett et al., 1989). Reductions in style morph diversity may also be attributed, in part, to genetic bottlenecks caused by drift in small populations. In this context, it is worth noting that small population sizes are common in *E. paniculata* with approximately 57% of populations ($N=110$) containing less than 100 individuals (see Fig. 5, Barrett et

al., 1989). Thus, declines in the value of E appear to result from both the stochastic loss of style morphs and the spread of selfing variants. Both processes are favored in small populations and can result in alterations in the mating system leading to increased levels of inbreeding.

The significance of demographic factors to outcrossing rates in populations of *E. paniculata* was most strongly revealed using partial correlations. The relationships observed, however, differed between Brazil and Jamaica. This finding appears to be the result of the strong regional differences in the frequency of selfing variants between the two regions. In Jamaica, all mid-styled plants on the island are selfing variants and these predominate in virtually all populations. With morph diversity thus constrained, population size and plant density become significantly correlated with variation in outcrossing rate. In contrast, in N. E. Brazil reproductive factors dominated the analysis of partial correlations, presumably because of the wide range of population morph structures present in the region.

Demographic factors such as the size and density of populations can affect mating patterns in plant populations by influencing levels of inbreeding. Population genetic theory predicts that inbreeding will be intensified in small populations, owing to the increase in the probability of identity by descent. In addition, higher levels of homozygosity, due to the variance effect of small populations, will increase the likelihood of matings to relatives (Kimura and Crow, 1963). The relationship between plant density and levels of inbreeding are less straightforward and, in animal pollinated plants, will depend on the interactions between pollinator behavior and population structure (Antonovics and Levin, 1980). In *E. paniculata*, we examined the relationship between both population size and plant density and the intensity of biparental inbreeding by comparing single- and multi-locus estimates of outcrossing rate. For both demographic variables, there was no significant association with biparental inbreeding. Our failure to detect any relationships may be due to the insensitivity of the measure of biparental inbreeding employed (see Waller and Knight, 1989), as well as sampling errors associated with estimating outcrossing rates. In addition, since populations of *E. paniculata* can change dramatically in size from year to year (Husband and Barrett, unpublished data), the levels of inbreeding that we measured may not reflect the size of populations from which progenies were sampled. Accurate estimates of biparental in-

breeding can only come from investigations of temporal variation in both demographic and genetic parameters of populations, particularly in species for which population size fluctuations are common.

3. Mating Patterns and Population Genetic Structure

The results of our investigation of the genetic consequences of variable outcrossing rates in *E. paniculata* were strongly influenced by the marked regional differentiation in population genetic structure of the species. Although all four genetic parameters that we investigated were significantly correlated with variation in \hat{t} , the association with P and N_a was largely a result of the low levels of genetic diversity present in Jamaica. Associations of these two parameters with \hat{t} were only evident in the pooled data set and were absent at the regional level. In contrast, as theory would predict, H_o and \hat{f} were strongly correlated with variation in \hat{t} for populations in both regions, despite their contrasting evolutionary histories. Our results clearly demonstrate the prime importance of mating system in organizing genetic variability within plant populations and extend to the intraspecific level patterns observed in large scale surveys of unrelated species (Loveless and Hamrick, 1984).

The inbreeding coefficient compares the observed frequency of heterozygotes to that expected under random mating and, in *E. paniculata*, was negatively correlated with outcrossing rate. Since a variety of factors can influence the inbreeding coefficient, it is of value to compare this measure to the related statistic, F_{eq} , which is the amount of inbreeding expected at equilibrium for a given outcrossing rate. The difference between these two measures (ΔF) should be negligible if the mating system is the sole determinant of inbreeding in a populations. In *E. paniculata*, the mean ΔF was 0.066 (range = -0.343 – 0.374 ; $se = 0.035$) with 20 of the 30 populations that were examined displaying a deficiency of heterozygotes. In addition, ΔF was significantly correlated with outcrossing rate ($\rho = 0.546$, $p < 0.01$); populations with high \hat{t} estimates exhibit a marked deficiency whereas populations with low estimates showed an excess (Fig. 6). This pattern was first revealed from interspecific comparisons and has been referred to as the 'heterozygosity paradox' (Brown, 1979). Interestingly, the mean ΔF values for Brazilian and Jamaican populations of *E. paniculata* (0.13 and -0.06 , respectively) are similar to values obtained from Brown's survey of out-

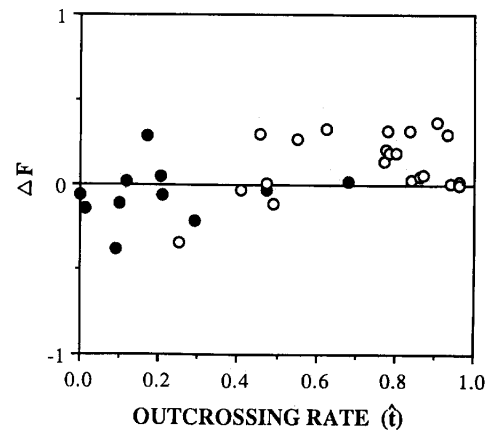


Fig. 6. The association between outcrossing rate (\hat{t}) and the deviation in \hat{f} from that expected at equilibrium (ΔF) in 30 populations of *Eichhornia paniculata*. Open circles represent populations from N. E. Brazil, solid circles are from Jamaica.

breeding and inbreeding species (0.16 and -0.08 , respectively). Several demographic and genetic factors can influence variation in ΔF (reviewed by Brown, 1979), but unfortunately few empirical studies have examined their relative importance in natural plant populations.

We have investigated the causes and consequences of variation in the mating system of a tropical, disjunct quatic plant. Both reproductive and demographic features of populations contribute significantly to variation in outcrossing rate. Not only do these features of populations interact, but the nature of the interactions vary, depending on the geographical context in which populations occur. In wide ranging species, such as *E. paniculata*, the evolutionary forces operating on mating systems are likely to be diverse and will depend not only on local ecological factors, but also on the evolutionary history and regional characteristics of populations.

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Appendix

Summary of the locations, outcrossing rates, demographic, reproductive, and population genetic parameters for the 11 Jamaican and 21 Brazilian populations investigated in this study. *Electrophoretic data from Glover and Barrett (1987).

Pop.	Location	\hat{f}	E	M	N	d	P	H_o	N_e	\hat{f}
JAMAICA										
J12*	Angels, St. Catherine	0.470	0.382	0.85	150	2	9.5	0.025	2.0	0.33
J14*	Rhynsbury, Clarendon	0.290	0.424	0.83	500	3	14.3	0.023	2.0	0.34
J15*	Pridee, Clarendon	0.680	0.740	0.56	500	3	14.3	0.050	2.0	0.21
J16	George's Plain, Westmoreland	0.169	0.000	1.00	48	1	3.7	0.000	2.0	1.00
J17	Meylersfield, Westmoreland	0.101	0.000	1.00	2000	5	11.1	0.010	2.3	0.71
J19	Milk River, Clarendon	0.014	0.748	0.47	17	2	11.1	0.005	2.0	0.83
J22	Osborne, Clarendon	0.118	0.270	0.90	222	3	7.4	0.007	2.0	0.81
J24	Angels, St. Catherine	0.093	0.293	0.89	28	2	7.4	0.016	2.0	0.45
J25	Spanish Town 1, St. Catherine	0.206	0.000	1.00	265	3	11.1	0.017	3.0	0.60
J26	Spanish Town 2, St. Catherine	0.002	0.000	1.00	50	4	3.7	0.001	2.0	0.94
J27	Old Pera, St. Thomas	0.203	0.319	0.88	1000	4	11.1	0.016	2.0	0.71
BRAZIL										
B5*	Recife, Pernambuco	0.960	0.997	0.00	1000	4	35.0	0.110	2.6	0.04
B7*	Vitória, Pernambuco	0.840	0.865	0.00	160	3	19.0	0.078	2.3	0.11
B8*	Murici, Alagoas	0.470	0.000	1.00	55	1	4.7	0.008	2.0	0.37
B9*	U. D. Palmares, Alagoas	0.490	0.578	0.74	110	2	30.0	0.070	2.0	0.23
B10*	Arapiraca, Alagoas	0.770	0.293	0.89	40	1	25.0	0.079	2.2	0.27
B11*	Quixadá, Ceará	0.940	0.992	0.00	1000	5	30.0	0.120	2.2	0.05
B31	Fortaleza, Ceará	0.454	0.711	0.00	13	4	34.8	0.033	2.1	0.67
B32	Fortaleza, Ceará	0.780	0.960	0.00	99	3	34.8	0.070	2.3	0.45
B33	Maranguape, Ceará	0.903	0.972	0.00	113	5	30.4	0.058	2.3	0.43
B34	Maranguape, Ceará	0.800	0.988	0.00	2500	4	30.4	0.090	2.3	0.30
B39	Quixadá, Ceará	0.783	0.984	0.00	2000	4	43.5	0.115	2.2	0.32
B43	Quixadá, Ceará	0.550	0.977	0.00	100	3	39.1	0.075	2.3	0.56
B46	Quixadá, Ceará	0.870	0.800	0.10	500	3	22.2	0.052	2.2	0.13
B55	Patos, Paraíba	0.251	0.710	0.00	200	5	8.7	0.017	2.0	0.26
B56	Patos, Paraíba	0.860	0.990	0.07	2000	4	20.8	0.054	2.3	0.13
B58	B. Jardim, Pernambuco	0.930	0.948	0.20	1400	4	26.1	0.042	2.7	0.33
B59	B. Jardim, Pernambuco	0.837	0.745	0.33	22	2	21.7	0.035	2.5	0.41
B63	Garunhuns, Pernambuco	0.407	0.000	1.00	10000	3	7.4	0.011	2.5	0.39
B77	Gravata, Pernambuco	0.778	0.989	0.00	680	3	30.4	0.057	2.3	0.34
B78	Gravata, Pernambuco	0.622	0.797	0.00	30	2	26.1	0.041	2.4	0.57
B81	Vitória, Pernambuco	0.961	0.892	0.00	54	2	47.4	0.157	—	—