Evolution of floral display in *Eichhornia paniculata* (Pontederiaceae): genetic correlations between flower size and number

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Keywords:

Eichhornia paniculata;
floral display;
Pontederiaceae;
quantitative genetics;
size–number trade-offs.

Abstract

The evolution of floral display is thought to be constrained by trade-offs between the size and number of flowers and inflorescences. We grew in the glasshouse 60 maternal families from each of two Brazilian populations of the annual herb, *Eichhornia paniculata*. We measured flower size, daily flower number, and total flower number per inflorescence, and two indices of module size, leaf area and age at flowering. We also assessed the size and number of inflorescences produced over 6 weeks. All floral traits exhibited significant heritable variation, some of which was due to genetic variation in module size. Genetic (maternal family) correlations between daily and total flower number did not differ from 1.0, indicating that display size (daily flower number) cannot evolve independently from total flower number per inflorescence. Genetic correlations between flower size and daily flower number ranged from negative to positive ($r = -0.78$ to $+0.84$), depending on population and inflorescence. Positive correlations occurred when variation in investment per inflorescence was high so that some families produced both larger and more flowers. These correlations became zero when we controlled for variation in module size. Families that flowered later produced fewer, larger inflorescences ($r = -0.33, -0.85$). These data support theoretical predictions regarding the combined effects of variation in resource acquisition and allocation on traits involved in trade-offs, and they emphasize the hierarchical organization of floral displays. Our results imply that patterns of resource allocation among inflorescences influence evolutionary changes in flower size and number per inflorescence.

Introduction

The finite nature of resources places a universal constraint on reproductive output or fitness. Because resources are finite, increases in one fitness component are thought to occur at the expense of one or more others. For example, investment in traits promoting current reproduction is often at the expense of traits promoting survival and future reproduction (Bell & Koufopanou, 1986; Snow & Whigham, 1989; Roff, 1992; Stearns, 1992). Another widespread trade-off is between the size and number of repeated parts or products. Theoretical models predict offspring number (n) to be inversely proportional to investment per offspring (s) according to the relation $n \propto E/s$, where E is the energy available for seed production (Smith & Fretwell, 1974; Lloyd, 1987). Lloyd (1987) suggested that his general model predicting optimal offspring size applies to any structure that is produced repeatedly on an individual, e.g. leaves, flowers and fruit. Size-number trade-offs have since been demonstrated among eggs and

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live offspring per litter (review by Roff, 1992), seeds per fruit (reviews by Roff, 1992; Méndez, 1997), and pollen grains per flower (Vonhof & Harder, 1995) and indeed appear to occur generally.

The ubiquity of trade-offs between size and number has influenced our perception of the enormous variation in floral display, the size, number and arrangement of flowers in animal-pollinated plants. Theoretical models considering the evolution of floral display often assume inverse relations between flower size and number (Cohen & Dukas, 1990; Morgan, 1993; Sakai, 1993; Harder & Barrett, 1996) or inflorescence size and number (Schoen & Dubuc, 1990; Fishbein & Venable, 1996; Venable, 1996). In these models, the optimal floral display reflects both pollination biology and mating systems, but size–number trade-offs place an important constraint on the combinations of flower size and number that can evolve. Empirical evidence for such trade-offs is limited, however. Flower size and number sometimes vary negatively among varieties or species (Sato & Yahara, 1999; Worley *et al*., 2000), between sexes (Delph, 1996), or after artificial selection on flower size or number (Meagher, 1994). In contrast, strong negative phenotypic or genetic relations between flower size and number within species were only detected in four of the nine studies reviewed by Worley & Barrett (2000).

The frequent occurrence of nonsignificant or positive correlations between flower size and number within species suggests that variation in resource levels may often obscure trade-offs (cf. Van Noordwijk & de Jong, 1986; Houle, 1991). Indeed, several studies have shown that genetic variation in resource status contributes to positive correlations among plant reproductive traits (e.g. Robertson *et al*., 1994; Fenster & Carr, 1997; Sugiyama & Bazzaz, 1998; but see Andersson, 1996; Campbell, 1997). Variation in the resources available for flowering may reflect variation in the resource status of individuals and/or variation in total allocation to flowering. The latter possibility implies that resource allocation is hierarchical. For example, resources may be allocated to flowering vs. vegetative growth, and the resources invested in flowering subdivided between flower size and number. Individuals that invest more resources in flowering may produce both more and larger flowers, thus obscuring the trade-off between flower size and number. The potential of hierarchical allocation to cause positive correlations between traits involved in trade-offs has been discussed in the context of female vs. male allocation (de Laguerie *et al*., 1991; Koelewijn & Hunscheid, 2000) and in general terms (de Jong, 1993). Possible effects of variation in resource status and of hierarchical allocation on genetic correlations between flower size and number have not been considered.

The modular structure of plants makes a hierarchy of trade-offs seem particularly likely (Venable, 1996). For example, flowers are displayed in inflorescences, which often mature a few flowers each day, and most plants produce multiple inflorescences over their lifetime. Thus, trade-offs between size and number could be manifested at several levels. The number of flowers open each day (daily flower number) is most relevant to display size and to the effects of flower number on pollinator attraction and mating systems (Harder & Barrett, 1996). However, to the extent that modules form physiological units (Watson, 1986), allocation to flower size vs. number may occur at the inflorescence level, i.e. between the size and total number of flowers produced by an inflorescence. If some genotypes display many flowers over a few days and others display a few flowers each day over a longer period, total flower number and daily flower number may not necessarily have the same relation with flower size. Finally, the size and number of inflorescences produced may be negatively related (Schoen & Dubuc, 1990; Venable, 1996). In species that produce flowers sequentially, delaying inflorescence production and increasing the time between inflorescences should allow plants to produce inflorescences with both more and larger flowers. To our knowledge, no study has examined the correlation between inflorescence size and number, even though several theoretical models assume a trade-off (Schoen & Dubuc, 1990; Fishbein & Venable, 1996; Venable, 1996). In addition, studies investigating the correlation between flower size and number have focused on a single level of organization (e.g. whole plant, within inflorescence), rather than considering multiple levels.

Here we investigate genetic correlations between flower size and number in two populations of the bee-pollinated annual *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). Glasshouse studies under uniform conditions have revealed genetic differentiation for flower size and total flower number per inflorescence among populations from north-eastern Brazil (Barrett, 1985). If trade-offs between flower size and number have an important influence on floral display in *E. paniculata*, they should be apparent both within and among populations. In a previous study, additive genetic correlations and correlated responses to selection did not strongly support a trade-off between flower size and number per inflorescence (Worley & Barrett, 2000). If trade-offs do indeed occur, the limited evidence for them could reflect variable investment in flowering, or trade-offs occurring at the whole plant rather than the module level. Here, we assess the possibility of trade-offs between inflorescence size and number. *Eichhornia paniculata* plants produce successive modules, each with a single inflorescence that produces a new cohort of one-day flowers each day (Richards & Barrett, 1984). Within inflorescences, we examine genetic correlations between flower size and both daily and total flower number. Our approach allows us to compare multiple estimates of the correlation between flower size and number, as well as factors thought to influence this correlation, e.g. module size.

We use correlations based on maternal families to estimate genetic correlations. These estimates differ from additive genetic correlations because they may include effects of dominance, epistasis and the maternal environment. However, analysis of one of our study populations for which we had three generations of pedigree information (Worley & Barrett, 2000) indicated that maternal identity accounted for only 2.5–7.5% of total variation in the traits we measured, and did not significantly change genetic correlations. Therefore, examining correlations based on maternal families should give a reasonable indication of the genetic correlations between flower size and number.

We assess the occurrence of floral trade-offs in *E. paniculata* with the following specific questions. (1) How does variation in module size influence genetic correlations between flower size, daily flower number and total flower number? This question encompasses two related issues. First, how much variation in the three floral traits is attributable to genetic variation in leaf area and age at flowering, our indices of module size? Second, does controlling for variation in module size reveal negative correlations between flower size and number? (2) Do correlations between size and number depend on the measure of flower production used: daily flower number per inflorescence; total number per inflorescence; or total inflorescence production? (3) Are genetic correlations among flower size, flower number and plant size similar for the two populations measured?

Methods

Study species

Eichhornia paniculata is an emergent aquatic native to the Neotropics, especially north-east Brazil and the Caribbean. Populations are short-lived with annual or rarely perennial life-histories, depending on how long the ephemeral ponds or ditches they occupy remain wet. Because plants in the field are very short-lived, selection is likely to act most strongly on early flower production and seed set. Plants typically grow in monospecific stands and are often even-aged due to synchronous germination following rain. The species is easily grown in the glasshouse and can be raised from seed to flowering in 3–4 months (see Barrett, 1985; Barrett & Husband, 1997; for detailed descriptions of the natural history).

Vigorous *E. paniculata* plants produce new reproductive shoots every 7–10 days. Each reproductive shoot or module consists of an elongated internode, and an inflorescence subtended by two bracts. One bract is greatly reduced and the other has a large cordate lamina, which is the only leaf-like structure on the module (Richards & Barrett, 1984). The compound inflorescence usually produces 10–100 flowers over 7–18 days, and each flower lasts 6–8 h (Morgan & Barrett, 1989). Here, we refer to the number of flowers open each day as daily flower number, and the number of flowers produced by an inflorescence as total flower number. Flowers are self-compatible and bee pollinated; most Brazilian populations are tristylous and largely outcrossing, including the populations that we used as seed sources for this experiment (see Barrett & Husband, 1990). These were B104 and B181, located about 50 km apart near the towns of Lajedo and Agrestina, respectively, in the state of Pernambuco.

Experimental design and data collection

Mature seed was collected from both populations in 1994. We planted seeds from 60 maternal families within each population on 8 March 1995, in a University of Toronto glasshouse maintained between 25 and 40 °C. On 24–26 April, we transplanted six plants from each maternal family into individual pots for a total of 720 plants in the experiment (2 populations × 60 maternal families × 6 plants). We measured height at transplant and arranged the plants in a randomized block design with trays as blocks. Trays were required because *E. paniculata* is an emergent aquatic and grows best when the roots and lower stems are submerged. In May, each tray contained 30 plants in 2.25″ pots and, after plants were moved to 3″ pots (29–31 May), each tray contained 15 plants. Plants were fertilized once weekly in May and twice weekly from June through August by adding dissolved 20:20:20 N:P:K fertilizer ($\sim$16 g L^{-1}) to the water in each tray. Fertilizer and watering regimes were identical for all plants throughout the experiment, although we increased fertilizer volume from 30 mL to 70 mL per tray to keep pace with plant growth.

We measured flower size, daily flower number and total flower number on the first two inflorescences produced by each plant. All plants were harvested on 14–15 July, and total inflorescence production over the 6-week flowering period was recorded for 240 plants in each population (four plants per family). To assess flower size, we used digital calipers to measure to the nearest 0.1 mm the width and length of the perianth of three flowers open on the fourth day of flowering. We multiplied length by width to estimate perianth area, which corresponded well to the dry weight of individual flowers ($R^2 = 0.65$, $P < 0.001$, $N = 69$: regression of perianth area on dry mass). Perianth areas were averaged to give mean flower size for each inflorescence. We counted the number of open flowers on the fourth day of flowering to assess daily flower number because peak flower production is generally on day 4 (Morgan & Barrett, 1989). Genetic variation in flower production and trade-offs between flower size and number are most likely to be detectable on this day, rather than early or late in flowering when few flowers are open. We estimated plant size by the area of the large bract subtending each

inflorescence, and by the age at flowering. For age at flowering, day 1 was the day that the first plant flowered and subsequent days were numbered consecutively until all plants had produced two inflorescences. All plants increased at least two-fold in height and girth during the experiment, indicating that plants that flowered later accumulated more resources during flowering. Bract area (hereafter referred to as leaf area) should provide an estimate of resource availability during both development and flowering. The bract and inflorescence develop concurrently and the bract likely supplies photosynthates to the inflorescence during flowering because it is the only leaf-like structure on the module.

Data analysis

Population differentiation and changes between inflorescences

Before analysing genetic correlations between flower size and number, we assessed differences in flower size, daily flower number, total flower number, and indices of module size between populations and inflorescences. We initially fitted repeated-measures models (PROC GLM: SAS, 1997) with block, population, and family within population as between-subject effects and inflorescence as the within-subject effect. These analyses allowed us to determine whether trait values differed significantly between inflorescences, and whether changes between inflorescences were similar for the two populations. However, population- and inflorescence-level differences in floral traits could reflect differences in relative allocation to size vs. number, or differences in module size. To test the latter possibility, we analysed flower size, daily flower number and total flower production separately for each inflorescence by fitting mixed models using restricted maximum likelihood (PROC MIXED: SAS, 1997). We analysed all traits in response to block, population (fixed effects), family within population (random effect), leaf area and age at flowering (covariates). With the exception of those including family, for which there was insufficient replication, we included all possible two–way interactions in the initial models. Non-significant interactions involving covariates were dropped using backwards elimination ($\alpha = 0.05$). We indicate partial regression coefficients with the letter b and their standard errors with s_b. These coefficients indicate the response of the dependent variable to one unit change in a specific independent variable, while all other independent variables remain constant.

Analyses of inflorescence size and number were conducted using mixed models (PROC MIXED: SAS, 1997) with block and population as fixed effects and family within population as a random effect. Inflorescence size was estimated by multiplying flower size by total flower number, and the average size of the two inflorescences measured on each plant was used for analysis.

Genetic parameters

Genetic analyses were conducted using the VCE Reml package by Neumaier & Groeneveld (1998; ftp://192.108.34.1). This package has several advantages over estimates of variance components based on least-squares analyses (e.g. regression, ANOVA). First, it estimates variance components using a restricted maximum-likelihood approach, Reml. The Reml approach deals well with statistically unbalanced data and nontraditional crossing designs (Shaw, 1987; Falconer & Mackay, 1996). Second, VCE calculates variances of the maximum-likelihood estimates, which can be used to calculate their statistical significance. Finally, and most importantly from our perspective, covariates can be specified in the Reml models to estimate heritabilities of, as well as genetic and environmental correlations among, floral traits that are independent of phenotypic and genetic variation in plant size.

We used family membership in the first generation to estimate broad-sense heritability (H^2) and correlations among maternal families. Genetic parameters were estimated separately for each population and inflorescence, allowing us to compare multiple estimates. The Reml models included tray (= block) and maternal family. The proportions of variation explained by maternal family were doubled to obtain broad-sense heritabilities (Falconer & Mackay, 1996). The analysis also gave estimates of genetic correlations based on maternal families. We assessed the significance of individual estimates within each analysis using single sample t-tests. Significance tests of heritability estimates were one-tailed, whereas tests of genetic correlations between measured traits were two-tailed because correlations could be positive or negative. Because each analysis involved multiple tests of significance we calculated α-levels within analyses using the sequential Bonferroni technique (Rice, 1989).

We first calculated correlation matrices for all five inflorescence-level traits, and then for the three floral traits with the two estimates of module size (leaf area, age at flowering) included as covariates in the Reml models. The first approach allowed us to assess H^2 for each floral trait and the two size indices, along with maternal-family (genetic) correlations between floral traits and leaf area or age at flowering. The second indicated how much of the genetic variation in each floral trait was independent of genetic variation in leaf area and age at flowering, as well as how much the relations among floral traits were influenced by variation in module size. Accounting for genetic variation in module size is expected to reveal trade-offs between traits that depend on resource status. Therefore significance tests of correlations among flower size and daily or total flower number were one-tailed in the analyses which specified the indices of module size as covariates. Separate correlation matrices were calculated for inflorescence size and number because these were measured on a subset of the plants.

Results

Variation in floral traits and indices of module size

Population differentiation and changes between inflorescences

Repeated measures analysis indicated that all traits differed significantly between populations (population effect: $F_{1,470+} > 12$, $P < 0.001$ for all comparisons), with the exceptions of daily and total flower number in inflorescence 1 (population effect: both $F_{1,110+} < 0.5$, $P > 0.5$; Fig. 1). All trait values increased significantly between the first and second inflorescence (inflorescence effect: all $F_{1,470+} > 25$, $P < 0.001$; Fig. 1). Changes in trait values between inflorescences differed significantly between populations (population × inflorescence interaction: $F_{1,470+} > 10$, $P < 0.001$). Increases in flower size were greater in B181 (Fig. 1a) whereas increases in both measures of flower number and both indices of module size were greater in B104 (Fig. 1b–d). As a result, in the second inflorescence, plants from B181 produced fewer, larger flowers than plants from B104 (compare Fig. 1a,b). This pattern of population divergence was consistent with negative genetic correlations between flower size and number, although higher flower numbers in B104 could reflect the larger size of these plants.

After controlling for variation in leaf area and age at flowering, plants from B181 still produced larger flowers (Population effect in Table 1, Fig. 1a). However, these plants also produced slightly more flowers on inflorescence 1 than plants of equivalent leaf area and age from B104 (Population effect in Table 1, e.g. mean daily no. B181 = 4.7, LSE = 4.64, USE = 4.78; B104 = 4.6, LSE = 4.48, USE = 4.63). These contrast with the means in Fig. 4.1. Neither daily nor total flower production differed between populations in inflorescence 2 (Table 1), indicating that the population-level differences in flower number evident in Fig. 1 result from differences in the indices of module size.

Plants in B104 produced significantly fewer inflorescences than plants in B181 (mean inflorescence number ± SE: B181 = 5.7 ± 0.09, B104 = 4.9 ± 0.09, $t_{107} = 4.57$, $P < 0.001$), and their inflorescences were slightly smaller than in B181. However, the difference in inflorescence size was only marginally significant (mean inflorescence size ± SE: B181 = 35.4 ± 0.70 cm^2, B104 = 33.5 ± 0.72 cm^2, $t_{101} = 1.82$, $P < 0.1$).

Broad-sense heritability

Almost all traits exhibited significant heritable variation (Tables 2 and 3). Although heritability estimates for each trait differed between inflorescences and populations, most changes were of relatively small magnitude. Estimates of H^2 for each trait differed more between populations than between inflorescences (Table 2). H^2 estimates for inflorescence production were similar in the two populations, but the heritability estimate for inflorescence size was only significant in population B181 (Table 3).

Heritability estimates for floral traits on each inflorescence were generally unaffected by accounting for leaf area and age at flowering (Table 2), although size adjustments reduced the variance attributable to maternal family (Fig. 2). H^2 estimates remained similar in almost all cases because size adjustments also reduced residual variation, i.e. phenotypic variation not attributable to family. Although most H^2 estimates were little affected by size adjustment, reductions in the variance

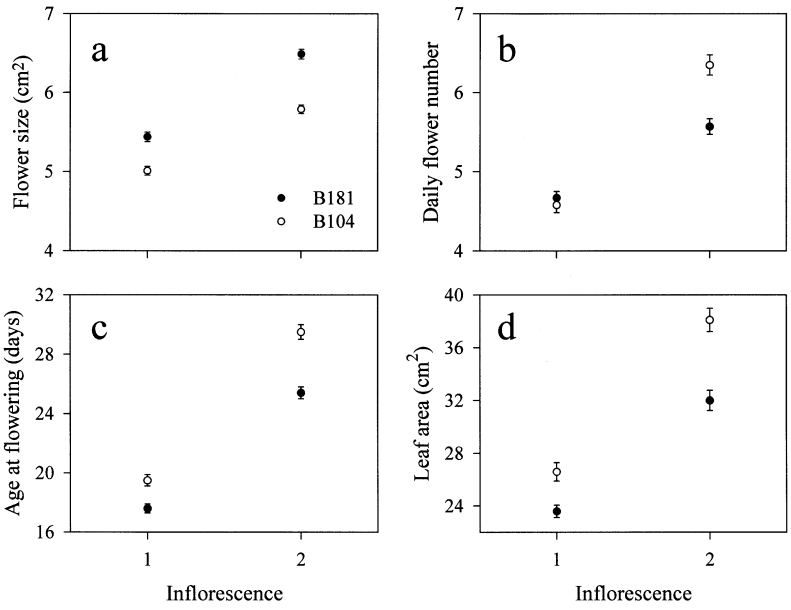

Fig. 1 Population and inflorescence means ± 1 SE for (a) flower size, (b) daily flower number, (c) age at flowering, and (d) leaf area in glasshouse-grown populations of *Eichhornia paniculata*. All means involved $N \approx 350$ plants. Means for total flower number are not shown. Trends were very similar to those shown for daily flower number (see Appendix 1).

Table 1 Mixed model analyses of factors influencing floral variation in glasshouse-grown *Eichhornia paniculata* plants. Sample sizes for inflorescence 1 were $N = 702$ for flower size and daily number, and $N = 707$ for total number. In inflorescence 2, $N = 695$ for flower size and daily number, and $N = 696$ for total number. All analyses initially included the two size indices (leaf area, age at flowering) and two- and three-way interactions. The analysis of daily flower number included total flower number as a covariate. Nonsignificant terms involving covariates were dropped using backwards elimination ($\alpha = 0.05$) and only terms that were significant in at least one analysis are included in the table. The random family effects were tested with a likelihood ratio test. Partial regression coefficients and standard errors for significant covariates are given in italics under the relevant F statistic.

	Flower size		Daily flower number		Total flower number	
Effect	Inflorescence 1	Inflorescence 2	Inflorescence 1	Inflorescence 2	Inflorescence 1	Inflorescence 2
Block	$F_{47,585} = 1.13$	$F_{47,572} = 1.61^{**}$	$F_{47,600} = 1.15$	$F_{47,598} = 1.50^{*}$	$F_{47,577} = 1.71^{**}$	$F_{47,491} = 2.41^{***}$
Population	$F_{1,108} = 48.62^{***}$	$F_{1,114} = 150.25^{***}$	$F_{1,106} = 2.34^{*}$	$F_{1,118} = 0.90$	$F_{1,111} = 2.82\dagger$	$F_{1,118} = 0.14$
Block × Population	$F_{47,585} = 0.94$	$F_{47,573} = 0.84$	$F_{47,599} = 0.81$	$F_{47,498} = 1.20$	$F_{47,576} = 1.02$	$F_{47,491} = 0.81$
Family (Population)	$G_{120} = 9.17^{**}$	$G_{120} = 14.41^{**}$	$G_{120} = 0.14$	$G_{120} = 0.00$	$G_{120} = 23.39^{***}$	$G_{120} = 16.48^{**}$
Leaf area	$F_{1,485} = 4.89^{*}$ *0.010 (0.0043)*	$F_{1,595} = 43.14^{***}$ *0.018 (0.0027)*	–	$F_{1,598} = 9.52^{***}$ *0.003 (0.0010)*	$F_{1,600} = 0.00$ *0.000 (0.0093)*	$F_{1,480} = 127.23^{***}$ *0.030 (0.0027)*
Age at flowering	$F_{1,552} = 125.94^{**}$ *0.085 (0.0076)*	$F_{1,541} = 108.49^{***}$ *0.053 (0.0051)*	–	–	$F_{1,608} = 9.63^{**}$ *– 0.038 (0.0122)*	$F_{1,480} = 116.63^{***}$ *0.055 (0.0051)*
Leaf area × Age at flowering	–	–	–	–	$F_{1,598} = 19.44^{***}$ *0.002 (0.0004)*	–
Total flower number	–	–	$F_{1,432} = 431.53^{***}$ *0.278 (0.0130)*	$F_{1,486} = 414.99^{***}$ *0.250 (0.0122)*	–	–

$\dagger P < 0.1$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Table 2 Broad-sense heritability estimates for measured (H^2) and size-adjusted (H^2_{adj}) floral traits in glasshouse-grown populations of *Eichhornia paniculata*. Heritabilities of leaf area and age at flowering are also shown. Separate analyses were conducted on each population and inflorescence (e.g. B181–1 refers to population B181, inflorescence 1). The significance of estimates within each analysis was assessed using α-levels calculated according to the sequential Bonferroni technique (Rice, 1989). Estimates that remained significant after Bonferroni correction are in bold type, and standard errors are in parentheses. Significant differences between measured and size-adjusted estimates are indicated using asterisks. Heritabilities were estimated using VCE 4.2 (see methods).

	Flower size		Daily flower number		Total flower number		Leaf area	Age at flowering
Population–Inflorescence	H^2	H^2_{adj}	H^2	H^2_{adj}	H^2	H^2_{adj}	H^2	H^2
B181–1	**0.32** (0.066)	**0.30** (0.070)	**0.14** (0.046)	**0.07** (0.036)	**0.27** (0.070)	**0.26** (0.080)	**0.20** (0.064)	**0.76** (0.088)
B181–2	**0.35** (0.070)	**0.33** (0.078)	0.08 (0.046)	0.04 (0.060)	**0.22** (0.064)	**0.40*** (0.086)	0.14 (0.062)	**0.87** (0.084)
B104–1	**0.19** (0.056)	0.06* (0.062)	**0.26** (0.058)	**0.19** (0.072)	**0.34** (0.070)	**0.38** (0.086)	**0.42** (0.082)	**0.57** (0.084)
B104–2	**0.20** (0.068)	**0.12** (0.062)	**0.23** (0.066)	0.06* (0.044)	**0.31** (0.078)	**0.15*** (0.068)	**0.27** (0.072)	**0.61** (0.086)

$^{*}P < 0.05$.

attributable to maternal family (Fig. 2) indicate that some genetic variation in floral traits reflects genetic variation in module size.

Correlations between floral traits and module size

Strong positive correlations between inflorescence-level floral traits and both leaf area and age at flowering were evident in analyses of phenotypic and genetic variation. Analyses including leaf area and age at flowering explained 16% more of the phenotypic variation in flower size and 20% more of that in total flower number than models without these indices of module size (F-statistics and slopes in Table 1). Maternal-family correlations were also almost all positive (Table 4). Correlations between floral traits and size indices were more significant and of greater magnitude in B104 than in B181. Correlations were also generally higher in the second than the first inflorescence of B104 plants (e.g. compare correlations between daily or total flower number and size indices, Table 4). Overall, the positive relations between floral traits and module size indicated that plants that flowered later and produced larger leaves also had more, larger flowers per inflorescence.

Correlations between size and number

Maternal-family correlations between flower size and number varied widely between inflorescences and populations. Flower size varied negatively with both total and daily number in inflorescence 1 of B181 plants, but not in inflorescence 2 (Table 4). Genetic correlations

Table 3 Broad-sense heritabilites (diagonal) and maternal-family correlations (off diagonal) between inflorescence size (flower size × total flower number per inflorescence) and the number of inflorescences produced over 6 weeks by glasshouse-grown *Eichhornia paniculata*. Estimates are from analyses of maternal families performed with VCE 4.2 (see Methods). Parameters were estimated separately for each population, and the significance of estimates within each analysis was assessed using α-levels calculated according to the sequential Bonferroni technique (Rice, 1989). Estimates that remained significant after Bonferroni correction are in bold type, and standard errors are in parentheses.

Population	Inflorescence size	Inflorescence number
Inflorescence size		
B181	**0.22** (0.098)	–0.33 (0.229)
B104	0.16 (0.098)	**–0.85** (0.183)
Inflorescence number		
B181		**0.43** (0.104)
B104		**0.53** (0.100)

between floral traits were positive in plants from B104 and, as for B181, closer to +1 in inflorescence 2 than in inflorescence 1 (Table 4). The differences in correlation coefficients between inflorescences and between B181 and B104 appeared to reflect increased variation in overall investment per inflorescence relative to variation in flower size and number (Fig. 3). For example, in inflorescence 2 of B104, variation in investment per inflorescence was relatively high so that some individuals produced both larger and more flowers. By contrast, in inflorescence 1 of B181, variation in investment per inflorescence was low compared to variation in allocation to flower size vs. daily flower number so that a negative correlation between these two traits was apparent (Fig. 3).

Controlling for variation in module size before calculating correlations between flower size and daily or total number removed the positive correlations, but did not reveal negative correlations between these floral traits (Table 5). However, the high standard errors of the estimates reduced our ability to detect negative genetic correlations. To illustrate this possibility we calculated minimum detectable correlations (Zar, 1996), given each standard error and assuming a power of 0.80 and $\alpha = 0.05$. These calculations indicated that only estimates of $r < -0.5$ between flower size and daily flower number, and of $r < -0.4$ between flower size and daily flower number would have been statistically significant (Table 5).

Maternal-family correlations between inflorescence size and number were negative for both populations (Table 3, Fig. 4), and suggest that trade-offs between size and number occur at the inflorescence level. However, in B181, the estimated correlation was not significant, and in B104 the estimated heritability of inflorescence size did not differ significantly from zero (Table 4a).

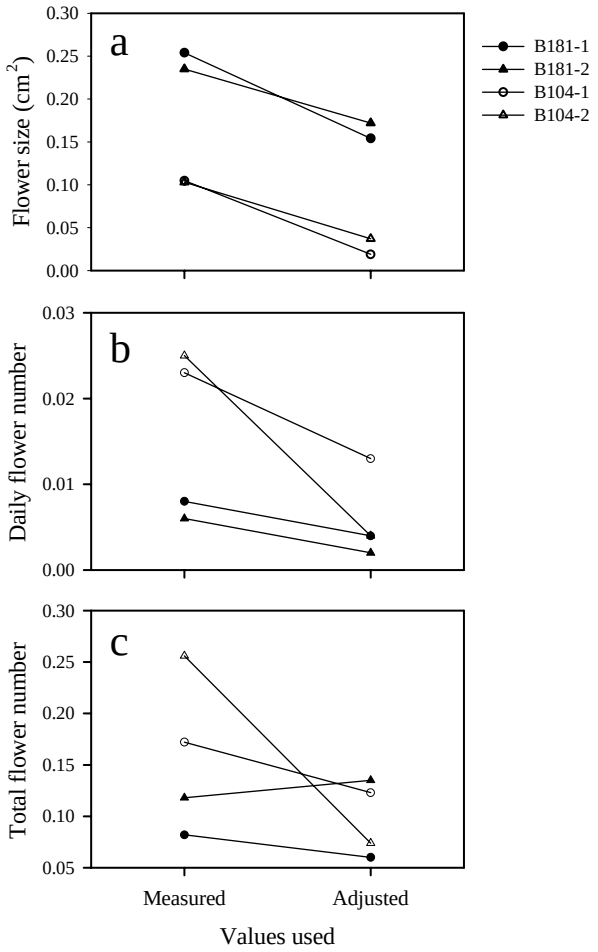

Fig. 2 Variance components for maternal families for (a) flower size, (b) daily flower number and (c) total flower number in glasshouse-grown populations of *Eichhornia paniculata*. Variance components were calculated using measured values and data that had been adjusted for variation in leaf area and age at flowering. The ratios of maternal-family variance to total phenotypic variance were doubled to obtain the broad-sense heritability estimates shown in Table 3. All analyses were performed with VCE 4.2.

Daily and total flower number

Our results indicated that the same genes contribute to variation in daily and total flower number. First, analyses of phenotypic variation showed daily flower number to be closely associated with total number, and analyses including total number (Table 1) explained 23–36% more variation in daily number than those excluding total number. Second, daily flower number was not significantly affected by family in analyses including total flower number (Table 1), indicating that family effects on daily flower number are associated with total flower number. Also, analyses of daily flower number involving size indices but not total number yielded results very similar to analyses of total flower number (results not

Table 4 Maternal-family correlations among floral characters and indices of module size in glasshouse-grown populations of *Eichhornia paniculata*. Correlations were estimated using VCE 4.2 (see methods). Parameters were estimated separately for each population and inflorescence. The significance of estimates within each analysis was assessed using α-levels calculated according to the sequential Bonferroni technique (Rice, 1989). Standard errors are in parentheses and the estimates that remained significant after Bonferroni correction are in bold type.

	Daily flower number	Total flower number	Leaf area	Age at flowering
Flower size				
B181–1	**−0.78** (0.140)	−0.46 (0.172)	0.21 (0.201)	**0.61** (0.102)
B181–2	0.20 (0.267)	−0.06 (0.196)	0.21 (0.212)	**0.54** (0.100)
B104–1	0.38 (0.189)	0.44 (0.173)	**0.85** (0.091)	**0.82** (0.082)
B104–2	**0.84** (0.130)	**0.65** (0.155)	**0.55** (0.164)	**0.86** (0.085)
Daily flower number				
B181–1		**0.88** (0.082)	0.29 (0.234)	**−0.55** (0.164)
B181–2		**0.91** (0.089)	**0.71** (0.201)	0.12 (0.229)
B104–1		**0.90** (0.055)	0.29 (0.154)	0.14 (0.155)
B104–2		**0.95** (0.037)	**0.81** (0.110)	**0.83** (0.085)
Total flower number				
B181–1			**0.47** (0.169)	−0.24 (0.156)
B181–2			0.51 (0.187)	0.16 (0.163)
B104–1			**0.58** (0.110)	**0.41** (0.122)
B104–2			**0.90** (0.076)	**0.73** (0.087)
Leaf area				
B181–1				0.40 (0.153)
B181–2				0.29 (0.214)
B104–1				**0.93** (0.033)
B104–2				**0.79** (0.094)

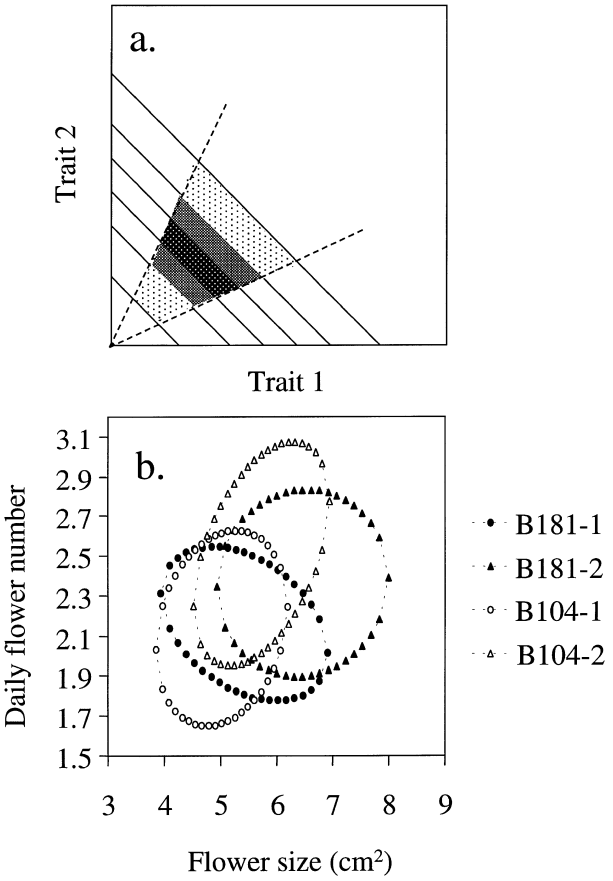

Fig. 3 (a) Diverse correlations between traits involved in a trade-off can occur under variable resource allocation and acquisition (Van Noordwijk & de Jong, 1986). The solid lines represent a trade-off between traits 1 and 2, which moves further from the origin as resource levels increase. The dashed lines represent relative allocation to the two traits and demarcate the limits of population-level variation in allocation. When variation in resource levels is high relative to variation in allocation (lightly shaded area) a positive correlation occurs between the two traits. When variation in resource levels is lower, measured relations between the traits may be nonsignificant (medium shading) or negative (dark shading). (b) Observed correlations between flower size and number in *E. paniculata* are consistent with Van Noordwijk and de Jong's predictions. A negative correlation between flower size and number occurred in inflorescence 1 of B181. In B104, and the second inflorescence of both populations, variation in the resources invested per inflorescence was higher and correlations between flower size and number were nonsignificant or positive. The ellipses encompass 95% of the variation in maternal family means for each inflorescence (formulae in Sokal & Rohlf, 1981).

shown). Third, all estimates of the correlation between daily and total flower number had confidence intervals spanning 1.0 (Table 4), even after controlling for variation in leaf area and age at flowering (Table 5). Finally, heritability estimates of daily number were very small or zero after measurements were adjusted for total flower number, as were heritability estimates for the proportion of flowers matured on day 4 (results not shown). These results indicate that almost all the genetic variation in daily number corresponded to that for total flower number.

Discussion

Although evolutionary biologists increasingly recognize the influence of floral displays on mating patterns in plants (e.g. Schoen & Dubuc, 1990; Morgan, 1993; Harder & Barrett, 1995; Fishbein & Venable, 1996), few studies have investigated the genetics of traits contributing to variation in floral display. In this study, genetic correlations between flower size and number per inflorescence in *E. paniculata* changed from negative to positive in response to increased variation in investment per inflorescence (Fig. 3). These results are consistent with predictions regarding the combined effects of variation in resource acquisition and allocation on traits involved in trade-offs (van Noordwijk & de Jong, 1986; Houle, 1991). Negative genetic correlations between inflorescence size and number indicate that trade-offs occurred between, as well as within, modules and

Table 5 Maternal-family correlations, *r*, among size-adjusted floral traits in glasshouse-grown *Eichhornia paniculata* plants. Also shown are minimum detectable correlations, δ (Zar, 1996), given the standard error of each estimate, $\alpha = 0.05$ and power $(1 - \beta) = 0.8$. All analyses were performed with VCE 4.2 (see methods), and were conducted separately for each population and inflorescence. Standard errors are in parentheses and estimates in bold type differ significantly from zero. The significance of estimates within each analysis was assessed using α-levels calculated according to the sequential Bonferroni technique (Rice, 1989).

	Daily flower number		Total flower number	
	r (SE)	δ	*r* (SE)	δ
Flower size				
B181–1	**−0.77** (0.214)	−0.54	**−0.41** (0.201)	−0.51
B181–2	0.22 (0.225)	−0.57	−0.07 (0.156)	−0.39
B104–1	0.46 (0.229)	−0.58	0.00 (0.240)	−0.60
B104–2	0.49 (0.426)	−1.07	−0.06 (0.343)	−0.86
Daily flower number				
B181–1			**0.89** (0.125)	–
B181–2			**0.96** (0.051)	–
B104–1			**0.88** (0.063)	–
B104–2			**0.84** (0.184)	–

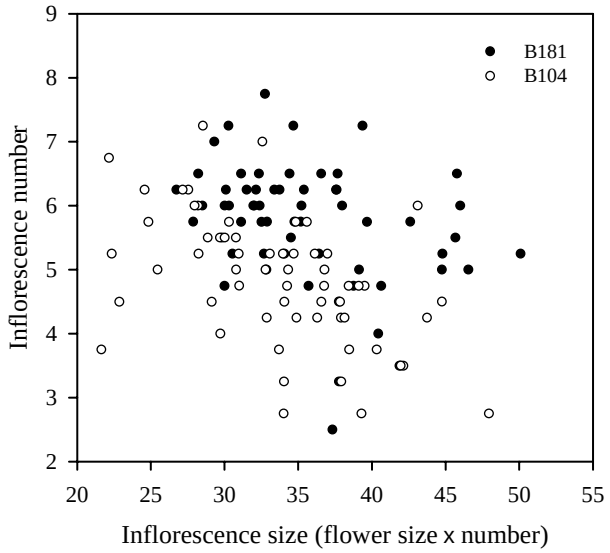

Fig. 4 Correlations between number and size of inflorescences produced by glasshouse-grown *Eichhornia paniculata*. Inflorescence size was estimated by multiplying flower size by flower number for each of the first and second inflorescence produced, and taking the average. Data points are means for maternal families. Estimated genetic (maternal-family) correlations and broad-sense heritabilities are in Table 3.

provide one of the first empirical examples of an allocation hierarchy (cf. de Laguerie *et al*., 1991; de Jong, 1993). Variation in inflorescence size and number per plant influences genetic correlations between flower size and number per inflorescence. Such patterns will complicate the evolution of floral display because factors affecting inflorescence size may alter evolutionary trajectories of flower size and number per inflorescence. Finally, the tight genetic correlation between daily and total flower number per inflorescence indicates that the fraction of flowers displayed each day (the unit of pollinator attraction) cannot evolve independently from the total number of flowers on an inflorescence. We discuss these results and their implications in more detail below.

Effects of module size on flower size and number

Genetic correlations between flower size and both daily and total flower number per inflorescence in *E. paniculata* were strongly influenced by genetic variation in module size. First, the variance in flower size and number attributable to maternal family decreased after accounting for variation in leaf area and age at flowering (Fig. 2). Second, increased flower size and number between inflorescence 1 and 2 corresponded to increased leaf area and age at flowering (Fig. 1), indicating that larger modules produced both more and larger flowers. Third, increased magnitude and significance of positive correlations between floral traits and module size were associated with increasingly positive correlations between flower size and number, from the first to the second inflorescence, and between B181 and B104 (Table 4, Fig. 3b). Fourth, controlling for variation in module size removed all positive correlations between flower size and number (Table 5). These results indicate that genetic variation in module size contributed to the positive correlations between floral traits in *E. paniculata*, as we found in our previous study (Worley & Barrett, 2000). Although other studies have demonstrated positive correlations between plant size and flower number (e.g. Mazer, 1989; Herrera, 1991; Meagher, 1992; Mitchell, 1994; Conner *et al*., 1996) and between plant size and flower size (Meagher, 1992; Andersson, 1996), effects of genetic variation in module size on correlations between flower size and number have not previously been investigated.

Interestingly, correlations between flower size and both measures of flower number were closer to +1 in the second than in the first inflorescence for both populations. Although temporal variation in genetic correlations among plant reproductive traits has been documented (Mazer & Delesalle, 1996), factors causing such variation have not been examined. In this study, increased variation in overall allocation to flowering in the second inflorescence altered genetic correlations between flower size and number (Fig. 3b). Time and increases in plant size are likely to magnify genetic differences both in the ability to acquire resources and in rates of growth and development. In nature, selection should strongly reduce genetic variation in resource

acquisition. However, glasshouse or laboratory conditions differing substantially from the wild may expose genotype–environment interactions leading to inflated or altered estimates of genetic correlations (Aastveit & Aastveit, 1993; Lynch & Walsh, 1998). Some empirical comparisons suggest that genetic correlations estimated in the wild differ from those in the lab, especially those involving life-history traits (Roff, 1996). However, other studies involving plant reproductive traits have found no difference between genetic correlations estimated in the glasshouse and field (Young *et al*., 1994). It is possible that the genetic correlations we measured between flower size and number differed from those in wild populations. On the other hand, wild *E. paniculata* populations germinate synchronously and grow in dense monospecific stands similar to those we measured in the glasshouse, suggesting the two environments may be grossly similar.

Correlations between size and number

Although controlling for leaf area and age at flowering accounted for some genetic variation in resource status (Fig. 2), it never revealed negative maternal–family correlations between flower size and number (Table 5). Limited power may partially account for the lack of significant correlations between size-adjusted data, as indicated by the large standard errors (Table 5). However, negative correlations between flower size and both daily and total flower number occurred for inflorescence 1 of B181, even without size adjustments. The diverse correlations between flower size and number (Fig. 3) support the idea that trade-offs between flower size and number are only evident when allocation to flowering varies little, or when variation in allocation between flower size and number is high (cf. de Laguerie *et al*., 1991). These circumstances may have occurred in the first inflorescence of B181. Interestingly, in inflorescence 1 of B181, variation in floral traits was less closely related to variation in resource status than in inflorescence 2 or B104 (Table 4), perhaps because flower size and number were more influenced by trade-offs (see also Fig. 3b). The alternative interpretation, that flower size and number are not genetically related, is discussed by Worley & Barrett (2000).

Plants that delayed flowering produced inflorescences with more and larger flowers, and had longer intervals between inflorescences. This pattern resulted in negative correlations between maternal-family means for inflorescence size and number (Fig. 4). Although the correlations were not all statistically significant, they did support a genetically based trade-off between inflorescence size and number. This negative correlation is likely a manifestation of the trade-off between age and size at first reproduction which has been demonstrated in *Brassica campetris* (Dorn & Mitchell-Olds, 1991) and *Arabidopsis thaliana* (Mitchell-Olds, 1996). In general, selection should favour early reproduction by *E. paniculata* because plants are often killed by drought when they have only produced a few inflorescences (S. C. H. Barrett, personal observation). Thus selection for early flowering may counter selection for large floral displays.

The occurrence of a trade-off between inflorescence size and number has additional implications for the evolution of floral display. First, the pattern implies a hierarchy of allocation, first between inflorescence size and number and then between flower size and number within inflorescences, which seems a natural consequence of *E. paniculata*'s modular structure (cf. Venable, 1996). Second, it raises the possibility that genetic variation for flowering time influences the sign of genetic correlations between flower size and number. The suitability of *E. paniculata* habitat for growth depends on rainfall, which is highly variable in its native range (Barrett & Husband, 1997; Husband & Barrett, 1998). Variable habitat duration may cause high genetic variation in flowering time, and also in inflorescence size because modules that flower later are usually larger. If some genotypes produce both larger and more flowers per inflorescence, genetic correlations between flower size and number will be positive, even in the presence of an underlying trade-off. Although trade-offs will still influence the evolution of flower size and number, positive genetic correlations are likely to alter evolutionary trajectories (cf. Via & Lande, 1985; A. C. Worley unpublished data).

Daily and total flower number are controlled by the same genes

All relevant data in this study supported the conclusion that the same genes control daily and total flower number in *E. paniculata*. Additive genetic correlations and corrrelated responses to selection on daily flower number also supported this conclusion (Worley & Barrett, 2000). We were intrigued by this result because we expected genetic variation in the fraction of flowers matured each day. The close dependence of daily flower number on total flower number may reflect developmental phenology in *E. paniculata*. Inflorescences are initiated and develop rapidly, within 3–5 weeks, and flowers are initiated in a stereotyped sequence, followed immediately by anthesis (see Richards & Barrett, 1984). This situation could apply to other species that initiate and mature flowers rapidly. In contrast to *E. paniculata*, many perennial plants differentiate flowers well in advance of expansion. This situation may provide greater opportunity for variation in the proportion of flowers matured each day. Differences in display size and the scope for independent evolution of daily and total flower number may thus depend partly on developmental phenology.

The genetic correspondence between daily and total flower number raises interesting questions about the

evolution of flower number per inflorescence in *E. paniculata*, because different selective pressures are likely to affect each flower count. Daily flower number influences pollinator attraction and patterns of pollen dispersal (Barrett *et al*., 1994; Harder & Barrett, 1995, 1996). In species with more prolonged development than *E. paniculata*, daily number can also affect the intensity of resource expenditure by influencing the temporal distribution of flowering and fruiting. Total flower number sets an upper limit on reproductive potential (Lloyd, 1980) and, in species with animal-dispersed fruit, may also influence the attractiveness of the infructescence (Howe & Smallwood, 1982). Daily and total flower number may not evolve independently in *E. paniculata*, although phenotypic responses suggest the perfect genetic correlation between daily and total flower number could be disrupted by fruit set (Morgan & Barrett, 1989). Our genetic data suggest that plants cannot increase their attractiveness to pollinators by maturing more flowers each day without also increasing total flower number. Evolution of daily and total flower number must therefore reflect the net effects of selection on both flower counts. More data are needed to determine whether the joint evolution of daily and total number per inflorescence is truly constrained.

Acknowledgments

We thank D. Houle and S. Mazer for advice; T. Sarkissian, S. Wright, H. S. Yoon, L. Jesson and B. Cole for help in the glasshouse; E. Elle, J. Gibson and G. Vandervoort for help with VCE; and G. Bell, A. Case, J. Conner, S. Mazer, M. Morgan and two anonymous reviewers for insightful comments on an earlier version of the manuscript. This project was funded by a Natural Sciences and Engineering Research Council of Canada research grant to S.C.H.B. and an Ontario Graduate Scholarship to A.C.W.

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Received 24 December 2000; accepted 11 January 2001

Appendix 1 Summary statistics including standard deviations (SD) and coefficients of variation (CV) for floral traits in glasshouse populations of *Eichhornia paniculata* for each population and inflorescence. Means and standard errors are plotted in Fig. 1. Note that means for daily and total flower number are square-root transformed here, but were back-transformed for Fig. 1.

	Inflorescence 1				Inflorescence 2			
Trait	*N*	Mean	SD	CV	*N*	Mean	SD	CV
(a) B181								
Flower size (cm^2)	358	5.44	1.153	21	354	6.49	1.16	18
Daily flower number	357	2.16	0.358	16	354	2.36	0.394	17
Total flower number	355	5.43	0.822	15	352	6.36	1.084	17
Leaf area (cm^2)	355	23.6	8.92	38	352	32.0	14.35	45
Age at flowering	358	17.6	5.68	32	354	25.4	7.48	29
(b) B104								
Flower size (cm^2)	354	5.01	1.063	21	345	5.71	1.021	18
Daily flower number	357	2.14	0.424	20	346	2.52	0.470	19
Total flower number	356	5.47	1.014	19	346	6.78	1.322	19
Leaf area (cm^2)	356	26.6	13.3	50	345	38.1	16.45	43
Age at flowering	359	19.5	7.18	37	346	29.5	9.18	31