

VARIATION IN THE MATING SYSTEM OF *EICHHORNIA PANICULATA*  
(SPRENG.) SOLMS. (PONTEDERIACEAE)

DEBORAH E. GLOVER<sup>1</sup> AND SPENCER C. H. BARRETT

Department of Botany, University of Toronto, Toronto, Ontario M5S 1A1, Canada

**Abstract.**—A multilocus procedure was used to estimate outcrossing rates from allozyme data in nine populations of *Eichhornia paniculata* from NE Brazil and Jamaica. The populations were chosen to represent stages in a proposed model of the evolutionary breakdown of tristily to semi-homostyly; they differed in morph structure (trimorphic, dimorphic, or monomorphic) and floral traits likely to influence the mating system. The interpopulation range in outcrossing rate,  $t$ , was 0.96–0.29. Two additional populations from Jamaica, composed exclusively of self-pollinating, semi-homostylous, mid-styled plants, were invariant at 21 isozyme loci, precluding estimates of outcrossing frequency.

Trimorphic populations from Brazil had uniformly high outcrossing rates of 0.96–0.88. Values for the floral morphs within populations were not significantly different. A controlled pollination experiment, comparing the competitive ability of self and cross pollen using the *Got-3* marker locus, provided evidence that the maintenance of high outcrossing rates in trimorphic populations results from the prepotency of cross pollen and/or the selective abortion of selfed zygotes.

Morph-dependent variation in  $t$  was detected within a dimorphic population with the L morph outcrossing with a frequency of 0.76 in comparison with 0.36 in the M morph. The difference in the mating system of floral morphs results from modifications in position of short-level stamens in flowers of the M morph resulting in automatic self-pollination. The occurrence of *E. paniculata* populations composed exclusively of self-pollinating, mid-styled variants is thought to be associated with the spread of genes modifying stamen position. The high level of self-fertilization demonstrated in the M morph would allow automatic selection of these genes, augmented by fertility assurance in the absence of specialized pollinators.

Received November 14, 1985. Accepted July 8, 1986

Comparative studies of closely related plant species with contrasting breeding systems have provided evidence in support of the generalization that predominant self-fertilization is usually a derived condition in the angiosperms (Stebbins, 1957, 1974; Crowe, 1964; Grant, 1975; Jain, 1976; De Nettancourt, 1977). Heteromorphic incompatibility systems provide particularly useful experimental material for studies concerned with the evolution of selfing, since the genetic modifications and direction of change in mating behavior are often readily interpretable (Ernst, 1955; Dowrick, 1956; Baker, 1966; Charlesworth and Charlesworth, 1979; Shore and Barrett, 1985). Within each of the three tristylous families, trimorphic incompatibility has broken down repeatedly giving rise to autogamous population systems (Ornduff, 1972; Lewis, 1975; Barrett, 1979; Charlesworth, 1979; Ganders, 1979). The most common pathway involves relaxation and eventual loss of the incompatibility system followed by

changes in the relative positions of reproductive organs. The resulting phenotypes, known as semi-homostyles, have one set of anthers adjacent to the stigma and, as a result, are largely self-pollinating.

The evolutionary breakdown of tristily is particularly evident in the diploid, emergent aquatic, *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Populations of this species exhibit floral modifications ranging from complete tristily in NE Brazil to semi-homostyly on the island of Jamaica (Barrett, 1985a, 1985b). Surveys of style-morph frequency and studies of the reproductive ecology of populations in these two regions have enabled the formulation of a model of the breakdown process (Barrett, 1985b, unpubl.). Figure 1 illustrates the proposed stages in the breakdown of tristily to semi-homostyly. Of the 45 populations of *E. paniculata* surveyed, 22 were trimorphic, 9 were dimorphic, and 14 were monomorphic. Dimorphic populations are composed of long- and mid-styled plants, and monomorphic populations are fixed for a range of self-pollinating, semi-homostylous, mid-styled variants. The occurrence of modified mid-

<sup>1</sup> Current address: Department of Botany and Plant Sciences, University of California, Riverside, CA 92521.

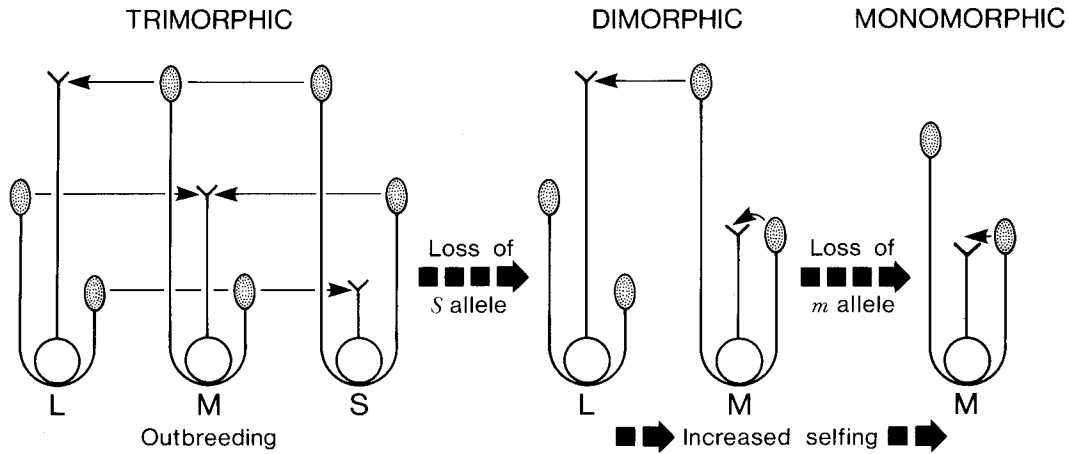


FIG. 1. Hypothetical stages in the breakdown of tristyliness to semi-homostyly in *Eichhornia paniculata*. Arrows indicate predominant matings. Note modifications in short-stamen position of the M morph and general reduction in size of reproductive organs associated with the evolution of selfing. After Barrett (1985b).

styled variants in varying frequency in dimorphic populations suggests that this morph replaces the long-styled morph as a result of the automatic selection of selfing genes and fertility assurance (Barrett, 1985b).

The model of the breakdown of tristyliness makes various assumptions about the mating system of populations of *E. paniculata* and predicts that the species should display both wide interpopulation variation in outcrossing rates as well as morph-specific differences in the levels of selfing in dimorphic populations (Fig. 1). To investigate these hypotheses we undertook an examination of mating patterns in 11 populations of *E. paniculata* using allozyme data. Populations chosen for study represented the range of morph frequencies found in the species and exhibited variation in floral traits likely to influence the mating system. We predicted that 1) trimorphic populations would be largely outcrossing, 2) dimorphic populations might display variable levels of outcrossing, depending on morph ratios and the degree of floral modifications, and 3) monomorphic populations would be largely self-fertilizing. Finally, since *E. paniculata* is self-compatible and progeny arrays using the style length loci as genetic markers indicated high levels of outcrossing in a tristylous population (Barrett et al., 1986), the possibility of prepotency of cross pollen over self pollen was examined in a pollination

experiment conducted under glasshouse conditions.

#### MATERIALS AND METHODS

##### *Field Sampling*

Seed families were collected in May–June 1982 from six populations of *Eichhornia paniculata* in NE Brazil (B) and five populations located on the island of Jamaica (J) during January 1983. The distribution of *E. paniculata* and location of populations used in the study are shown in Figure 2. Populations sampled in NE Brazil were chosen to represent the range of floral morph structures observed for the region, whereas in Jamaica all populations encountered during the 1983 season were sampled.

Within each population, floral morph frequencies were determined by direct count, or estimated from a sample of randomly chosen plants. Seeds from individual maternal genotypes were collected and stored separately. To avoid repeated sampling of genets, a minimum distance of 2 m between inflorescences was used. The number of seed families obtained from each population ranged from 10–65, depending on population size. For each family, between 8–30 individuals were grown under uniform glasshouse conditions in the spring of 1983 and winter of 1984. Three to four weeks after germination, seedlings were transplanted individually into 5.5 cm or 10.5 cm

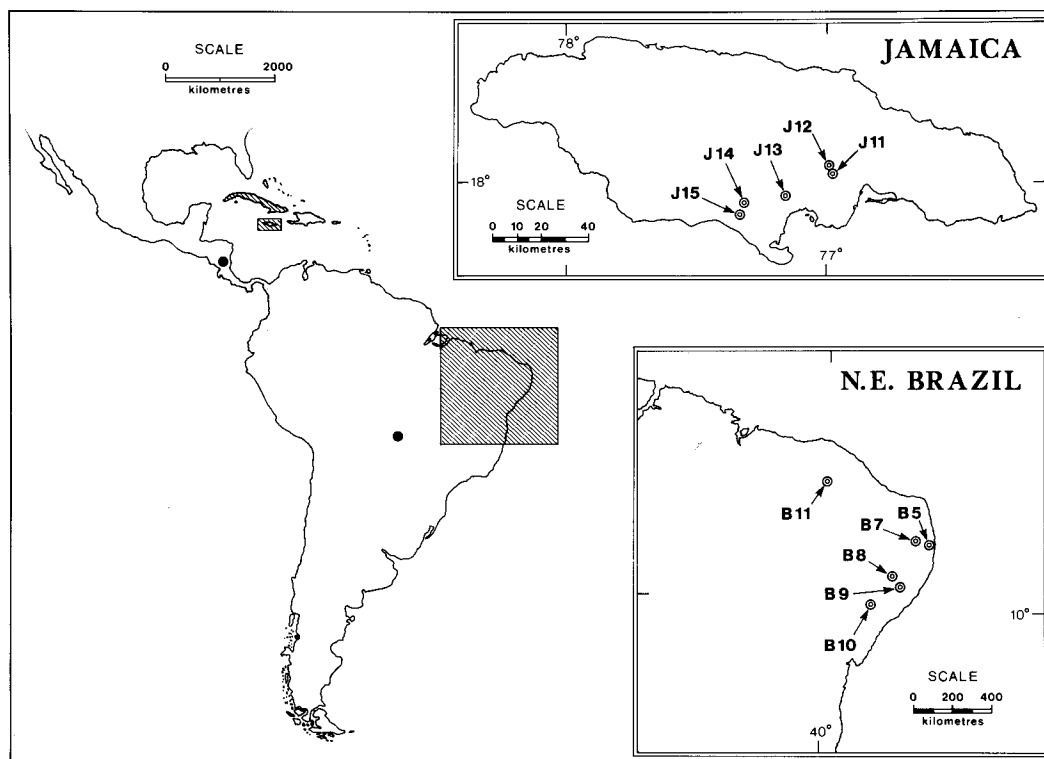


FIG. 2. Distribution of *Eichhornia paniculata* and location of populations sampled in NE Brazil and Jamaica.

pots. Mortality following germination was negligible.

#### *Electrophoretic Procedures*

Allozyme data were obtained using horizontal starch gel electrophoresis. Mature buds were found to provide the highest enzyme activity in *E. paniculata*. Accordingly, plants were grown until flowering, and buds were collected on the morning of a particular run. The buffer systems and references for staining procedures are given in Glover (1985). Only polymorphic loci were used for the estimation of mating-system parameters. Individuals were scored for 4–6 loci in trimorphic populations and 3–5 loci in dimorphic populations. Of the three monomorphic populations, two from Jamaica were invariant at all loci examined, and the remaining population from Brazil was polymorphic for one locus. The enzyme systems used were: diaphorase (DIA), glutamate dehydrogenase (GDH), malate dehydrogenase (MDH), peroxidase (PER), glutamate-oxaloacetate transaminase (GOT), phospho-

glucomutase (PGM), acid phosphatase (ACP), and phosphoglucose isomerase (PGI). For a given enzyme system, the most anodally migrating isozyme was designated 1, the next 2, and so on. Inheritance studies to document simple Mendelian control of the observed variation are presented in Glover (1985).

The number of progeny screened in each population ranged from 80–1,480. Larger sample sizes (1,480, 431, 547) were obtained for populations B5, B11 and B9, respectively, to enable a comparison of the outcrossing rates of the floral morphs. The remaining sample sizes are given in Tables 1 and 2.

#### *Mating System Estimation*

The multilocus model of Ritland and Jain (1981) was used to obtain mating-system parameters. The outcrossing rate ( $t$ ) was estimated via the Newton-Raphson method, and the pollen gene frequency ( $p$ ) was estimated by the expectation-maximization method. The most likely maternal genotype

TABLE 1. Location, floral morph frequency, approximate size, habitat, and number of seed families collected from 11 populations of *Eichhornia paniculata* from NE Brazil and Jamaica. L: long-styled morph; M: mid-styled morph; S: short-styled morph.

Designation	Location	Morph frequency			Approximate size	Habitat	Number of families collected
		L	M	S			
B5	Recife, Pernambuco, Brazil	0.35	0.36	0.29	1,000	roadside ditch	65
B7	Vitória de S. Antão, Pernambuco, Brazil	0.46	0.46	0.08	160	drainage ditch	15
B11	Quixadá, Ceará, Brazil	0.27	0.36	0.37	1,000	lakeshore	30
B9	União dos Palmares, Alagoas, Brazil	0.26	0.74*	—	110	low lying pasture	30
B10	Arapiraca, Alagoas, Brazil	0.11	0.89*	—	40	seasonal marsh	10
B8	Murici, Alagoas, Brazil	—	1.00*	—	55	pasture	10
J15	Pridee, Clarendon, Jamaica	0.44	0.56*	—	500	wet pasture	10
J14	Rhynsbury, Clarendon, Jamaica	0.17	0.83*	—	500	wet pasture	10
J12	Angels, St. Catherine, Jamaica	0.15	0.85*	—	150	seasonal pool	10
J11	Angels, St. Catherine, Jamaica	—	1.00*	—	100	seasonal pool	10
J13	Rosewell, Clarendon, Jamaica	—	1.00*	—	75	seasonal pool	10

\* Populations contain self-pollinating M individuals with modifications of the short stamen position.

of each family was inferred by the method of Brown and Allard (1970). Within populations B5, B11, and B9, estimates of  $t$  were computed for each floral morph. Single-locus estimates for all loci were also computed in all populations. Differences between outcrossing rates were tested using chi-square heterogeneity tests (Bailey, 1961).

#### Pollen Competition Experiment

Polymorphism at the *Got-3* locus was used to investigate competition between self and outcross pollen in genotypes originating from tristylous population B5. In tristylous species with sporophytic self-incompatibility, the strength of incompatibility varies with the stamen level of the pollen source. The highest level of seed set is obtained with pollen from anthers at a level equivalent to the stigma (termed "legitimate pollination"). Although *E. paniculata* is self-compatible (Barrett, 1985a) pollen originating from different anther levels was also compared to assess the possible occurrence of residual (weak) self-incompatibility. Three plants (either homozygous *aa* or *bb*) of each floral morph (L, M, and S) received three pollination treatments giving a total of 27 (3 genotypes  $\times$  3 floral morphs  $\times$  3 treatments) progeny arrays in the experiment. All pollinations were carried out on potted plants under glasshouse conditions between 8:00 A.M. and 12:30 P.M. on June 13, 1984. Treatment orders were assigned randomly to individuals and flowers. To control for the possibility of developmental effects, only inflorescences on their first or second day

of flowering were used. Two flowers per genotype received each treatment and were individually tagged. All other flowers on the inflorescence were removed. The pollination treatments used were: 1) self pollen from the nearest anther level to the stigma (long-level anthers in M flowers) versus outcross pollen from the nearest anther level to the stigma (long-level anthers in M flowers) in another individual of the same floral morph (illegitimate pollination); 2) self pollen as in treatment 1 versus outcross pollen from the anther level of equivalent height as the stigma in an individual of one of the other two floral morphs (legitimate pollination); 3) outcross pollen as in treatment 1 versus outcross pollen as in treatment 2. The second

TABLE 2. Multilocus estimates of outcrossing rate ( $t$ ) in populations of *Eichhornia paniculata* from NE Brazil and Jamaica. T: trimorphic; D: dimorphic; M: monomorphic.

Population	Morph structure	N (families/plants)	Number of loci used	$t$	SE
Brazil					
B5	T	65/1,480	6	0.96	0.025
B7	T	15/229	4	0.84	0.086
B11	T	27/432	5	0.94	0.023
B9	D	25/547	5	0.49	0.027
B10	D	7/85	5	0.77	0.067
B8	M	10/155	1	0.47	0.140
Jamaica					
J15	D	10/80	3	0.68	0.087
J14	D	10/80	3	0.29	0.070
J12	D	10/80	2	0.47	0.090

TABLE 3. Multilocus estimates of outcrossing rate ( $t$ ) for the floral morphs in populations of *Eichhornia paniculata* from NE Brazil. L: long-styled morph; M: mid-styled morph; S: short-styled morph.

Population	Floral morph	$N$ (families/plants)	$t$	SE	Heterogeneity $\chi^2$	$P$
B5	L	20/480	0.98	0.021	3.21 (2 <i>d.f.</i> )	ns
	M	23/520	0.97	0.018		
	S	22/480	0.93	0.022		
B11	L	9/156	0.95	0.040	5.71 (2 <i>d.f.</i> )	ns
	M	9/135	1.01	0.025		
	S	9/141	0.90	0.048		
B9	L	6/115	0.78	0.060	37.24 (1 <i>d.f.</i> )	$\leq 0.001$
	M*	19/432	0.36	0.032		

\* Population contains individuals with modifications of short stamen position.

pollen donor was of the alternate homozygous genotype at *Got-3* in all treatments.

Prior to pollination, long- and mid-level anthers were removed from S flowers, and long-level anthers were removed from M flowers. In order to expose the stigma of S flowers, slits were cut in the perianth and the upper portion gently folded back. Corresponding slits were cut in L and M flowers. For each flower, one anther load from each of the pollen sources was brushed over  $\frac{1}{2}$  the stigmatic surface with fine forceps. One anther produces several thousand pollen grains, so ample pollen was provided to ensure fertilization of all ovules (average ovule number is 94–118). In the two flowers that received the same treatment, the order of application was reversed. Mature seed was collected 11 days after pollination on June 24.

Progeny sample sizes are presented in Table 5. Mature buds were electrophoresed and scored for *Got-3*. Under the null hypothesis of no difference in competitive ability of the two pollen sources a 1:1 ratio of homozygous to heterozygous genotypes is expected. Ratios were tested using  $G$ -tests for goodness-of-fit and heterogeneity.

## RESULTS

Floral-morph frequencies, estimated population size, habitat type, and the number of seed families collected in each locality are presented in Table 1.

### Outcrossing Rates

Multilocus estimates of  $t$  in populations of *E. paniculata* ranged from 0.96–0.29. In NE Brazil, trimorphic populations had sim-

ilar outcrossing rates of 0.96, 0.84, and 0.94 for populations B5, B7, and B11, respectively (Table 2). Dimorphic populations in Brazil (B9 and B10) displayed intermediate values of 0.49 and 0.77, respectively. In Jamaica estimates of  $t$  for dimorphic populations ranged from 0.68–0.29. The only monomorphic population for which an estimate was obtained is B8, with  $t = 0.47$  (Table 2). Chi-square heterogeneity tests were performed on  $t$  values from trimorphic and dimorphic populations. Trimorphic populations were not significantly heterogeneous in outcrossing rates ( $\chi^2 = 2.07$ ,

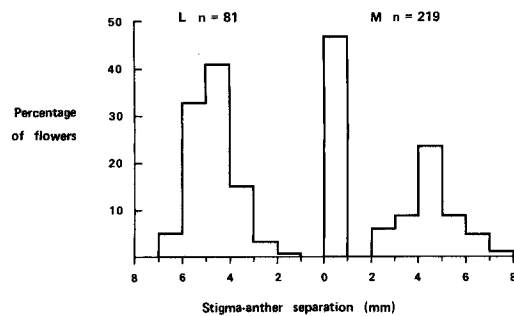


FIG. 3. Breakdown of herkogamy (separation between stigma and anthers) in the M morph of *Eichhornia paniculata* in population B9. The distributions are of the distance separating the stigma and the nearest mid-level anther in the L morph and the nearest "short-level" anther in the M morph. Flowers with no separation between the reproductive organs self-pollinate automatically. Three flowers were sampled from a single inflorescence from 27 and 73 plants of the L and M morph, respectively, grown under uniform glass-house conditions. Individual M plants produce both modified and unmodified flowers (see Barrett, 1985a), thus contributing to the discontinuous distribution for the M morph.

TABLE 4. Comparison of multilocus estimates of  $t$  with the minimum variance mean of single-locus estimates for populations of *Eichhornia paniculata* from NE Brazil and Jamaica.

Population	Number of loci	$t$ (multi-locus estimate)	$t$ (Mean of single-locus estimates)	Difference
Brazil				
B5 (L morph)	6	0.98	0.95	0.03
B5 (M morph)	6	0.97	0.91	0.06
B5 (S morph)	6	0.93	0.95	-0.02
B7	4	0.84	0.78	0.06
B11	5	0.94	0.89	0.05
B9	5	0.49	0.45	0.04
B10	5	0.77	0.67	0.10
Jamaica				
J15	3	0.68	0.50	0.18
J14	3	0.29	0.14	0.15
J12	2	0.47	0.44	0.03

$d.f. = 2$ , ns), whereas dimorphic populations were ( $X^2 = 29.58$ ,  $d.f. = 5$ ,  $P \ll 0.001$ ).

Table 3 presents outcrossing rates for the floral morphs within three Brazilian populations. For the two large, trimorphic populations estimates of  $t$  range from 0.90–1.01. Within each of the two populations, the floral morphs do not differ significantly in outcrossing rate (B5:  $X^2 = 3.21$ ,  $d.f. = 2$ , ns; B11:  $X^2 = 5.71$ ,  $d.f. = 2$ , ns) (Table 3). In contrast, estimates of  $t$  for the floral morphs within dimorphic population B9 were significantly different. The L morph exhibited an outcrossing rate of 0.78, while the M morph outcrossed with a frequency of 0.36 ( $X^2 = 37.24$ ,  $d.f. = 1$ ,  $P \ll 0.001$ ) (Table 3). This difference in mating system between the floral morphs is the result of a breakdown of herkogamy (stigma-anther separation) in the M morph resulting in the automatic self-pollination of flowers (Fig. 3).

There are other factors, in addition to self-fertilization, that can contribute to a departure from random mating ( $t = 1.0$ ) in plant populations. Spatial structuring of populations, assortative mating, or variation in outcrossing among maternal genotypes can all bias estimates of  $t$  downward (Clegg, 1980; Ennos and Clegg, 1982; Ritland and Ganders, 1985). Inbreeding caused by population structuring can be partly detected by a comparison of single-locus estimates with multilocus estimates. Inbreed-

ing will cause a greater downward bias in single-locus estimates compared to multilocus estimates. Since single-locus estimates vary in accuracy depending on allele frequencies and frequency of maternal genotypes, the means were computed by weighting estimates by the inverse of their variance. The differences (Table 4) are small in most populations, and no consistent deviation is evident. However, for populations B10, J14, and J15 differences are positive and relatively large.

#### Pollen Competition

The results of the pollination experiment are presented in Table 5. All pollinations resulted in ample seed, and high levels of germination and establishment were obtained in all replicates.  $G$ -tests of independence between treatments 1 and 2 revealed no significant association between the origin of pollen (stamen level) and the number of outcrossed progeny, either overall ( $G = 1.48$ ,  $d.f. = 1$ , ns) or by morph (L:  $G = 2.44$ ,  $d.f. = 1$ , ns; M:  $G = 0.063$ ,  $d.f. = 1$ , ns; S:  $G = 0.401$ ,  $d.f. = 1$ , ns). Hence, there is no evidence that "residual incompatibility" influences the competitive ability of outcross pollen types (legitimate or illegitimate) in comparison with self pollen. In subsequent analyses, data for treatments 1 and 2 were combined.

If the abilities of self and outcross pollen to fertilize ovules were not significantly different, the resulting progeny would segregate in a 1:1 ratio for homozygous and heterozygous genotypes at the *Got-3* locus. Pooling across morphs, a  $G$ -test of the observed segregation reveals a statistically significant lack of fit to a 1:1 distribution ( $G = 25.62$ ,  $d.f. = 1$ ,  $P \ll 0.001$ ). Almost twice as many progeny resulted from fertilizations by outcrossed pollen. Significant heterogeneity is evident among morphs ( $G_{\text{net}} = 16.25$ ,  $d.f. = 2$ ,  $P < 0.001$ ) and among genotypes ( $G_{\text{net}} = 22.71$ ,  $d.f. = 8$ ,  $P < 0.01$ ).

An unplanned test of the homogeneity of replicates testing for goodness-of-fit was used to delimit homogeneous sets of replicates (Sokal and Rohlf, 1981). The simultaneous test procedure identified a subset of five genotypes (31 L, 6 M, 42 M, 23 S, and 15 S) which together have a significant excess of outcrossed progeny ( $G = 11.75$ ,  $d.f. = 1$ ,

TABLE 5. Frequency of self and outcross progeny following controlled pollination of plants from tristylous population B5 of *Eichhornia paniculata*. All pollinations were carried out under glasshouse conditions. Mature buds were scored for genotype at *Got-3*. See text for a description of treatments. Self = progeny from self pollen, detectable as homozygous *aa* or *bb* at *Got-3*. Cross = progeny from outcross pollen, detectable as heterozygous *ab* at *Got-3*. Cross (1) = progeny from outcross pollen, detectable as homozygous *aa* or *bb* at *Got-3*. Cross (2) = progeny from outcross pollen, detectable as heterozygous *ab* at *Got-3*.

Maternal genotype	Floral morph	Treatment 1			Treatment 2			Treatment 3		
		Self	Cross	Total	Self	Cross	Total	Cross (1)	Cross (2)	Total
31	L	0.63	0.37	16	0.32	0.68	25	0.48	0.52	29
4	L	0.66	0.34	29	0.57	0.43	23	0.56	0.44	25
25	L	0.30	0.70	10	0	1.00	1	0.57	0.43	28
	Total	0.58	0.42	55	0.43	0.57	49	0.54	0.46	82
6	M	0.57	0.43	28	0	1.00	28	0.26	0.74	23
33	M	0.33	0.67	12	0.22	0.78	9	0.33	0.67	15
42	M	0.17	0.83	23	0.78	0.22	27	0.68	0.32	28
	Total	0.38	0.62	63	0.36	0.63	64	0.45	0.35	66
23	S	0.35	0.65	26	0.29	0.71	14	0	1.00	23
15	S	0.33	0.67	27	0.50	0.50	16	0.20	0.88	15
30	S	0.13	0.87	23	0.03	0.97	27	0.71	0.29	28
	Total	0.28	0.22	76	0.23	0.77	57	0.35	0.65	66
	Total	0.39	0.61	194	0.34	0.66	170	0.45	0.55	214

$P < 0.001$ ). Of the remaining four genotypes, three have significant excesses of outcrossed progeny (25 L, 33 M, 30 S), and family 4 L has a progeny ratio not significantly different from 1:1.

Among-family heterogeneity is not unexpected when the pollen source is from only one or two donors per pollination. However, it is clear from the data that outcross progeny are disproportionately represented in families in which competition between self and cross pollen was investigated.

Treatment 3 was designed to compare the competitive ability of two sources of outcross pollen (legitimate and illegitimate outcrosses). There was no significant difference from a 1:1 ratio overall ( $G = 1.87$ ,  $d.f. = 8$ ,  $P \ll 0.001$ ), although segregation among families was heterogeneous ( $G_{\text{het}} = 52.88$ ,  $d.f. = 8$ ,  $P \ll 0.001$ ). This result confirms the absence of residual trimorphic incompatibility favoring legitimate over illegitimate cross pollinations in *E. paniculata*.

#### DISCUSSION

Plant mating systems have traditionally been classified under broad headings (e.g., outbreeders, selfers, apomicts) based on inferences from floral traits and the patterns of intra- and interpopulation variation in morphological characters. During the last

decade, the availability of electrophoretic markers has enabled workers to obtain estimates of outcrossing rate in a large number of plant species (reviewed in Schemske and Lande, 1985). These quantitative studies have uncovered a wealth of variability both within and between species. Broad interpopulation ranges in  $t$ , as well as species with intermediate levels of selfing and outcrossing, have been observed (examples reviewed in Schemske and Lande, 1985; but see also Phillips and Brown, 1977; Jain, 1978; Rick et al., 1978; Hauptli and Jain, 1985; Ritland and Ganders, 1985). A major unresolved question concerns the relative contribution of genetic and environmental factors to the maintenance of this variation. On theoretical grounds, Lande and Schemske (1985) predict that equilibrium populations will rest at the extremes of predominant selfing or predominant outcrossing. If this were true, observed variation in  $t$  should always be attributable to nonequilibrium conditions or to environmental and demographic factors such as variation in pollinator service or plant density.

In *Eichhornia paniculata*, the observed outcrossing rates among populations ranged from 0.96–0.29. However, the highly autogamous semi-homostylous populations of *E. paniculata* from Jamaica undoubtedly

have considerably lower outcrossing rates. The complete monomorphism of the 21 loci examined in these populations precluded quantitative analysis of their mating systems. If we assume that these populations are predominantly self-fertilizing, then the mating system of *E. paniculata* populations spans the full range from highly selfing to predominantly outcrossing. This variation is associated with the breakdown of tristily to semi-homostyly within the species.

The uniformly high outcrossing rates observed in trimorphic populations (0.96–0.84) are unusual for a self-compatible, herbaceous species, and are comparable to outcrossing levels reported in long-lived gymnospermous trees. In the only other self-compatible heterostylous species for which data are available (*Amsinckia spectabilis*), Ganders et al. (1985) estimated outcrossing rates of 0.47 and 0.53 in two distylous populations. Although morphological tristily may function to promote some degree of cross-pollination, the disproportionate representation of outcrossed progeny in the glasshouse study (Table 5) suggests that prepotency of outcross pollen may be a major factor maintaining high outcrossing rates in trimorphic populations. However, since detailed observations of pollen-tube growth were not made, this conclusion is tentative. An alternative explanation is that the selective abortion of selfed embryos may occur. Since *E. paniculata* possesses multi-ovulate flowers, this is a distinct possibility. Studies are in progress to examine these phenomena.

The processes that result in variable outcrossing rates among dimorphic populations of *E. paniculata* are of central interest to the evolution of selfing via semi-homostyly in the species. Present-day populations may represent stages in the breakdown of tristily from large outcrossing trimorphic populations to predominantly inbreeding populations monomorphic for variants of the M morph. The breakdown model proposed by Barrett (1985b) postulates that loss of the *S* allele (and hence the S morph) results primarily from stochastic influences on population size in conjunction with random dispersal (see also Heuch, 1980), while loss of the *m* allele (and hence the L morph) is associated with the spread of genes mod-

ifying the position of short-level stamens in the M morph. For dimorphic populations the model predicts a mating asymmetry between the L and M morphs, with the former primarily outcrossing and the latter exhibiting a high degree of self-fertilization.

In only one dimorphic population (B9) were sample sizes sufficient to compare the mating patterns of the L and M morphs. Data from this population were in accord with the proposed model of mating-system evolution. The L morph outcrossed with a frequency of 0.78, whereas only 36% outcrossing was detected in the M morph. The contrast in mating system between the two morphs is undoubtedly the result of a shift in stamen position of the M morph. Since not all plants, or flowers within plants, automatically self-pollinate in the M morph, some degree of outcrossing occurs. Outcrossing rates in the M morph are likely to be strongly influenced by both the genetic and environmental factors governing the floral instability of genotypes. A detailed consideration of the nature of floral instability in *E. paniculata* and factors influencing intraplant variation in floral form will be presented elsewhere.

In some dimorphic populations of *E. paniculata*, it is possible that a protected polymorphism for selfing and outcrossing genotypes (Crosby, 1949; Maynard Smith, 1978) may be maintained. A stable equilibrium would be most easily obtained with high levels of inbreeding depression in progeny of M individuals and pollen discounting (Holsinger et al., 1984). However, pollen discounting is probably not a major obstacle to the spread of selfing genotypes in *E. paniculata* since long-level stamens of modified M flowers are unaffected by the change in position of "short-level" stamens and thus can continue to contribute genes to the outcrossed pollen pool. Studies of inbreeding depression are currently in progress to assess its significance to the evolution of mating systems in this species.

In the absence of counterselection, a single gene which confers an increased rate of self-fertilization will rapidly spread to fixation in populations where it arises (Fisher, 1941). In *E. paniculata*, modification of short-stamen position in the M morph is governed by relatively few recessive genes



(Barrett, unpubl.). Any fertility advantage to self-pollinating variants of the M morph owing to poor pollinator service would further augment automatic selection for selfing genotypes (Barrett, 1985b). It is significant that all monomorphic populations observed in NE Brazil and Jamaica are composed exclusively of this morph, and these populations are invariably small and inhabit temporary pools and ditches.

Individuals of *E. paniculata* that are self-pollinating would be favored in establishment on Jamaica following long-distance dispersal. This presumably accounts for the predominance of self-pollinating M variants on the island. Of 15 populations sampled on Jamaica, 12 were composed exclusively of this morph, and the remaining three were dimorphic (L, M) with modified mid-styled individuals predominating (Barrett, 1985b). *Eichhornia paniculata* is serviced by long-tongued, solitary bees in Brazilian populations (Barrett, 1985a), and the absence of a specialized pollinator fauna on Jamaica may also contribute toward selective pressures that favor self-pollination.

The lack of electrophoretically detectable variation in the monomorphic Jamaican populations (J11 and J13) may well be a consequence of the combined effects of isolation (lack of gene flow in an insular habitat) and inbreeding. Morphological studies indicate that monomorphic Jamaican populations are completely autogamous when grown under glasshouse conditions and that they show features typical of selfing relatives of primarily outcrossing taxa (Barrett, 1985a). These include lower pollen/ovule ratios, smaller, less showy flowers, and fewer flowers per inflorescence. Individuals in monomorphic Brazilian populations are characterized by similar, but less dramatic, alterations in floral traits. These populations may be more recently derived from tristylous ancestors, so that selection and drift have not altered floral traits and the reproductive economy of populations to the extent that is evident in Jamaica. The intermediate outcrossing estimate (0.47) obtained for population B8 is in accord with this suggestion. Individuals from this population displayed varying degrees of floral modification, with genotypes producing a mixture of modified and unmodified flowers.

The evolutionary processes responsible for the breakdown of tristily in *E. paniculata* may not be restricted to the species. In the closely related *E. crassipes* (Mart.) Solms, parallel patterns of floral-morph frequency occur (Barrett, 1977; Barrett and Forno, 1982), and similar modifications that increase selfing of the M morph (but not the L morph) have been documented in a dimorphic population (Barrett, 1979). Elsewhere, morph-dependent variation in the mating system has been associated with polymorphism for flower color in *Lupinus nanus* Dougl. (Horovitz and Harding, 1972) and *Ipomoea purpurea* (Roth.) (Brown and Clegg, 1984); presence or absence of ray florets in *Senecio vulgaris* L. (Marshall and Abbott, 1982); gynodioecism in *Thymus vulgaris* L. (Valdeyron et al., 1977) and *Limnanthes douglasii* R. Br. (Kesseli and Jain, 1984); and distily and homostily in *Primula vulgaris* L. (Piper et al., 1984). These types of polymorphic systems may provide the most suitable material for experimental studies of mating-system evolution.

#### ACKNOWLEDGMENTS

We thank Petra Donnelly for assistance with electrophoresis, Joel Shore and George Sidrak for aid in securing field collections, Kermit Ritland for providing computer programs to analyze data, and Tony Brown for advice. The manuscript benefited from the constructive criticism of Jim Eckenwalder, Kermit Ritland, and Joel Shore. Research was funded by an operating grant from the Natural Sciences and Engineering Research Council of Canada to S. C. H. Barrett and a University of Toronto Open Fellowship to D. E. Glover.

#### LITERATURE CITED

- BAILEY, N. T. J. 1961. Introduction to the Mathematical Theory of Genetic Linkage. Clarendon, London, U.K.
- BAKER, H. G. 1966. The evolution, functioning, and breakdown of heteromorphic incompatibility systems. *Evolution* 20:349-368.
- BARRETT, S. C. H. 1977. Tristily in *Eichhornia crassipes* (Mart.) Solms (Water Hyacinth). *Biotropica* 9:230-238.
- . 1979. The evolutionary breakdown of tristily in *Eichhornia crassipes* (Mart.) Solms. (Water Hyacinth). *Evolution* 33:499-510.
- . 1985a. Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Biol. J. Linn. Soc.* 25:41-60.

- . 1985b. Ecological genetics of breakdown in tristylly, pp. 267–275. In J. Haeck and J. W. Woldendorp (eds.), *Structure and Functioning of Plant Populations II: Phenotypic and Genotypic Variation in Plant Populations*. North-Holland, Amsterdam, Neth.
- BARRETT, S. C. H., A. H. D. BROWN, AND J. S. SHORE. 1986. Disassortative mating in tristylous *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). *Heredity*. In press.
- BARRETT, S. C. H., AND I. W. FORNO. 1982. Style morph distribution in New World populations of *Eichhornia crassipes* (Mart.) Solms-Laubach (Water Hyacinth). *Aquat. Bot.* 13:299–306.
- BROWN, A. H. D., AND R. W. ALLARD. 1970. Estimation of the mating system in open-pollinated maize populations using isozyme polymorphisms. *Genetics* 66:133–145.
- BROWN, B. A., AND M. T. CLEGG. 1984. Influence of flower color polymorphism on genetic transmission in a natural population of the common morning glory, *Ipomoea purpurea*. *Evolution* 38:796–803.
- CHARLESWORTH, B., AND D. CHARLESWORTH. 1979. The maintenance and breakdown of distyly. *Amer. Natur.* 114:499–513.
- CHARLESWORTH, D. 1979. The evolution and breakdown of tristylly. *Evolution* 33:489–498.
- CLEGG, M. T. 1980. Measuring plant mating systems. *BioScience* 30:814–818.
- CROSBY, J. L. 1949. Selection of an unfavorable gene-complex. *Evolution* 3:212–230.
- CROWE, L. K. 1964. The evolution of outbreeding in plants. I. The angiosperms. *Heredity* 19:435–457.
- DOWRICK, V. P. J. 1956. Heterostyly and homostyly in *Primula obconica*. *Heredity* 10:219–236.
- ENNOS, R. A., AND M. T. CLEGG. 1982. Effect of population substructuring on estimation of outcrossing rate in plant populations. *Heredity* 48:283–292.
- ERNST, A. 1955. Self-fertility in monomorphic *Primulas*. *Genetica* 27:391–448.
- FISHER, R. 1941. Average excess and average effect of a gene substitution. *Ann. Eugen.* 11:53–63.
- GANDERS, F. R. 1979. The biology of heterostyly. *N. Zeal. J. Bot.* 17:607–635.
- GANDERS, F. R., S. K. DENNY, AND D. TSAI. 1985. Breeding systems and genetic variation in *Amsinckia spectabilis* (Boraginaceae). *Can. J. Bot.* 66:533–538.
- GLOVER, D. E. 1985. Mating systems and genetic variation in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). M.S. Thesis. Univ. Toronto, Toronto, Ontario, Canada.
- GRANT, V. 1975. *Genetics of Flowering Plants*. Columbia Univ. Press, N.Y.
- HAUPTLI, H., AND S. K. JAIN. 1985. Genetic variation in outcrossing rate and correlated floral traits in a population of grain amaranth (*Amaranthus cruentus* L.). *Genetica* 66:21–27.
- HEUCH, I. 1980. Loss of incompatibility types in finite populations of the tristylous plant *Lythrum salicaria*. *Hereditas* 92:53–57.
- HOLSINGER, K. E., M. W. FELDMAN, AND F. B. CHRISTIANSEN. 1984. The evolution of self-pollination in plants. *Amer. Natur.* 124:446–453.
- HOROVITZ, A., AND J. HARDING. 1972. Genetics of *Lupinus*. V. Intraspecific variability for reproductive traits in *Lupinus nanus*. *Botan. Gaz.* 133:155–165.
- JAIN, S. K. 1976. The evolution of inbreeding in plants. *Ann. Rev. Ecol. Syst.* 7:469–495.
- . 1978. Breeding system in *Limnanthes alba*: Several alternative measures. *Amer. J. Bot.* 65:272–275.
- KESSELI, R., AND S. K. JAIN. 1984. An ecological genetic study of gynodioecy in *Limnanthes douglasii* (Limnanthaceae). *Amer. J. Bot.* 71:775–786.
- LANDE, R., AND D. W. SCHEMSKE. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39:24–40.
- LEWIS, D. 1975. Heteromorphic incompatibility system under disruptive selection. *Proc. R. Soc. Lond. B* 188:247–256.
- MARSHALL, D. F., AND R. J. ABBOTT. 1982. Polymorphism for outcrossing frequency at the ray floret locus in *Senecio vulgaris* L. I. Evidence. *Heredity* 48:227–235.
- MAYNARD SMITH, J. 1978. *The Evolution of Sex*. Cambridge Univ. Press, Cambridge, U.K.
- NETTANCOURT, D. DE. 1977. *Incompatibility in Angiosperms*. Springer-Verlag, Berlin, W. Ger.
- ORNDUFF, R. 1972. The breakdown of trimorphic incompatibility in *Oxalis* section *Corniculatae*. *Evolution* 26:52–65.
- PHILLIPS, M. A., AND A. H. D. BROWN. 1977. Mating system and hybridity in *Eucalyptus pauciflora*. *Austral. J. Biol. Sci.* 30:337–344.
- PIPER, J. G., B. CHARLESWORTH, AND D. CHARLESWORTH. 1984. A high rate of self-fertilization and increased seed fecundity of homostyle primroses. *Nature* 310:50–51.
- RICK, C. M., M. HOLLE, AND R. W. THORP. 1978. Rates of cross-pollination in *Lycopersicon pimpinellifolium*: Impact of genetic variation in floral characters. *Plant Syst. Evol.* 129:31–44.
- RITLAND, K., AND F. R. GANDERS. 1985. Variation in the mating system of *Bidens menziesii* (Asteraceae) in relation to population substructure. *Heredity* 54:235–244.
- RITLAND, K., AND S. K. JAIN. 1981. A model for the estimation of outcrossing rate and gene frequencies using  $n$  independent loci. *Heredity* 47:35–52.
- SCHEMSKE, D. W., AND R. LANDE. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39:41–52.
- SHORE, J. S., AND S. C. H. BARRETT. 1985. Genetics of distyly and homostyly in *Turnera ulmifolia* L. (Turneraceae). *Heredity* 54:167–174.
- SOKAL, R. R., AND F. J. ROHLF. 1981. *Biometry*, 2nd Ed. Freeman, San Francisco, CA.
- STEBBINS, G. L. 1957. Self-fertilization and population variability in the higher plants. *Amer. Natur.* 41:337–354.
- . 1974. *Flowering Plant Evolution above the Species Level*. Belknap, Cambridge, MA.
- VALDEYRON, G., B. DOMEJE, AND PH. VERNET. 1977. Self-fertilisation in male-fertile plants of a gynodioecious species *Thymus vulgaris*. *Heredity* 39:243–249.