J. evol. Biol. 6: 591–608 (1993)

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Multiple origins of self-fertilization in tristylous *Eichhornia paniculata* (Pontederiaceae): Inferences from style morph and isozyme variation

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Key words: Mating-system evolution; origins of self-fertilization; tristyly; isozymes; genetic differentiation; *Eichhornia paniculata.*

Abstract

Populations of *Eichhornia paniculata* (Pontederiaceae) exhibit a wide range of mating systems, from predominant outcrossing to high levels of self-fertilization. The origin of self-fertilization in this tristylous species is associated with the loss of style-length morphs from populations and the spread of self-pollinating, floral variants. We examined geographic variation in style morph and allozyme frequencies to determine whether the loss of style morphs and transition to selfing could have multiple origins in *E. paniculata.* Surveys of floral variation in 167 populations from six states in northeastern Brazil revealed that at least one style morph was absent from 29.3%. Non-trimorphic populations occurred in all states and ranged in frequency from 9% in Ceará to 68% in Alagoas. Selfing variants occurred in 8.5% and 55% of trimorphic and non-trimorphic populations, respectively, and were distributed among five of six states with primary concentrations in Alagoas and Pernambuco. A comparison of electrophoretic variation at 24 isozyme loci in 28 trimorphic, 13 dimorphic and 3 monomorphic populations indicated that non-trimorphic populations contained 84% of the allelic variation present in trimorphic populations and were markedly differentiated from one another. Analyses of genetic distance and the distribution of rare alleles indicated that non-trimorphic populations were often more similar to neighbouring trimorphic populations than to one another. Populations with selfing variants occurred at low frequency in three genetically distinct parts of the range. These results, in combination with genetic and morphological evidence suggest that style morphs are lost repeatedly

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from populations of *E. paniculata* and that selfing variants may have originated on at least three separate occasions in northeastern Brazil.

Introduction

The transition from outcrossing to self-fertilization has occurred on numerous occasions among herbaceous flowering plant families (Stebbins, 1957, 1974; Jain, 1976; Wyatt, 1988). Explanations for the origin of self-fertilization are often inferred from geographical patterns of mating-system variation and various demographic and ecological correlates (Baker, 1955; Rick et al., 1977; Lloyd, 1980; Schoen, 1982; Wyatt, 1986; Barrett and Shore, 1987). However, interpreting this variation may be difficult in the absence of information on the historical relationships among populations, since the extent of past colonization and migration events will determine to what degree different populations behave as independent evolutionary units (Avise et al., 1987; Slatkin and Maddison, 1989). The evolution of self-fertilization is of particular interest in this regard because selfing appears to be more readily achieved than other breeding-system changes (Raven, 1978; Grant, 1981; Gottlieb, 1984; Barrett and Shore, 1987; Wyatt, 1988) such as the shift to apomixis (Marshall and Brown, 1981), dioecy (Donoghue, 1989) and various self-incompatibility systems (Olmstead, 1989; Lloyd and Webb, 1991). For any taxon or group of conspecific populations, it is therefore important to determine whether selfing has single or multiple origins, and, if the latter, whether common causal mechanisms may be involved.

In the annual, emergent aquatic *Eichhornia paniculata* (Pontederiaceae), the evolution of self-fertilizing populations results from the breakdown of its predominantly outcrossing, tristylous breeding system (Barrett et al., 1989). Increased self-fertilization in populations appears to be associated with the change in stylemorph structure from trimorphism through dimorphism to monomorphism and the spread of self-pollinating, floral variants (Barrett, 1985a, b). Earlier surveys of the geographic distribution of non-trimorphic populations indicated that self-fertilization was more commonly found at the periphery of the species range (Barrett et al., 1989). The marginal distribution and small size of many of these populations led to the suggestion that stochastic forces such as founder events and genetic drift play a prominent role in mating-system evolution in the species (Barrett, 1989).

Theoretical studies of stochastic forces indicate that style morphs should be readily lost by sampling error from small tristylous populations (Heuch, 1980; Barrett et al., 1989; Eckert and Barrett, 1992). In *E. paniculata*, dimorphic populations are smaller on average than trimorphic populations; however it is not clear whether different dimorphic populations are the result of separate evolutionary events and, furthermore, whether morph loss is always associated with the evolution of self-fertilization. To investigate these issues we have undertaken an historical analysis of the evolution of self-fertilization by determining the genetic relationships between *E. paniculata* populations with contrasting morph structure using allozyme data.

Here we 1) describe the geographical distribution of style morphs in *E. paniculata* populations in northeastern Brazil, 2) examine the association between population morph structure and the presence of selfing variants, 3) compare levels of genetic variability in trimorphic and non-trimorphic populations, and 4) examine the genetic relationships among trimorphic and non-trimorphic populations and among populations with and without self-pollinating variants. The results are used to address the question of the origin of self-fertilization in *E. paniculata*. Specifically, we evaluate the alternate hypotheses that the breakdown of tristyly leading to predominant self-fertilization has had a single origin followed by subsequent migration of selfing populations to other parts of the species range or whether selfing has had multiple origins. Comparative data on population genetic structure are also used to assess the role of stochastic forces in the evolution of self-fertilization in *E. paniculata*.

Materials and methods

Population survey of style morph variation

We surveyed 167 populations of *E. paniculata* in northeastern Brazil for the occurrence of long-, mid- and short-styled morphs (hereafter L, M, S morphs). Morph frequencies were estimated from a complete census of inflorescences in populations less than 250 individuals, and from at least 100 inflorescences in larger populations. *E. paniculata* occurs in discrete pools and ditches so populations are readily distinguished. Because plants are not clonal and flower synchronously, estimates of morph representation could be made unambiguously on a single visit. Populations were classified as either trimorphic, dimorphic or monomorphic depending on the number of style morphs. In addition, the frequency of self-pollinating variants of the M morph was recorded in each population. These variants are morphologically distinct and readily identified in the field. Details of the floral biology (Seburn et al., 1990; Barrett and Harder, 1992) and mating system (Barrett et al., 1989; Barrett and Husband, 1990) of these variants are presented elsewhere.

Populations were sampled during the period 1982–1989 along most major roadways in the states of Ceará, Paraíba, Pernambuco, Alagoas, Sergipe and Bahia (Fig. 1). For populations sampled more than once during this period, we report only the initial estimate of morph frequencies. Most populations occurred in a band running parallel to the coast, on the eastern margin of the dry interior. Despite extensive searches, no populations were found in Rio Grande do Norte and only six in the neighbouring state of Paraíba.

Electrophoretic analysis

Geographic patterns of isozyme variation, were examined in 44 populations of *E. paniculata* using starch gel electrophoresis. Here we assumed that isozyme variants

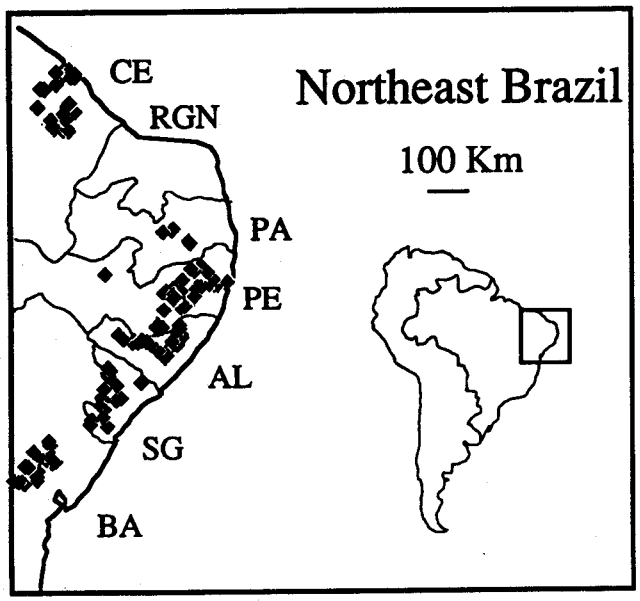

Fig. 1. Map of the 167 populations of *Eichhornia paniculata* surveyed for morph frequencies in six states in northeastern Brazil (AL – Alagoas; BA – Bahia; CE – Ceará; PA – Paraíba; PE – Pernambuco; SG – Sergipe). No populations were found during surveys of Rio Grande do Norte (RGN).

at a large number of loci can be treated as selectively neutral and hence their distribution should reflect the historical patterns of colonization and migration among populations. Populations were sampled in six states (Alagoas 9 pops., Bahia 3 pops., Ceará 12 pops., Paraíba 3 pops., Pernambuco 11 pops., Sergipe 6 pops.) corresponding to the major area of distribution of the species in Brazil (Tab. 1). Open pollinated seed families were collected from 4–59 (mean = 26 families/population) randomly selected individuals from each population, depending on its size. Following germination, between 1–20 (mean = 6) seedlings per family were transplanted into individual pots and grown to flowering under glasshouse conditions.

Flower buds were extracted in a 0.02 M Na_2HPO_4 buffer (pH 7.4) containing DL-dithiothreitol. Enzymes were resolved on two buffer systems: aspartate amino transferase (AAT), glutamate dehydrogenase (GDH), triose phosphate isomerase (TPI) and diaphorase (DIA) on a lithium-borate buffer system; aconitase (ACO), acid phosphotase (ACP), isocitric dehydrogenase (IDH), malate dehydrogenase (MDH), peroxidase (PER), phosphoglucose dehydrogenase (PGD), phosphoglucose isomerase (PGI), and shikimic dehydrogenase (SkDH) on a histidine-citrate buffer system. For further details of electrophoretic methods, see Glover and Barrett (1987).

The number and types of alleles present, the number of alleles per locus (k_a) and the percentage of loci that were polymporphic (*PLP*) were compared among populations. A locus was designated as polymorphic if the frequency of the most common allele was less than 0.95. Levels of genetic variation (k_a, *PLP*) were compared among trimorphic, dimorphic and monomorphic populations in a one-way ANOVA. Both variables met the assumption of normality; however, *PLP* was arc-sine transformed to reduce heterogeneity of variances. To account for any

Table 1. Summary of the 44 populations of *Eichhornia paniculata* sampled in northeastern Brazil for allozyme variation. Included are the population code, locality and number of families/progeny screened for each population.

Population	Location	Families/progeny
B31	Fortaleza 1, Ceará	12/44
B32	Fortaleza 2, Ceará	8/69
B33	Maranguape 1, Ceará	7/48
B34	Maranguape 2, Ceará	59/604
B35	Maranguape 3, Ceará	15/150
B39	Quixadá 1, Ceará	20/172
B41	Quixadá 3, Ceará	39/88
B43	Quixadá 4, Ceará	13/50
B44	Quixadá 5, Ceará	33/164
B46	Quixadá 7, Ceará	25/25
B49	Quixadá 12, Ceará	22/55
B54	Pacajus, Ceará	34/166
B55	Patos 1, Paraíba	31/80
B56	Patos 2, Paraíba	68/417
B58	Belo Jardim 1, Pernambuco	35/186
B59	Belo Jardim 2, Pernambuco	22/196
B62	Jupi 3, Pernambuco	29/123
B63	Garunhuns 1, Pernambuco	47/274
B68	Capela 1, Alagoas	10/70
B69	Murici 1, Alagoas	40/386
B70	Murici 2, Alagoas	25/145
B71	União dos Palmarés 1, Alagoas	35/300
B72	Ibateguara, Alagoas	18/110
B75	Agrestina 1, Pernambuco	18/177
B77	Gravatá 1, Pernambuco	21/204
B78	Gravatá 2, Pernambuco	22/131
B81	Vitoria 3, Pernambuco	21/95
B85	Limoeiro 1, Pernambuco	36/155
B86	Limoeiro 2, Pernambuco	4/23
B88	Umbuzeiro 2, Paraíba	28/140
B100	Campina Grande 1, Paraíba	25/98
B115	Capela 2, Alagoas	22/255
B117	Anadia 3, Alagoas	11/58
B120	Itabaiana, Sergipe	37/320
B123	Arauá 1, Sergipe	17/143
B124	Arauá 2, Sergipe	52/363
B147	Poco Redondo, Sergipe	26/158
B149	Curituba 1, Alagoas	15/15
B151	Mte Alegre 1, Sergipe	32/124
B152	Mte Alegre 2, Sergipe	17/117
B158	Mte Alegre 8, Sergipe	30/101
B165	Feira de Santana 1, Bahia	21/146
B168	Feira de Santana 4, Bahia	10/15
B169	Feira de Santana 5, Bahia	16/30

geographic differences in genetic variation, we conducted a two-way ANOVA with morph structure and state as fixed effects. In addition, we compared allozyme diversity in six trimorphic-dimorphic population pairs, representing each state. Populations in each pair were separated by less than 10 km and are therefore likely to have similar genetic backgrounds. The similarity between each trimorphic and dimorphic population was assessed by comparing the percentage of loci polymorphic and the total number of alleles, including those shared and those exclusive (U_a) to each population. One potential problem with this comparison is that sample sizes for dimorphic populations are generally smaller than for trimorphic populations, because of their differences in population size (Husband and Barrett, 1992). However, sampling was undertaken in proportion to the number of individuals in populations so the probability of detecting rare alleles is comparable. As a result, differences among trimorphic and dimorphic populations is unlikely to be an artifact of sampling but a result of differences in diversity.

Relationships among populations were inferred using genetic distances derived from allele frequency data (Nei, 1978) and the unweighted pair-group clustering method (UPGMA, Sneath and Sokal, 1973). Gene frequencies and genetic distances were computed using the BIOSYS program (Swofford and Selander, 1981). Standard errors for each genetic distance estimate were calculated using methods outlined in Ritland (1989). For our purposes, all population clusters represented by a branch in the dendogram whose length is greater than twice the standard error are considered statistically distinct. The correlation between genetic distance and geographic distance matrices were measured using Kendall's tau statistic and tested against a theoretical distribution generated by 2000 random permutations of the genetic distance matrix (Dietz, 1983).

Results

Survey of the style-length polymorphism

Of the 167 populations sampled in northeastern Brazil, 118 (70.7%) contained all three style morphs, 42 (25.1%) contained two, and seven (4.2%) were fixed for a single morph (Tab. 2). Most dimorphic populations (85.7%) contained L and M morphs and all monomorphic populations were fixed for the M morph. Self-pollinating variants of the M morph occurred in 8.5% of trimorphic populations, 50% of dimorphic populations, and in all but one (85.7%) of the monomorphic populations (Tab. 3). When present, the variants were infrequent in trimorphic populations (5% of individuals), were more frequent in dimorphic populations (37%), and were fixed in monomorphic populations (Tab. 3).

Geographic patterns

Trimorphic populations were abundant throughout the region sampled and were most frequent, relative to non-trimorphic populations, in the most southern (Bahia

Table 2. Style morph structure of *Eichhornia paniculata* populations surveyed in six states in northeastern Brazil during 1982–1989. Values are the percentage of populations from a given state in each category (n = the number of populations sampled in each state).

State	n	Trimorphic	Dimorphic			Monomorphic		
		LMS	LM	LS	MS	L	M	S
Alagoas	22	18.0	68.0	4.0	0.0	0.0	9.0	0.0
Bahia	23	78.0	9.0	9.0	4.0	0.0	0.0	0.0
Ceará	46	91.0	9.0	0.0	0.0	0.0	0.0	0.0
Paraíba	6	67.0	33.0	0.0	0.0	0.0	0.0	0.0
Pernambuco	52	69.0	23.0	0.0	0.0	0.0	8.0	0.0
Sergipe	18	78.0	6.0	6.0	6.0	0.0	6.0	0.0
Total	167	70.6	21.6	2.4	1.2	0.0	4.2	0.0

Table 3. Distribution and frequency of self-pollinating variants of the M morph in trimorphic, dimorphic and monomorphic populations of *Eichhornia paniculata* in northeastern Brazil.

	Trimorphic	Dimorphic	Monomorphic	Total
n	118	42	7	167
Pops. with self-pollinating variants (%)	10 (8.5)	21 (50.0)	6 (85.7)	37 (22.2)
Mean frequency in occupied pops.	0.05	0.37	1.00	0.39
Mean frequency in all pops.	0.005	0.191	0.857	0.086

and Sergipe) and northern (Ceará) states (Tab. 2, Fig. 2A). Dimorphic populations were represented in every state examined ranging from 8.7% of all populations in Ceará (n = 46 pops.) to 68% in Alagoas (n = 22 pops.; Tab. 2, Fig. 2B). The major concentration of dimorphic populations (67% of all dimorphic pops.) occurred in the neighbouring states of Alagoas and Pernambuco.

Eighty-six percent of dimorphic populations contained L and M morphs; these occurred in all states surveyed. Dimorphic populations with L and S, or M and S morphs were few in number and restricted to the southern states of Alagoas, Sergipe and Bahia (Tab. 2). All but one monomorphic population occurred in the central region of Alagoas and Pernambuco (Fig. 2C). The single outlier, found in Sergipe state, contained a few individuals and was the only monomorphic population with no self-pollinating variants.

Self-pollinating variants of the M morph were widely distributed in northeastern Brazil, occurring in five of the six states surveyed (Fig. 2D). Primary concentrations were evident in Alagoas and Pernambuco, with sporadic occurrences in Ceará, Paraíba and Sergipe. Only two of the 46 populations surveyed in the northern state of Ceará contained self-pollinating variants.

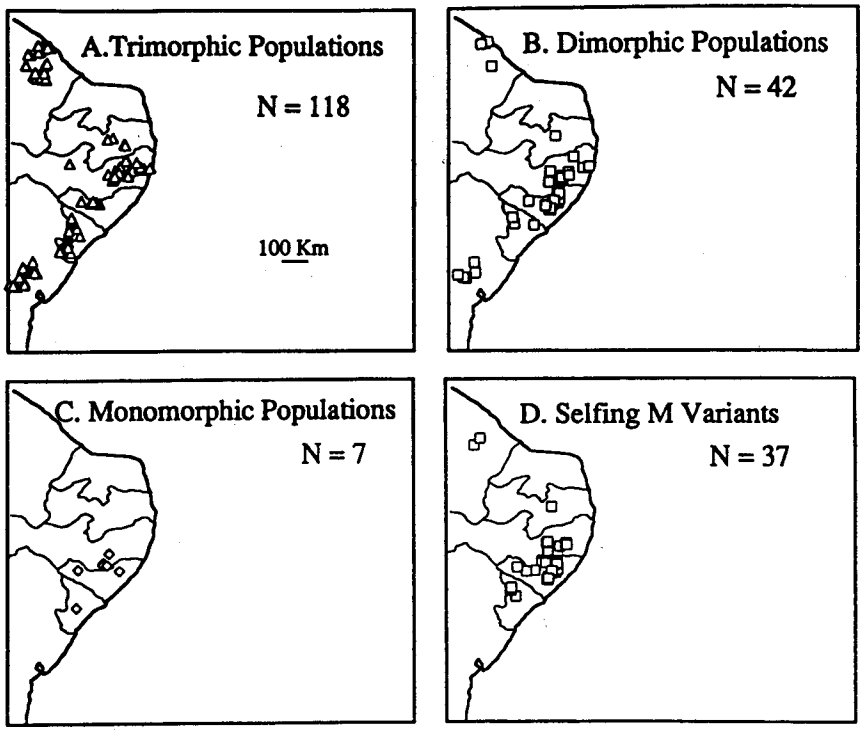

Fig. 2. Maps of northeastern Brazil showing the distribution of A) trimorphic, B) dimorphic and C) monomorphic populations sampled from 1982 to 1989. Map D shows the distribution of populations with self-pollinating variants of the M morph.

Electrophoretic analysis

Twenty (83.3%) of the 24 isozyme loci surveyed were polymorphic in at least one of the 44 populations examined. The loci *Tpi-1*, *Pgi-1*, *Skdh-1*, and *Skdh-2* were each monomorphic for the same allele in all populations. A total of 58 alleles were revealed among the loci; most were either widespread or very rare. For example, twenty-two (37.9%) alleles occurred in all 44 populations; fifteen (25.9%) occurred in three or fewer populations, four of which were found in only a single population. Allele frequency data are available from the authors on request.

Morph structure and genetic diversity

Trimorphic populations as a group were polymorphic at 20 loci (83.3%) and contained 57 of the 58 alleles documented in *E. paniculata* populations. Dimorphic populations were polymorphic at 16 loci (66.7%) and contained 49 alleles, 48 of which were shared with trimorphic populations. Eight polymorphic loci (25%) and 32 alleles were revealed in monomorphic populations. Differences in allele richness among trimorphic, dimorphic and monomorphic populations primarily involved the number of rare alleles encountered. Of the seven alleles unique to trimorphic populations, five were found in only one or two populations. One allele was unique

to dimorphic populations, occurring in only a single population (B75) in the central part of the range. There were no alleles unique to monomorphic populations.

Of the 44 populations surveyed for electrophoretic variation, 16 contained self-pollinating variants of the M morph, 9 of these were dimorphic with L and M morphs. Collectively, populations with self-pollinating variants contained 44 (76%) of the alleles identified in the allozyme survey of northeastern Brazil. Two alleles were unqiue to populations with self-pollinating variants; one occurred in two populations (*Tpi-2a* in B58 and B59), while the second occurred in a single population (*Aat-1a* in B75). Of the 14 alleles not found in these populations, 50 percent were well represented (>5 populations) in populations without self-pollinating variants of the M morph.

The percentage of loci that were polymorphic per population ranged from 4.2 to 45.8% and averaged 28.5. Each locus contained from one to four alleles per population with a mean of 1.3 among all populations. Populations with different numbers of morphs differed in levels of isozyme variation (Tab. 4). Based on a one-way ANOVA, the proportion of loci that are polymorphic ($F = 10.9$, $P < 0.001$) and number of alleles/locus ($F = 8.8$, $P < 0.001$) varied significantly among populations differing in morph number. To separate the effects of morph structure from regional differences in genetic variation, we partitioned the variation in genetic diversity among two main effects, morph structure and state, as well as their interactions using a two-way ANOVA. Alagoas was omitted from the analysis since there were no allozyme data from trimorphic populations in this state. Both state and morph structure accounted for significant variations in genetic diversity (Tab. 5). The levels of polymorphism (*PLP*) and allele richness (k_a) were higher in the most northern and southern states (mean $PLP = 34.6\%$ and 40.3%; $k_a = 1.4$ and 1.53 in Ceará and Bahia, respectively) than in the central states of the range of *E. paniculata* (mean $PLP = 24.7\%$; $k_a = 1.3$). The mean *PLP* for trimorphic populations (33.0%, $SE = 1.7$) was significantly greater ($F = 6.5$, $P < 0.05$) than in non-trimorphic populations (22.1%, $SE = 2.7$). A similar trend was evident with

Table 4. Isozyme polymorphism and allele richness of trimorphic, dimorphic and monomorphic populations of *Eichhornia paniculata* in northeastern Brazil.

	Trimorphic	Dimorphic	Monomorphic
n	28	13	3
Polymorphic loci (%)			
all populations	83.3	66.7	25.0
mean per pop. (*SE*)	33.0 (1.64)	22.1 (2.73)	13.9 (3.67)
range	16.7–45.8	4.2–37.5	8.3–20.8
Allele number			
all populations	57	49	32
% of all alleles	98.3	84.5	55.2
mean per pop. (*SE*)	32.7 (0.44)	30.1 (0.90)	27.3 (0.88)
range	28–37	25–28	26–29
unique alleles	7	1	0
mean alleles/locus (*SE*)	1.36 (0.02)	1.25 (0.04)	1.13 (0.04)

Table 5. Effects of morph structure and geographic location on the percentage of loci polymorphic (*PLP*, arcsine transformed) and number of alleles per locus among populations of *Eichhornia paniculata* from northeastern Brazil. Analysis is based on a two-way ANOVA, excluding Alagoas state.

Source of Variation	*DF*	*F*	*P*
Percentage of Loci Polymorphic			
Morph structure	1	6.5	0.0167
State	4	4.2	0.0092
Interaction	4	0.9	0.4830
residual	26		
Alleles per locus (k_a)			
Morph structure	1	6.2	0.0195
State	4	7.9	0.0004
Interaction	4	1.6	0.2046
residual	26		

respect to the number of alleles per locus ($F = 6.2$, $P < 0.05$). The non-significant interaction indicated that trimorphic populations were consistently more variable than non-trimorphic populations, regardless of geographic location. However, the mean difference in *PLP* between trimorphic and non-trimorphic populations was much larger in the north and central states (Ceará, Paraíba, Pernambuco; 14.64%) than in the southern states (Sergipe and Bahia: 0.83%).

Comparisons of genetic diversity in neighbouring trimorphic and dimorphic population pairs from six regions in northeastern Brazil confirmed the relationship between genetic diversity and population morph structure (Tab. 6). In all cases, trimorphic populations contained more alleles and as many or more polymorphic loci as dimorphic populations. Each population pair, on average, shared 84.1% of their alleles. Allozymes in dimorphic populations largely comprised a subset of the allozymes in neighbouring trimorphic populations, with an average of 1.2 unique

Table 6. Comparison of allozyme diversity between pairs of adjacent trimorphic and dimorphic populations of *Eichhornia paniculata* in northeastern Brazil. Each pair is compared in terms of the percentage of loci that are polymorphic (*PLP*), number of alleles present (N_a), and the number of alleles that are not found in the other population (U_a).

Population pairs	Trimorphic *PLP*	Trimorphic N_a	Trimorphic U_a		Dimorphic *PLP*	Dimorphic N_a	Dimorphic U_a
B35	33.3	32	1	B31	29.2	31	0
B56	20.8	30	6	B55	4.2	25	1
B58	37.5	35	8	B59	16.7	28	1
B151	29.2	31	4	B147	25.0	30	3
B158	29.2	34	4	B152	29.2	31	1
B169	41.7	39	3	B168	41.7	37	1
Mean	33.5	32.0	4.3		30.3	24.3	1.2

alleles per population. Nearly four times as many alleles (4.3 alleles per population) were exclusive to trimorphic populations.

Genetic relationships

The average genetic distance among *E. paniculata* populations was 0.104 ($SD = 0.048$) and ranged from 0.002 to 0.385. A UPGMA cluster analysis revealed three broad groups of populations. Two clusters, which distinguish populations in the northern part of the range from those in the south, comprise 43 of the 44 populations (Fig. 3). A third divergent group was represented by a single population from the central part of the range (B149). Within the two major groupings, populations were clustered with others from the same geographic area. A comparison between the genetic and geographic distance matrices, using Kendall's tau

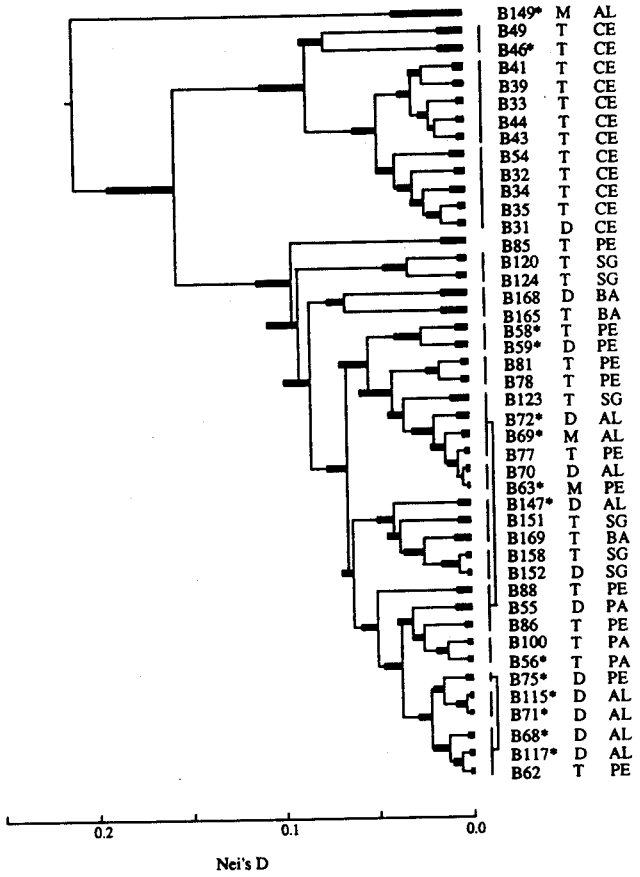

Fig. 3. Genetic relationships among 44 populations of *Eichhornia paniculata* from northeastern Brazil. The UPGMA dendrogram of Nei's genetic distance (1978) was based on 24 isozyme loci. Shaded bars on dendrogram branches represent one standard error (Ritland, 1989). The morph structure (T – trimorphic, D – dimorphic, M – monomorphic) and state (AL – Alagoas, BA – Bahia, CE – Ceará, PA – Paraíba, PE – Pernambuco, SE – Sergipe) are listed for each population. Asterisks indicate the populations containing self-pollinating variants of the M morph. Vertical bars adjacent to the population codes identify statistically distinct clusters of populations, based on the criteria that the branch length for that cluster is greater than twice the standard error.

statistic, indicated that genetic distance was positively correlated with geographic distance ($K_c = 16\,792$, $P < 0.00001$).

Non-trimorphic populations were represented in the two major clusters of the dendrogram and often had greater similarity with neighbouring trimorphic populations than with other dimorphic populations (Fig. 3). Within the two major groups, seven of the fourteen statistically significant clusters in the dendrogram that had more than one population contained both dimorphic and trimorphic populations (dimorphic-trimorphic populations: B31-35; B59-58; B72/B55–B123/B88/B86; B75/B68/B117–B62; B147–B151/B169; B152-158; B168-165). In each case, dimorphic populations occurred in relatively close proximity (20 km or less in five clusters, 100 km for the remaining groups). The relationship between local trimorphic and non-trimorphic populations revealed in the dendrogram was reflected in the distribution of rare alleles. Five different allozymes were identified that were shared by only two populations, one trimorphic and the other dimorphic for style morph. An additional allozyme was shared by a single group consisting of a trimorphic, dimorphic and monomorphic population. One trimorphic-dimorphic pair (B55-56) was also identified as genetically similar since it contained alleles which were locally distinct, although they were found in populations from other states. These two populations were separated by less than 5 km.

The dendrogram indicated that populations with self-pollinating variants were not a single, genetically homogeneous group. Self-pollinating variants occurred in nine statistically distinct clusters, or groups, of populations in northeastern Brazil (Fig. 3; B46; B56; B58/59; 71/115; B72/69; 70/63; 68/117/75; B147/151; B149). Population B46, one of two populations in the north that contained self-pollinating variants, was markedly differentiated from all other populations with self-pollinating variants (Fig. 3). Population B149 was also substantially different from all other populations. Both of these populations were distinguishable by the presence of diagnostic allozymes at two loci that were not present in other populations with self-pollinating variants. All but one of these diagnostic allozymes were also common in neighbouring populations with no self-pollinating variants. In the southern states, four statistically significant groups of populations with self-pollinating variants were identifiable based on the presence or absence of diagnostic alleles (Tab. 7; Fig. 3). In all cases, diagnostic alleles occurred at a single locus, and were unique to those groups. The remaining populations with self-pollinating variants could not be distinguished on the basis of diagnostic alleles. These included populations from four of the dendrogram clusters which contained populations from the center of the range in Alagoas and Pernambuco.

Discussion

Populations of *E. paniculata* exhibit a wide range of floral conditions associated with mating-system variation. Populations can be trimorphic, dimorphic, or monomorphic for style length and, in addition, may contain various frequencies of self-pollinating variants. Outcrossing rates range from 0.002 to 0.960 ($n = 32$ populations) and are positively correlated with morph number and negatively

Table 7. Diagnostic alleles that distinguish groups of *Eichhornia paniculata* populations in northeastern Brazil with self-pollinating variants. Each group was identified from the dendrogram and *SE* estimates of branch lengths (see Fig. 3). Also present are the number of populations in which diagnostic alleles are found. This includes populations with and without self-pollinating variants.

Populations	State	Diagnostic Alleles	Number of Populations
B46	Ceará	*Pgi 2-d*	12
		Aat 3-d	2
B58/59	Pernambuco	*Tpi 2-a*	2
B75/68/117	Pernambuco	*Aat 3-a*	1
B71/115	Alagoas	*Aat 2-a*	2
B147/151	Alagoas	*Aco 1-a, c*	2
B149	Alagoas	*Pgd 2-a*	4
		Acp 2-c	5

correlated with the frequency of self-pollinating variants within populations (Barrett and Husband, 1990). Our survey of 167 populations indicated that self-pollinating variants occur nonrandomly with respect to the number of style morphs in populations. Only 8.5% of trimorphic populations surveyed contained self-pollinating variants compared to 50.0% of dimorphic and 85.7% of monomorphic populations. These results indicate that self-pollinating variants are almost always associated with the loss of morphs. While self-pollinating variants are not excluded from trimorphic populations the initial loss of style morphs appears to be an independent yet necessary step required for them to spread. However, the loss of morphs is not always associated with the spread of self-pollinating variants. When examining the origins of self-fertilization in *E. paniculata*, therefore, we consider initial loss of morphs from populations and the spread of self-pollinating variants separately.

Our investigation of genetic diversity and the genetic relationships among populations of *E. paniculata* in northeastern Brazil indicated that non-trimorphic populations do not form a homogeneous group. Rather, non-trimorphic populations were scattered throughout the dendrogram based on genetic distance, frequently clustering with trimorphic populations in close geographic proximity. For dimorphic and monomorphic populations to be maintained in close geographical proximity to trimorphic populations where there is potential for gene flow to introduce missing morphs suggests that morph loss may have occurred quite recently. This pattern suggests that the loss of style morphs from trimorphic populations has occurred repeatedly and is an ongoing process in the species. Multiple occurrences of morph loss would also explain why non-trimorphic populations collectively contained 84% of all the allelic variation described for populations in northeastern Brazil. Most of the remaining alleles were extremely rare, often occurring in only single trimorphic populations. The absence of these alleles from the survey of non-trimorphic populations may simply be a product of having sampled fewer populations (19 dimorphic + monomorphic vs 28 trimorphic pops.).

Repeated morph loss is also supported by the geographical survey of style-morph structure, which revealed the presence of non-trimorphic populations in all six

states (Tab. 2). The majority of these populations were characterized by an absence of the S morph (Tab. 2), supporting theoretical predictions that this morph is most susceptible to stochastic loss from populations owing to population bottlenecks and founder events (Heuch, 1980; Barrett et al., 1989; Eckert and Barrett, 1992). Electrophoretic analyses of neighbouring trimorphic and dimorphic populations provide additional evidence that stochastic processes play a role in the origin of non-trimorphic populations. Dimorphic populations had fewer polymorphic loci, and the allozymes found were largely a subset of those in nearby trimorphic populations. Reduced diversity in dimorphic populations may be a result of their smaller average population size or their recent derivation from trimorphic populations. While we cannot exclude selective mechanisms of morph loss based on this data, the parallel reductions in allozyme richness, population size and morph representation (Husband and Barrett, 1992) are consistent with morph loss through population bottlenecks or founder events (Nei et al., 1975).

Earlier field studies in northeastern Brazil indicated that non-trimorphic populations were primarily concentrated at the southern periphery of the area surveyed in Alagoas state and virtually all contained self-pollinating variants (Fig. 2, Barrett et al., 1989). However, the more extensive survey reported here indicated that populations to the south of this area in Sergipe and Bahia were primarily trimorphic and those which were dimorphic rarely contained self-pollinating variants. This indicates that the primary concentration of populations with self-pollinating variants is geographically central in distribution, a pattern which appears contrary to the distribution of selfing populations in several other species and to theoretical predictions based on ecological marginality and reproductive assurance hypotheses (reviewed in Jain, 1976; Lloyd, 1980; Wyatt, 1988). Why selfing populations in *E. paniculata* occur more commonly in the center of the Brazilian range is unclear; however, we note that populations in Alagoas and parts of Pernambuco are often smaller, less dense and shorter lived than elsewhere (Barrett et al., 1989 and B. C. Husband, unpubl. data) suggesting that the ecological conditions in this region may be less suitable for the persistence of *E. paniculata*. Under these conditions, self-pollinating variants may have fitness advantages over floral morphs from trimorphic populations. Field studies would be required to evaluate this hypothesis.

Does the origin of self-pollinating variants in northeastern Brazil parallel the repeated loss of style morphs in *E. paniculata* populations? Based on our study of the genetic relationship among populations in northeastern Brazil, self-pollinating variants occurred in nine statistically distinct groups, eight of which occur in the center of the range (Fig. 3). These groups could represent populations in which the independent origin of self-pollinating variants has occurred. Alternatively, these populations may have become electrophoretically differentiated subsequent to the spread of self-pollinating variants. Groups containing populations B58/59, B71/115, B147/151 and B75 were each distinguishable by diagnostic allozymes at a single locus; otherwise, they shared the same alleles. Because these diagnostic allozymes were restricted to one or two populations, it is most likely that these allelic differences have only recently arisen through gene flow or mutation. Since self-pollinating variants may have existed in these populations prior to differentiation,

without further evidence their origin cannot be identified as separate from the larger, central group of selfing populations.

Two groups, each containing a single population with self-pollinating variants (B46, B149) were, however, very distinct electrophoretically from all other groups containing self-pollinating populations and from one another (Fig. 3). Their differentiation is reflected in markedly different allele frequencies and the presence of diagnostic alleles at two loci in both populations. Although these alleles were not found in other populations with self-pollinating variants, they commonly occurred in trimorphic populations nearby. This pattern contrasts with that evident from the diagnostic alleles in the four selfing groups discussed above, where the diagnostic alleles were restricted in distribution to populations with self-pollinating variants. If we assume that the widespread versus restricted distribution of alleles is a function of the time since their origin and opportunities to spread through gene flow, then the diagnostic alleles in B149 and B46 likely arose sufficiently long ago to have spread among several nearby populations. Since these populations are so divergent from any other population with self-pollinating variants, it is likely that they have not shared genes with other selfing populations through gene flow or common ancestry in the recent past. This evidence, combined with the geographically isolated and short-lived nature of most *E. paniculata* populations (Husband, 1991), suggests that it is most likely that populations B46 and B149 are historically distinct from other populations with self-pollinating variants and represent separate origins of self-fertilization.

An alternative interpretation is that self-pollinating variants arose only once. If this were true the present distribution of self-pollinating populations can only be explained if self-pollinating variants were widespread at one time and now are represented by a few relictual populations which are highly differentiated and isolated from one another. This idea seems less likely than the multiple origin hypothesis for two reasons. First, populations with self-pollinating variants are not widespread in northeastern Brazil. Second, the likelihood that self-pollinating variants would be reduced to one or two populations in two geographically distinct regions is extremely low. Additional evidence for the independent origin of self-pollinating variants in these populations comes from morphological and genetic studies.

Controlled crosses among self-pollinating variants from different parts of the geographic range of *E. paniculata* in northern Brazil indicate that different recessive modifier genes are responsible for alterations to stamen level in the mid-styled morph (C. B. Fenster and S. C. H. Barrett, unpubl. data). Crosses of genotypes from population B46 with self-pollinating variants from the central part of the range resulted in unmodified F_1 plants identical in appearance to unmodified individuals of the mid-styled morph. In contrast, crosses among self-pollinating variants from different populations within the center of the range produced only modified plants, suggesting similar genetic factors were responsible for stamen elongation and self-pollination. The distinct nature of genetic control of selfing in population B46 therefore supports an hypothesis of independent origin.

Morphological studies of stamen modification in population B149 also corroborate the electrophoretic evidence for the independent origin of selfing in this

population. All individuals comprised a variant form of the M morph in which all three short-level stamens were elongated so their anthers were adjacent to the stigma. This condition differs from virtually all other populations with self-pollinating variants in northeastern Brazil, which contain variants with a single short-level stamen adjacent to the stigma (Seburn et al., 1990; Barrett and Harder, 1992). In addition, flowers from population B149 are smaller in size and more darkly pigmented than flowers from other populations with self-pollinating variants. These differences in floral morphology among variants of the M morph are maintained under glasshouse conditions and are transmitted from parents to progeny, indicating a genetic basis for the floral variation. Because of the distinct phenotype of these self-pollinating variants and the marked differentiation of allozymes in this population from those in the remainder of Brazil, we suggest that self-fertilization in this population may also represent a separate evolutionary origin.

Inferences from electrophoretic data are likely to lead to an underestimate of the number of origins of self-fertilization in *E. paniculata*. The majority of populations containing self-pollinating variants, were indistinguishable from one another based on diagnostic alleles. While self-pollinating variants may have arisen and spread repeatedly among these populations, the relatively low levels of diversity, ongoing exchange of genes among populations and high concentration of populations with self-pollinating variants in this region make such events unresolvable using allozyme techniques and would require molecular markers to more accurately determine the historical relationships among populations (Avise et al., 1987; Quattro et al., 1991). Until such data are available, the most parsimonious interpretation is that self-pollinating variants in this central group of populations have had a single origin and subsequently spread throughout central parts of northeastern Brazil. If this interpretation is correct, then self-pollinating variants in *E. paniculata* have originated on at least three separate occasions in northeastern Brazil. This conclusion rests on several different lines of evidence including information on style morph and electrophoretic variation, and morphological and genetic data on the self-pollinating variants.

Acknowledgements

We thank William Cole, Jan Conn and Martin Morgan for field assistance, Kermit Ritland for the use of his computer program for estimating genetic distances, and the Natural Sciences and Engineering Research Council of Canada for financial support in the form of an operating grant to SCHB.

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Received 8 November 1991;
accepted 21 January 1993.
Corresponding Editor: J. Travis