

THE DISSOLUTION OF A COMPLEX GENETIC POLYMORPHISM:  
THE EVOLUTION OF SELF-FERTILIZATION IN TRISTYLOUS  
*EICHHORNIA PANICULATA* (PONTEDERIACEAE)

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**Abstract.**—*Eichhornia paniculata* (Pontederiaceae) displays a wide range of outcrossing levels as a result of the dissolution of the tristylous genetic polymorphism and the evolution of semihomostyly. Population surveys, comparison of fitness components of the style morphs, and computer simulations were used to investigate the breakdown of tristyly and the selective mechanisms responsible for the evolution of self-fertilization. Of 110 populations surveyed in northeast Brazil and Jamaica, 53% were trimorphic, 25% were dimorphic, and 22% were monomorphic for style morph. The short (S) morph was underrepresented in trimorphic populations and absent from nontrimorphic populations. The mid (M) morph predominated in dimorphic populations and was the only morph in monomorphic populations. Stamen modifications promoting selfing, associated with semihomostyle evolution, were largely confined to the M morph. They were rare in trimorphic populations, common in dimorphic populations, and often fixed in monomorphic populations.

Stochastic simulations and comparisons of fruit set in natural populations indicate that founder events, population bottlenecks, and lowered fertility of the S morph due to an absence of long-tongued pollinators can each account for loss of the S morph from trimorphic populations. A reduced level of disassortative mating can accentuate the rate at which the S morph is lost by both random and deterministic processes. Nontrimorphic populations occur at the geographical margins of the region surveyed and tend to be smaller and less dense than trimorphic populations. These observations and the higher fruit set of the M morph relative to the L morph in dimorphic populations suggest that reproductive assurance, favoring selfing variants of the M morph under conditions of low pollinator service, has been of primary importance in the origin of most monomorphic populations. Where pollinator service is reliable, however, automatic selection of selfing genes, aided by mating asymmetries between the morphs, can cause the M morph to spread to fixation in dimorphic populations.

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The evolution of self-fertilization is the most prominent and well studied mating-system change in flowering plants. Numerous biosystematic studies have documented the shift from outcrossing to selfing in herbaceous groups (reviewed in Stebbins [1974], Jain [1976], Wyatt [1988], and Barrett [1989a]), and a large body of theoretical work has examined the population-genetic conditions and selective forces that account for the spread of selfing variants in outcrossing populations (Fisher, 1941; Crosby, 1949; Nagylaki, 1976; Maynard Smith, 1978; Lloyd, 1979; Wells, 1979; Holsinger et al., 1984; Lande and Schemske, 1985; Uyenoyama, 1986). Despite this rich literature, few plant groups have been studied in sufficient detail to provide a comprehensive picture of the processes responsible for the evolution of self-fertilization. Because

most of the theory has not been tested empirically, there is still uncertainty as to the relative importance of factors such as reproductive assurance (Baker, 1955), automatic selection of selfing genes (Fisher, 1941), inbreeding depression (Charlesworth and Charlesworth, 1987), and pollen discounting (Holsinger, 1988a) for different groups of plants.

Heterostylous breeding systems (distyly and tristyly) provide convenient experimental material for studies of mating-system evolution. The dissolution of these complex genetic polymorphisms by homostyle formation represents a paradigm for studies of the evolution of self-fertilization in plants. This is because the direction of evolutionary change is usually unambiguous, genetic modifications are often simply inherited, and alterations in floral morphology that influence mating behavior can usually be detected without difficulty under field conditions (Ganders, 1979; Barrett, 1989b). Most heterostylous genera contain

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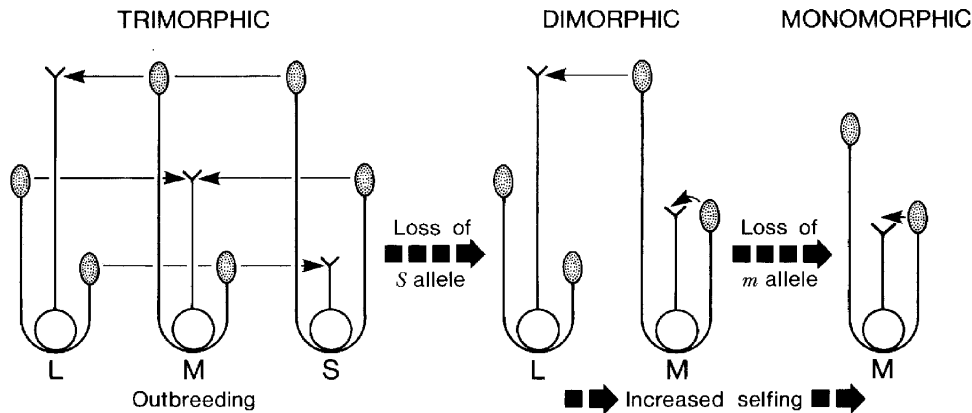


FIG. 1. Evolutionary breakdown of tristyliness to semihomostyly in *Eichhornia paniculata* (after Barrett [1985b]). Arrows indicate predominant matings within populations. Note the modification in the short-level stamen position of the M morph in dimorphic and monomorphic populations. Genotypes for the three floral morphs are *ssmm* (L morph); *ssMm* and *ssMM* (both M morph); and *SsMm*, *SsMM*, and *Ssmm* (all S morph).

homostylous taxa that have originated from the breakdown of the floral polymorphism (Darwin, 1877; Ernst, 1955; Baker, 1966; Ornduff, 1972; Barrett and Shore, 1987). In most cases, homostyle evolution is accompanied by reductions in the size and attractiveness of floral organs, acquisition of a self-pollinating habit, and development of reproductive isolation. However, in some species, homostyles of recent origin can be found intermixed with outcrossing morphs in various frequencies (Bodmer, 1960; Ganders, 1975; Barrett, 1979; Charlesworth and Charlesworth, 1979), providing opportunities for investigation of the dynamics of mating-system change.

*Eichhornia* comprises eight species of freshwater aquatic and palustral herbs in the monocotyledonous family Pontederiaceae. Three species possess showy, entomophilous outcrossed flowers and are primarily tristylous; the remaining five species are small-flowered, largely monomorphic for floral traits, and predominantly self-pollinating. Several lines of evidence (reviewed in Barrett [1988]) indicate that the major pathway of mating-system evolution in the genus involves the multiple breakdown of tristyliness to give rise to selfing, semihomostylous (with one set of stamens adjacent to the stigma) variants, races, and species.

Genetic studies of the inheritance of tristyliness in *Eichhornia* indicate that the poly-

morphism is controlled by two linked, diallelic loci (*S* and *M*), with *S* epistatic to *M* (Barrett, unpubl.). With this inheritance, equal style-morph fitnesses, and legitimate mating between the morphs, isoplethy (1:1:1 morph frequencies) is the only possible equilibrium in large populations (Heuch, 1979). This expectation provides a starting point for studies concerned with the maintenance and breakdown of tristyliness, and surveys of style morph frequencies in natural populations can provide insights into the mechanisms responsible for mating-system change in heterostylous plants (e.g., Lewis, 1975; Weller, 1979).

Preliminary surveys of style-morph frequencies in *Eichhornia paniculata* in conjunction with studies of the floral biology (Barrett, 1985a) and mating systems of populations (Glover and Barrett, 1986; Barrett et al., 1987) have enabled the formulation of a model of the breakdown process (Fig. 1). The model involves two key stages 1) loss of the *S* allele and, thus, the short-styled (S) morph from populations and 2) loss of the *m* allele and, hence, the long-styled (L) morph. These events result in changes in population structure from floral trimorphism through dimorphism to monomorphism and are accompanied by the spread and fixation of selfing semihomostylous variants of the mid-styled (M) morph (Barrett, 1985b).

In this paper, we provide evidence in support of the breakdown model by presenting a large body of survey data on frequencies of style morphs in populations of *E. paniculata* from northeast Brazil and Jamaica. We then examine the selective forces that are likely to account for the breakdown of tristily and the evolution of self-fertilization. Two approaches are used. We first examine whether fitness differences between the floral morphs or alleles governing style length are evident in *E. paniculata*. Comparisons of the female fertility and mating systems of style morphs and of the transmission of alleles at style-length loci are undertaken using field and greenhouse studies. Data from these empirical studies are then used in computer simulations to investigate the influence of random and deterministic forces on style-morph frequencies and the spread of differing variants in dimorphic populations.

#### MATERIALS AND METHODS

##### *Sampling of Style-Morph Frequencies*

The frequencies of style morphs were sampled in 110 populations of *Eichhornia paniculata*. Populations were sampled in northeast Brazil during May–June 1982 and 1987 and in Jamaica during December–January 1979, 1983, and 1987. These periods correspond to peak flowering in the two regions. Virtually all populations located in northeast Brazil and Jamaica were sampled. Populations of *E. paniculata* are short-lived, and although the same sites were visited during different sampling periods, few populations persisted (northeast Brazil  $N = 4$ ; Jamaica  $N = 2$ ) to enable resampling of morph frequencies. Morph frequencies from the first sample of six populations that were resampled were used in calculations. In small populations (<100 individuals) the style lengths of all flowering plants were recorded, whereas in larger populations, a random sample of plants was obtained. Since *E. paniculata* is an annual or short-lived perennial with no clonal propagation, the identification of genets is unambiguous.

Plants were classified as either long-, mid-, or short-styled (hereafter L, M, and S), and floral modifications promoting self-pollination were recorded. Modifications are

largely confined to the M morph and involve elongation of filaments of short-level stamens so that they are positioned adjacent to mid-level stigmas (Seburn et al., 1989). An index of morph evenness ( $E$ ) based on Simpson's index of ecological diversity (Simpson, 1949) was calculated for each population following formulas developed by Nei (1973).  $E$  ranges from 0 (monomorphic populations) to 1 (trimorphic with equal frequencies of the style morphs). The locality, morph frequencies, and demographic information (see below) for each population are available upon request from the senior author.

In all Brazilian populations sampled for morph frequency, the number of reproductive individuals was estimated. Since flowering in *E. paniculata* tends to be synchronous and since plants flower continuously, these estimates represent the majority of individuals in most populations. At each population, the density ( $D$ ) of plants was assessed by 2–3 independent observers using a five-point scale. The scale corresponds to the occupation of suitable sites in a habitat: 1 represents populations composed of scattered, widely-spaced plants; 2 is similar but with sporadic clumping of plants; 3 indicates that the population consists of many small clumps; 4 indicates that the site has filled in to the point where only a few, large clumps of high density are distinguishable; and 5 corresponds to a continuous dense population. The density classes are correlated with plant number, but in an exponential fashion ( $\log N = 0.824D + 2.397$ ,  $R^2 = 0.338$ ,  $P < 0.001$ ). Although the density scale is qualitative, close agreement between the observers was obtained at all sites. The relationships between population size, density class, and morph frequency were examined using a Kruskal-Wallis nonparametric test of significance.

##### *Empirical Studies*

*Transmission Genetics.*—To investigate whether the reduced frequencies of the S morph in trimorphic populations and the L morph in dimorphic populations could result from selection against  $S$  and  $m$  alleles at the gametophytic level, controlled crosses were performed using plants of known genotype, and progeny arrays were compared

to Mendelian expectations. Two sets of crosses were conducted: with 12 plants of the S morph that were heterozygous at the S locus and with 12 plants of the M morph that were heterozygous at the M locus. Plants of the S morph (*Ss*—) originated from trimorphic population B5 from northeast Brazil, and M plants (*ssMm*) originated from two dimorphic populations (J14 and J15) from Jamaica. No interpopulation crosses were conducted. Each plant was selfed and crossed as a pollen parent to the L morph. In this way, selection at the gametophytic level can be assessed (see Baker, 1975), since pollen grains carrying *S* or *s* alleles from the S morph and *M* or *m* alleles from the M morph are compared on styles of the L morph (*ssmm*).

**Fertility Selection.**—Differences in the fertility of style morphs can arise for a variety of reasons and may contribute to selection for or against a particular style morph. Two specific types of female fertility variation might be expected in heterostylous species. The first involves a reduction in fertility in the absence of specialized long-tongued pollinators (Charlesworth, 1979). This is most likely to involve the S morph, because of its concealed female reproductive organs (Beach and Bawa, 1980; see Fig. 1). A second form of fertility variation may occur in populations that receive sporadic pollinator service and in which self-pollinating variants (homostyles) occur. In these circumstances, variants may experience a fitness advantage as a result of reproductive assurance (Barrett, 1979; Piper et al., 1986).

To investigate whether female fertility differences occur among style morphs of *E. paniculata*, percentage fruit set was measured in 12 populations of contrasting structure in northeast Brazil (ten populations) and Jamaica (two populations). Six populations were trimorphic, five were dimorphic, and one was monomorphic for style length. In nontrimorphic populations, the frequency of self-pollinating M variants was also recorded. Pollinator observations were made at five of the six trimorphic populations (B5, B22, B34, B42, and B46). Specialized long-tongued solitary bees, primarily *Florilegus festivus* and *Ancyloscelis* spp., were observed feeding on nectar at all trimorphic populations except B22, an iso-

lated population occurring in central Pernambuco (Fig. 2). At this population, the only flower visitors were pollen-collecting generalist bees with *Trigona* spp. abundant and the honey bee *Apis mellifera* occurring infrequently. Observations of their foraging behavior indicated that most feeding activity involved pollen collection from long- and mid-level stamens. Accordingly, it was predicted that the S morph may set less fruit than the other two morphs. Detailed records of pollinators were not undertaken in nontrimorphic populations, since pollinator densities in both northeast Brazil and Jamaica were low and pollinator visitation was sporadic. Observations made in Jamaica over three field seasons indicate very low levels of generalist pollinator activity (primarily *Apis mellifera* and Syrphidae) and a notable absence of specialist long-tongued pollinators.

To establish whether intrinsic differences in female fertility occur among the style morphs of *E. paniculata*, a pollination program was conducted under greenhouse conditions on plants of each style morph in two trimorphic populations (B5 and B11) from northeast Brazil. Hand self-pollinations and legitimate cross-pollinations were undertaken, and the number of seeds produced per capsule was recorded for each pollination treatment per plant. Analysis of variance was used to compare the fertility of style morphs in field and greenhouse studies. Details of ANOVA and sample sizes are given below.

**Mating-System Variation.**—Different patterns of mating in the style morphs can have a major influence on morph frequencies (Charlesworth, 1979). Previous work on outcrossing rates in *E. paniculata* demonstrated a morph-specific difference in mating system in a dimorphic population (B9) from northeast Brazil. While the L morph was largely outcrossing, the M morph experienced a high degree of self-fertilization (Glover and Barrett, 1986; Barrett et al., 1987). To examine whether this pattern is of more general significance in the species, outcrossing rates in three Jamaican dimorphic populations (J14, J15, and J27-1) were estimated using isozyme markers, following methods detailed in Glover and Barrett (1986). Progeny sizes and the numbers of

marker loci used are indicated in Table 5. In contrast to population B9, in which a mixture of modified and unmodified M plants occurred, all M plants in the three populations were self-pollinating variants of the M morph.

#### Computer Models

Having determined the pattern of style-morph frequencies in natural populations of *E. paniculata*, and whether morph-specific differences in fertility and mating system occur, computer models were used to establish the range of population structures that are likely in tristylous systems and to explore the consequences of the empirical observations for mating-system evolution. Two classes of model were employed. Stochastic simulations were used to investigate the effects of genetic drift on population structure and style-morph frequencies. Of particular importance was to establish whether random processes could account for the decrease in frequency and loss of the S morph from trimorphic populations. A second class of model involved deterministic calculations which examined the effects of morph-specific differences in fertility and mating system on morph frequencies. Both models initially assume self-compatibility and no inbreeding depression upon selfing. Outcrossing occurs with probability  $t = 0.99$ , while self-fertilization occurs with probability  $1 - t$ . Outcross matings were disassortative (legitimate) with probability  $d = 0.95$  and random with probability  $1 - d$ . These values are in agreement with estimates of outcrossing and disassortative mating in trimorphic populations (Glover and Barrett, 1986; Barrett et al., 1987). The S and M loci were assumed to be tightly linked (Barrett, unpubl.).

*Effects of Small Population Size.*—A series of simulations were run in which trimorphic populations were initiated with 10, 20, 30, or 40 individuals. Genotypes were chosen to initiate a population with probabilities proportional to their frequencies in a large, disassortatively mating population

at equilibrium. Mating occurred by choosing a maternal plant at random and assigning self, random outcross, or disassortative paternal parentage. If random outcross mating occurred, then a paternal parent was chosen at random from the population, whereas if disassortative mating occurred, a plant of a different morph was chosen. Gametes were selected from the maternal and paternal parents to form progeny. This process was repeated until the number of progeny sampled equalled the population size in the next generation. Population sizes were kept constant, and the mating system parameters described above were used. Morph frequencies were determined every tenth generation. Each simulation was continued for 100 generations and replicated 200 times.

*Morph-Specific Fitness Differences.*—Processes occurring in large populations were described using a mathematical formulation of the mating system. The vector  $\mathbf{g}$  denotes the frequency of the 10 possible style morph genotypes in the population, and the  $10 \times 4$  matrix  $\mathbf{A}$  represents frequencies of the four gametes ( $sm$ ,  $sM$ ,  $Sm$ , and  $SM$ ) segregating from each of these genotypes. Gametes contributed by genotypes of the L, M, and S morphs to the pollen pool are represented as vectors  $\mathbf{l}_p$ ,  $\mathbf{m}_p$ , and  $\mathbf{s}_p$ , respectively. Using the S morph as an example, the  $j$ th element of  $\mathbf{s}_p$  is calculated as

$$s_{pj} = \sum_i g_i w_{pi} A_{ij} \quad (1)$$

where the summation is over all genotypes of a given morph, and  $w_{pi}$  is the paternal fitness of the  $i$ th genotype. Matings are described using the vectors  $\mathbf{t}$  and  $\mathbf{d}$  corresponding to the probability of outcrossed and disassortative mating, respectively, for each genotype. These parameters are used to form the  $4 \times 4$  matrix  $\mathbf{B}$  in which  $B_{kl}$  represents the frequency with which matings occur between maternal gamete  $k$  and paternal gamete  $l$ . Again using the S morph as an example, these elements are given by Equation (2) (below)

$$\begin{aligned} B_{S,kl} = & s_{pl} \sum_i [(1 - t_i) g_i w_{mi} A_{ik}] \\ & + (l_{pl} + m_{pi}) \sum_i [t_i d_i g_i w_{mi} A_{ik}] \\ & + (l_{pl} + m_{pi} + s_{pi}) \sum_i [t_i (1 - d_i) g_i w_{mi} A_{ik}] \end{aligned} \quad (2)$$

where  $w_{m_i}$  is the maternal fitness of the  $i$ th genotype. The three terms in this expression correspond to self, disassortative, and random mating, respectively. The complete matrix  $\mathbf{B}$  includes Equation (2) summed over all morphs, with terms describing mating appropriately restructured to reflect changing genotypic membership in the self, random, and disassortative mating classes. Genotype frequencies in the next generation,  $\mathbf{g}'$ , are calculated from  $\mathbf{B}$  and are normalized to sum to 1. Mating-system asymmetries and morph-specific differences in fertility were incorporated into the model by altering values of  $t$ ,  $d$  and  $w_m$ .

Genetic modifications of the mating system of *E. paniculata* are particularly evident in dimorphic populations (Fig. 1). Selfing phenotypes of the M morph with altered short-level stamens occur in most populations at various frequencies. Modifications are largely restricted to the M morph and appear to be under relatively simple genetic control involving one or a few recessive alleles (Barrett, unpubl.). To examine the spread of the selfing variants in dimorphic populations, we assumed that a recessive allele at a single locus,  $A$ , is responsible for increased levels of self-fertilization and that the expression of the allele is confined to the M morph. Calculations involved a modification of the program described above. Specifically, the correspondence between genotype and phenotype was altered so that individuals with genotype  $aaM-$  express the variant M phenotype, while  $AaM-$  or  $AAM-$  genotypes correspond to unmodified M plants. All  $---mm$  genotypes express the unmodified L phenotype. Only the selfing rate of variant M genotypes was altered, other mating-system parameters being identical to those used in the unmodified M and L phenotypes. Models examined the consequences of different rates of selfing and disassortative mating on the spread of the variant M morph and the effects of fixed rates of inbreeding depression on equilibrium frequencies.

## RESULTS

### Population Survey

*Geographical Patterns.*—The geographical distribution of trimorphic, dimorphic, and monomorphic populations of *E. paniculata* sampled in northeast Brazil is illus-

trated in Figure 2. Most populations are concentrated in the north in Ceará state or to the south in Pernambuco and Alagoas. Since *E. paniculata* is an aquatic species, large areas of the arid caatinga region of northeast Brazil are unsuitable for persistence of populations, due to prolonged periods of drought (Nimer, 1972). The most significant feature of the geographical distribution of populations is the nonrandom occurrence of dimorphic and monomorphic populations. The majority of the 26 non-trimorphic populations occur at the southern periphery of the region surveyed, in an area where trimorphic populations are absent.

The distribution of dimorphic and monomorphic populations of *E. paniculata* in Jamaica is illustrated in Figure 3, with frequencies of the L and M morphs indicated for dimorphic populations. The L morph occurs sporadically at low frequency in populations in St. Thomas, St. Catherine's, and Clarendon parishes and is absent from the western end of the island.

*Style-Morph Frequencies.*—*Eichhornia paniculata* exhibits a wide range of population structures with considerable heterogeneity in style-morph frequencies among populations. Of the 110 populations sampled in northeast Brazil and Jamaica, 53% were trimorphic, 25% were dimorphic, and the remaining 22% were monomorphic for style length. Among the 58 trimorphic populations sampled, only 31% contained the style morphs at equal frequency. All dimorphic populations of *E. paniculata* were composed of the L and M morphs, and all monomorphic populations were fixed for the M morph. While the majority of populations (69%) sampled in northeast Brazil were trimorphic, absence of the S morph in Jamaica precludes formation of trimorphic populations, and monomorphic populations were most commonly encountered (71%). Table 1 presents the average frequencies of style morphs in populations of *E. paniculata* in northeast Brazil and Jamaica. Among trimorphic populations, there was a significant deficiency of the S morph, while the L and M morphs were, on average, equally frequent. Among dimorphic populations in both regions, the average frequency of the M morph was 2–3 times higher than that of the L morph.

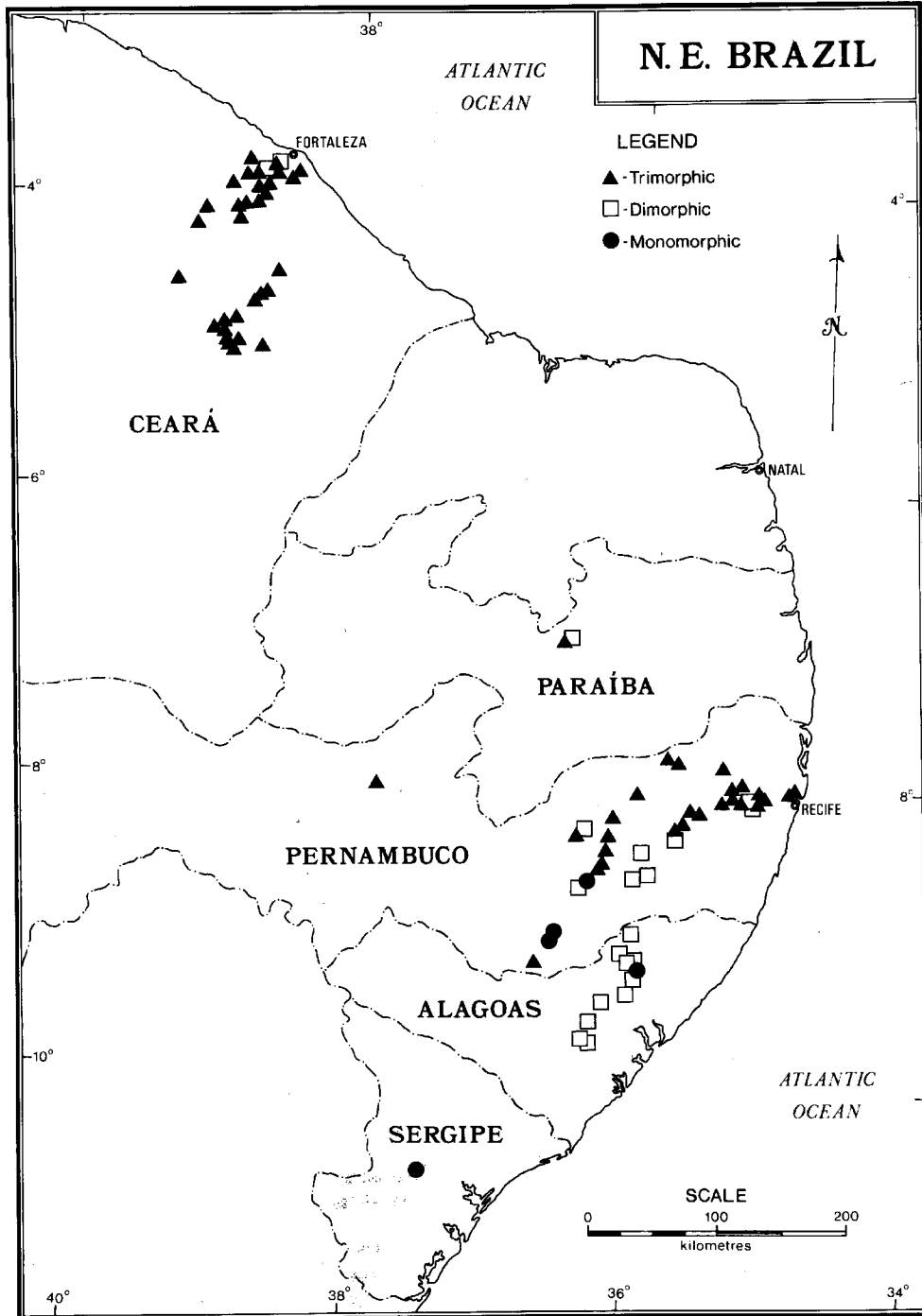


FIG. 2. Geographical distribution of *Eichhornia paniculata* populations sampled for style morph frequency in northeast Brazil. Triangles are trimorphic populations, squares are dimorphic populations, and circles are

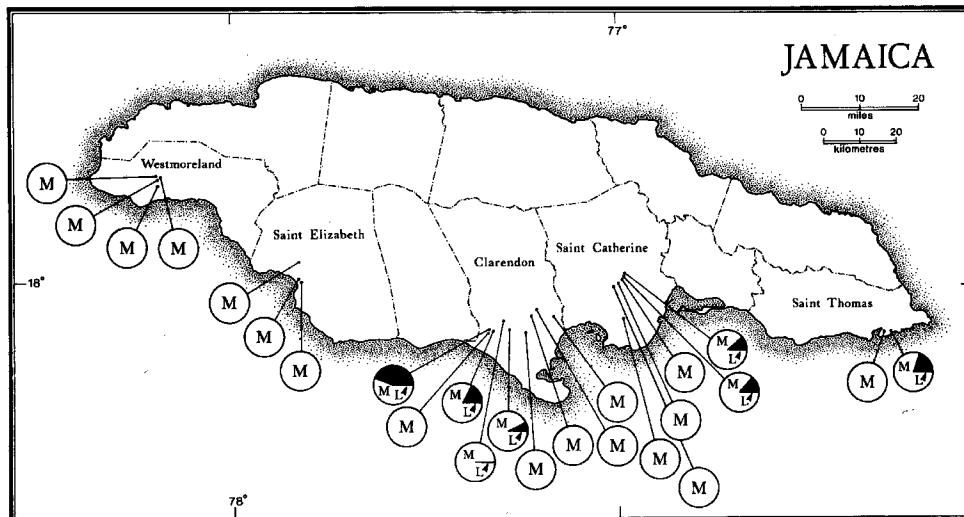


FIG. 3. Geographical distribution of *Eichhornia paniculata* populations sampled for style-morph frequency in Jamaica and the relative frequency of the L and M morphs within dimorphic populations. All plants of the M morph in Jamaica are self-pollinating variants.

Genetic modifications in stamen length that influence mating patterns in the M morph were nonrandom in distribution. Only four trimorphic populations contained self-pollinating variants of the M morph at frequencies above 1%. In populations B46, B56, B58, and B61, variants represented 5.0%, 2.4%, 9.6%, and 1.9% of individuals sampled in populations, respectively. Of the remaining trimorphic populations, 52 contained no variants, and two had sporadic variants at very low frequencies. Nontrimorphic populations exhibited a markedly different pattern. On Jamaica, all plants of the M morph are modified, and in northeast Brazil, 16 of 21 dimorphic populations contained self-pollinating variants at various frequencies (see below), and three of five monomorphic populations were fixed for self-pollinating variants. Among nontrimorphic populations containing self-pollinating variants of the M morph from northeast Brazil, there was a highly significant negative correlation between the frequency of the L morph and the frequency of M plants that were self-pollinating vari-

ants (Fig. 4). While genetic modifications to reproductive organs were frequently encountered in the M morph, they were less commonly observed in the L and S morphs.

**Population Size and Density.**—Although population sizes tend to be small, regardless of style-morph frequency, there was a marked tendency for dimorphic and monomorphic populations to be smaller in size than trimorphic populations (Fig. 5). While 80% of nontrimorphic populations contained fewer than 100 individuals, the corresponding value for trimorphic populations was 37%. The difference in distributions of sizes between the two classes of population structure is significantly different (Kolmogorov-Smirnov  $D = 0.411$ ,  $P < 0.01$ ). The majority of Jamaican populations contain fewer than 100 individuals.

Populations of *E. paniculata* in northeast Brazil with different style-morph structure also differ in plant density. Values of evenness of the style morphs declined significantly with decreasing plant density (Table 2). Trimorphic populations were often composed of large numbers of plants at high

←  
monomorphic populations. Population B22, which was used for comparisons of fruit set of the style morphs, is the isolated population in central Pernambuco.



TABLE 1. Average frequencies of the style morphs in populations of *Eichhornia paniculata* from northeast Brazil and Jamaica.

Region	Style-morph structure	Number of populations	Frequencies		
			L	M	S
Northeast Brazil	trimorphic	58	0.374	0.370	0.256
	dimorphic	21	0.336	0.664	—
	monomorphic	5	—	1.000	—
Jamaica	dimorphic	7	0.211	0.789	—
	monomorphic	19	—	1.000	—

density, whereas dimorphic and monomorphic populations were typically composed of a smaller number of scattered individuals at low density.

#### Empirical Data

*Transmission Genetics.*—Controlled crosses using heterozygous genotypes of the S and M morphs of *E. paniculata* provided no evidence of transmission biases favoring particular style-length alleles during the gametophyte phase of the mating cycle. Crosses to the L morph yielded 1:1 progeny ratios, with no significant heterogeneity among individual families (Table 3). However, significant deviations from the 3:1 expectation were obtained in self-pollinations of the S and M morphs. Among the 12 selfed families of the S morph, four of the five showing significant deviation displayed deficiencies of the S morph. Significant deficiency in the frequency of the L morph was evident in three of 12 selfed M families. The deficiencies of the S and L morphs observed in some families following self-pollination may result from diploid selection during megasporogenesis or early embryo development. Alternatively, such effects may result from variation in male gametic fitness of the S and m alleles in different maternal environments following self-pollination.

*Fertility Variation.*—Surveys of fruit set in populations of *E. paniculata* revealed statistically significant differences in female fertility between the style morphs in six of the 11 polymorphic populations examined (Table 4). In populations B34 and B22, fruit set of the S morph was significantly lower than that of the L and M morphs, and in population B5, the M morph set more fruit than the L and S morphs. The most dramatic differences in fruit set among style

morphs was recorded in population B22, the only trimorphic population observed that was visited exclusively by short-tongued, generalist pollinators. The S morph set 34.1% and 28.0% less fruit than the L and M morphs, respectively.

In all five dimorphic populations of *E. paniculata*, mean fruit set of the M morph was greater than that of the L morph, although the difference was statistically significant in only three populations. The smallest difference (3.4%) was recorded in population B55, in which no selfing variants of the M morph occurred. In populations B66 and B65, the mean percentage fruit set of the M morph was 12.8% and 20.3% greater than the L morph, respectively. Both populations contained a mixture of modified and unmodified M plants. The largest differences in fruit set between the L and M morphs occurred in the Jamaican populations J27-1 and J27-2 (32.2% and 55.6%, respectively), in which all M plants were self-pollinating variants.

Seed set from controlled self- and cross-pollinations of the three style morphs in two tristylous populations of *E. paniculata* is illustrated in Figure 6. The most significant finding concerns the low seed set obtained from self-pollination of the S morph. This pattern was observed in both populations and is consistent with data collected in two additional tristylous populations (Barrett, 1985a). Collectively, the results suggest that reduced seed set following self-pollination of the S morph is a general phenomenon in the species.

*Mating Patterns in Dimorphic Populations.*—Morph-specific differences in outcrossing rate were detected in all three dimorphic populations of *E. paniculata* examined. In each population, the L morph

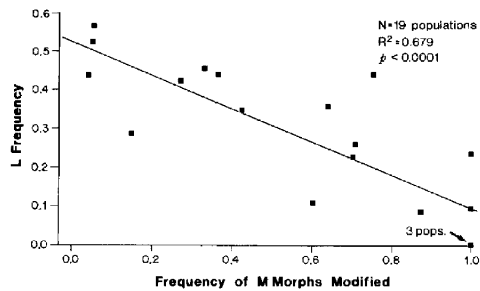


FIG. 4. The relationship between the frequency of the L morph and the frequency of M plants that were self-pollinating variants in 19 nontrimorphic populations of *Eichhornia paniculata* from northeast Brazil.

was predominantly outcrossing, whereas the M morph experienced high levels of self-fertilization (Table 5). The large difference in outcrossing rates between morphs in each population results from genetic modifications in the position of short-level stamens in the M morph. The modifications facilitate automatic self-pollination of mid-level stigmas. Differences in outcrossing rate between the L and M morphs in Jamaican populations were considerably larger than previously reported for a dimorphic population from northeast Brazil (Table 5). The difference probably results from the different degrees of genetic modification to the M morph in the two regions.

#### Computer Models

*Effects of Small Population Size.*—The frequencies of trimorphic populations observed in simulations with various population sizes are plotted against number of generations since population initiation in Figure 7A. With a population size of 10 individuals, the frequency of trimorphic populations decreased exponentially until generation 65, when all but one of the 200 initial populations had lost one or more of the style morphs. Increasing population size to 20 individuals resulted in a more gradual and linear loss of trimorphism, while only 11% of trimorphic populations with 30 individuals had become dimorphic or monomorphic after 100 generations. Virtually all populations initiated with 40 individuals remained trimorphic throughout the simulation.

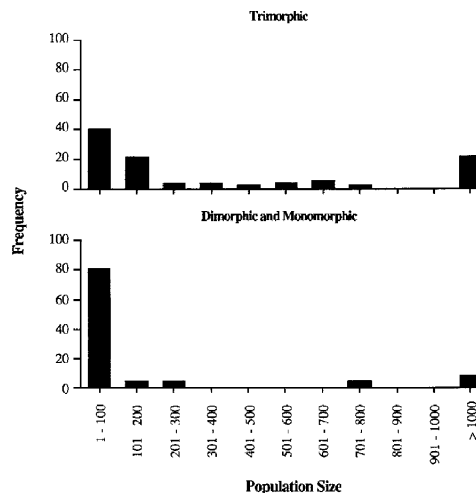


FIG. 5. Size distributions in trimorphic ( $N = 58$ ) and nontrimorphic ( $N = 26$ ) populations of *Eichhornia paniculata* surveyed in northeast Brazil.

Alleles at style-morph loci resist chance loss through genetic drift in population sizes that are surprisingly small compared to theoretical expectations based on alleles at neutral loci. When simulations were run with the amount of disassortative mating reduced to  $d = 0.50$ , populations became dimorphic or monomorphic more rapidly (Fig. 7B). This result is particularly evident in populations of 30 individuals. High levels of disassortative mating maintain trimorphism in small populations.

Population structures observed after 100 generations of high and intermediate levels

TABLE 2. Mean evenness ( $E$ ) of style-morph frequencies and standard deviations for 58 populations of *Eichhornia paniculata* from northeast Brazil classified by density.  $E$  is calculated using Simpson's Index and ranges from 0 (monomorphic) to 1 (trimorphic). See Materials and Methods for a description of the density classes. Differences among density classes were significant in a Kruskal-Wallis nonparametric test ( $P < 0.0001$ ).

Density	$N$	$E$	SD
1	10	0.340	0.337
2	17	0.769	0.127
3	20	0.809	0.293
4	24	0.902	0.148
5	13	0.932	0.076

TABLE 3. Transmission of *S* and *m* alleles following controlled selfing and crossing of heterozygous individuals of the *S* and *M* morphs of *Eichhornia paniculata*. Plants of known genotype were selfed and crossed to the *L* morph, and progeny ratios were compared with the 3:1 and 1:1 expectations, respectively, for the two-locus model of the inheritance of tristylly.

A) Heterozygous <i>S</i> ( <i>Ss</i> —):							
Selfed				Crossed with <i>L</i> morph			
Number of families	Total progeny	Number of <i>S</i>	Number of non- <i>S</i>	Number of families	Total progeny	Number of <i>S</i>	Number of non- <i>S</i>
12	1,170	837	333	12	1,325	646	679
Deviation from 3:1, $G = 7.26$ , $P = 0.0070$ Heterogeneity, $G = 33.3$ , $P = 0.0006$				Deviation from 1:1, $G = 0.82$ , $P = 0.6391$ Heterogeneity, $G = 11.9$ , $P = 0.3753$			
B) Heterozygous <i>M</i> ( <i>ssMm</i> ):							
Selfed				Crossed with <i>L</i> morph			
Number of families	Total progeny	Number of <i>L</i>	Number of <i>M</i>	Number of families	Total progeny	Number of <i>L</i>	Number of <i>M</i>
12	653	124	529	12	672	329	343
Deviation from 3:1, $G = 13.4$ , $P = 0.0004$ Heterogeneity, $G = 31.8$ , $P = 0.0010$				Deviation from 1:1, $G = 0.29$ , $P = 0.5961$ Heterogeneity, $G = 19.1$ , $P = 0.0623$			

of disassortative mating are summarized in Table 6. High disassortative mating in populations of 10 individuals resulted in most populations (91%) becoming dimorphic, while only 9% became monomorphic. Among dimorphic populations, about 60% lost the *S* morph, 34% lost the *M* morph, and the remainder lost the *L* morph. Increasing population size decreased the number of populations that became dimorphic;

however, among dimorphic populations, the proportion that contained the *L* and *M* morphs and the proportion containing the *L* and *S* morphs remain approximately equal to those observed in simulations with populations of size 10. Decreasing the strength of disassortative mating to  $d = 0.50$  results in an increase in the proportion of dimorphic and monomorphic populations, but the relative frequencies of different dimorphic

TABLE 4. Mean fruit set, standard deviation, and sample size of style morphs in 12 populations of *Eichhornia paniculata* from northeast Brazil and Jamaica. Floral modifications to the *M* morph were observed in all non-trimorphic populations except B55. One-way ANOVA was conducted on arcsine-transformed data, but results are reported as untransformed means. Means in the same row with different superscripts are significantly different at  $P < 0.05$ .

Popu- lation	Morph									<i>P</i>
	<i>L</i>			<i>M</i>			<i>S</i>			
	Mean	SD	<i>N</i>	Mean	SD	<i>N</i>	Mean	SD	<i>N</i>	
Trimorphic:										
B88	95.3	5.2	30	95.3	5.7	30	95.2	8.5	30	0.726
B42	93.8	5.0	20	95.3	3.9	20	92.0	6.6	20	0.462
B46	94.8	4.5	20	94.5	7.4	35	91.9	5.2	9	0.363
B34	97.1 <sup>a,b</sup>	3.4	27	98.5 <sup>a</sup>	2.3	27	95.4 <sup>b</sup>	3.6	18	0.003
B5	77.2 <sup>b</sup>	8.8	40	88.3 <sup>a</sup>	6.5	40	78.0 <sup>b</sup>	11.1	40	<0.0001
B22	71.0 <sup>a</sup>	13.2	40	65.0 <sup>b</sup>	9.7	40	46.8 <sup>c</sup>	17.9	40	<0.0001
Dimorphic:										
B55	95.3	4.9	8	98.7	1.9	10	—	—	—	0.059
B66	71.0	25.2	16	83.8	15.8	16	—	—	—	0.101
B65	75.4 <sup>a</sup>	15.4	6	95.7 <sup>b</sup>	5.5	10	—	—	—	0.007
J27-1	53.9 <sup>a</sup>	21.2	40	86.1 <sup>b</sup>	10.5	40	—	—	—	<0.0001
J27-2	12.6 <sup>a</sup>	11.8	30	68.0 <sup>b</sup>	22.8	30	—	—	—	<0.0001
Monomorphic:										
B63	—	—	—	98.4	3.1	31	—	—	—	—

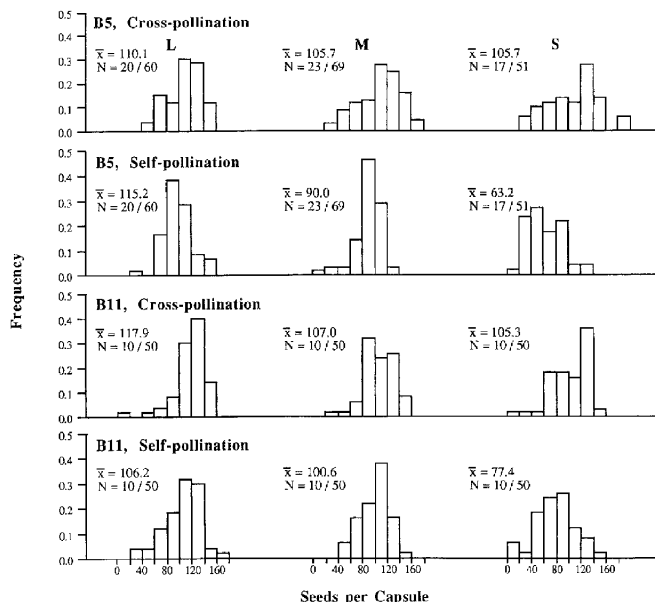


FIG. 6. Distributions of seeds per capsule after self- and cross-pollinations of style morphs of *Eichhornia paniculata* in two trimorphic populations. All population, morph, and pollination-treatment combinations were analyzed using one-way ANOVAs; self- and cross-pollinations for each morph were compared using linear contrasts (B5: L,  $F = 6.07$ ,  $P = 0.014$ ; M,  $F = 11.19$ ,  $P = 0.001$ ; S,  $F = 60.52$ ,  $P < 0.0001$ ; B11: L,  $F = 4.69$ ,  $P = 0.031$ ; M,  $F = 1.36$ ,  $P = 0.244$ ; S,  $F = 25.68$ ,  $P < 0.0001$ ). Sample sizes indicated are the numbers of plants and fruits per morph.

population structures observed in natural populations are expected to remain approximately constant.

Style-morph frequencies in small trimorphic populations of fixed size (10–40) with high ( $d = 0.95$ ) and intermediate ( $d = 0.50$ ) levels of disassortative mating showed no consistent bias as a result of drift (results not shown). Simulations in which population sizes were assigned at random each generation from the observed size distribution in northeast Brazil gave qualitatively similar results to those with populations of fixed size (Morgan, 1988). These results suggest that stochastic loss of the S morph occurs rapidly in natural populations.

**Morph-Specific Fitness Differences.** — Calculations involving fertility variation among style morphs indicate that a reduction in female fertility of the S morph reduces its frequency in populations, while frequencies of the L and M morphs are increased by equal amounts (Fig. 8). Since *E. paniculata* is highly self-compatible, in-

creased levels of random mating might be anticipated in populations serviced by generalist pollinators. Calculations in which disassortative mating is reduced to  $d = 0.50$  result in a more rapid loss of the S morph from populations (Fig. 8). This demonstrates the importance of disassortative

TABLE 5. Multilocus outcrossing rates ( $t$ ) of style morphs in dimorphic populations of *Eichhornia paniculata* from northeast Brazil and Jamaica.

Pop-ulation	Number of loci	Morph	$N$ (plants/family)	$t$	SE
B9*	5	L	115/6	0.78	0.060
		M	432/19	0.36	0.032
J14	2	L	262/10	0.86	0.060
		M	685/21	0.22	0.026
J15	2	L	355/13	0.99	0.054
		M	507/18	0.12	0.018
J27	3	L	89/14	0.61	0.075
		M	74/14	0.02	0.016

\* Data from Glover and Barrett (1986).

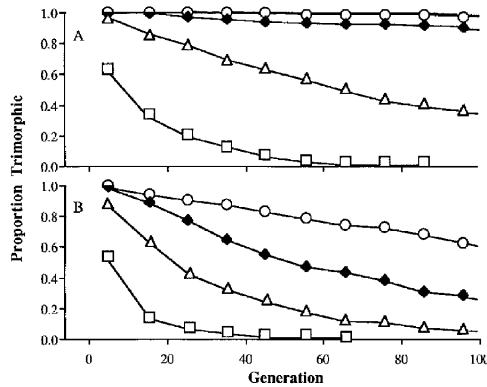


FIG. 7. The effect of small population size on style-morph frequencies in *Eichhornia paniculata* populations under different levels of disassortative mating. Simulations were conducted with A) high ( $d = 0.95$ ) and B) intermediate ( $d = 0.50$ ) levels of disassortative mating. Population sizes:  $N = 10$  (squares),  $N = 20$  (triangles),  $N = 30$  (diamonds), and  $N = 40$  (circles).

mating in maintaining morph frequencies in the face of strong morph-specific fertility differences.

The model of mating-system evolution illustrated in Figure 1 involves loss of the L morph from dimorphic populations in association with the spread of genetic modifications promoting self-fertilization in the M morph. Calculations indicate that when an allele that increases the selfing rate of the M morph is introduced into a disassortatively mating dimorphic population in the absence of inbreeding depression, the allele spreads to fixation. However, since expression of the selfing allele is restricted to the M morph, fixation of the selfing allele does

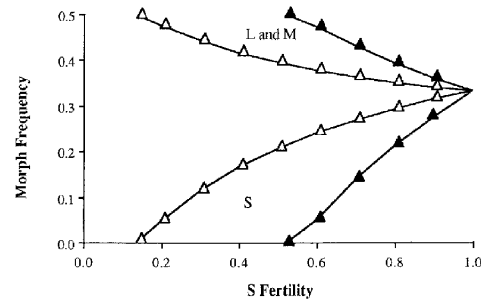


FIG. 8. Style-morph frequencies in populations of *Eichhornia paniculata* with reduced female fertility of the S morph under high ( $d = 0.95$ ; open symbols) and intermediate ( $d = 0.50$ ; filled symbols) levels of disassortative mating.

not necessarily result in loss of the L morph. When the variant M morph selfs at a low rate (e.g., 0.15), it increases in frequency and displaces unmodified M plants from the population. However, this process has little effect on the frequency of the L morph. Higher selfing rates in the modified M morph decrease the equilibrium frequency of the L morph, as shown in Figure 9. When  $d = 0.95$ , the modified M morph must self with a probability greater than 0.68 before the L morph is lost from equilibrium populations. A selfing rate of 0.55 will result in loss of the L morph when  $d = 0.50$ .

Alleles causing greater self-fertilization in the M morph increase in frequency when fixed rates of inbreeding depression ( $\delta$ ) are included in the model. High levels of disassortative mating allow selfing alleles to spread when  $\delta$  is greater than  $1/2$ . This contrasts with the analytic results of Lloyd

TABLE 6. Effect of small population size on style-morph structure in populations of *Eichhornia paniculata* after 100 generations of high ( $d = 0.95$ ) and intermediate ( $d = 0.50$ ) levels of disassortative mating. Tabled values are percentages of populations with the indicated structure.

$d$	Population size	Percentage							
		Monomorphic			Dimorphic			Trimorphic	
		L	M	S	LM	LS	MS	LMS	
0.95	10	8.5	0.5	0.0	53.5	30.5	7.0	0.0	
	20	0.0	0.0	0.0	39.0	23.5	3.0	34.5	
	30	0.0	0.0	0.0	5.0	5.5	0.5	89.0	
	40	0.0	0.0	0.0	1.5	0.0	0.0	98.5	
0.50	10	63.5	9.5	7.0	11.5	5.0	3.5	0.0	
	20	6.0	1.0	0.0	43.0	37.0	7.5	5.5	
	30	0.0	0.0	0.0	41.0	28.0	5.0	26.0	
	40	0.0	0.0	0.0	21.5	16.0	2.0	60.5	

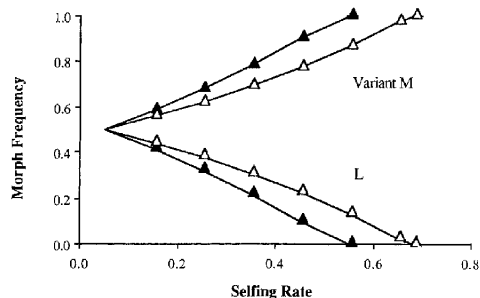


FIG. 9. Style-morph frequencies in dimorphic populations of *Eichhornia paniculata* with different rates of self-fertilization in the variant M morph under high ( $d = 0.95$ ; open symbols) and intermediate ( $d = 0.50$ ; filled symbols) levels of disassortative mating.

(1979), who demonstrated that alleles increasing the amount of competing self-fertilization in hermaphroditic species will spread only if  $\delta$  is less than  $1/2$ . In Lloyd's model, the effects of inbreeding depression are countered by the twofold advantage of self-fertilization (Fisher, 1941). In the model developed here, the selfing variant enjoys an additional advantage associated with the inheritance of style length and the frequency-dependence of disassortative mating. At least two-thirds of the progeny from self-fertilization of the variant M morph will be the M or variant M phenotype as a simple consequence of the genetic basis of heterostyly. Inbreeding depression in self-fertilized progeny therefore causes a greater reduction in the frequency of the M and variant M morphs, compared to the L morph. However, the effect of inbreeding depression is partly countered at mating. The M and variant M morphs are at lower frequency than in the absence of inbreeding depression and so benefit from the frequency-dependent mating advantage associated with outcrossing to the L morph. The net effect is to allow selfing alleles to spread at values of  $\delta$  greater than  $1/2$ .

When the variant M morph does spread, fixed rates of inbreeding depression influence equilibrium morph frequencies (Fig. 10). The effect of inbreeding depression on morph frequencies is greater when  $d = 0.50$  than when  $d = 0.95$ , and at high rather than low selfing rates. The effects of altering linkage between the style-length locus and the

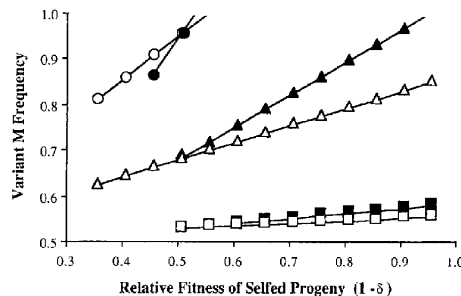


FIG. 10. Equilibrium frequencies of the variant M morph calculated under different mating-system parameters and fixed levels of inbreeding depression. Results are shown when the variant M morph selfs at  $s = 0.05$  (squares),  $s = 0.50$  (triangles), and  $s = 0.95$  (circles) under high ( $d = 0.95$ ; open symbols) and intermediate ( $d = 0.50$ ; filled symbols) levels of disassortative mating.

selfing locus are minimal for both rates of spread and equilibrium morph frequencies.

#### DISCUSSION

The evolutionary breakdown of tristily in *Eichhornia paniculata* involves a loss of style morphs from populations and the spread of genetic modifications in stamen position that increase selfing rates. In contrast to many mating-system shifts in flowering plants, the processes responsible for these changes are still active in contemporary populations, facilitating microevolutionary studies of the dynamics of mating-system change. The model of the breakdown process in *E. paniculata* illustrated in Figure 1 was formulated after a survey of 48 populations revealed: 1) a deficiency of the S morph in trimorphic populations and its complete absence from nontrimorphic populations and 2) underrepresentation of the L morph in dimorphic populations and fixation of the M morph in monomorphic populations (Barrett, 1985b). These patterns have remained unchanged following surveys of an additional 62 populations, indicating that the model is robust and represents the most likely evolutionary pathway to floral monomorphism and self-fertilization in *E. paniculata*. If this is accepted, then the next step is to determine the selective processes responsible for the breakdown of tristily. The remainder of the dis-

discussion therefore focuses on the genetic and ecological factors responsible for the transition from floral trimorphism through dimorphism to monomorphism.

#### *Loss of the S Morph*

A variety of selective mechanisms could potentially lead to loss of the S morph from tristylous populations of *E. paniculata*. Simulation studies conducted here and those of Heuch (1980) indicate that founder events and periodic population bottlenecks would be more likely to result in loss of the S allele from populations than to lead to the loss of any of the remaining three alleles, particularly in populations with weak disassortative mating. An absence of specialized long-tongued pollinators could affect the maternal fitness of the S morph as a result of its concealed female reproductive parts. Genetic factors, including gametophytic selection against the S allele, viability selection against SS— zygotes following inbreeding, or greater inbreeding depression in the S morph than in the other morphs, could all potentially influence the fitness of the S morph and, hence, its frequency in natural populations.

Controlled cross-pollinations provided little evidence that the reduced representation of the S morph in natural populations of *E. paniculata* results from a transmission bias against the S allele during the gametophytic stage of the mating cycle. Controlled self-pollinations, however, revealed a reduction in fertility of the S morph in comparison to the L and M morphs and a deficiency of S plants in some selfed families. At this stage, it is not known whether these effects are related or what underlying cause(s) are responsible. Inbreeding depression resulting from the sheltering of deleterious genes at the S locus (Strobeck, 1980), selection against SS— zygotes (Mather and de Winton, 1941) or weak self-incompatibility are possible explanations. To what extent these factors could influence the fitness of the S morph in natural populations is unclear. It is possible that, in small populations experiencing inbreeding, the S morph may suffer disproportionately, affecting its frequency relative to the L and M morphs. More work on inbreeding effects in the S morph is required, however, to es-

tablish whether such phenomena have any significance with regard to the breakdown of tristylous.

Founder events most likely account for the absence of the S morph from most dimorphic populations of *E. paniculata* encountered in the survey. Since the dominant S allele governing the expression of the short style is only carried by plants of the S morph, separate introduction(s) of this morph are necessary for it to become established in a region. Electrophoretic evidence suggests that a small number of long-distance dispersal events were involved in the colonization of Jamaica by *E. paniculata* (Glover and Barrett, 1987). These do not appear to have involved the S morph. The related tristylous *E. crassipes* displays a similar pattern of style-morph distribution to that found in *E. paniculata*. The S morph is absent from many parts of the New and Old World range, presumably as a result of repeated founder events (Barrett, 1977).

Although founder events probably account for the absence of the S morph of *E. paniculata* from Jamaica, and possibly from the southern periphery of the area sampled in northeast Brazil, more complex forces appear to be responsible for its underrepresentation in many trimorphic populations of the species. Periodic fluctuations in patch size and local extinctions of the S morph on a neighborhood scale may account for the deficiency of the S morph in many trimorphic populations. In addition to these random processes, however, selective factors mediated by pollinators also appear to play a role, either in combination with the effects of small population size or acting alone. The reduced fertility of the S morph in population B22 indicates that this morph is particularly susceptible to decreased frequencies of specialist long-tongued pollinators. Populations most likely to be affected are those that are small or isolated or that occur at the margins of the pollinator's range. Computer calculations demonstrate that decreased fertility of the S morph can lead to its loss from trimorphic populations, especially when the strength of disassortative mating is reduced. Changes in mating patterns may be most likely to occur in the absence of specialist pollinators because *E. paniculata* is self-compatible, and disassor-

tative mating will be enhanced by precise pollen placement and transfer to the appropriate stigma surface.

The above considerations suggest that dimorphic populations of *E. paniculata* originate in two distinct ways. Local extinction of the S morph through population bottlenecks or an absence of specialized long-tongued pollinators can convert trimorphic populations to dimorphic populations composed of the L and M morphs. Among the sample of trimorphic populations, 6.5% were characterized by frequencies of the S morph below 5%. In these circumstances, random processes with or without pollinator-associated selection could lead to the origin of floral dimorphism. Colonization events involving M plants of genotype *ssMm* are also likely to give rise to dimorphic populations composed of the L and M morphs. Self-fertilization of heterozygous M plants in modified variants, either by generalist pollinators or through autonomous means, would result in segregating progeny in ratios similar to those observed in many dimorphic populations. This suggests that at least some of these populations have recently originated and have been founded by heterozygous M plants.

#### *Loss of the L Morph*

Two distinct selective mechanisms can account for the origin of floral monomorphism from dimorphism in *E. paniculata*: reproductive assurance and automatic selection. Reproductive assurance favoring variants of the M morph under conditions of low pollinator service is the most likely mechanism resulting in the establishment of monomorphic populations. Even with reliable pollinators, however, automatic selection of the M morph can occur as a result of mating asymmetries between the morphs. Although the evolutionary processes are different for each of these mechanisms, both involve the establishment and spread of genes modifying short-level stamen position in the M morph. The prevalence of these modifications in dimorphic populations of *E. paniculata* suggests that the transition from dimorphism to monomorphism occurs repeatedly in both northeast Brazil and Jamaica.

Several lines of evidence support the hy-

pothesis that reproductive assurance favors the evolution of populations fixed for selfing variants of the M morph. Comparisons of fruit set in the L and M morphs of dimorphic populations demonstrated significant advantages for the M morph. Differences in fertility were related to the frequency of modified M plants in populations. Populations sampled from Jamaica, where all M plants are modified and specialized pollinators are absent, exhibited large differences in fruit set between the morphs. In contrast, in a dimorphic population from northeast Brazil in which no genetic modifications were apparent, the two morphs were equally fertile. Simulation studies indicate that, with the fertility differences and patterns of mating observed in Jamaica, the M morph will spread to fixation within populations.

The geographical distribution of dimorphic and monomorphic populations of *E. paniculata* is also consistent with the reproductive-assurance hypothesis. Establishment on Jamaica following long-distance dispersal probably favored the self-pollinating variants that predominate on the island. Subsequent spread of *E. paniculata* in Jamaica has largely involved monomorphic populations (Fig. 3). These have probably arisen from repeated colonization by M plants homozygous at the *M* locus (*ssMM*). The high level of selfing demonstrated in the M morph in Jamaica increases the frequency of homozygous M plants and favors initiation of monomorphic populations. The fact that all monomorphic populations are composed of the M morph rather than the L morph indicates the advantages of automatic self-pollination for population establishment.

In northeast Brazil, the majority of dimorphic and monomorphic populations were concentrated at the southern periphery of the region surveyed. This pattern and the fact that populations tend to be smaller and less dense than in other parts of the Brazilian range suggest that the ecological conditions in this zone are less favorable for persistence of trimorphic populations. Small populations composed of scattered individuals may be less likely to attract specialized pollinators, or pollinator service in general may be less reliable at low density. In either situation, selfing variants would be at a se-



lective advantage as a result of reproductive assurance. The association between selfing and low-density conditions is a recurrent theme in the mating-system literature, although surprisingly few empirical studies of plant populations have provided evidence bearing on the relationship. This is because selfing and outcrossing plants are most often ecologically segregated, and their performances are therefore difficult to compare. Polymorphic systems, such as heterostyly, provide convenient experimental systems for investigating the reproductive-assurance hypothesis when heterostylous and homostylous morphs occur within populations (Piper et al., 1986).

While many monomorphic populations of *E. paniculata*, particularly in Jamaica, probably originate from colonization events involving selfing variants of the M morph, evolutionary processes operating within dimorphic populations can potentially convert these populations to floral monomorphism. The dynamics of dimorphic populations are of particular significance to broader issues concerned with the equilibrium properties and evolutionary significance of mixed mating systems (Lande and Schemske, 1985; Uyenoyama, 1986; Waller, 1986; Holsinger, 1988b). Once selfing variants arise in dimorphic populations, it is of importance to determine whether a stable equilibrium of selfing and outcrossing morphs can occur or, alternatively, whether the variants will inevitably spread to fixation. The available evidence indicates that the maintenance of a dimorphic mixed mating system in *E. paniculata* can only occur under restricted conditions and that the spread of the M morph is a more likely outcome.

Selfing variants of the M morph exhibit several important fitness advantages over the L and unmodified M morphs. We have already considered the most obvious of these, reproductive assurance in the absence of pollinators, in the context of colonization. However, even when pollinator service is reliable, modified M plants also experience a fitness advantage as a result of their mating patterns. This is because genes that promote self-fertilization bias their own transmission through the mating cycle and thus tend to be automatically selected (Fish-

er, 1941). As illustrated in Figure 1, selfing variants are capable of transmitting genes via pollen and ovules through selfing, as well as through outcrossing to the L morph. In contrast, the L morph suffers under this mating system, since it acts principally as a female in matings with selfing variants due to the fact that its pollen is incapable of effectively outcompeting the selfed pollen of the M variant. While we have verified that the maternal outcrossing rates of the L and M morph in four dimorphic populations are consistent with the mating model illustrated in Figure 1, we have yet to measure the male fertility of the two morphs to confirm that the M morph has higher male fitness. Experiments with electrophoretic markers, similar to those undertaken by Schoen and Clegg (1985), are currently in progress to enable us to do this.

Pollen discounting (Holsinger et al., 1984) and inbreeding depression (Charlesworth and Charlesworth, 1987) can potentially prevent the spread of selfing variants in outbreeding populations. However, these factors appear to be of minor significance in dimorphic populations of *E. paniculata*. Long-level anthers of selfing variants are unaffected by genetic changes in the position of the "short-level" stamens and, hence, continue to contribute genes to the outcross-pollen pool. Inbreeding depression in dimorphic populations appears to be mild enough that it does not present a major obstacle to the spread of selfing variants (P. Toppings and S. C. H. Barrett, unpubl.). It is possible that population bottlenecks, which contribute to the loss of the S morph, reduce genetic loads sufficiently that, if selfing variants do arise in populations, they are automatically selected, unless lost through drift when at low frequency.

Surveys of morph frequencies in nontrimorphic populations of *E. paniculata* in northeast Brazil provide data that are in accord with the above model of mating-system change. Among 19 populations sampled, there was a significant negative relationship between the frequency of the L morph and the proportion of M plants that were self-pollinating variants (Fig. 4). This suggests that at least some populations are at different stages in the transition from dimorphism to monomorphism. However,

long-term censusing of morph frequencies within these populations would be required to provide conclusive evidence that the dimorphic mixed mating system of *E. paniculata* cannot be maintained as an evolutionarily stable state.

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