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# Floral variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae) II. Effects of development and environment on the formation of selfing flowers

Spencer C. H. Barrett[1] and Lawrence D. Harder[2]

[1]*Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2*
[2]*Department of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4*

*Key words:* Floral instability; position effects; stress conditions; logistic regression; *Eichhornia paniculata*; selfing flowers; heterostyly.

**Abstract**

Genotypes of the mid-styled morph of tristylous *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae) exhibit developmental instability in the position of short-level stamens under both field and glasshouse conditions. Elongation of one of the stamens to a position adjacent to the stigma results in automatic self-pollination of flowers. This modification initiates subsequent changes to floral morphology leading to the evolution of predominant self-fertilization in *E. paniculata*. The influence of genetic, developmental and environmental factors on the expression of stamen instability was investigated in experiments with genotypes from two populations from northeast Brazil and interpopulation hybrids. Genotypes from the three sources differed significantly in the degree and pattern of stamen instability expressed under uniform growing conditions. Significant position effects in the production of modified flowers were detected among genotypes using logistic regression techniques. Modified flowers were most frequently produced on later inflorescence branches in the flowering sequence and at proximal flower positions within an inflorescence branch. However, these patterns were complex, varying among genotypes and experimental conditions. Stamen modification increased in clones grown under water stress or at high temperature, demonstrating a significant environmental component to floral instability.

## Introduction

The evolutionary transition from outcrossing to high levels of self-fertilization in angiosperms is usually accompanied by major changes in floral morphology (Darwin, 1877; Ornduff, 1969; Wyatt, 1983; Richards, 1986). Alterations may involve reduction in the size and showiness of floral organs (Grant and Grant, 1965; Lloyd, 1965), shifts in position of reproductive parts (Rick et al., 1978; Garnock-Jones, 1981), modification in patterns of sex allocation (Schoen, 1982; Cruden and Lyon, 1985), and changes in the timing of developmental processes (Guerrant, 1989). The syndrome of traits associated with high levels of self-fertilization is probably governed by many genes, and shifts to a predominantly autogamous breeding system are likely to occur in stages over long periods.

In heterostylous plants, acquisition of a self-pollinating habit usually occurs before other floral changes that characterize the selfing syndrome have evolved. This is because predominant selfing can originate in a single step through recombination in the supergene that governs the floral polymorphism (Ernst, 1955; Dowrick, 1956; Shore and Barrett, 1985) or by modifier genes with large effects on reproductive organ position (Mather, 1950; Mather and DeWinton, 1941; S. C. H. Barrett, unpubl. data). Gradual reductions in flower size and changes in sex allocation patterns may then follow if selection favors the evolution of predominant self-fertilization (Ornduff, 1972; Barrett, 1988; Morgan and Barrett, 1989). This evolutionary sequence differs from that in many non-heterostylous plants where reductions in flower size may be the cause rather than the consequence of increased self-fertilization (Guerrant, 1989).

*Eichhornia paniculata* (Pontederiaceae) displays a wide range of mating systems from predominant outcrossing to high levels of self-fertilization (Barrett and Husband, 1990). This variation is associated with high self-fertility in the species and the evolutionary breakdown of trisyly to semi-homostyly (Barrett et al., 1989). In common with other heterostylous plants, changes in the position of reproductive organs initiate the evolution of selfing in *E. paniculata*. The modifications differ, however, from those reported in homostylous variants of other heterostylous groups. Unlike *Primula*, where all homostylous flowers autonomously self-pollinate, genotypes of *E. paniculata* vary in the capacity of their flowers to self-pollinate. This variation arises because of developmental instability in stamen position and results in plants with differing frequencies of self-pollinating flowers. Floral variation of this type thereby affects the selfing rate of style morphs and population genetic structure (Glover and Barrett, 1986; Barrett and Husband, 1990).

Seburn et al. (1990) demonstrated differences in the instability of short-level stamen position among genotypes of the mid-styled morph of *E. paniculata*, with filaments of the two lateral short-level stamens exhibiting the greatest variability. Within a flower the elongation of one of the three short-level stamens to a position adjacent to mid-level stigmas results in autonomous self-pollination of flowers (Fig. 1). An issue not addressed in their study concerned the non-genetic sources of variation that influence patterns of stamen modification. Observations have indicated that both developmental and environmental factors influence patterns of

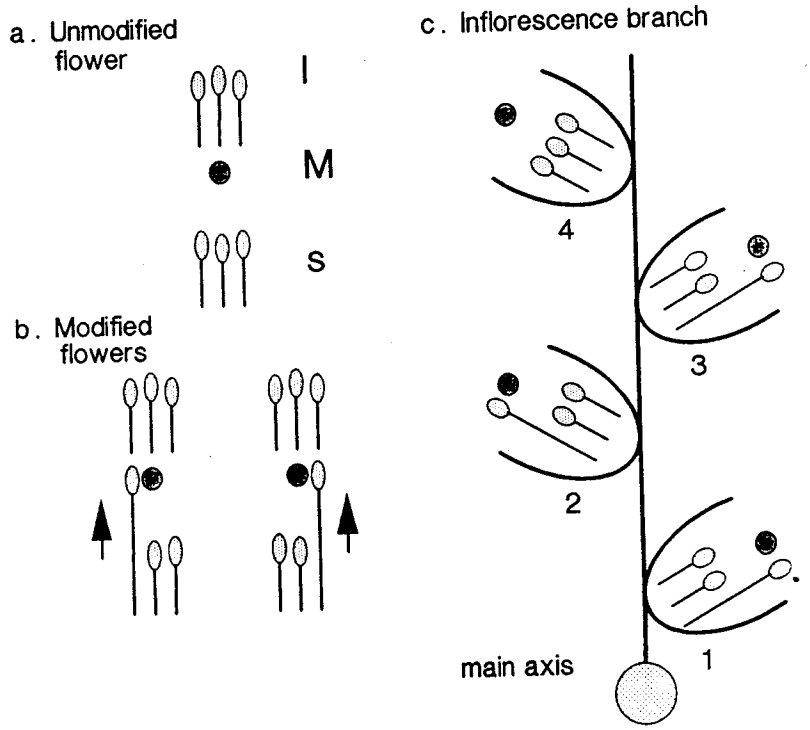


**Fig. 1.** Schematic illustration of stamen modifications in flowers of the mid-styled morph of *Eichhornia paniculata* investigated in this study. a) unmodified flower showing long-level stamens (l) above and short-level stamens (s) below the mid-level stigma (M). b) modified flowers showing elongation of either of the two lateral stamens to a position adjacent to the mid-level stigma as a result of increased filament length. c) Inflorescence branch with four flowers in order of flowering (1 first, 4 last). The first three flowers are modified; the distal flower is unmodified. There is 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 the lower-level stamen is elongated. Hence modified as well as modifiable stamens can be readily identified.

floral variation in *E. paniculata*. Because inflorescences produce both selfing and non-selfing flowers it is of interest to determine whether these contrasting floral phenotypes are produced randomly with respect to development, or whether positional effects, of the type reported in cleistogamous species (Lord, 1981; Ellstrand et al., 1984), are evident. Field and glasshouse observations have also suggested that unfavourable growing conditions, particularly water stress or high temperatures, promote the production of self-pollinating flowers in genotypes displaying floral instability. This observation is in accord with previous work on both plants and animals demonstrating a higher incidence of developmental instability under stress conditions (Lerner, 1954; Beardmore, 1960; Griffing and Langridge, 1963; Huether, 1968; Levin, 1970).

To investigate factors influencing intraplant variation in the occurrence of self-pollinating flowers we initiated the present study of *E. paniculata*. Our investigation had three major objectives: 1) to document variation within and between genotypes in inflorescence characters and patterns of floral instability; 2) to determine whether self-pollinating flowers are produced randomly within and between inflorescences; and 3) to examine whether stress increases the production of self-pollinating flowers. Our findings suggest that the traditional view of the flower as a highly canalized, repeated structure needs revision.

## *Materials and Methods*

The experiments described in this study were conducted under uniform glasshouse or growth chamber conditions using genotypes of *E. paniculata* that originated from three sources. Some genotypes were randomly selected from seed collected in two populations from northeast Brazil (B3, dimorphic; B4, monomorphic). Field observations of plants in the two populations indicated a high degree of short-level stamen instability. The remaining genotypes were $F_1$ interpopulation hybrids (H) between genotypes originating from population B2 (Brazil) and population J3 (Jamaica). The hybrids had high vegetative vigour allowing cloning of material, were fully fertile, and possessed modified flowers of the type observed in populations B3 and B4 (see below). To facilitate comparisons, all genotypes were of the mid-styled morph. Localities and details of the floral biology of source populations are given in Barrett (1985), and information on the glasshouse culture, growth, floral development and reproductive biology of *E. paniculata* is provided in Barrett (1985), Richards and Barrett (1984), and Morgan and Barrett (1989).

### *Sampling and Classification of Flowers*

As described in Seburn et al. (1990), within a given flower either (but usually not both) of the lateral short-level stamens may be modified by elongation of the free filament (Fig. 1). Flowers were initially classified into four classes: 1) unmodified flowers, which did not display an observable increase in filament length; 2) partially modified flowers, which had an elongated filament but in which the anther did not contact mid-level stigmas; 3) modified flowers, which had a fully elongated filament allowing the anther to contact mid-level stigmas resulting in automatic self-pollination; and 4) abnormal flowers, which had unexpanded floral organs and appeared bud-like. Filament length in short-level stamens of genotypes exhibiting floral instability usually varies bimodally, rather than continuously (Fig. 2; also see Seburn et al. 1990). As a result, most flowers can be classified as either unmodified (1) or modified (3) with relatively few falling into the partially modified (2) class. Owing to the relatively small numbers of partially modified flowers that were recorded in subsequent samples, classes 2 and 3 were pooled in the statistical

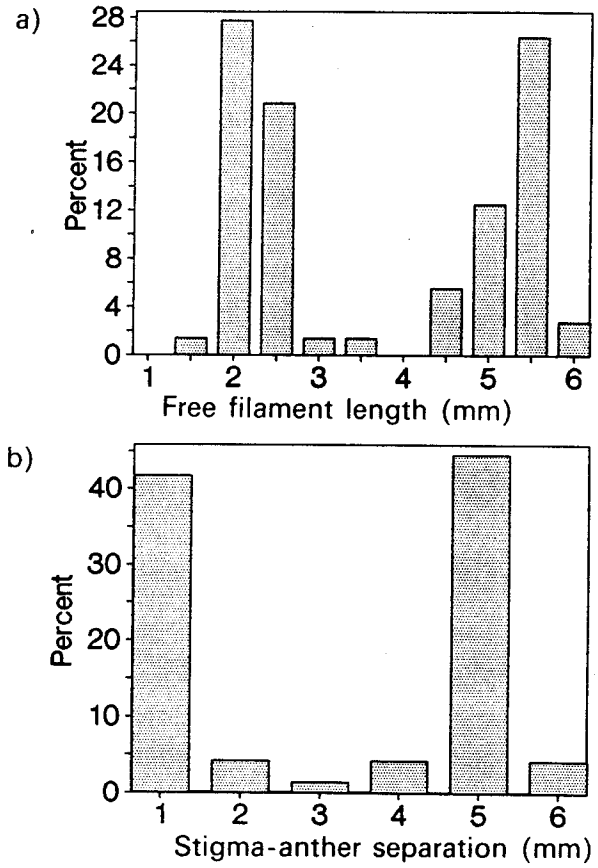


**Fig. 2.** Distribution of a) free filament length and b) stigma-anther separation in a random sample of 72 flowers from genotype H-2 of *Eichhornia paniculata* (based on 6 flowers from each of 2 inflorescences on 6 ramets). Each distribution is bimodal, reflecting the production of unmodified and modified flowers.

analysis of data described below, unless otherwise noted. These classes are hereafter referred to as modified flowers.

*Experiments*

Five experiments were conducted to determine population, genotypic, developmental and environmental sources of variation in the formation of modified flowers.

*Experiment 1*

To survey the extent of short-level stamen instability in *E. paniculata* grown under uniform glasshouse conditions, we compared 20 genotypes from each of the

three populations. All flowers on three consecutive inflorescences were scored according to the classification scheme described above. Results from this experiment established that instability in stamen position is expressed under uniform growing conditions as well as in the field.

*Experiment 2*

Two genotypes were then randomly selected from each population to investigate the relationships between flowering patterns, inflorescence characteristics and floral modification. Over three months all flowers on twelve consecutive inflorescences from each of the six genotypes were classified as above. We recorded each flower's position on an inflorescence (branch number [most basal branch = 1] and position on a branch [most proximal flower = 1]) and the date of anthesis. Plants were grown in 10 cm pots in a glasshouse between 25–30° C and fertilized every two weeks.

*Experiment 3*

To investigate further the details of intraplant variation in the production of modified flowers, genotype H-2 was cloned into six ramets which were grown in 10 cm pots for four months under uniform growing conditions at 25–30° C. This genotype was chosen from the six above because it displayed high variability in stamen position and could be cloned readily. All flowers on six consecutive inflorescences on each of the six ramets were scored for flower type, branch number, bud position, and date of flowering.

*Experiment 4*

To investigate the influence of stressful growing conditions on stamen modification, genotype H-2 was cloned into a further 12 ramets; six were grown in 10 cm pots and the remainder in 5 cm pots for four months. Plants in large pots were fertilized regularly and water levels maintained at the soil surface. In contrast, plants in small pots were not fertilized and were subjected to periodic drought. Hereafter we refer to these two treatments as unstressed and stressed, respectively. The contrasting treatments resulted in large differences in plant size. Plant height and the length and width of the largest leaf (technically a bract) were then measured and all flowers on two consecutive inflorescences produced by the 12 ramets were scored as above. After the six unstressed ramets had produced two inflorescences, fertilizer was withheld and the plants were subjected to drought conditions for two weeks. A third inflorescence was then scored. Observations of floral instability under glasshouse conditions suggested that high temperatures (e.g. > 30° C) promoted stamen modifications. In experiment 4, involving stress and non-stress conditions, glasshouse temperatures were lower (20–25° C) to enable comparisons of plants in experiments 2 and 3 and those in the unstressed treatment in experiment 4.

*Experiment 5*

Five more ramets of H-2 were grown in a growth chamber at 20° C [12 hours light] to investigate the effect of cooler growing conditions on floral modification.

Plants were grown in 10 cm pots, fertilized every two weeks and all flowers on six consecutive inflorescences were scored as above.

*Statistical Analysis*

Logistic regression (Cox and Snell, 1989) was used to determine whether modified flowers were systematically produced within and between genotypes, ramets, and inflorescences. This technique relates a dichotomous response (unmodified vs. modified flower) to a group of independent variables, which can be either continuous or categorical. Specifically, logistic regression depicts the natural logarithm of the odds that a flower is modified (odds = Pr[modified flower]/Pr[unmodified flower]) as a linear function of $k$ independent variables ($V_{ik}$), so that

$$\ln [\text{odds}] = b_o + \sum b_k V_{ik},$$

where the $b_k$ constants estimate the effect of a unit change in the respective variable, and $b_o$ (intercept) represents the ln[odds] expected when all $V_{ik} = 0$. The probability that a particular combination of conditions resulted in a modified flower can be estimated by the logistic transformation,

$$\Pr [\text{modified}] = \exp\left(b_o + \sum b_k V_{ik}\right) \Big/ \left[1 + \exp\left(b_o + \sum b_k V_{ik}\right)\right].$$

We used a stepwise selection procedure (BMDPLR; Dixon, 1983) to select the set of independent variables that contributed significantly ($P < 0.05$) to minimizing the difference between the observed and predicted probabilities that a given set of conditions resulted in a modified flower. The basic variables representing inflorescence characters that we considered included: inflorescence number; branch number; flower position along a branch; and the number of days elapsed since a given inflorescence produced its first flower (flowering day). Several quadratic and interaction terms were also considered to describe nonlinear influences, including: inflorescence$^2$; branch$^2$; position$^2$; inflorescence × branch; inflorescence$^2$ × branch; inflorescence × branch$^2$; inflorescence × position; inflorescence$^2$ × position; inflorescence × position$^2$; branch × position; branch$^2$ × position; and branch × position$^2$. Crossed (experiment 2 – population (B3, B4, H); experiment 3 – ramet; experiment 4 – stress treatment) and nested categorical effects (experiment 2 – genotypes within populations; experiment 4 – ramets within stress treatments) were represented by series of indicator variables (Neter et al., 1985). Several two-factor interaction terms between these categorical effects and inflorescence characteristics were also included as potential predictors in the logistic regression model.

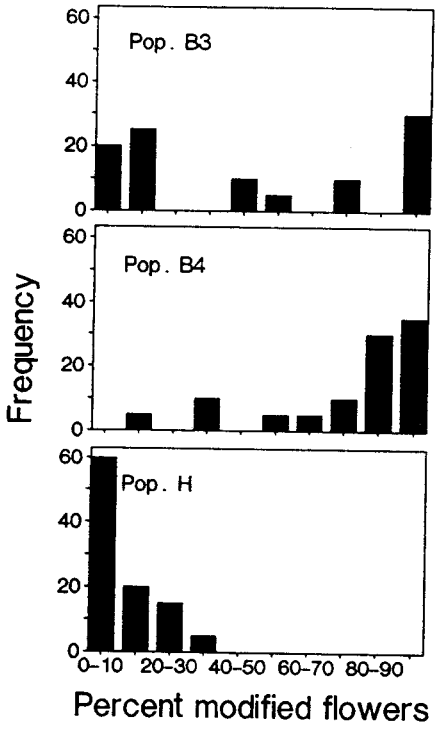


**Fig. 3.** Frequency distributions of the percentage of modified flowers per plant from samples of 20 genotypes in three populations (B3, B4, H) of *Eichhornia paniculata* in experiment 1. All flowers on three consecutive inflorescences per genotype were classified according to the criteria discussed in the Methods section. The three populations differed significantly in the frequency of modified flowers produced by individual genotypes (Kruskal-Wallis $H = 21.629$, 2 df 2, $P < 0.001$).

## Results

### *Experiment 1*

The three experimental populations (B3, B4, H) each exhibited considerable variation among genotypes in the degree of short-level stamen modification (Fig. 3). In addition, the three populations differed from each other in the degree of modification exhibited by genotypes: plants from population B4 usually produced modified flowers (median percent of modified flowers = 86.2, inter-quartile range = 72.9–94.5); population B3 plants exhibited a wide range of phenotypes (median = 47.7, inter-quartile range = 10.2–91.0); and hybrid plants (H) seldom produced modified flowers (median = 8.4, inter-quartile range = 3.0–18.0).

**Table 1.** Characteristics of inflorescences produced by the six genotypes of *Eichhornia paniculata* in Experiment 2 (mean ± SE, based on 12 inflorescences per genotype). Means and standard errors for flower production are based on square-root transformed data. The proportions of modified flowers are based on the number of flowers indicated in parentheses.

| Population and genotype | Number of flowers per inflorescence | Number of branches per inflorescence | Median number of flowers per branch | Proportion of modified flowers |
|---|---|---|---|---|
| Pop. B3 | | | | |
| B3-1 | 20.1<br>18.1–22.2 | 11.8 ± 0.45 | 1.6 ± 0.15 | 0.810<br>(242) |
| B3-2 | 41.6<br>36.3–47.3 | 14.3 ± 0.68 | 3.3 ± 0.41 | 0.906<br>(500) |
| Pop. B4 | | | | |
| B4-1 | 29.4<br>26.2–32.8 | 14.7 ± 0.38 | 2.1 ± 0.26 | 0.496<br>(351) |
| B4-2 | 34.7<br>32.2–37.2 | 12.5 ± 0.40 | 2.9 ± 0.23 | 0.198<br>(415) |
| Hybrid (H) | | | | |
| H-1 | 45.1<br>39.5–51.0 | 11.5 ± 0.67 | 4.1 ± 0.36 | 0.029*<br>(561) |
| H-2 | 33.4<br>29.1–38.0 | 9.6 ± 0.79 | 3.5 ± 0.32 | 0.468<br>(417) |

*Experiment 2*

The six genotypes chosen from populations B3, B4, and H differed significantly in the number and patterns of flower production (Table 1). Population affiliation did not significantly affect the numbers of flowers per inflorescence, branches per inflorescence, or median numbers of flowers per branch (analyses of variance using genotypes within populations as the error term, $P > 0.25$ in all cases). Within populations, the respective genotypes produced significantly different numbers of branches per inflorescence (*a priori* contrasts, $P < 0.025$ in all cases); however, only genotypes B3–1 and 2 differed from one another in flower production per inflorescence and the median number of flowers produced per branch (*a priori* contrasts, $P < 0.001$ in both cases). Total flower production did not vary significantly between inflorescences ($F = 0.84$, 11 and 33 df, $P > 0.5$), nor was there a significant population × inflorescence interaction ($F = 1.09$, 22 and 33 df, $P > 0.25$).

Production of modified flowers varied considerably between populations, between genotypes within populations, between inflorescences within genotypes and within inflorescences (Fig. 4). Of the 2528 flowers for which we recorded flower condition: 54.2% (1370) were unmodified, 9.5% (240) were partially modified, 34.7% (876) were modified and 1.7% (42) failed to expand. Because of the small number of flowers in this last category and the possibility that they arise by a different

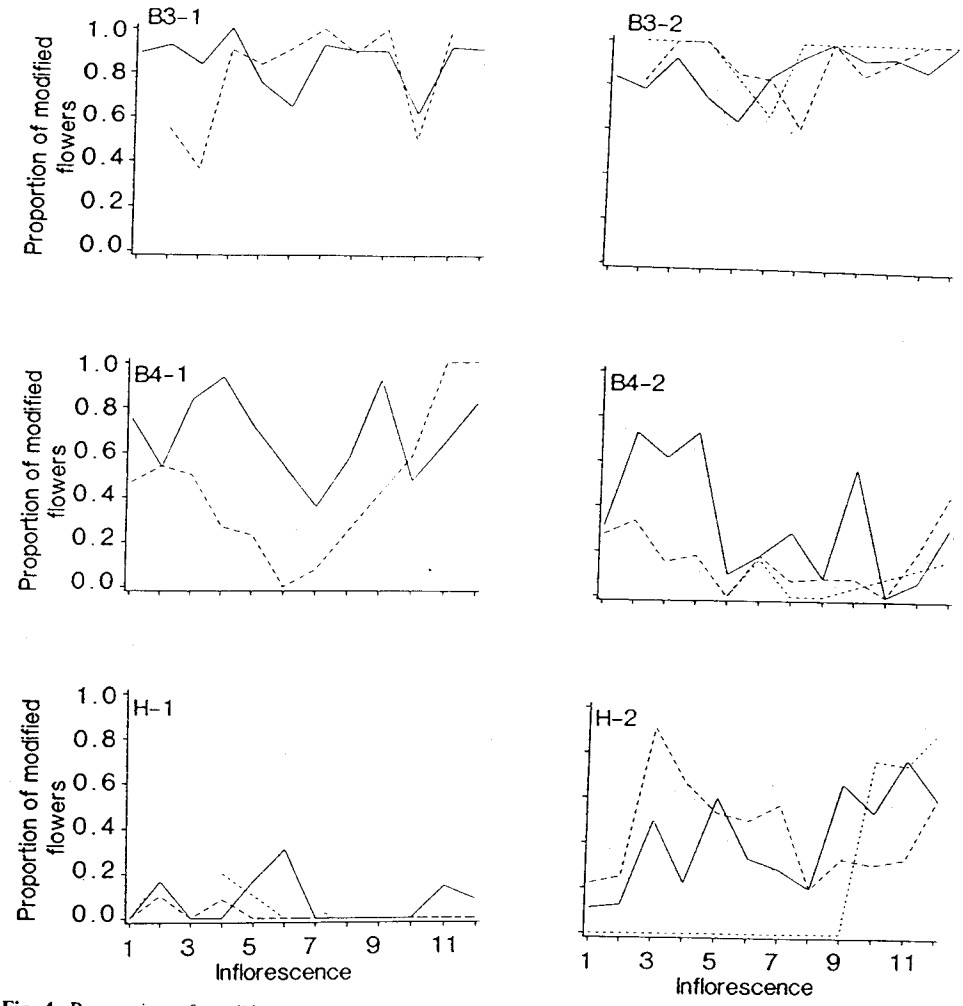


**Fig. 4.** Proportion of modified flowers produced at the first four positions along an inflorescence branch for 12 consecutive inflorescences by six genotypes of *Eichhornia paniculata* in experiment 2. The proportions were calculated over all branches within an inflorescence. Only observations based on at least three flowers are illustrated. Position 1 (most proximal) – solid line; position 2 – long dashes; position 3 – dotted line; position 4 – short dashes.

developmental process than the other floral modifications, they were excluded from further analysis.

Genotypes B3-1,2 produced modified flowers much more frequently than genotypes from the other groups (Table 1, Fig. 4). In contrast, hybrid genotype H-1 rarely produced modified flowers. Genotypes B4-1,2 produced intermediate frequencies of modified flowers and exhibited considerable variation both within and between inflorescences. A similar pattern was evident in genotype H-2, with the

**Table 2.** Logistic regression coefficients for variables that contributed significantly ($P < 0.05$) in describing the likelihood of a modified flower being produced for the six genotypes of *Eichhornia paniculata* studied in Experiment 2. The regression coefficients estimate the effect of a single unit increase of the respective variable on ln(Pr[modified flower] Pr[modified flower]).

| Effect | B3 | | B4 | | Hybrid | |
|---|---|---|---|---|---|---|
| | B3-1 | B3-2 | B4-1 | B4-2 | H-1 | H-2 |
| Intercept | 0.784 | 0.784 | 1.761 | 1.051 | −2.699 | −1.675 |
| Inflorescence | −0.076 | −0.076 | −0.344 | 1.325 | −0.086 | 0.200 |
| Inflorescence$^2$ | 0.004 | 0.004 | 0.019 | 0.019 | −0.022 | −0.022 |
| Position | 0.261 | 0.684 | −0.383 | −0.383 | −0.092 | −0.092 |
| Position$^2$ | – | – | – | – | −0.054 | 0.054 |
| Branch | −0.024 | −0.024 | −0.065 | −0.065 | 0.089 | 0.089 |
| Branch$^2$ | – | – | 0.003 | 0.003 | – | – |
| Inflorescence × Position$^2$ | −0.054 | −0.054 | −0.054 | −0.054 | −0.054 | −0.054 |
| Inflorescence$^2$ × Position | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 | 0.005 |

frequency of modified flowers varying greatly between successive inflorescences (Fig. 4). The tendency for the largest variance in modified flower production to be associated with intermediate mean frequencies is consistent with a binomial process.

Logistic regression analysis of the occurrence of modified flowers detected significant differences between populations ($F = 17.50$, 2 and 2464 df, $P < 0.001$), between genotypes from population B4 ($F = 4.74$, 1 and 2465 df, $P < 0.05$), and between the two hybrid genotypes ($F = 9.62$, 1 and 2465 df, $P < 0.005$). The final models for each genotype (Table 2) all include effects due to inflorescence, branch, flower position, and an interaction between inflorescence and flower position. Typically, the probability of producing a modified flower declined with successive inflorescences (Fig. 5). The effect of flower position within a branch varied between genotypes: modified flowers were more likely in proximal positions for genotypes from population B4; whereas proximal flowers had the lowest expected frequency of modification in genotypes from population B3. These effects of flower position were generally less pronounced for later inflorescences, hence the inflorescence × position interaction. The regression coefficients (Table 2) indicate that the probability of producing a modified flower declined from proximal to distal branches within an inflorescence, except in the hybrid genotypes (but see below).

### *Experiment 3*

The six ramets of genotype H-2 studied in experiment 3 produced from 169 to 218 flowers (mean = 186.7, SD = 16.97) and individual inflorescences produced from 14 to 44 flowers (mean = 31.1, SD = 5.96). There was no consistent pattern in

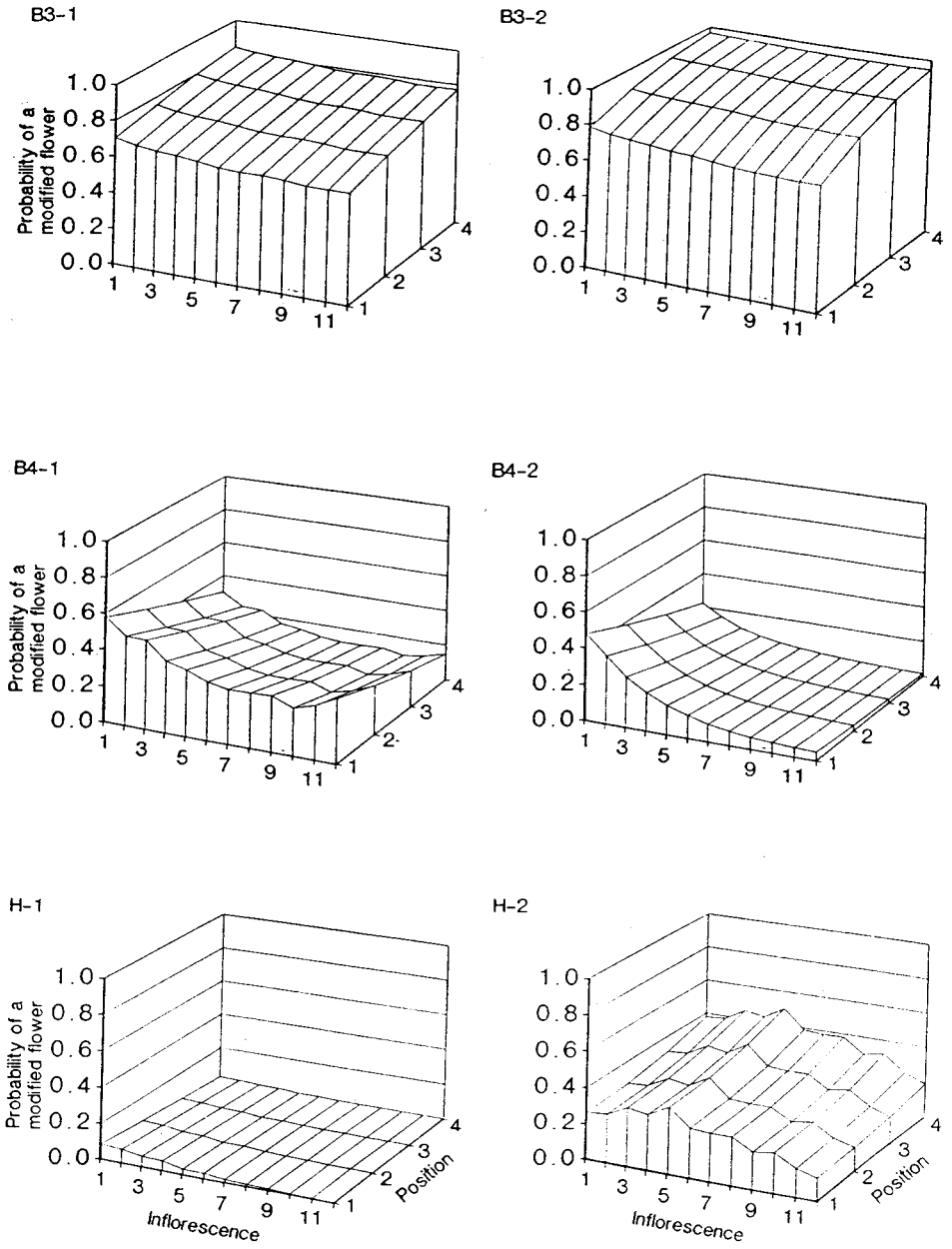


**Fig. 5.** Predicted probability of a modified flower at the first four positions along an inflorescence branch for 12 successive inflorescences in six genotypes of *Eichhornia paniculata* in experiment 2. Data are based on the logistic regression statistics presented in Table 2. The probabilities are averaged over all branches within an inflorescence. The irregular response surfaces result because inflorescences produced different numbers of branches.

the number of flowers produced by successive inflorescences within a ramet. Each of the six inflorescences developed from 7 to 11 branches: lower branches produced from 3 to 5 flowers, with the number of flowers per branch gradually declining up the inflorescence.

Most (63.8%) of the 1120 flowers produced were unmodified with respect to the filament lengths of short-level stamens. Of the remaining flowers, 32.4% were modified and 3.8% failed to expand. Two particular ramets produced 29 (69.0%) of the abnormal flowers, of which 24 were produced by the sixth inflorescence. No pattern was evident in the occurrence of the remaining abnormal flowers. Modified flowers were clearly not randomly distributed with respect to inflorescence within a ramet, branch within an inflorescence, or position along a branch. For example, 10 of the 15 modified flowers on second branches occupied the most proximal position on the branch. In addition, the proportion of modified flowers increased from 11.6% (15/129) on second branches to 25.9% (30/116) on sixth branches and 48.1% (25/52) on tenth branches.

The final logistic regression model (Table 3a) includes only a small subset of the available independent variables and indicates that inflorescence, branch and

**Table 3.** Logistic regression statistics for variables that significantly contributed ($P < 0.05$) in describing the likelihood of modified flower production in Experiments 3 and 4 conducted on genotype H-2 of *Eichhornia paniculata*. The regression coefficients estimate the effect of a single unit increase of the respective variable on ln(Pr[modified flower/Pr[unmodified flower]). The stress treatments were indicated by a dichotomous variable (stress = −1, not stressed = 1).

| Effect | Coefficient | Standard error |
|---|---|---|
| a) Experiment 3 | | |
| Branch | 0.22 | 0.023 |
| Inflorescence × Position | −0.81 | 0.063 |
| Inflorescence$^2$ × Position | 0.09 | 0.007 |
| Inflorescence × Position$^2$ | 0.07 | 0.011 |
| b) Experiment 4 – stress treatment | | |
| Stress | −1.26 | 0.169 |
| Branch | 0.14 | 0.050 |
| Position | −2.25 | 0.294 |
| Position$^2$ | 0.33 | 0.059 |
| Ramet 8 | 1.10 | 0.357 |
| Ramet 9 | −1.22 | 0.414 |
| c) Experiment 4 – stress treatment on initially unstressed ramets | | |
| Stress | −3.54 | 0.672 |
| Position | −1.72 | 0.285 |
| Stress × Position | 1.68 | 0.393 |
| Branch × Position$^2$ | −0.01 | 0.006 |
| Ramet 2 | 1.16 | 0.515 |
| Ramet 3 | 2.37 | 0.643 |
| Ramet 5 | −4.56 | 1.188 |

position all influenced the likelihood of a flower being modified (the five flowers in position 5 were excluded from this analysis). Specifically, proximal flowers on branches were more likely to be modified and this tendency increased with successive branches in an inflorescence (Fig. 6).

In addition, flowers on first and sixth inflorescences were more likely to be modified than flowers on intervening inflorescences. The most likely location for a modified flower on a given branch level was in the most proximal position on first inflorescences. Modified flowers occurred with equivalent frequency on all six ramets.

*Experiment 4*

Stress dramatically affected both vegetative and floral characteristics in ramets of genotype H-2. Stressed ramets produced shorter flowering stems (Table 4: $F = 30.17$, 1 and 10 df, $P < 0.001$; based on repeated-measures ANOVA) and the leaf subtending the inflorescence was shorter ($F = 68.43$, 1 and 10 df, $P < 0.001$) and narrower ($F = 45.72$, 1 and 10 df, $P < 0.001$) than in unstressed ramets. In addition, inflorescences on stressed ramets produced only one third as many flowers relative to unstressed ramets (Table 4: $F = 39.34$, 1 and 10 df, $P < 0.001$). The decreased flower production resulted primarily because inflorescences on stressed ramets produced fewer flowers per branch; branch production was less affected by stress (see sample sizes in Table 4).

Logistic regression analysis indicated the same pattern of production of modified flowers within an inflorescence as in the previous experiment (Table 3b, Fig. 7). Unlike the previous experiment, the probability of producing a modified flower did not change significantly from one inflorescence to the next; however, the stress experiment involved only two inflorescences per ramet, so there was little opportunity for this effect to be realized.

Logistic regression analysis also indicated a significant effect of stress on the probability of flower modification (Table 3b). Few flowers on unstressed ramets were modified; whereas almost one third of the flowers on stressed ramets exhibited some modification (Table 4). Stress appears to have uniformly increased the probability of modification (see Table 5), since interaction terms between stress treatment and branch or flower position did not significantly improve the regression model ($P > 0.1$ in both cases). Two of the stressed ramets (ramets 8 and 9) differed significantly from the remaining stressed ramets in the proportion of modified flowers (ramet 8, 54.8%; ramet 9, 11.9%; remaining ramets 31.9%). The biological basis for these differences are unknown.

After the six unstressed ramets had produced two inflorescences, they were subjected to severe water stress and then a third inflorescence was scored. The response to this stress, both in flower production and the proportion of modified flowers, varied greatly between ramets (Tables 3c and 6). Over all ramets, the application of water stress dramatically increased the probability of a modified flower at the most proximal position on an inflorescence branch. This response is reflected in the significant stress × position interaction detected by logistic regression analysis (Table 3c).

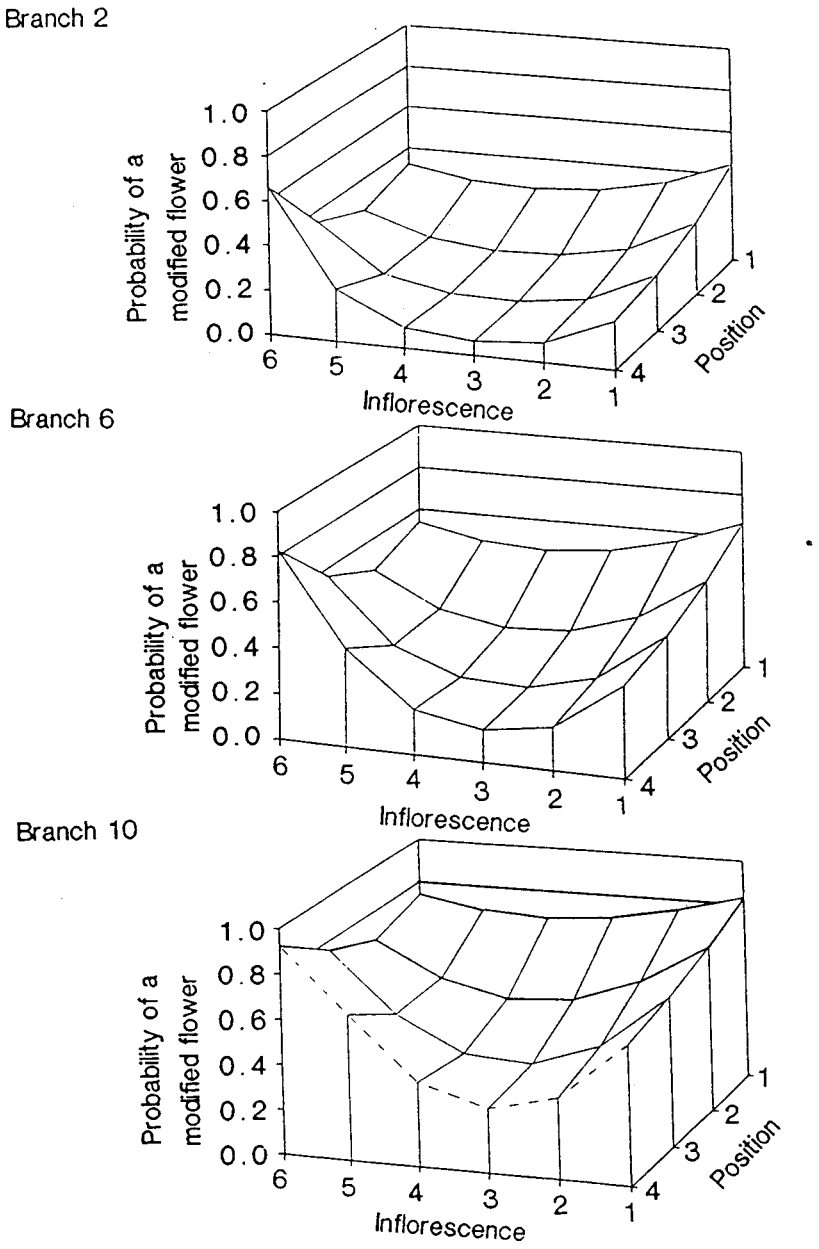


**Fig. 6.** Predicted probability of a modified flower being produced by genotype H-2 of *Eichhornia paniculata* in experiment 3 in relation to: position along the branch, branch number within an inflorescence, and inflorescence within a ramet. Data are based on the logistic regression statistics presented in Table 4a. Note that proximal flowers within a branch are toward the rear of the figure and proximal inflorescences are toward the right. The dashed lines for branch 10 indicate extrapolation beyond the data: no tenth branch produced four flowers.

**Table 4.** Inflorescence characteristics for ramets of *Eichhornia paniculata* genotype H-2 grown under different treatments (mean ± SE). Data are based on the first two inflorescences produced per ramet, with 10 inflorescences per treatment for all characters except flower production and the proportion of modified flowers (n = 12). The numbers in parentheses for the proportion of modified flowers indicate the total number of flowers.

| | Experiment 3 | Experiment 4 | |
|---|---|---|---|
| | | Unstressed | Stressed |
| Height | – | 36.0 ± 1.67 | 20.9 ± 1.09 |
| Bract length (cm) | – | 11.7 ± 0.42 | 5.2 ± 0.42 |
| Bract width (cm) | – | 13.8 ± 0.79 | 4.8 ± 0.55 |
| Number of flowers | 28.8 ± 2.1 | 45.4 ± 3.4 | 15.8 ± 1.9 |
| Proportion of modified flowers | 0.376 (338) | 0.033 (545) | 0.312 (189) |

Based on the first two inflorescences produced per ramet, the ramets used for experiment 3 produced significantly more flowers than stressed ramets in experiment 4, but significantly fewer flowers than unstressed ramets (see Table 4: based on Tukey's multiple comparisons in a repeated-measures design). Ramets in experiment 3, and those receiving the stressed treatment in experiment 4, produced equivalent proportions of modified flowers (Table 4: likelihood ratio chisquare, $G = 2.16$, 1 df, $P > 0.1$): together they produced a significantly greater proportion of modified flowers than unstressed plants in experiment 4 ($G = 200.91$,1 df, $P < 0.001$). The relative frequency of partially modified and modified flowers did not differ among the different experiments or treatments ($G$4.13, 1 df, $P > 0.1$).

*Experiment 5*

The five ramets of H-2 grown in a growth chamber at 20° C showed very little floral modification. Of the total of 685 flowers sampled on six consecutive inflorescences per ramet all except 6 were unmodified. Of these, four were fully modified and two failed to expand. The four modified flowers were all produced by a single ramet.

## Discussion

Populations of *E. paniculata* exhibit diverse floral variation associated with evolutionary breakdown of tristyly to semi-homostyly. The stamen modifications investigated in this study are the most significant aspects of floral variation relevant to the evolution of self-fertilization because they involve the specific mechanisms that govern the facility for spontaneous self-pollination. Modifications are infrequent in trimorphic populations but occur commonly in dimorphic and monomorphic populations (Barrett et al., 1989). They are usually restricted to the mid-styled

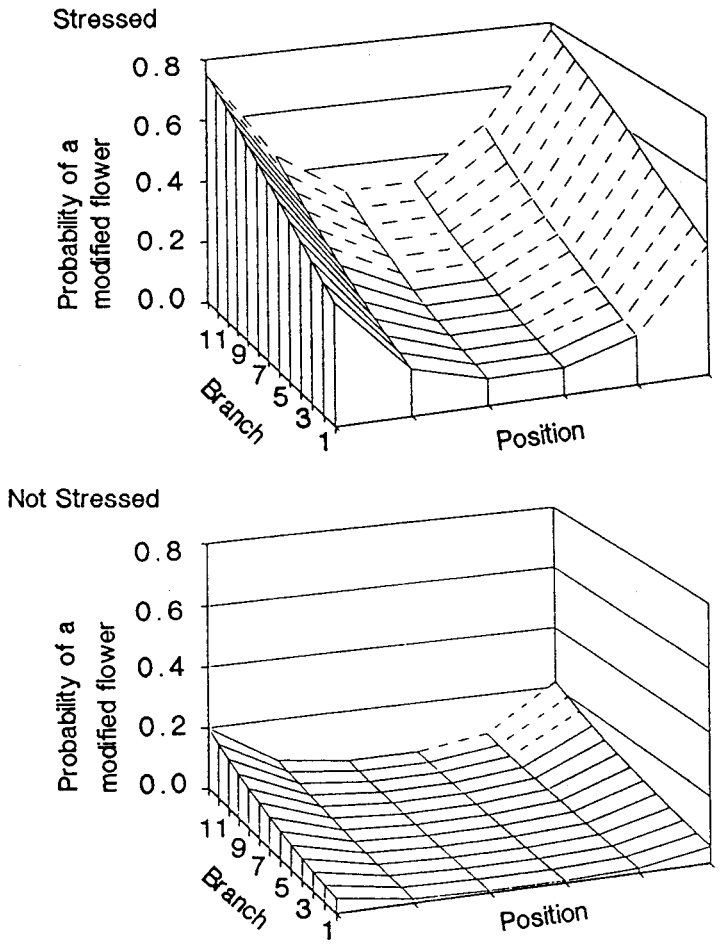


**Fig. 7.** Predicted probability of a modified flower being produced by genotype H-2 of *Eichhornia paniculata* in experiment 4 in relation to: stress treatment, branch number and position along the branch. Data are based on the logistic regression statistics presented in Table 4b. Dashed lines indicate extrapolation beyond the data.

morph and involve changes in the position of short-level stamens. Genetic studies indicate that a small number of recessive genes govern stamen modifications (S. C. H. Barrett, unpubl. data). Analysis of these modifications is complicated, however, because not all flowers borne within a single inflorescence necessarily display the same phenotype (Seburn et al., 1990). In this study we attempted to determine the sources of variation in short-level stamen modification, with particular emphasis placed on developmental and environmental factors.

The experimental material used was deliberately chosen to include genotypes of contrasting pedigree and levels of heterozygosity. In this way it was possible to

**Table 5.** Observed proportions of modified flowers in Experiment 4 on selected branches of *Eichhornia paniculata* in relation to position along the branch, and stress treatment. Numbers in parentheses indicate sample size.

| Branch | Position | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Nonstressed | | | | | | |
| 2 | .000 (12) | .000 (12) | .083 (12) | .083 (12) | .000 (9) | .000 (5) |
| 4 | .083 (12) | .083 (12) | .000 (12) | .000 (12) | .000 (9) | .000 (4) |
| 6 | .091 (11) | .000 (11) | .091 (11) | .000 (11) | .143 (7) | .000 (1) |
| 8 | .000 (11) | .000 (11) | .000 (11) | .200 (10) | .000 (6) | – |
| 10 | .250 (8) | .000 (8) | .125 (8) | .000 (7) | .000 (1) | – |
| 12 | .000 (1) | .000 (1) | .000 (1) | .000 (1) | – | – |
| Stressed | | | | | | |
| 2 | .417 (12) | .111 (9) | .167 (6) | .000 (1) | .000 (1) | – |
| 4 | .500 (12) | .200 (10) | .000 (4) | .000 (1) | – | – |
| 6 | .545 (11) | .111 (9) | .667 (3) | .000 (1) | – | – |
| 8 | 1.000 (3) | .500 (2) | – | – | – | – |
| 10 | 1.000 (1) | 1.000 (1) | – | – | – | – |

evaluate generally the relationships between heterozygosity and floral instability. Populations B3 and B4 were respectively dimorphic and monomorphic for style length. Genotypes with self-pollinating flowers were common in B3 and fixed in B4. These differences in floral biology directly influence the levels of inbreeding and amounts of heterozygosity in *E. paniculata* populations (Glover and Barrett, 1987; Barrett and Husband, 1990). Genotypes sampled from populations B3 and B4 are therefore likely to be inbred, perhaps to different degrees, with lower levels of heterozygosity than normally occur in tristylous populations. In contrast, the hybrid genotypes synthesized from inter-population crosses are probably heterozygous at many gene loci. The two parental populations are geographically and morphologically differentiated and fixed for different alleles at several isozyme loci (S. C. H. Barrett, unpubl. data).

Despite the different genetic backgrounds of our experimental material, each of the three populations of *E. paniculata* displayed considerable developmental instability

**Table 6.** Observed proportions of modified flowers on the second inflorescence of nonstressed ramets of *Eichhornia paniculata* in Experiment 4 and on an inflorescence on the same ramets after two weeks of water stress in relation to: ramet, position along the branch, and stress treatment. Numbers in parentheses indicate sample size.

| Ramet | Position | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Nonstressed | | | | | | |
| 1 | .000 (10) | .000 (10) | .000 (10) | .000 (10) | .000 (7) | .000 (2) |
| 2 | .000 (10) | .000 (10) | .000 (10) | .100 (10) | .143 (7) | – |
| 3 | .200 (5) | .400 (5) | .000 (5) | .000 (4) | – | – |
| 4 | .091 (11) | .000 (11) | .000 (11) | .000 (10) | .000 (9) | .000 (4) |
| 5 | .000 (10) | .000 (10) | .000 (10) | .000 (10) | .000 (9) | .000 (2) |
| 6 | .000 (12) | .000 (12) | .083 (12) | .167 (12) | .090 (11) | .000 (4) |
| Stressed | | | | | | |
| 1 | .833 (11) | .000 (11) | .000 (9) | – | – | – |
| 2 | .692 (13) | .154 (13) | .000 (1) | – | – | – |
| 3 | 1.000 (6) | – | – | – | – | – |
| 4 | .500 (14) | .000 (14) | .000 (12) | – | – | – |
| 5 | .000 (8) | .000 (8) | .000 (7) | – | – | – |
| 6 | .615 (13) | – | – | – | – | – |

in short-level stamen position. This indicates that developmental instability in *E. paniculata* can be associated with both inbreeding and outbreeding effects. Wide crossing may have disrupted gene combinations that canalize normal development (Dobzhansky, 1950; Wallace and Vetukhiv, 1955; Levin, 1970; Vrijenhoek and Lerman, 1982), whereas inbreeding can lead to the fixation of recessive genes capable of destabilizing developmental pathways (Lerner, 1954; Mather, 1950; Paxman, 1956). In either case the resulting variant floral phenotypes were similar in appearance suggesting that similar developmental mechanisms are involved in modifying stamen position.

The two natural populations from northeast Brazil differed significantly in the degree of stamen modification (Fig. 3). Most flowers produced by genotypes from the monomorphic population (B4) were self-pollinating, whereas in the dimorphic

population (B3) more flowers were unmodified than modified. These differences agree with what is known of the floral biology and mating systems of monomorphic and dimorphic populations of *E. paniculata* (Seburn et al., 1990; Barrett and Husband, 1990). In contrast, the vast majority of flowers produced by hybrid genotypes were unmodified. The parental populations of the hybrids differed in floral traits: genotypes from the trimorphic population B2 were completely unmodified, whereas those from the monomorphic population J3 were fixed for the semi-homostylous condition, with all three stamens at the mid-level position. The rarity of modified mid-styled flowers in $F_1$ plants is consistent with recessive gene control of stamen modification. However, that most genotypes produced small but varying numbers of modified flowers indicates that the genes controlling stamen position in hybrids exhibit variable expressivity. This effect is likely associated with the divergent genetic backgrounds of parental genotypes.

The comparisons of populations and genotypes in this study, and by Seburn et al. (1990), demonstrate a genetic component to floral instability in *E. paniculata*. However, the patterns of instability expressed by genotypes result from complex interactions between development and the environmental conditions under which they are grown. Analysis of non-genetic sources of variation in stamen modification requires detailed records to be made of the type and developmental position of all flowers borne by inflorescences on plants grown under varying conditions. By treating the inflorescence as a metapopulation of flowers (e.g. Ellstrand et al., 1984) and using statistical approaches, such as logistic regression analysis, we revealed considerable developmental variation in stamen modification. In addition, by cloning genotype H-2 and subjecting the resulting ramets to contrasting growth conditions, we demonstrated a significant environmental influence on stamen modification.

Non-random patterns of stamen modification occurred in each of the six genotypes compared in experiment 2 (Table 2 and Fig. 5). Logistic regression revealed effects due to inflorescence, branch number, and flower position resulting in strong position-dependent effects on the production of self-pollinating flowers. Ellstrand et al. (1984) also reported that position effects influence the formation of self-pollinating flowers in a study of chasmogamous and cleistogamous flowers in the cymose inflorescences of *Collomia grandiflora*. Self-pollinating cleistogamous flowers were produced at the beginning of anthesis, followed by a transition to chasmogamous flowers. Similar position effects have been reported in other cleistogamous taxa (Lord, 1981; Campbell et al., 1983). Developmental changes in floral function within inflorescences also commonly occur in species with diclinous flowers. For example, staminate flowers of many monoecious and andromonecious species often occur distally within spicate or umbellate inflorescences, respectively. As a result, they often flower after most female function has occurred within an inflorescence (e.g. Lovett-Doust, 1980; Thomson and Barrett, 1981; Diggle, 1988).

The position effects found in *E. paniculata* are considerably more subtle than have been previously reported in taxa with cleistogamous and diclinous flowers. No clear temporal transition between the floral phenotypes was evident during inflorescence flowering, and the underlying patterns could only be detected using statistical techniques. Furthermore, even though self-pollinating flowers were produced

non-randomly with respect to inflorescence development, different patterns were observed among genotypes (Fig. 5) and experiments (compare genotype H-2 in Figs. 5 and 6). While the probability of producing self-pollinating flowers declined with successive inflorescences for all genotypes in experiment 2, more complex patterns were evident for genotype H-2 in experiment 3. The effect of flower position within a branch varied between genotypes: proximal positions produced more self-pollinating flowers in hybrid genotypes and those from population B4, but not in genotypes from population B3 (Fig. 5).

These results suggest that the position effects on stamen modification found in *E. paniculata* do not involve programmed developmental changes of the type evident in taxa with cleistogamous and diclinous flowers. Instead, the signals involved are apparently more sensitive to both internal and external factors and, as a result, the likelihood of modification is less predictable. This type of variability has usually been viewed as maladaptive and ascribed to "random developmental accidents" resulting from a breakdown in canalization (Lerner, 1954; Thoday, 1956). However, our study indicates that stamen modifications are clearly not randomly distributed. Furthermore, under ecological conditions favouring self-pollination, the alterations in floral phenotypes may be of adaptive significance (see below).

The physiological mechanisms regulating stamen modification in *E. paniculata* are unknown. Changes in stamen position result primarily from filament elongation, although modified stamens tend to be inserted slightly higher on the floral tube. Modifications of filament length occur through cell elongation, not cell division, and involve rapid changes in filament length approximately 24 h prior to anthesis (Richards and Barrett, 1991). In other flowering plants, changes of this type result from gradients of hormones, particularly auxin and gibberellic acid (Greyson and Tepfer, 1967; Koning, 1983a,b; Pharis and King, 1985; Jones and Koning, 1986; Koning and Raab, 1987). If hormones control filament elongation in *E. paniculata*, the gene(s) regulating synthesis apparently acts relatively late in floral development. This may account for why stamen modifications occur independently of other changes to floral phenotype when they initially arise in populations and why they have no apparent negative pleiotropic effects on fitness.

Stressful conditions increased the frequency of stamen modification. Unfertilized, drought-stressed ramets produced more self-pollinating flowers than ramets that were given an abundant water supply and fertilizer (Table 5 and Fig. 7). This difference was not simply a consequence of the smaller inflorescences of the stressed ramets and the associated higher proportion of proximal flower positions in the sample. When previously unstressed ramets experienced two weeks of drought stress, the frequency of modified flowers increased significantly (Table 4), despite large inflorescences relative to the continually stressed ramets. This response to a briefer stress further suggests that stamen modifications are determined relatively late in floral development, and certainly well after inflorescences are initiated (see Richards and Barrett, 1984).

The effect of stressful growing conditions on stamen modification in *E. paniculata* may have ecological significance. Populations in northeast Brazil inhabit temporary pools and ditches throughout the arid caatinga region. Drought is a dominant

feature of this area which has one of the least predictable rainfall regimes in the world (Nimer, 1972). Populations of *E. paniculata* are short-lived, primarily because of frequent desiccation of aquatic habitats. Assured reproduction through self-fertilization may therefore facilitate response to deteriorating conditions, particularly at low population densities or when pollinator service is unreliable. The ability of self-pollinating variants of the mid-styled morph to adjust the frequency of flowers capable of autonomous self-pollination in response to environmental conditions may have favoured the spread and fixation of this morph. This ability would be particularly beneficial in non-trimorphic populations, which typically have lower plant densities and are smaller than trimorphic populations (Barrett et al., 1989).

High temperatures ( $> 30^\circ$ C) tended to increase the frequency of stamen modification in *E. paniculata*. When genotype H-2 was grown in a growth chamber at $20^\circ$ C the incidence of stamen modification dropped to negligible levels. Under field conditions in northeast Brazil temperatures during the flowering season range from $25-40^\circ$ C with cooler temperatures less frequent. Because stamen modifications occur commonly in non-trimorphic populations, temperatures in the field are clearly in the range that permits expression of the trait. However, further studies would be required to determine whether temperatures within the range encountered in the field significantly influence the frequency of stamen modification in natural populations.

Gottlieb (1984) argued that in plants major genes that exert their influence relatively late in development often regulate morphological changes of evolutionary significance. Genes governing late developmental changes may be less likely to produce negative pleiotropic effects on fitness than genes acting earlier in development. This may be particularly likely in heterostylous plants because many of the traits distinguishing the style morphs are determined relatively late in development (Richards and Barrett, 1984, 1987). Although genetic changes to floral architecture may involve relatively minor developmental alterations (e.g. involving heterochrony), such modifications can have profound effects on the reproductive biology of variant genotypes. In *E. paniculata*, stamen modifications increase the rate of self-pollination. Under certain ecological conditions this change appears to be adaptive and leads to the evolutionary breakdown of tristyly to semi-homostyly. The shift in breeding system is closely associated with morphological divergence and speciation in the Pontederiaceae (Eckenwalder and Barrett, 1985; Barrett 1989). Although the evolution of reproductive isolation is a complex process, the initial genetic changes that initiate reproductive character divergence may involve relatively minor alterations to the position of reproductive organs. In *E. paniculata*, modifications to filament length of a single stamen in the mid-styled morph apparently set these changes in motion.

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