

THE EVOLUTIONARY BREAKDOWN OF TRISTYLY
IN *EICHHORNIA CRASSIPES* (MART.) SOLMS
(WATER HYACINTH)

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Received February 14, 1978. Revised July 27, 1978

Evolutionary changes in the breeding systems of heterostylous plants have been documented in several unrelated flowering plant families. Modifications include the replacement of one type of outcrossing mechanism by another, such as the evolution of distyly from tristily in the Lythraceae (Lewis and Rao, 1971) and Oxalidaceae (Ornduff, 1972; Weller, 1976) and the origin of dioecism from distyly in the Rubiaceae (Baker, 1958) and Menyanthaceae (Ornduff, 1966a). More frequently heterostylous systems break down in the direction of increased self-fertilization; the commonest pathway to this condition is by the formation of self-compatible homostyles (Crosby, 1949; Baker 1966; Ornduff, 1972). Despite continued interest in heterostylous systems, little is known about the selective pressures which cause these changes and the advantages conferred by alterations in breeding system often are not apparent.

Tristyly has been documented in the Lythraceae, Oxalidaceae and Pontederiaceae (Vuilleumier, 1967). In the last family, tristily occurs in two of the eight genera. In *Pontederia*, the two species which have been studied experimentally (*P. cordata*, *P. rotundifolia*) exhibit trimorphic incompatibility, strong pollen trimorphism and populations which frequently contain a mixture of the three style forms. The breeding system of *Eichhornia azurea* resembles that of *Pontederia* species, self-incompatibility is accompanied by strong pollen trimorphism and populations are frequently trimorphic. In these species, tristily presumably functions as an outcrossing mechanism (Ornduff,

1966b; Barrett, 1977a, 1978). In contrast, the tristylous breeding system of *Eichhornia crassipes* is largely non-functional due to the predominantly monomorphic structure of populations and high levels of self-compatibility in the species. Although seed production occurs in the majority of populations, sexual reproduction is restricted due to the absence of suitable ecological conditions for seed germination and seedling establishment (Barrett, 1977b). The absence of sexual reproduction in many populations (Mulcahy, 1975; Barrett, 1977c) provides limited opportunities for evolutionary modifications of the breeding system. As a result, in asexual populations of *E. crassipes* the morphological features of tristily are retained despite relaxation of selective pressure responsible for the evolution and maintenance of floral polymorphism.

Although vegetative propagation is the principal means of reproduction in *E. crassipes*, sexual reproduction occurs in some seasonal habitats, particularly in the native range of the species in tropical South America. Here vegetative parts may be periodically destroyed and seeds play an important role in re-establishing populations (Barrett, 1977b). In sexual populations of *E. crassipes*, opportunities are provided for adjustments in the breeding system to meet local conditions. Seed set in populations of *E. crassipes* is submaximal due to low pollinator service and "inefficient" pollinators (Barrett, 1977b, c) and therefore modifications which would increase fecundity are likely to be at a greater selective advantage.

The purpose of this study is to docu-

ment the floral morphology, compatibility relationships and reproductive biology of a sexually reproducing population of *E. crassipes* in Costa Rica. Evidence is presented which suggests that floral trimorphism may break down in the direction of increased inbreeding in sexually reproducing populations.

MATERIALS AND METHODS

The sexually reproducing population of *Eichhornia crassipes* used for this investigation occurs in an extensive area of marshlands at Palo Verde, Guanacaste Province, Costa Rica. Field studies were undertaken during November, 1975. The marsh is surrounded by savanna vegetation and is bordered on its southwest edge by the Rio Tempisque. Due to the marked dry season in Guanacaste Province, parts of the marsh at Palo Verde are seasonal. Cycles of sexual reproduction in *E. crassipes* are associated with water level fluctuations in the marsh. Warm, shallow water permits seed germination and seedling establishment.

The population of *E. crassipes* consists of long- and mid-styled forms. In order to estimate the representation and seed production of floral forms, inflorescences and infructescences were gathered at 5 m intervals along several transects which bisected the marsh. Measurements of the reproductive organs of 25 flowers of each style form were also made. In order to determine pollen loads, one hundred stigmas were collected from each of the floral forms in the population after the completion of pollinator activity at 2:00 P.M. The samples of stigmas from each style form were acetolysed for 15 minutes in nine parts acetic anhydride:one part concentrated sulfuric acid, centrifuged, washed and the number of pollen grains was estimated. The acetolysis method was chosen because it is the most accurate method of determining pollen loads (Ganders, 1975a) although, due to the pooling of samples, it is not possible to estimate pollen loads of individual stigmas. The pollen production of floral forms was obtained by

the use of a hemacytometer (see Lloyd, 1965, for method).

The pattern of mating of floral forms in the population was estimated by progeny tests of naturally pollinated plants. Progenies from 25 individuals from the population were grown to flowering and the style forms were recorded. The average number of progeny grown from individuals plants was 36.6. The *M* locus (Francois, 1964; Barrett, 1977c) controlling the inheritance of mid- versus long-styled forms in *E. crassipes* was used as a marker gene. The frequency of homozygous recessives (long-styled forms) in the population was obtained during the sampling for population structure. Estimates of the proportions of homozygous dominants and heterozygotes in the population were obtained from progeny tests. Mid-styled plants that produced families with only mid-styled forms were considered to be homozygous dominants; families containing both mid- and long-styled forms were considered to be from heterozygotes. Frequencies of long-styled plants in long- and heterozygous mid-styled progenies were compared with expected values from complete selfing, random and complete disassortative mating (see Ganders, 1975b, for method). Insufficient fruits from semi-homostylous mids (see later) were collected to progeny test this group separately from "normal" mids.

An artificial pollination program was undertaken at Berkeley, California, during 1976 in order to determine the compatibility relationships of long- and mid-styled forms obtained from the population at Palo Verde. Sixty-five long-styled plants and 84 mid-styled plants were utilized in the crossing program. Plants were grown to flowering from seed and self- and cross-pollinations were conducted in a pollinator-free glasshouse with the aid of forceps. Capsule and seed production from the various pollen-carpel combinations were compared by analysis of variance. Autogamous seed set in floral forms was determined by recording capsule and seed set of undisturbed inflorescences.

Measurements of the reproductive organs of a single flower from each of 100 long- and mid-styled plants grown from seed from Palo Verde were compared with measurements from flowers of other *E. crassipes* populations. Comparisons involved nine clones of diverse origins grown under glasshouse conditions. In addition, measurements of floral parts were made on four natural populations of *E. crassipes*. Three populations were monomorphic for style form and were asexual (Arenal, Aranjuez, Costa Rica and Andytown, Florida), the remaining population (Wallisville, Texas) was composed of 69.6% long and 30.4% ($N = 483$) mid-styled forms and contained a limited number of seedlings during August, 1976. Details of the compatibility relationships and seed fecundity of glasshouse-grown clones and natural populations are presented elsewhere (Barrett, 1977c). The sizes of 100 pollen grains of each floral form at Palo Verde were measured with the aid of an ocular micrometer and compound microscope.

RESULTS

Floral morphology.—The sample of inflorescences ($N = 1272$) collected from Palo Verde yielded 1020 mid-styled and 252 long-styled forms, representing frequencies of 0.802 and 0.198 respectively. There are no short-styled forms in the population. Although flowers can be unequivocally assigned to a floral form, mid-styled flowers exhibit a wide variation in stamen and style configuration. Some inflorescences are composed of conventional mid-styled flowers whereas in others the stigma is adjacent to the long anther level (Fig. 1). This condition is known as semi-homostyly.

There were no significant differences in the sizes of reproductive organs from field or glasshouse samples from Palo Verde (Table 1). Mid-styled plants from other populations of *E. crassipes* do not show the same range of values for stigma-anther separation exhibited by the Palo Verde population. No semi-homostylous flowers



FIG. 1. Flower of mid-styled form of *Eichhornia crassipes* with 2 mm separation between stigma and anthers of long stamen whorl. Glasshouse grown plant, seed from Palo Verde, Costa Rica.

were observed in other populations of *E. crassipes* although the Arenal population (Costa Rica) exhibited values for mean stigma-anther separation which approached semi-homostyly.

Figure 2 illustrates the variation in distance between the stigma and nearest anther level in mid- and long-styled forms from Palo Verde. The range of variation is considerably lower in long-styled flowers than in mid-styled flowers. The mean distance from stigma to the nearest anther level is 10.4 mm in long-styled flowers and 4.5 mm in mid-styled flowers. There is a correspondence in the height of the short anther levels in mid- and long-styled forms and between the lengths of mid-styles and the mid-stamen level of long-styled forms. However, long anthers of mid-styled forms are below the level of stigmas of long-styled flowers. Hence the variation in distance between stigmas and anthers of mid-styled forms is a result of differences in the length of long stamens rather than the length of mid-styles.

There was some variation in the lengths of reproductive organs on a single inflorescence. However, the range of variation

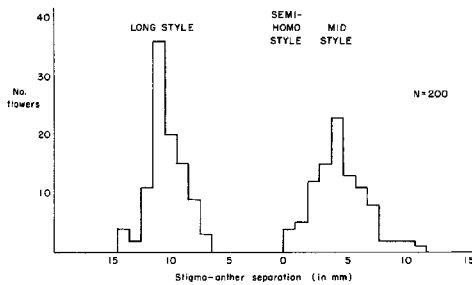


FIG. 2. Stigma-anther separation classes in flowers of *Eichhornia crassipes* from Palo Verde, Costa Rica.

was small compared to the total variation exhibited for a particular measurement.

In both mid- and long-styled forms, the mean sizes of pollen grains from the two anther levels are significantly different. Size dimorphism is weak with a considerable overlap in the range of pollen sizes from different anther levels. In common with most tristylous plants, pollen grains from the short anther levels of mid- and long-styled forms are similar in size. How-

ever, unlike the conventional situation, pollen from the mid anthers of long-styled plants does not differ significantly in size from pollen of the long anther level of mid-styled forms (Table 2). Associated with this correspondence in size of pollen is a similarity in the amount of pollen produced by the two style forms. There is no significant difference in the pollen production of the mid and long anther levels of long- and mid-styled plants ($F_{1,17} = 1.07$, N.S.). The short anther levels of long- and mid-styled flowers produce 34.7% and 37.1% more pollen respectively than the mid and long anther levels of these style forms.

Autogamy.—Floral forms of *E. crassipes* exhibit striking differences in their facility for autogamous seed set under glasshouse conditions (Table 3). Long-styled plants produced only one capsule from 195 flowers left undisturbed. Autogamy in flowers of mid-styled populations ranged from 0.0–66.9% capsule set. The highest value was obtained from mid-styled plants from Palo Verde with a 0–4

TABLE 1. Length of reproductive organs (\bar{x} and standard deviation in mm) in flowers of *Eichhornia crassipes*.

Population	Style form	Sample size	Style length	Long stamen length	Mid stamen length	Short stamen length	Stigma-anther sep.	Range
Field Measurements								
Costa Rica, Palo Verde	M	25	24.6 ± 2.3	28.9 ± 3.0	—	15.6 ± 1.5	4.3	0–8
Costa Rica, Palo Verde	L	25	35.4 ± 0.7	—	24.9 ± 0.9	15.8 ± 0.9	10.5	9–11
Costa Rica, Arenal	M	25	24.9 ± 1.3	28.6 ± 1.4	—	13.4 ± 1.2	3.7	2–5
Costa Rica, Aranjuez	L	25	32.5 ± 1.4	—	21.2 ± 1.2	11.0 ± 2.3	11.3	9–14
Texas, Wallisville	M	50	24.8 ± 1.3	32.0 ± 1.6	—	17.2 ± 1.1	7.2	4–12
Texas, Wallisville	L	50	32.8 ± 1.1	—	25.4 ± 1.5	15.9 ± 1.3	7.4	5–11
Florida, Andytown	M	25	25.9 ± 1.0	33.6 ± 1.1	—	17.2 ± 1.3	7.7	6–10
Glasshouse Measurements								
Costa Rica, Palo Verde	M	100	25.7 ± 1.7	30.2 ± 2.1	—	15.7 ± 1.8	4.5	0–16
Costa Rica, Palo Verde	L	100	34.7 ± 1.6	—	24.3 ± 1.4	15.2 ± 1.3	10.4	7–14
California, Stockton	M	25	25.9 ± 0.6	32.3 ± 0.7	—	16.1 ± 0.9	6.4	6–9
Louisiana, Orleans P.	M	25	24.8 ± 1.5	31.3 ± 1.3	—	16.5 ± 0.8	6.5	5–11
Florida, Hosfoud	M	25	25.4 ± 0.5	31.4 ± 0.8	—	15.4 ± 1.0	6.0	5–7
Mexico, Xochimilco	M	25	25.6 ± 0.7	32.0 ± 0.7	—	16.9 ± 1.0	6.4	6–7
S. Brazil, Porto Alegre	M	25	25.3 ± 0.6	31.6 ± 0.9	—	16.6 ± 1.0	6.3	5–7
Sudan, Khartoum	M	25	25.8 ± 0.8	31.4 ± 0.9	—	17.2 ± 0.7	5.6	5–6
Zaire, Congo River	M	25	25.4 ± 0.6	31.4 ± 1.2	—	16.6 ± 1.1	6.0	4–7
India, Calcutta	M	25	26.1 ± 0.7	33.5 ± 1.0	—	17.4 ± 0.8	7.4	6–9
Guyana, Mon Repos	M	25	24.4 ± 1.1	33.5 ± 1.0	—	17.3 ± 1.3	9.1	8–11

TABLE 2. Pollen size and production per flower in floral forms of *Eichhornia crassipes* from Palo Verde, Costa Rica.

Style form	Anther level		
	Long	Mid	Short
Long-styled			
Size (μm)	—	93.1 ± 3.5*	80.8 ± 3.7
Production (per fl.)	—	16,149 ± 1,265.7	24,987 ± 934.3
Mid-styled			
Size (μm)	92.7 ± 3.2	—	80.4 ± 3.8
Production (per fl.)	16,756 ± 1,287.4	—	26,659 ± 1,466.9

* \bar{x} and standard deviation.

mm separation between the mid stigma and long anther level. Self-pollination occurs during the withering of flowers. Anthers of the long stamens are pushed into contact with the mid stigma by the inflexion of perianth parts. The distance between the stigma and the anthers of the long stamen whorl influences the likelihood of successful pollination occurring in this way. This process does not occur in long-styled forms because of the difference in the relative position of reproductive organs.

Compatibility relationships.—Self-pollinations of long- and mid-styled forms of *E. crassipes* resulted in the production of near maximum numbers of capsules, indicating a high degree of self-compatibility in the two floral forms (Table 4). There was no significant difference in seed production using either anther whorl in self-pollinations. The interform illegitimate

crosses $M \times s/L$ and $L \times s/M$ also resulted in levels of seed production similar to those from self-pollinations conducted on each style form. Hence there was little difference within floral forms in the amount of seed produced by the three classes of illegitimate pollinations. However, mid-styled plants produced 29.6% more seed than long-styled plants in total illegitimate pollinations.

Legitimate pollinations in both style forms resulted in increased levels of seed production compared with illegitimate pollinations. However only in long-styled forms was this difference statistically significant (Table 4). In common with illegitimate crosses, legitimate crosses of mid-styled plants were more productive of seed than those of long-styled plants.

Although there was no difference in behavior of pollen from either anther level in self-pollinations of the two style forms,

TABLE 3. Autogamous seed set in *Eichhornia crassipes*.

Origin	Style form and stigma-anther separation (mm)	No. fls. undisturbed	No. fls. producing capsules	% capsule set	Total seeds produced	Av. seed set per flower
Palo Verde	Long 7-14	195	1	0.5	59	0.3
Palo Verde	Mid 4-8	197	18	9.1	791	4.0
Palo Verde	Mid 0-4	127	85	66.9	6,161	48.5
California	Mid 6-9	152	15	9.9	683	4.5
Louisiana	Mid 5-11	72	8	11.1	507	7.0
Florida	Mid 5-7	104	4	3.9	95	0.9
Mexico	Mid 6-7	147	9	6.1	340	2.3
S. Brazil	Mid 5-7	113	4	3.5	198	1.8
Sudan	Mid 5-6	98	6	6.1	311	3.2
Congo	Mid 4-7	91	9	9.9	405	4.5
Calcutta	Mid 6-9	64	6	9.4	125	2.0
Guyana	Mid 8-11	63	0	0	0	0

TABLE 4. *Compatibility relationships of floral forms of Eichhornia crassipes from Palo Verde, Costa Rica.*

Cross	No. of indiv. tested	No. fls. pollin.	No. fls. prod. caps.	% capsule set	Av. seed set per pollin.	SE of mean
LONG-STYLED						
<i>Illegitimate</i>						
L-1. L × m/L	16	102	102	100.0	94.1	10.6
L-2. L × s/L	7	51	50	98.1	78.9	8.2
L-3. L × s/M	21	158	157	99.4	85.6	4.8
<i>Legitimate</i>						
L-4. L × 1/M	21	134	134	100.0	125.2	8.0
MID-STYLED						
<i>Illegitimate</i>						
M-1. M × 1/M	18	109	108	99.1	129.1	8.7
M-2. M × s/M	26	159	155	97.5	122.2	12.1
M-3. M × s/L	19	138	138	100.0	124.8	8.0
<i>Legitimate</i>						
M-4. M × m/L	21	126	125	99.2	142.9	6.4

ANALYSIS OF VARIANCE.

Comparison	F	Degrees of freedom	Level of significance
L-1, 2, 3 versus L-4	18.61	1, 63	$\dot{P} = <.001$
M-1, 2, 3 versus M-4	2.62	1, 82	N.S.
L-1, 2 versus M-1, 2	8.29	1, 65	$P = .008$
L-4 versus M-4	2.96	1, 40	N.S.
L-3 versus L-4	17.88	1, 40	$P = <.001$
M-3 versus M-4	3.2	1, 38	$P = .09$

in cross-pollinations (legitimate and illegitimate) a significant difference in seed production was obtained (Table 4). Legitimate pollinations were more productive of seed than interform illegitimate pollinations. This pattern was most marked in the long-styled form where pollen from the long anther level of mid-styled plants produced 31.6% more seed than pollen from the short anther level. The differences in seed production following legitimate and interform illegitimate pollinations in mid- and long-styled forms indicate the presence of weak self-incompatibility in *E. crassipes*.

Pollination and seed set.—The major insect visitors to flowers of *E. crassipes* at Palo Verde are introduced honey bees (*Apis mellifera* L.) and small social bees of the Meliponidae. Both bee groups were observed collecting pollen from the two anther levels of long- and mid-styled

forms. Neither bee group was observed feeding on nectar. Foraging bees tended to visit most flowers on an inflorescence before departing.

Pollen loads of naturally pollinated stigmas of *E. crassipes* varied among the floral forms at Palo Verde. Due to the overlap in the range of pollen sizes from different anther levels, it is not possible to discriminate between the pollen types of *E. crassipes*. However, since the floral forms are thoroughly self- and cross-compatible, all pollen grains are capable of effecting fertilization. Mid-styled flowers with the smallest stigma-anther separation received more total pollen grains than the other floral types ($N = 29586$, \bar{x} no. grains per stigma = 295.9). The amount of pollen deposited on stigmas was intermediate in mid-styled forms with a 4–8 mm stigma-anther separation ($N = 19142$, \bar{x} no. grains per stigma = 191.4) and stigmas of

TABLE 5. Capsule and seed production of floral forms of *Eichhornia crassipes* at Palo Verde, Costa Rica.

Style form	Long-styled	Mid-styled
Total flowers sampled	529	689
% capsules set	72.2	80.1
\bar{x} fls. per inflor. ± s.e.m.	5.5 ± 0.1	5.2 ± 0.1
\bar{x} seeds per inflor. ± s.e.m.	189.8 ± 17.0	262.4 ± 19.0
\bar{x} seeds per capsule ± s.e.m.	52.6 ± 2.1	67.3 ± 2.6
\bar{x} seeds per flower ± s.e.m.	34.5 ± 1.9	50.7 ± 2.4

long-styled plants received the lowest numbers of pollen grains ($N = 11921$, \bar{x} no. grains per stigma = 119.2). These results suggest that the distance separating reproductive organs in floral forms strongly influences the amount of pollen deposited on stigmas.

The natural level of seed production of mid-styled plants at Palo Verde was significantly different from that of long-styled plants (Table 5). The average numbers of seeds per inflorescence ($t = 2.85$, $P = .005$), capsule ($t = 4.4$, $P = <.001$), and flower ($t = 5.24$, $P = <.001$) were all significantly higher in mid-styled plants than in long-styled plants. The average numbers of flowers produced on an inflorescence of both forms were similar.

Mating system.—A total of 914 progeny was grown to flowering from the 25 families obtained from Palo Verde. Of these

568 were mid-styled (62.1%) and 346 (37.9%) were long-styled. Nine of the 14 mid-styled families segregated long-styled plants indicating that they were heterozygous at the M locus. Frequencies of long-styled plants in segregating families of long- and heterozygous mid-styled plants are presented in Table 6. These frequencies are compared with expected values based on progeny ratios from complete selfing, random and disassortative mating. Of the 430 progeny raised from the long-styled plants, 66.3% were long-styled. This frequency lies mid-way between the expectations for selfing and random mating. All long-styled plants, except one, segregated a small number of mid-styled progeny. The exceptional plant produced 49 long-styled plants, suggesting that the individual may have been predominantly self-fertilized. The net selfing component of total matings in long-styled plants is 38.7%. The frequency of long-styled plants in progenies of heterozygous mid-styled plants is not significantly different from the expectations for selfing and random mating. Since the expectations for selfing and random mating are not significantly different from one another little can be deduced about the mating pattern of mid-styled plants, except that the amount of disassortative mating was low.

DISCUSSION

The syndrome of morphological and physiological features associated with heterostyly is thought to be governed by a

TABLE 6. Pattern of mating in long- and mid-styled forms of *Eichhornia crassipes* at Palo Verde, Costa Rica.

Parents	Long-styled progeny				Total progeny
	Observed	Expected (selfing)	Expected (random)	Expected (disassortative)	
Long-styled					
No.	285.0	430.0	193.5	138.5	430.0
%	66.3	100.0	45.0	32.2	100.0
Heterozygous mid-styled					
No.	61.0*	74.8*	67.3*	149.5	299.0
%	20.4	25.0	22.5	50.0	100.0

* Not significantly different at the 1% level.

supergene (Mather, 1950; Lewis, 1954; Dowrick, 1956). This locus is visualized as a group of contiguous genes, each responsible for the various sub-characters of heterostyly. Since the component genes are tightly linked, they usually segregate as a single unit or block. Within a supergene, rare crossing over can result in the formation of novel floral forms in a single generation. In many normally heterostylous taxa, flowers occur that have the style length of one floral form combined with the anther level of the other form. These floral types are termed homostyles, are usually self-compatible and are predominantly self-fertilizing.

Although supergenes controlling heterostyly have been demonstrated in distylous plants there is no experimental evidence of their occurrence in tristylous species. Evolutionary modifications in the tristylous syndrome of *E. crassipes* appear to be the result of genetic mechanisms other than those involving recombination in supergenes. Modifications in subcharacters of tristily appear to result from gradual changes in expression rather than through structural changes in the heterostyly supergene. Mid-styled forms at Palo Verde exhibit continuous variation in the length of the long stamen whorl. This variation may be caused by the action of modifier genes rather than that of major genes which govern the sub-characters of tristily. Mather (1950) demonstrated the presence of modifier genes in *Primula sinensis* which were capable of altering stamen and style lengths, resulting in homostyly. The genes segregated independently of the *S* gene and were considered to be polygenic in character and to constitute part of the background genotype of the species. Variability in the expression of heterostyly also occurs in *Amsinckia spectabilis*, where it may be due to modifier genes and possibly environmental factors (Ganders, 1975c).

In 4% of the mid-styled flowers sampled from Palo Verde, the upper set of anthers is adjacent to the stigma. This type of configuration of reproductive organs has been

termed semi-homostyly, rather than homostyly, since not all the anthers in a flower are adjacent to the stigma (Stout, 1925; Lewis, 1954; Ornduff, 1972). In those taxa which have been examined in detail, homostylous and semi-homostylous forms are considered to be secondarily derived from heterostylous ancestors (Crosby, 1949; Baker, 1966; Ornduff, 1972). François (1964) reported mid-styled flowers of *E. crassipes* in which the long level anthers were situated close to mid stigmas. He interpreted this condition as an indication that floral trimorphism was still in the process of being developed in *E. crassipes*. I prefer the view that the semi-homostylous condition in *E. crassipes* represents a derived reproductive trait. This view is based partly on analogy with other heterostylous groups. It is also supported by the relatively restricted occurrence of semi-homostyly in a sexual population of *E. crassipes* and by the presence in the native range of fully developed floral trimorphism in the species and in related taxa in the family (Barrett, 1977b, 1978).

Uchida (1953) described anomalous flowers of *E. crassipes* from Japan in which the anthers and stigmas were at the same level. However flowers of this type occurred on the same inflorescence as "normal" flowers indicating that in this circumstance, semi-homostyly had arisen as a result of abnormalities in development. In the present study, the range of variation in the lengths of stamens and styles of flowers on an inflorescence was small. Furthermore, measurements of floral parts of plants grown under field and uniform glasshouse conditions were similar. This indicates that the variation in length of reproductive organs has a major genetic component.

One problem concerns the difference in the degree of heterostyly expressed in the two floral forms at Palo Verde. While a reduction in the spatial separation of anthers and stigmas is apparent in the mid-styled form, there is little evidence of any change from the typical heterostylous condition in long-styled plants. Evolution of

semi-homostyly in the long-styled form would involve either the lengthening of the mid anther level or the shortening of the long style. It is possible that alterations of this type are prevented by developmental constraints. Alternatively, the floral morphology of long-styled plants may be maintained by selection.

Insect visitors to flowers of *E. crassipes* at Palo Verde were abundant during November, 1975. Nevertheless, the seed set of naturally pollinated plants was $\frac{1}{3}$ – $\frac{1}{2}$ that of artificially pollinated plants grown under glasshouse conditions. This suggests that seed production at Palo Verde is sub-maximal, at least during part of the year. This is probably due to ill-adapted and "inefficient" pollinators which limit fecundity in many populations of *E. crassipes*, particularly in the species' adventive range (Barrett, 1977c). Pollen collecting insects are more likely to cause pollination if the stigma is in close proximity to anthers. The data on pollen loads of naturally pollinated flowers supports this view. The results illustrate that an increase in pollen deposition accompanies a reduction in the distance separating reproductive organs. In addition, the facility for autogamous seed set is greatly enhanced by the floral morphology of mid-styled plants, particularly in those individuals with stigmas and anthers in close proximity. These factors probably account for the greater level of natural seed production in mid- than in long-styled forms. Although seed set at Palo Verde was sub-maximal during November, 1975, the values for mid-styled plants were the highest obtained in a survey of seed production in nineteen New World populations of *E. crassipes*. Details of the survey are presented elsewhere (Barrett, 1977c).

A further difference between the two floral forms emerges from the controlled crossing program. In all pollination types, the seed productivity of mid-styled plants was consistently higher than that of long-styled plants. The basis for this difference in reproductive potential is not known.

The differences in floral morphology and seed set between style forms at Palo Verde suggest that mid-styled plants are at a selective advantage compared to long-styled plants. Little is known of the history of *E. crassipes* at Palo Verde marsh, although it is likely that the species is introduced to the region. Standley (1928) considered *E. crassipes* to be adventive in Central America and native to the South American tropics (and see Sculthorpe, 1967, p. 462). The absence from Central America of the short-styled form of *E. crassipes* and of specialized pollinators of the species such as *Ancyloscelis gigas* (Barrett, 1977b) supports this view. Under an ill-adapted and perhaps limited pollinator regime it is likely that floral modifications which increase fecundity would be at a selective advantage. Selection for autogamy would therefore seem to be a plausible explanation for the occurrence of semi-homostyly at Palo Verde. Whether or not the mid-styled segment of the population at Palo Verde is currently evolving towards semi-homostyly is not known. It is possible that an equilibrium among the floral forms has been achieved.

The pattern of mating of the floral forms at Palo Verde provides evidence of the breakdown in function of heterostyly as an outcrossing mechanism in *E. crassipes*. Both floral forms experienced very low levels of disassortative mating. This was particularly surprising in the case of the long-styled form. Its low frequency in the population, combined with a floral morphology which in comparison with mid-styled forms would seem less likely to result in self-pollination, suggested high levels of disassortative mating. This expectation was not met. There was little difference between the mating patterns of long- and mid-styled plants. The low level of disassortative mating probably results from the pattern of insect visitation and strong self-compatibility. Foraging bees tended to visit most flowers on an inflorescence before departing.

In addition to floral morphology, other characters in *E. crassipes* exhibit devia-

tions from the orthodox heterostylous condition. In tristylous species, size dimorphism of pollen is substantially greater between the two anther whorls in mid- than in long- and short-styled forms (Dulberger, 1970; Ornduff, 1972; Weller, 1976). This is because there is normally a relationship between anther level and pollen size, and mid-styled forms possess the extreme anther levels (i.e. long and short). However, the mid- and long-styled forms of *E. crassipes* at Palo Verde exhibit similar pollen size differences. Size dimorphism is identical in both forms and the diameter of pollen from the mid anther level of long-styled flowers is similar to that from the long anther level of mid-styled plants (Table 2). Associated with these pollen size relationships is a similarity in the amount of pollen produced by the upper anther levels of the two style forms. This is in contrast to other tristylous species in which pollen production has been estimated, where mid level anthers produce substantially more pollen than long level anthers (Ornduff, 1975; Weller, 1976).

The most profound modifications of the tristylous breeding system of *E. crassipes* involve alterations in the compatibility relationships of floral forms. In the majority of heterostylous taxa, a physiological self-incompatibility system accompanies floral heteromorphism (Vuilleumier, 1967). However, in *E. crassipes* high levels of self-compatibility have been demonstrated in many populations (François, 1964; Mulcahy, 1975; Barrett, 1977b, c). The results of the present study are consistent with these findings; all illegitimate pollinations of long- and mid-styled plants from Palo Verde produced large amounts of seed. Although this indicates the presence of self-compatibility, evidence for weak or residual self-incompatibility in *E. crassipes* was also obtained. Illegitimate pollinations were less productive of seed than legitimate pollinations. Reduced seed set in self-pollinations can be due to weak self-incompatibility, but this effect may also result from the abortion of developing

embryos due to inbreeding depression (Brink and Cooper, 1947; Lloyd, 1968). Previous studies have not enabled a distinction to be made between these factors. However, since interform illegitimate pollinations between plants from Palo Verde yielded levels of seed set similar to those from self-pollinations, inbreeding depression phenomena are unlikely to be involved.

The high levels of self-compatibility currently exhibited by *E. crassipes* may represent the result of a relaxation in the strength of self-incompatibility. In general, where closely related taxa exhibit self-incompatibility and self-compatibility, the latter is considered to be derived (Lewis, 1954; Stebbins, 1957). Other tristylous taxa in the Pontederiaceae possess incompatibility systems of various strengths (Ornduff, 1966b; Barrett, 1977a, 1978), suggesting that trimorphic incompatibility was perhaps an ancestral condition in *Eichhornia*. Further evidence for the breakdown of trimorphic incompatibility in *E. crassipes* concerns the weakening of pollen heteromorphism in the species. A weakening and eventual loss of pollen heteromorphism is concomitant with breakdown in self-incompatibility in other heterostylous taxa (Baker, 1966; Ornduff, 1972). Trimorphic incompatibility in *Pontederia cordata*, *P. rotundifolia* and *Eichhornia azurea* is accompanied by strong pollen trimorphism (Ornduff, 1966; Barrett, 1977a, 1978), whereas in *E. crassipes*, pollen heteromorphism is weakly developed.

In *E. crassipes* the functioning of tristily as an outcrossing mechanism is disrupted as a result of the evolution of vigorous clonal propagation and the free-floating habit (Barrett, 1977b). These traits give *E. crassipes* prolific colonizing ability but favor the establishment of populations which are monomorphic or contain a predominance of one style form. High levels of self-compatibility further restrict outcrossing by allowing illegitimate fertilizations to occur. In ecological circumstances favorable for sexual repro-

duction, floral morphology may be subject to evolutionary modification, particularly in populations visited by an ill-adapted pollinator fauna. Perhaps the most likely outcome under these circumstances is the breakdown of floral trimorphism and the evolution of autogamy. The Palo Verde population may exemplify a transitional stage in the evolutionary sequence from outbreeding to inbreeding in the species. Progeny tests of semi-homostylous plants would provide an opportunity to verify this suggestion.

Elsewhere in *Eichhornia*, an autogamous, semi-homostylous race is reported in the normally tristylous *E. azurea* (Barrett, 1978) and *E. heterosperma*, *E. natans* and *E. diversifolia* are exclusively semi-homostylous (Barrett, unpubl.). In *Pontederia*, four species are tristylous and a single semi-homostylous species (*P. parviflora*) is considered to be derived from a tristylous ancestor (Lowden, 1973). This suggests that breakdown of floral trimorphism has occurred on a number of occasions in the Pontederiaceae and in each case has resulted in the evolution of inbreeding groups. Due to the limited occurrence of sexual reproduction in *E. crassipes*, evolutionary modifications of floral trimorphism are likely to occur at a considerably slower rate than in other heterostylous taxa.

SUMMARY

Although vegetative propagation is the principal means of reproduction in *Eichhornia crassipes*, sexual reproduction does occur in certain seasonal habitats. A sexually reproducing population of *E. crassipes* at Palo Verde, Costa Rica, is composed of 80.2% mid- and 19.8% long-styled forms. The expression of heterostyly differs in the two forms. Wide variation in the length of the long stamen whorl occurs in mid-styled forms, whereas long-styled forms exhibit the conventional configuration of reproductive organs. In 4% of mid-styled flowers, the long anther level is adjacent to the stigma, a condition known as semi-homostyly. Size dimor-

phism of pollen from the two anther levels of each style form is identical. Both floral forms are highly self-compatible, but differences in seed set following legitimate and inter-form illegitimate pollinations demonstrate the presence of weak or residual self-incompatibility. Autogamous seed set is greatly enhanced by the close proximity of anthers and stigma in semi-homostylous flowers. Long-styled flowers set 0.5% capsules autogamously compared with 0.0–66.9% in mid-styled flowers. In naturally pollinated flowers, an increase in pollen deposition accompanies a reduction in the distance separating anthers and stigmas. Seed production in artificial and naturally pollinated flowers is significantly higher in mid- than in long-styled forms. Progeny tests of naturally pollinated plants indicate that both floral forms experience low levels of disassortative mating.

The Palo Verde population of *E. crassipes* may exemplify a transitional stage in the evolutionary sequence from outbreeding to inbreeding in the species. Selection for autogamy during periods of unreliable or inefficient pollinator activity may account for the evolution of semi-homostyly in *E. crassipes*.

ACKNOWLEDGMENTS

I thank Herbert Baker, Robert Ornduff and Stephen Weller for comments on an earlier draft of the manuscript. I am grateful to Suzanne Barrett, Irene Baker and Donald Seaman for assistance with data collection and to Beryl Simpson for providing a Japanese translation. Field studies were supported by N.S.F. grant GB40747X to H. G. Baker.

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