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Floral variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae): I. Instability of stamen position in genotypes from northeast Brazil

Carolyn N. L. Seburn[1,3], Timothy A. Dickinson[1,2] and Spencer C. H. Barrett[1]

[1]*Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2, and* [2]*Department of Botany, Royal Ontario Museum, 100 Queen's Park, Toronto, Ontario, Canada M5S 2C6.* [3]*Present address: Department of Biological Sciences, University of Windsor, Windsor, Ontario, Canada N9B 3P4.*

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Abstract

Floral instability in the mid-styled morph of tristylous *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae) was investigated in 14 genotypes from three Brazilian populations of contrasting morph structure (trimorphic, dimorphic, and monomorphic) and levels of inbreeding. Floral instability was described using uni- and multivariate methods, in terms of the degree and pattern of variation and covariation in 14 floral measurements from plants grown under uniform glasshouse conditions. The greatest variability was exhibited by filaments of the two lateral short-level stamens, within and between genotypes from the dimorphic and monomorphic populations. Elongation of one of the stamens to a position adjacent to the stigma results in automatic self-pollination of flowers. Genotypes were found to differ more in the pattern of covariation between measurements than in multivariate variability. Differences between genotypes in the correlation structure of floral traits were most pronounced in the monomorphic population, and primarily involved measurements of unmodified, rather than modified, stamens. There was no consistent relationship between multivariate variability and whether genotypes produced modified flowers. This pattern suggests that short-stamen modification arises from changes at specific loci rather than through generalised floral instability brought about by genome-wide homozygosity.

Introduction

Plants exhibit considerable phenotypic variation as a result of genetic differentiation, environmentally-induced influences on growth and reproduction, and both

programmed changes and random accidents during the course of development. Understanding the relative importance of these factors in determining the patterns of phenotypic variation in plant populations is a major focus of research in plant evolutionary biology (Bradshaw, 1965; Lloyd, 1984; Scheiner and Goodnight, 1984; Schlichting, 1986). Population level studies of variation often neglect the details of intraplant variation, perhaps because of difficulties in determining whether such variation is adaptive or non-adaptive (although see Huether, 1969; Ellstrand, 1983). Programmed changes in development, such as heterophylly (Sculthorpe, 1967), or the production of heteroblastic leaf types (Dengler, 1983; Richards, 1983) or of cleistogamous and chasmogamous flowers (Lord, 1981), have usually been considered to have some functional significance whereas developmental instability in repeated structures is most commonly viewed as the result of mistakes in development, and hence maladaptive (Lerner, 1954; Jinks and Mather, 1955; Paxman, 1956; Rendel, 1959; Levin, 1970).

In comparison with most vegetative organs of angiosperms the flower is usually considered among the least prone to non-genetic influences on variation. Many floral characters not only remain relatively constant within individuals but also exhibit a stereotyped plan among populations, species and often families. Much of angiosperm taxonomy is based on floral characters because of their relative uniformity within taxonomic groups (Stebbins, 1951; Berg, 1959). The functional basis of floral constancy is usually explained in relation to the pollination system and requirements for efficient pollen transport. In animal pollinated plants wide variation in the size and position of reproductive organs can potentially lead to considerable losses in reproductive potential. Following this view canalized floral development and the constancy in expression of floral traits result from strong stabilizing selection mediated by the size and behaviour of specific pollinator groups.

Among the many angiosperm pollination mechanisms, heterostyly represents one of the most specialized, in terms of the importance of the relative positions of reproductive parts. The reciprocal placement of stamens and styles in the floral morphs of heterostylous species is generally viewed as a mechanism to promote insect-mediated pollen transfer between anthers and stigmas of equivalent height (Darwin, 1877, Ganders, 1979). For such a system to function effectively it is of prime importance that a high degree of constancy in the size and position of reproductive organs occurs within the floral morphs. While most heterostylous species exhibit minor variation in the size and position of reproductive parts, others, in which the polymorphism has become evolutionarily modified, can show striking variation in stamen and style length (e.g. Lewis and Rao, 1971; Barrett and Shore, 1987). This research is concerned with a heterostylous species that possesses considerable variation in reproductive structures both within and between individuals and populations. The variation is associated with the breakdown of heterostyly to semi-homostyly and the resultant shift in mating system from outcrossing to selfing.

Eichhornia paniculata (Spreng.) Solms (Pontederiaceae) is a short-lived perennial or annual of seasonal marshes, pools and ditches in northeast Brazil and the Caribbean islands of Cuba and Jamaica. Populations vary in their floral biology and mating systems and range from outcrossing tristylous populations to highly

selfing semi-homostylous populations (Glover and Barrett, 1986; Barrett et al., 1989). Accompanying the shift to selfing are alterations in a range of reproductive attributes including inflorescence and flower size, the showiness of perianth parts, the size and production of pollen grains, and other aspects of floral allocation (Barrett, 1985a, 1988; Morgan and Barrett, 1989). The breakdown of tristyly in *E. paniculata* is manifested by changes from floral trimorphism, through dimorphism to monomorphism. These changes are associated with increased levels of inbreeding, and a reduction in the genetic diversity and levels of heterozygosity within populations (Glover and Barrett, 1987).

Field studies indicate that populations of *E. paniculata* exhibit considerable developmental instability in the expression of floral traits (Barrett, 1985a). These include the number and symmetry of tepals, pollen sterility, and the degree of filament elongation in short-level stamens. The magnitude of this variation varies among regions, populations, and individuals within populations. In general, populations on Jamaica display higher levels of floral instability than those from Brazil, and floral constancy is in general higher in tristylous populations than in dimorphic and monomorphic populations (Barrett, 1985b and unpubl. data). These observations suggest that the occurrence of floral instability in *E. paniculata* is associated with the breakdown of tristyly and the consequent evolution of inbreeding in the species.

This study employs a range of quantitative techniques to describe the nature of floral instability in genotypes obtained from three populations of *E. paniculata* from northeast Brazil. This description involves determining not only whether genotypes differ in the expression of certain measurable floral traits, but also whether they differ in the amount of variation exhibited by the traits. The first objective can readily be met by cloning genotypes and growing them under uniform glasshouse conditions; the resulting data are then analyzed by comparing the variances of genotypes with those of clones within genotypes. The usual uni- and multivariate methods for measuring variability, however, do not lend themselves to similar comparisons. Variability was therefore measured by transforming the multivariate data into a Levene statistic (Levene, 1960; Van Valen, 1978; Schultz, 1985), so as to summarize deviations from genotype centroids as distances which can, in turn, be analyzed using nested analyses of variance. Graphical and other methods (Campbell, 1981; Dietz, 1983) were also used to compare not only genotype variability but also the correlation structure of the measurements within each genotype. The specific questions addressed were thus: (1) Are there significant genotypic differences in floral traits within and between populations? (2) Do genotypes differ in the amount of developmental instability, that is, is the amount of intraplant variation exhibited by floral traits different among genotypes? And (3), does the correlation structure of floral traits differ between genotypes and populations?

Materials and methods

Details of the growth, floral biology and development, and glasshouse culture of *Eichhornia paniculata* are described in Richards and Barrett, (1984), Barrett (1985a), and Morgan and Barrett, (1989).

Sampling

Six genotypes were randomly selected from each of two populations, one dimorphic (B9) and one monomorphic (B8; Table 1). Field observations indicated that the populations exhibited a high degree of floral instability in comparison with most trimorphic populations. Two genotypes were selected from a trimorphic population (B5), for baseline comparisons (Table 1). To facilitate comparisons between genotypes, all were of the mid-styled morph.

Three clones were propagated from each genotype and grown in soil in submerged, four inch pots in a fully randomized design under glasshouse conditions. Plants were fertilized weekly with soluble N-P-K. For each of three consecutive inflorescences that expanded during November and December 1986, the number of flowers per inflorescence was estimated and seven flowers were randomly collected from that number. From these, five were randomly chosen from each inflorescence resulting in a sample size of 45 flowers for each of the 14 genotypes. At the beginning of anthesis, when flowers were fully expanded, they were removed and preserved in FAA (formalin, glacial acetic acid, 70 % ethanol 5:5:90).

Data collection

Flowers were stained with acid fuchsin and dissected in 70 % ethanol using a Zeiss SV8 stereomicroscope. Fourteen measurements were recorded for each flower (Fig. 1; Table 2). Stamens are numbered one to six, counter-clockwise, beginning with the stamen associated with the tepal to the right of the banner tepal (Fig. 1). In the genotypes studied, the filament elongation described below typically occurs in only one of the two lateral short-level stamens (Fig. 1). Preliminary observations demonstrated a consistent relationship between the position of a flower, relative to that of the next younger one on the axis, and the side of the flower on which a lower-level stamen elongated. Thus, in some flowers stamen elongation occurred on the right side (stamen 1) while in others it occurred on the left (stamen 3); we

Table 1. Locality, style morph structure, outcrossing rates, and observed heterozygosity of *Eichhornia paniculata* populations from which genotypes used in this study originated. Data on outcrossing rates and heterozygosity were based on a survey of six, five, and one polymorphic loci in B5, B9, and B8, respectively. All quantitative data collected in 1983. Additional information on floral biology, ecology, and genetics of these populations is available in Glover and Barrett, (1986, 1987) and Morgan and Barrett, (1989).

Population code	Locality in Brazil	Style morph structure	Estimated population size	Outcrossing rate	Observed heterozygosity
B5	Recife, Pernambuco	trimorphic	~1000	0.96	11.3
B9	União dos Palmares, Alagoas	dimorphic	110	0.36	7.0
B8	Murici, Alagoas	monomorphic	55	0.47	0.8

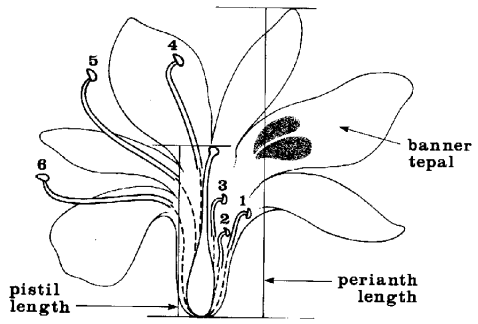

Fig. 1. Floral organization of *Eichhornia paniculata* showing the measurements made on dissected flowers (compare Table 2): perianth length (PERIANTH) and pistil length (PISTIL); measurements on stamens 1–6 comprised the total length from insertion of the floral tube to the anther, and free filament length (double line; STAM1F, STAM2F, STAM3F, STAM4F, STAM5F, STAM6F). Length of perianth from insertion to the attachment of the free filament (dashed line; STAM1A, STAM2A, STAM3A, STAM4A, STAM5A, STAM6A) was obtained by subtraction, so as to avoid logically correlated measurements.

consider these events to be developmentally equivalent. For this reason, measurements of these stamens (1 and 3, Fig. 1) were pooled so as to describe their overall pattern of variation in length (Fig. 2). Four additional measurements (ELONGA, ELONGF, UNELOA, UNELOF; Table 2) were derived from the pooled data in order to remove the influence of variation due solely to this positional effect (flower by flower, measurements of stamens 1 and 3 were recoded as belonging to the elongated stamen or the unelongated stamen, based on the stamen's free filament length).

Thus a total of four data sets were available for analysis: (1) all 14 measurements, as originally recorded; (2) the eight measurements of the four unmodified stamens (2, 4, 5, 6); (3) the four measurements of the two modifiable stamens (1, 3), as originally recorded; and (4) the four corresponding derived measurements. Except as noted, analyses were carried out on the unpooled data for stamens 1 and 3

Table 2. Measurements of *Eichhornia paniculata* used in data analysis (compare Fig. 1).

PERIANTH – shortest distance from insertion of the floral tube to tip of tepal lobe 3. PISTIL – pistil length. STAM1A, STAM2A, STAM3A, STAM4A, STAM5A, STAM6A – shortest distance from insertion of floral tube to attachment of free filament of stamens 1, 2, 3, 4, 5, 6 respectively. STAM1F, STAM2F, STAM3F, STAM4F, STAM5F, STAM6F – length of the free filament of stamens 1, 2, 3, 4, 5, 6 respectively. ELONGF – for any given flower, the larger of STAM1F and STAM3F. UNELOF – for any given flower, the smaller of STAM1F and STAM3F. ELONGA – equal to STAM1A or STAM3A, according to which stamen (1 or 3) had the larger value for free filament length (ELONGF). UNELOA – equal to STAM1A or STAM3A, according to which stamen (1 or 3) had the smaller value for free filament length (UNELOF).

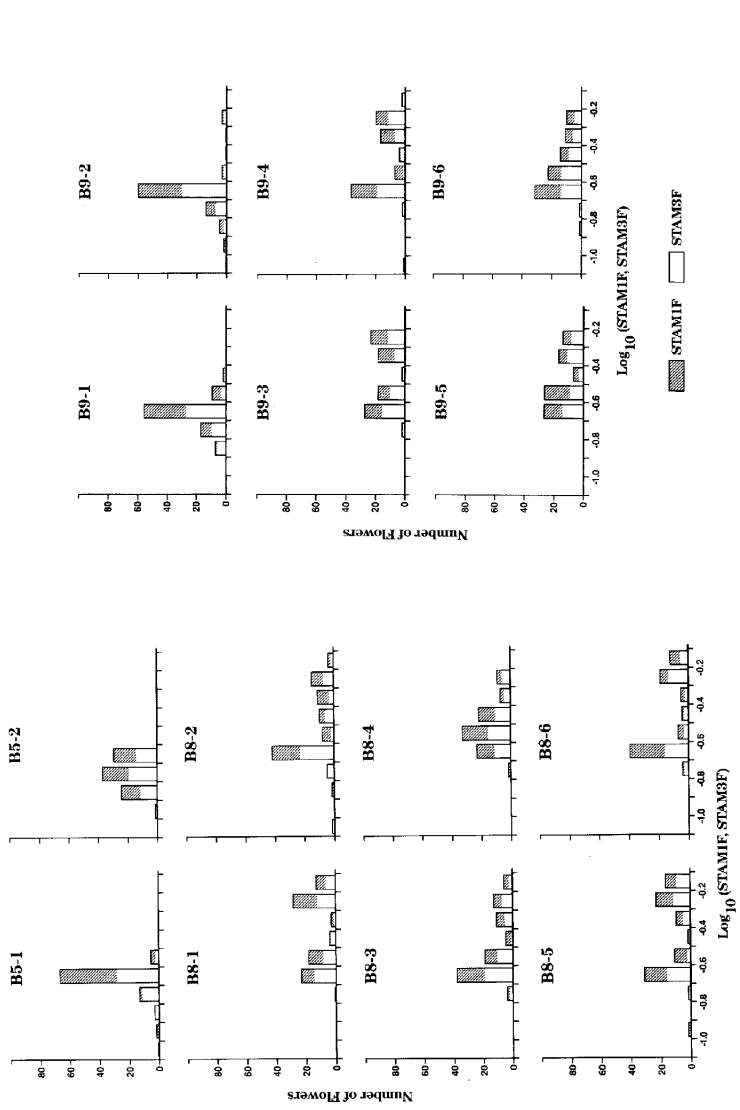

Fig. 2. Frequency histograms for $\log_{10}$-transformed free filament lengths of stamen 1 (STAM1F; □) and stamen 3 (STAM3F; □) for each of 14 genotypes of *Eichhornia paniculata* from northeast Brazil. The histogram for each genotype represents two measurements on each of 45 flowers.

(STAM1A, STAM1F, STAM3A, STAM3F; Fig. 1; Table 2). Measurements were $\log_{10}$-transformed to reduce the effect of differences in absolute size and to obtain variables that were more nearly normal in distribution. These $\log_{10}$-transformed data were used in all analyses described below unless otherwise indicated.

Data analysis

Genotypic differentiation with respect to each of the 14 floral measurements was examined by means of nested analyses of variance (ANOVAs) corresponding to the structure of the experiment (clones within genotypes, etc.). The F values reported for these analyses are the ratios of the mean squares for genotypes and clones.

Genotypic differentiation was also examined using multigroup principal components analysis based on the pooled within-groups covariance matrix **W**(M-PCA; Pimentel, 1979; Dickinson, Parker and Strauss, 1987). These analyses either used all 14 measurements together or compared the measurements of modified stamens (1 and 3) with those of the unmodified ones (2, 4–6; Table 5, 6). Dimensionality of the M-PCAs was evaluated using Frontier's broken-stick model criterion (Table D in Legendre and Legendre, 1983) for the proportion of the sum of the diagonal elements of **W** (i.e. the trace of **W**) accounted for by successive components. Where this criterion gave anomalous results (only a second or third axis found to be significant, but not previous ones) the proportion of variance accounted for by successive axes was compared with trace (**W**)/P, for analyses of P measurements (Legendre and Legendre, 1983).

Variability in floral measurements was described in terms of a mean total variance quantity $\bar{y}_M$ based on the Mahalanobis generalized distances between individual flowers and the centroids of the genotype they represented (y_{iM}; Van Valen, 1978). These were calculated from scores on significant M-PCA axes (Orlóci, 1978; Dickinson et al., 1987). Total variances were calculated separately for unmodified and modifiable stamens. For purposes of comparison, genotype covariance matrix determinants (generalized variances; Sokal, 1965; Goodman, 1968; Soulé, 1971) were calculated for the same subsets of measurements and compared using the F approximation of Bartlett's M (Pimentel, 1979).

Nested ANOVAs were also carried out on the y_{iM} values. In these analyses, and in those described above, two approaches were taken in order to control for the effects of departures from normality and homoscedasticity. First, in addition to carrying out the usual analyses, robust oneway ANOVA methods (Brown and Forsythe, 1974a, b) were used that test null hypotheses about homoscedasticity regardless of departures from normality, and about the equality of means regardless of heteroscedasticity. Second, normalizing transformations were applied to the y_{iM} values. The y_{iM} values were converted to Chi-squared probabilities with degrees of freedom equal to the number of significant M-PCA dimensions. Two- and three-level nested ANOVAs were then carried out on the normal probability quantiles corresponding to these probabilities (three-level analyses used program NESTAN, Rohlf, 1987).

The graphical method for comparing covariance matrices suggested by Campbell, (1981) involves plotting measurement correlations and variances within each genotype against an 'average' value obtained, in this case, from **W**. Patterns of relationship between the values for individual genotypes and the 'average' values are then described in terms of regression slopes and intercepts. The approach recommended by Dietz, (1983) for comparisons of resemblance matrices used a statistic, Kc, to test the null hypothesis that two resemblance matrices are independent. The significance of the value of Kc obtained is evaluated by means of a permutation test. A total of 91 pairwise comparisons of genotype correlation matrices were carried out for each of the four data sets described above, using a program written by E. J. Dietz. Correlation matrices were calculated with and without trimming of the genotype data, that is, removal of the effect of outlier flowers (Becker and Chambers, 1984).

To document the behavior of modified and unmodified flowers in the analyses, the sample of 630 flowers was split into unmodified and modified flowers, regardless of genotype. This was done by calculating a matrix of Euclidean distances from the measurements of the modifiable stamens (STAM1A, STAM1F, STAM3A, STAM3F), and using this for complete linkage clustering of the sample. At the highest level of dissimilarity, three groups were formed. The first of these consisted of two flowers which were outliers, belonging to genotype B8-5. The remaining groups comprised 280 unmodified flowers (including all the flowers representing genotype B5-1 and B5-2) and 348 modified flowers.

Unless otherwise indicated, the analyses described above were carried out using the functions and macros of the data analysis and graphics software package S (Becker and Chambers, 1984), running on the University of Toronto Department of Statistics Sun Unix system, and used either directly or incorporated in macros written by TAD.

Results

Differentiation of genotypes and populations

Univariate genotype comparisons

Significant differentiation of genotypes within populations B8 and B9 was found for most floral measurements (Table 3). Populations B8 and B9 were also found to differ significantly, but only in measures of floral size (PERIANTH and PISTIL, $p < 0.05$). After adjusting the α-probability level for simultaneous comparisons (from 0.05 to 0.05/14; Cooper 1968; Rice, 1989), however, population differentiation with respect to these measurements was also non-significant (Table 3).

In four genotypes (B5-1, B5-2, B9-1, B9-2) neither stamen 1 nor stamen 3 elongated, resulting in the unmodified mid-styled floral phenotype (unimodal distributions of free filament length for stamens 1 and 3, Fig. 2). These four genotypes are incapable of autonomous self-pollination. In the remaining genotypes either stamen 1 or stamen 3 elongated, in at least some flowers (Fig. 2). As

Table 3. Entries are F-values and variance components from nested ANOVAs within and between two Brazilian populations (B8, B9) of *Eichhornia paniculata*, for flower measurements, including derived measurements for stamens 1 and 3 (ELONGA, ELONGF, UNELOA, UNELOF). See Fig. 1 and Table 2 for explanation of measurements, and text for details. Asterisks indicate columnwise probabilities for rejecting null hypotheses of homogeneous means (Rice 1989); (a) variance component due to genotypes, (b) variance component due to clones, (c) variance component due to populations.

	Genotypes within B8			Genotypes within B9			Between B8 and B9		
		%[a]	%[b]		%[a]	%[b]		%[c]	%[a]
PERIANTH	10.8**	17.7	0.0	1.7	1.6	0.6	9.2	15.2	9.4
PISTIL	19.8***	37.5	2.0	14.5**	23.4	0.7	8.0	27.7	22.7
ELONGA	4.6*	11.2	3.7	13.7**	25.9	1.2	0.8	0.0	20.5
ELONGF	20.9***	30.8	0.0	38.2***	55.2	1.6	4.6	22.1	36.0
STAM2A	10.1**	19.4	1.1	13.8***	15.6	0.0	0.3	0.0	17.5
STAM2F	6.1*	11.8	1.1	15.5***	25.4	0.3	0.1	0.0	19.4
UNELOA	29.1***	27.2	0.0	15.4***	12.6	0.0	0.0	0.0	20.8
UNELOF	9.6**	14.5	0.0	22.5***	27.1	0.0	1.0	0.0	21.7
STAM4A	11.4**	26.5	2.9	7.4**	9.8	0.0	0.1	0.0	21.1
STAM4F	2.2	4.1	3.9	12.2**	24.6	1.7	0.3	0.0	16.2
STAM5A	25.0***	25.9	0.0	10.8**	9.0	0.0	0.0	0.0	18.1
STAM5F	1.5	2.0	6.1	9.5**	27.6	5.3	0.5	0.0	20.1
STAM6A	9.0**	23.2	3.9	12.3**	8.0	0.0	0.2	0.0	17.3
STAM6F	8.4**	14.1	0.0	8.6**	22.9	4.2	0.0	0.0	20.0

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

described above, which stamen elongated so as to produce the modified phenotype is related to the position of the flower in the inflorescence. In genotype B9-6 elongation of both stamens 1 and 3 in some flowers resulted in a strongly right-skewed distribution of free filament length (Fig. 2). In the remaining nine genotypes the distributions of free filament length for these stamens were markedly bimodal (Fig. 2), the bimodality being due to elongation of one or the other short-level stamens within a flower. Six genotypes can be considered fixed for the modification (B8-1, B8-5, B8-6, B9-3, B9-4, B9-5; Fig. 2). Stamen elongation in these genotypes appears to be a function of attached as well as free filament length (Fig. 3). In the other three genotypes with modified stamen phenotypes (B8-2, B8-3, B8-4; also B9-6; Fig. 2) only some flowers are modified. These genotypes therefore display developmental instability of the short-stamen level.

Multivariate genotype comparisons

The contrast between modified and unmodified flowers dominated the multivariate analyses of the data. Multigroup PCA of the 14 genotypes using all 14 measurements demonstrated this contrast in the space defined by the two significant components (accounting for 60 % of the trace of **W**). These components were associated primarily with the modifiable lower level stamens (1 and 3, Fig. 1). To examine this contrast in greater detail, M-PCAs were carried out separately on the

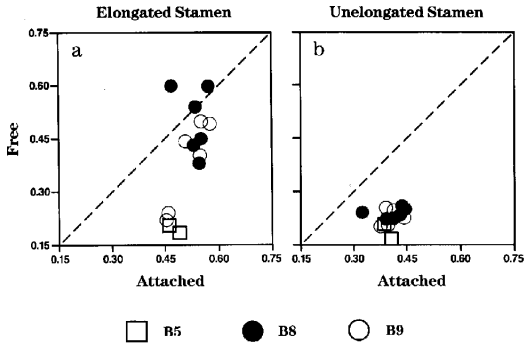

Fig. 3. Mean length of (a) elongated (ELONGA, ELONGF) and (b) unelongated (UNELOA, UNELOF) lower level stamens in 14 genotypes of *Eichhornia paniculata* from northeast Brazil. Genotype means are indicated by symbols: B5, □; B8, ●; B9, ○.

three additional data sets described above that comprise the measurements of the modifiable or the unmodified stamens (Table 4).

M-PCA based on measurements of modifiable stamens

The ordination based on the four modifiable stamen measurements demonstrated a separation of unmodified and modified flowers along either the non-significant second M-PCA axis (STAM1A, STAM1F, STAM3A, STAM3F; Fig. 4a; Table 4) or the first of two significant axes (ELONGA, ELONGF, UNELOA, UNELOF; Fig. 4b; Table 4). In both cases the centroids of the four genotypes with exclusively unmodified flowers (B5-1, B5-2, B9-1, B9-2) were segregated from those of the genotypes producing at least some modified flowers (Fig. 2). In the first analysis, the significant first M-PCA axis merely represents the distinction between flowers positioned on the left of the inflorescence axis and those positioned on the right. Correlations between STAM1F and STAM3F and scores on this axis have opposite signs (Fig. 4a; Table 4). The correlations between these measurements and M-PC2 have the same sign, and modified and unmodified flowers are separated along this axis (Fig. 4a; Table 4). Predictably, in the second analysis (Fig. 4b) ELONGF was correlated with the separation of floral types along M-PC1 (Table 4). The other measurements of the modifiable stamens were uncorrelated with this separation (Fig. 4b).

M-PCA based on measurements of unmodified stamens

In contrast, the ordination based on measurements of the unmodified stamens showed no such separation of unmodified and modified flowers, nor any comparable segregation of genotype centroids. In this analysis, with one exception (STAM2F; Fig. 4c; Table 4) it was measurements of the attached portion of the

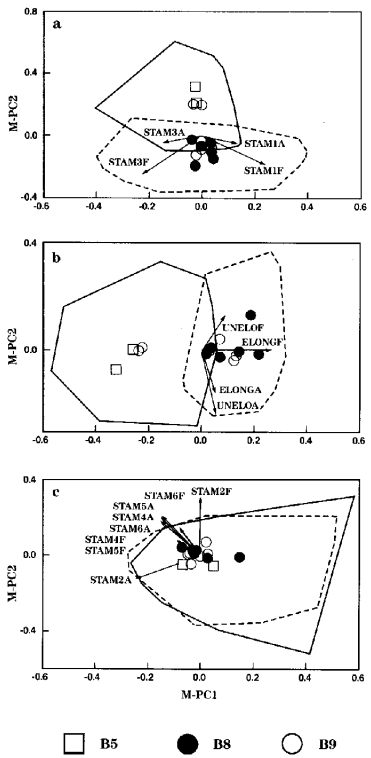

Fig. 4. Samples of 45 flowers from each of 14 genotypes of *Eichhornia paniculata* from northeast Brazil in the space of the first two axes of a multigroup principal components analysis (M-PC1, M-PC2). Measurements are based on modifiable stamens (a, b) and unmodified stamens (c). Genotype centroids are indicated by symbols: B5, □; B8, ●, B9, ○. The solid line convex hull encloses 280 unmodified flowers, while the dashed one encloses 348 modified flowers (regardless of genotype). Vectors represent the contribution of the measurement indicated to the scatter of flowers, in terms of the correlations between the measurement and M-PC1 and M-PC2 (Table 4).

stamen that had the greatest influence (STAM2A, STAM4A, STAM5A, STAM6A; Fig. 4c; Table 4).

Comparisons of variability

Covariance matrix determinants

The shift in floral organization from the unmodified phenotype (Fig. 2; B5-1, B5-2, B9-1, B9-2) to the modified one (Fig. 2; B8 genotypes, B9-3, B9-4, B9-5, B9-6)

Table 4. Means of 14 floral measurements for 14 genotypes of *Eichhornia paniculata* from northeast Brazil, and M-PCA results calculated separately from measurements of unmodified and modifiable (italicized entries) stamens. Means are shown back-transformed to their original units (cm).

	B5-1	B5-2	B8-1	B8-2	B8-3	B8-4	B8-5	B8-6	B9-1	B9-2	B9-3	B9-4	B9-5	B9-6	PC1[a]	PC2[a]	PC1[b]	PC1[c]	PC2[c]
PERIANTH	2.04	1.98	2.21	2.04	2.16	2.02	2.02	2.00	2.00	1.92	1.97	1.94	1.96	2.00					
PISTIL	1.09	1.15	1.09	0.97	1.11	1.11	0.94	1.07	1.20	1.15	1.16	1.05	1.14	1.18					
STAM1A	*0.45*	*0.44*	*0.50*	*0.45*	*0.47*	*0.50*	*0.42*	*0.47*	*0.43*	*0.41*	*0.50*	*0.51*	*0.46*	*0.49*			*0.49*	*0.19*	*−0.61*
STAM1F	*0.20*	*0.18*	*0.41*	*0.31*	*0.34*	*0.34*	*0.43*	*0.40*	*0.20*	*0.23*	*0.35*	*0.36*	*0.36*	*0.32*			*0.90*	*0.99*	*0.06*
STAM2A	0.25	0.29	0.29	0.25	0.27	0.27	0.21	0.26	0.27	0.25	0.28	0.30	0.25	0.24	−0.93	−0.32			
STAM2F	0.23	0.20	0.26	0.23	0.25	0.26	0.26	0.25	0.24	0.22	0.26	0.24	0.26	0.27	0.02	0.82			
STAM3A	*0.40*	*0.45*	*0.52*	*0.48*	*0.50*	*0.48*	*0.37*	*0.47*	*0.42*	*0.42*	*0.50*	*0.48*	*0.43*	*0.47*			*−0.54*	*0.19*	*−0.90*
STAM3F	*0.22*	*0.18*	*0.45*	*0.35*	*0.35*	*0.31*	*0.41*	*0.37*	*0.22*	*0.22*	*0.39*	*0.37*	*0.33*	*0.33*			*−0.84*	*0.33*	*0.49*
STAM4A	0.62	0.71	0.75	0.70	0.75	0.73	0.60	0.72	0.71	0.73	0.72	0.70	0.66	0.72	−0.57	0.55			
STAM4F	0.74	0.79	0.76	0.71	0.73	0.71	0.74	0.76	0.78	0.75	0.70	0.67	0.73	0.76	−0.31	0.39			
STAM5A	0.68	0.76	0.78	0.75	0.79	0.76	0.65	0.76	0.74	0.77	0.76	0.75	0.70	0.77	−0.55	0.56			
STAM5F	0.80	0.84	0.81	0.75	0.79	0.78	0.78	0.79	0.84	0.80	0.78	0.71	0.82	0.83	−0.31	0.34			
STAM6A	0.62	0.69	0.74	0.67	0.72	0.71	0.60	0.70	0.66	0.71	0.68	0.68	0.64	0.71	−0.56	0.50			
STAM6F	0.75	0.79	0.77	0.72	0.74	0.72	0.74	0.77	0.79	0.74	0.72	0.68	0.73	0.75	−0.30	0.41			

(a) Correlations between measurements of the unmodifiable stamens and the first two significant principal components of their pooled within-groups covariance matrix **W** that were used to calculate the total variance ($\bar{y}_M$) for these measurements (Table 5). These components accounted for 42 % and 25 % of trace (**W**), respectively.
(b) Correlations between measurements of the modifiable stamens (STAM1A, STAM1F, STAM3A, STAM3F) and the first significant principal components of their pooled within-groups covariance matrix **W** that was used to calculate the total variance ($\bar{y}_M$) for these measurements (Table 5). This component accounted for 64 % of trace (**W**).
(c) Correlations between measurements of the modifiable stamens (ELONGA, ELONGF, UNELOA, UNELOF) and the first two significant principal components of their pooled within-groups covariance matrix **W** that were used to calculate the total variance ($\bar{y}_M$) for these measurements (Table 5). These components accounted for 45 % and 27 % of trace (**W**), respectively.

Table 5. Correlation structure and variability displayed by stamens of 14 genotypes of *Eichhornia paniculata* from northeast Brazil. Entries are Individual-Average plot slope for variances (b_{var}) and correlations (b_{cor}), covariance matrix determinant ($|S|$), and untransformed total variance ($\bar{y}_M$). Stamen measurements are those of either unmodified stamens or modifiable stamens. Results of testing the equality of $|S|$ values are indicated, as are the results of nested ANOVAs of normalized y_{iM} values.

	unmodified stamens				modifiable stamens[a]			modifiable stamens[b]		
	b_{var}	b_{cor}	$\|S\|^c$	$\bar{y}_M^d$	b_{cor}	$\|S\|^e$	$\bar{y}_M^f$	b_{cor}	$\|S\|^g$	$\bar{y}_M^h$
B5-1	0.656	0.968	2490.0	1.259	−0.228	0.4	0.195	1.015	0.12	0.885
B5-2	1.067	1.069	31.6	1.076	0.037	0.1	0.256	2.091	0.01	0.785
B8-1	0.992	0.864	0.8	0.814	1.627	9.9	1.294	0.553	0.59	0.897
B8-2	1.159	1.099	39.4	1.176	1.013	41.2	0.982	0.617	4.64	1.517
B8-3	0.575	1.347	128.3	1.114	1.200	0.9	0.969	1.329	0.63	1.373
B8-4	1.045	0.603	9.5	1.136	0.611	6.9	0.579	1.699	0.57	1.424
B8-5	1.310	1.115	467.0	1.821	1.396	143.9	1.472	1.049	2.23	1.365
B8-6	0.823	0.940	1.9	0.737	1.494	15.8	1.302	1.276	0.55	1.326
B9-1	1.604	1.061	6.5	1.160	−0.422	0.4	0.208	1.958	0.09	1.127
B9-2	0.787	1.305	13.2	1.275	−0.045	6.9	0.344	1.471	1.29	1.358
B9-3	0.742	0.956	0.5	0.958	1.440	7.5	1.056	0.361	0.32	0.874
B9-4	1.096	1.044	24.4	1.105	1.259	19.8	1.220	0.677	1.88	1.117
B9-5	0.917	1.036	4.9	0.967	1.154	10.7	0.885	1.241	0.76	1.268
B9-6	1.129	0.843	255.1	1.133	0.991	13.6	0.786	0.887	1.87	1.351

(a) STAM1A, STAM1F, STAM3A, STAM3F (Table 2).
(b) ELONGA, ELONGF, UNELOA, UNELOF (Table 2).
(c) Entries are determinant $\times 10^{24}$; reject H_0: $\Delta_k = \Delta(k = 1 \ldots 14, p < 0.001; k = 1 \ldots 6$ (B8 and B9), $p < 0.001)$.
(d) Reject H_0 of the equality of the $\bar{y}_M$ values for the B8 genotypes ($p < 0.001$) but not the B9 ones ($p = 0.81$).
(e) Entries are determinant $\times 10^9$; reject H_0: $\Delta_k = \Delta(k = 1 \ldots 14, p < 0.001; k = 1 \ldots 6$ (B8), $p < 0.01; k = 1 \ldots 6$ (B9), $p < 0.001)$.
(f) Reject H_0 of the equality of the $\bar{y}_M$ values for both the B8 and B9 genotypes ($p < 0.001$).
(g) Entries are determinant $\times 10^9$; reject H_0: $\Delta_k = \Delta$ $(k = 1 \ldots 14, p < 0.001; k = 1 \ldots 6$ (B8), $p < 0.001; k = 1 \ldots 6$ (B9), $p < 0.001)$.
(h) Reject H_0 of the equality of the $\bar{y}_M$ values for both the B8 ($p < 0.001$) and B9 ($p < 0.05$) genotypes.

does not appear to be accompanied by a marked increase in floral instability. The usual measure of multivariate variability, the determinant of the covariance matrix (for each genotype) varies considerably between genotypes, but not in any consistent relation to whether a genotype produces modified flowers or not (Table 5). Different scalings of the determinant (Goodman, 1968; Soulé, 1971) do not alter this lack of concordance.

Individual-average plots

Much smaller differences in variability are suggested by plots of genotype variances against average variances (diagonal elements of **W**), for measurements of

unmodified stamens (slopes of I-A plot regressions, b_{var}; Table 5). Moreover, these differences are influenced by whether or not flower position on the inflorescence axis is taken into account, or not. Based on comparisons of the variances of all 14 measurements, slopes for the four genotypes with exclusively unmodified flowers (B5-1, B5-2, B9-1, B9-2; Fig. 2) were in the range 0.28–0.80 for the data set with STAM1A, STAM1F, STAM3A, and STAM3F (compared with 1.06–1.20 for the other B9 genotypes, and 0.92–1.46 for the B8 ones). For the data set with ELONGA, ELONGF, UNELOA, and UNELOF, the corresponding ranges were 0.42–1.24, 0.91–1.19, and 0.76–1.27, respectively. The modifiable stamens provided only four variances, vitiating regression-based comparisons.

Total variances

Dagnelie (1975) and Legendre and Legendre (1983) have suggested that the normality of y_{iM} values is indicative of the multivariate normality of the original data. Examination of y_{iM} values calculated from scores on all M-PCA axes indicates markedly right-skewed distributions within genotypes and for the total sample as a whole. Values of y_{iM} calculated from only the scores on significant M-PCA axes (Table 5) showed the same pattern. For this reason the nested ANOVAs of total variances are based on y_{iM} values transformed so as to have a normal distribution over the total sample. For measurements of unmodified stamens, only genotypes from population B8 differed significantly in variability (Table 5). Significant differences between genotypes with respect to measurements of modifiable stamens were most pronounced before variability within a genotype due to elongation of stamen 1 in some flowers, and of stamen 3 in others was removed by use of measurements ELONGA, ELONGF, UNELOA, and UNELOF (Table 5). Thus, when the variability of the four genotypes with exclusively unmodified flowers (B5-1, B5-2, B9-1, B9-2; Fig. 2) was compared with that of the remaining genotypes, a significant difference was found only with y_{iM} values calculated from measurements STAM1A, STAM1F, STAM3A, and STAM3F ($p = 0.0001$).

Comparisons of correlation structure

Individual-average plots

Plots of genotype correlations against average values (off-diagonal elements of the correlation matrix calculated from W) did not suggest the presence of major differences between genotypes in correlation structure, even when different subsets of measurements were used. Differences between genotypes in the correlations among measurements of unmodified stamens appear to have little to do with whether or not a genotype produced modified flowers (Table 5). With respect to measurements of the modifiable stamens, differences between genotypes that either do or do not produce modified flowers depend on whether or not flower position is taken into account (Table 5).

Table 6. Comparison of the correlation structure of *Eichhornia paniculata* genotypes from northeast Brazil. Entries are p values for Kendall's test of the pairwise independence of genotype correlation matrices for eight measurements of unmodifiable stamens. Italicized entries are those for which the p value is less than the critical value after correction for multiple comparisons (0.05/91 = 0.00055; Cooper 1968; Rice 1989).

B5-2	*0.0005*														
B8-1	*0.0005*	0.0025													
B8-2	0.0075	0.0150	0.0085												
B8-3	0.0055	0.0050	0.0040	0.0010											
B8-4	*0.0005*	*0.0005*	*0.0005*	0.0360	0.0060										
B8-5	0.0050	0.0240	0.0065	0.0035	0.0135	0.0020									
B8-6	0.0030	0.0200	0.0030	0.0300	0.0295	0.0020	0.0055								
B9-1	*0.0005*	0.0035	0.0010	0.0355	0.0225	*0.0005*	0.0015	0.0015							
B9-2	0.0010	0.0035	0.0015	*0.0005*	0.0015	*0.0005*	*0.0005*	0.0010	*0.0005*						
B9-3	0.0015	0.0035	0.0015	0.0025	0.0035	*0.0005*	*0.0005*	0.0045	*0.0005*	*0.0005*					
B9-4	0.0010	0.0015	0.0010	0.0325	0.0225	0.0040	0.0120	*0.0005*	0.0010	0.0040	0.0015				
B9-5	0.0015	0.0015	0.0020	0.0015	*0.0005*	0.0030	0.0025	0.0035	0.0025	*0.0005*	0.0065	0.0115			
B9-6	0.0010	0.0010	0.0015	0.0245	0.0090	0.0010	*0.0005*	*0.0005*	*0.0005*	*0.0005*	*0.0005*	*0.0005*	0.0060		
		B5-1	B5-2	B8-1	B8-2	B8-3	B8-4	B8-5	B8-6	B9-1	B9-2	B9-3	B9-4	B9-5	

Correlation matrix comparisons

The other approach taken to comparing genotypes with respect to the patterns of measurement inter-correlation was to test the null hypothesis of correlation matrix independence (Dietz 1983). Calculation of Kc statistics for 91 pairwise comparisons of genotypes, based on trimmed correlations between eight measurements of the unmodifiable stamens, led to rejection of the null hypothesis in 24 of 91 comparisons (Table 6), after adjusting the α-probability level for simultaneous comparisons (from 0.05 to 0.05/91; Cooper, 1968; Rice, 1989). Only one of 15 comparisons between B8 genotypes led to rejection of the null hypothesis. All other blocks of within or between population comparisons included at least four such rejections (Table 6). Thus, except within population B8, there was appreciable similarity between genotypes with respect to correlations between measurements of unmodified stamens.

The null hypothesis of correlation matrix independence was accepted for all comparisons between genotypes based on the four derived measurements of modifiable stamens (ELONGA, ELONGF, UNELOA, UNELOF); the original measurements yielded correlation matrices that showed even less relationship among genotypes. The four comparisons which most closely approached rejection of the null hypothesis ($p < 0.04$) were between some, but not all, of the genotypes with modified stamen phenotypes (B8-1 with B8-5 and B9-4; B8-5 with B9-4; B8-6 with B9-5; compare Fig. 2). The patterns of relationships found in the three subsets of the data were largely concealed in earlier analyses in which all 14 measurements were considered simultaneously.

The apparent discrepancy between the results of the correlation I-A plots (Table 5) and those of the correlation matrix comparisons (Table 6) is due to the descriptive nature of the former method. In this method, the elements of genotype correlation matrices are compared with an 'average' value using linear regression statistics. Moreover, in the present case, clustering of the majority of these 'average' values in a narrow range made the regression statistics very sensitive to the effect of the extreme values outside this range. In contrast, the comparisons made using the Kendall statistic have a probabilistic basis, and are unlikely to be affected by the distributional properties of the elements of the matrices being compared.

Discussion

Methods for the analysis of multivariate data have played a major role in our investigations of the patterns of floral variation displayed by genotypes of *E. paniculata* from northeast Brazil. These methods have the advantage that they take into account the inevitable covariation of measurements from different parts of a unitary structure such as a flower. In the present case, flowers represented by vectors of four or eight measurements were ordinated on the basis of properties of matrices of the measurements' variances and covariances. Correlations between individual measurements and the ordination axes allowed recovery of information about the role of particular measurements (Fig. 4; Table 4). Graphical methods

enabled us to separate and examine contrasts in variability and correlation structure, in these four- or eight-measurement data sets (Table 5). Additional methods made it possible to examine statistically these same contrasts between genotypes (Table 5, 6). In so doing we are not so much discovering discrepancies between the results of uni- and multivariate analyses (Willig, Owen and Colbert, 1986; Willig and Owen, 1987) as we are extracting and summarizing information from multivariate data more efficiently than is possible with strictly univariate methods. In our choice of methods, our aim was to avoid some of the disadvantages associated with comparisons of variability based on univariate statistics such as the coefficient of variation (Sokal and Braumann, 1980; Schultz, 1985), and with determinant-based multivariate comparisons (Van Valen, 1978).

The most striking feature of floral variability investigated in this study concerns the modifications in filament length of short-level stamens in the mid-styled morph. Elongation of either (only rarely both) of the two lateral stamens (1 and 3, Fig. 1) so that they take up a position adjacent to mid-level stigmas results in automatic self-pollination of flowers. In modified genotypes usually only a single stamen changes position within a flower and whether or not this is stamen 1 or 3 depends on its position relative to the next flower developing on the inflorescence axis. Illustrations of early inflorescence development in *E. paniculata* (Fig. 5–7 and 23 in Richards and Barrett, 1984) suggest that this positional effect could be related to interactions between growth centers, and their effect on the competence to become elongated of one stamen, but not the other. This competence appears to depend upon which stamen primordium is closer to the next younger flower developing on the same axis, and is not expressed until about 24 h before anthesis (S. C. H. Barrett, unpubl. data).

Genetic modifications to stamen position in the mid-styled morph occur infrequently in trimorphic populations (Barrett et al., 1989) and were not evident in flowers of the two genotypes from population B5 that were examined in this study. In dimorphic and monomorphic populations of *E. paniculata*, however, such modifications are widespread (Fig. 2).

In the dimorphic population (B9) three patterns of short-level stamen position were evident. The flowers of two genotypes (B9-1, B9-2) resembled those of mid-styled plants from trimorphic population B5 in that no modifications of stamen position were apparent. In contrast, some flowers sampled from genotype B9-6 were modified but the majority were not, resulting in a skewed distribution of free filament length (Fig. 2). This behaviour is commonly observed in natural populations of *E. paniculata* and was previously described as 'instability' in short-stamen level position (Barrett, 1985a, b). The remaining three genotypes from population B9 exhibited a different pattern of short-stamen level variability. Bimodal distributions of free filament length for lateral stamens 1 and 3 (Fig. 2) indicate that most flowers from these genotypes were modified, so that there were approximately equal numbers of elongated and unelongated stamens in our samples.

All genotypes of *E. paniculata* from monomorphic population B8 exhibited some degree of short-level stamen modification in comparison with the 'control' genotypes from population B5. Considerable bimodality in free filament length was

evident in three genotypes (B8-1, B8-5, B8-6), indicating that most flowers contained one elongated stamen. The remaining genotypes (B8-2, B8-3, B8-4) were 'unstable', exhibiting a mixture of modified and unmodified flowers.

The data on patterns of short-stamen level variability as well as univariate comparisons of floral trait measurements (Table 3) are consistent with what is known about the mating systems and genetic variability of the populations (Glover and Barrett, 1986, 1987). Population B9 is dimorphic for style length (L, M) and composed of a mixture of modified and unmodified mid-styled plants. A study of outcrossing rates in the population indicated that the L morph was mostly outcrossing ($t = 0.78$) whereas the M morph was mostly selfing ($s = 0.64$). Unfortunately, sample sizes did not permit separate estimates of outcrossing for modified and unmodified M plants. Levels of genetic diversity in population B9 were moderately high for the species and consistent with the mixed-mating system of the population. The genetic data, in conjunction with the patterns of variation exhibited by floral traits, suggest that the population has not experienced a long history of inbreeding and may be in the early stages of evolution towards increased levels of self-fertilization (see Barrett et al., 1989). In contrast population B8 contains little genetic polymorphism (one polymorphic isozyme locus of 21 screened) and was composed entirely of mid-styled plants exhibiting short-stamen modification. These data suggest the population may have been inbreeding for a longer period of time than populations B5 and B9. Data on the correlation structure of floral traits (see below) also supports this hypothesis.

From the viewpoint of developmental genetics the most curious feature of short-level stamen modification, especially in 'unstable' genotypes, is the relatively discontinuous nature of filament elongation in stamens which are modified (Fig. 3). This pattern suggests a threshold response in which competence to elongate is expressed in an all or nothing manner. The patterns in populations B8 and B9 thus suggest the following genetic model to account for short-stamen length variation. Stamen length modification is under the control of one or more major genes. Elongation to the mid-level position in lateral stamens results from mutation(s) at loci governing short-stamen position. The penetrance and expressivity of the mutants are sensitive to genetic background, so that the background in which the mutant genes occur determines the extent of floral modification of both individual flowers and genotypes. Both environmental and developmental factors may also influence stamen position to varying degrees, depending on genotype. Finally, modifier genes altering the precise position of the altered stamens may be selected under conditions where self-fertilization is favoured. In other populations of *E. paniculata*, particularly in Jamaica, all true short-level stamens are adjacent to mid-level stigmas indicating additional genetic adjustments have occurred that favour increased self-fertilization.

Controlled crosses and selfs of modified and unmodified mid-styled plants from a dimorphic population (B3) from northeast Brazil are consistent with a model of single recessive gene control of the altered short-level stamens (S. C. H. Barrett, unpubl. data). However, genetic background effects were evident in the crosses raising the possibility that part of the variation may be polygenically controlled, with a threshold response (see Rendel, 1967, 1979 and Falconer, 1981 for further discussion).

Major gene control of short-level stamen modification is suggested by the absence of significant differences in variability associated with fixation of stamen modification (b_{var}, $|S|$, $\bar{y}_M$ values, Table 5). If stamen modification in *E. paniculata* was simply due to an overall breakdown of developmental canalization associated with increased homozygosity (Lerner, 1954) we might have expected to find short-level stamen modification associated with increased variability in all of the floral measurements that were made. Our results are otherwise (nested ANOVAs of y_{iM} values; Table 5), suggesting specific changes in the genetic control of short-stamen position as opposed to generalized developmental instability brought about by inbreeding and the attendant increase in homozygosity. It is important to note that this explanation does not negate the role of inbreeding in bringing about the observed patterns of short-level stamen modification. Recessive genes that modify stamen position are more likely to be exposed to selection by inbreeding. Therefore their occurrence in dimorphic and monomorphic populations is likely to be associated with their mating systems and small size (Barrett et al., 1989).

Further evidence for the role of inbreeding in the origin of floral variation in *E. paniculata* is evident in the contrasts in correlation structure between the monomorphic population, B8, and the dimorphic population, B9 (Table 6). Genotype correlation matrices were compared pairwise in all combinations, resulting in a rejection of the null hypothesis of their independence in only one comparison between B8 genotypes, but in eight comparisons between B9 genotypes. This suggests that in the monomorphic population homozygosity at genes that reduce canalization has produced a wider array of developmental trajectories, each with its own correlation structure. The opportunities for fixing deleterious genes that influence developmental pathways are increased in small populations as a result of inbreeding and genetic drift. Population B8 was the smallest of the three populations that were examined in this study (Table 1). With small population size and moderate levels of self-fertilization, genetic drift is likely to play a significant role in structuring the dissolution of floral traits that make up the heterostylous syndrome.

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