Evolution, 54(5), 2000, pp. 1533–1545

EVOLUTION OF FLORAL DISPLAY IN *EICHHORNIA PANICULATA* (PONTEDERIACEAE): DIRECT AND CORRELATED RESPONSES TO SELECTION ON FLOWER SIZE AND NUMBER

ANNE C. WORLEY[1] AND SPENCER C. H. BARRETT[2]
Department of Botany, University of Toronto, 25 Willcocks Street, Toronto ON, M5S-3B2, Canada
[2]*E-mail: barrett@botany.utoronto.ca*

Abstract.—Trade-offs between flower size and number seem likely to influence the evolution of floral display and are an important assumption of several theoretical models. We assessed floral trade-offs by imposing two generations of selection on flower size and number in a greenhouse population of bee-pollinated *Eichhornia paniculata.* We established a control line and two replicate selection lines of 100 plants each for large flowers (S+), small flowers (S−), and many flowers per inflorescence (N+). We compared realized heritabilities and genetic correlations with estimates based on restricted-maximum-likelihood (REML) analysis of pedigrees. Responses to selection confirmed REML heritability estimates (flower size, $h^2 = 0.48$; daily flower number, $h^2 = 0.10$; total flower number, $h^2 = 0.23$). Differences in nectar, pollen, and ovule production between S+ and S− lines supported an overall divergence in investment per flower. Both realized and REML estimates of the genetic correlation between daily and total flower number were $r = 1.0$. However, correlated responses to selection were inconsistent in their support of a trade-off. In both S− lines, correlated increases in flower number indicated a genetic correlation of $r = -0.6$ between flower size and number. In contrast, correlated responses in N+ and S+ lines were not significant, although flower size decreased in one N+ line. In addition, REML estimates of genetic correlations between flower size and number were positive, and did not differ from zero when variation in leaf area and age at first flowering were taken into account. These results likely reflect the combined effects of variation in genes controlling the resources available for flowering and genes with opposing effects on flower size and number. Our results suggest that the short-term evolution of floral display is not necessarily constrained by trade-offs between flower size and number, as is often assumed.

Key words.—Artificial selection, *Eichhornia paniculata,* nectar, ovules, pollen, quantitative genetics, trade-offs.

Received June 8, 1999. Accepted April 10, 2000.

Flower size and number both influence the attractiveness of animal-pollinated plants (reviewed by de Jong and Klinkhamer 1994; Conner and Rush 1996; Harder and Barrett 1996). These two components of floral display can vary widely among populations, species and higher taxa. This observation, along with the recognition that finite floral resources cannot simultaneously contribute to increased flower size and number, has led to the expectation that trade-offs between these traits profoundly influence the evolution of floral display. Accordingly, theoretical models considering the evolution of floral display assume inverse relations between flower size and number (Cohen and Dukas 1990; Morgan 1993; Sakai 1995; Schoen and Ashman 1995; Harder and Barrett 1996) or between inflorescence size and number (Schoen and Dubuc 1990; Fishbein and Venable 1996; Venable 1996). In these models, the benefits of producing multiple flowers for pollinator attraction are countered both by resource costs and the potential transfer of self pollen between open flowers. The latter increases selfing between flowers (geitonogamy) and reduces pollen export (pollen discounting). Both mating costs are supported by convincing empirical evidence (de Jong et al. 1993; Harder and Barrett 1995; Snow et al. 1996).

Despite its apparent importance for floral evolution, few empirical studies have revealed a trade-off between flower size and number. Some species with unisexual flowers (dicliny) have one sex that produces smaller, more numerous flowers than the other (reviewed by Delph 1996). However, differences in allocation to flowering between the sexes may obscure trade-offs in diclinous species. Plants functioning as males often produce both more and larger flowers than plants functioning as females (Delph 1996), possibly because allocation to pollinator attraction is higher in males (cf. Bateman 1948; Charnov 1982). The influence of trade-offs between flower size and number on floral evolution is of particular interest in species that are monoecious or produce exclusively hermaphroditic flowers because these species are also subject to the mating costs of producing multiple flowers. Few studies have examined flower size and number within sexes of dimorphic species or in species with hermaphroditic flowers. Five of the nine species investigated did not show clear evidence of a trade-off between flower size and number (Table 1). Moreover, relevant genetic correlations have only been calculated for one species with hermaphroditic flowers. Andersson (1996) reported positive genetic and phenotypic correlations between flower number and the size of individual floral parts (Table 1). Thus, no study has demonstrated genetically based trade-offs between flower size and number in a hermaphroditic species.

If trade-offs between flower size and number occur generally, the frequent observation of positive or nonsignificant genetic correlations between floral traits (Table 1) implies that genes with positive or independent effects on each trait may often obscure trade-offs. Certainly, genes that increase resource acquisition should increase both flower size and number (van Noordwijk and de Jong 1986; Houle 1991). In addition, genes with positive pleiotropic effects on both traits (perhaps genes controlling allocation to flowering) may mask negative pleiotropy (genes controlling allocation among floral traits), unless variation in allocation to flower size versus

[1] Present address: Department of Botany, University of Washington, Box 355325, Seattle, Washington 98195-5325.

TABLE 1. Empirical studies investigating relations between flower size and number in flowering plants. Species for which mean flower size and number have only been compared between separate sexes are not included, because sex-specific differences in floral allocation may obscure trade-offs (see introduction). Unless otherwise indicated, all studies used corolla or perianth diameter to represent flower size.

Species	Sexual system	Measure of flower number	Type of evidence	Relation measured[1]	Reference
Silene latifolia	dioecious	total flower number	comparison of means between sexes	−	Delph 1996, Carroll and Delph 1996
		total flower number	genetic correlation	n.s.	Meagher 1992
		total flower number	response to selection	−	Meagher 1994
Solanum carolinense	andromonoecious	total flower number	genetic correlation	n.s.	Elle 1998[2]
			environmental correlation	n.s.	
		proportion male flowers	genetic correlation (one of three populations)	−	
			environmental correlation (two of three populations)	−	
Begonia involucrata	monoecious	total female flowers per inflorescence	phenotypic correlation	−	Schemske and Ågren 1995
Begonia oaxacana	monoecious	total female flowers per inflorescence	phenotypic correlation	n.s.	Schemske et al. 1996
Begonia semiovata	monoecious	total female flowers per inflorescence	phenotypic correlation	+	Ågren and Schemske 1995
			genetic correlation	n.s.	
		total male flowers per inflorescence	phenotypic correlation	+	
			genetic correlation	n.s.	
Raphanus sativus	hermaphroditic	flowers open on two consecutive days	phenotypic correlation	−	Stanton et al. 1991
Claytonia virginica	hermaphroditic	total flower number	phenotypic correlation	n.s.	Morgan 1998
Saxifraga granulata	hermaphroditic	total flower number	phenotypic correlation	+	Andersson 1996[3]
			genetic correlation	+	
Impatiens hypophylla	hermaphroditic	total flower number	comparison of means between selfing and outcrossing varieties	−	Sato and Yahara 1999[4]
		total flower number	phenotypic correlations within varieties	−	

[1] Negative relations are indicated by −, positive relations by +, and nonsignificant relations by n.s.
[2] The size of hermaphroditic flowers was used to indicate flower size in all calculations.
[3] Flower size per se was not measured, but petal width, style length, and stigma size were positively correlated with flower number.
[4] Measurement of investment per flower included dry mass of floral organs, nectar sugar secretion, and dry mass of seeds and pericarp.

number is high (de Laguerie et al. 1991; de Jong 1993). Thus, genetic variation in resource acquisition or in relative allocation to flowering may mask trade-offs between flower size and number. Some recent studies have controlled for phenotypic variation in resource status before conducting genetic analyses of plant reproductive characters. For example, genetic variation in resource status contributed to genetic variation in floral traits and a positive genetic correlation between male and female allocation in *Mimulus guttatus* (Robertson et al. 1994; Fenster and Carr 1997), but not in *Saxifraga granulata* (Andersson 1996) or *Ipomopsis aggregata* (Campbell 1997). The results for *M. guttatus* suggest that accounting for variation in resource levels may reveal how genetic variation in resource status influences genetic relations between traits.

Here we investigate genetic relations between flower size and number in the bee-pollinated annual *Eichhornia paniculata* (Pontederiaceae). Greenhouse studies under uniform conditions indicate that genetic differentiation for flower size and number occurs among populations from northeastern Brazil (Barrett 1985). The presence of substantial genetic variation in floral traits suggested that *E. paniculata* would be a suitable species in which to investigate floral trade-offs. We use artificial selection to generate plants with contrasting allocations to flower size and number and to investigate whether evolutionary increases in flower size cause decreases in flower number. Such short-term selection has revealed negative genetic correlations between flower size and number within sexes of dioecious *Silene latifolia* (Meagher 1992, 1994) and between ovule and anther production in *Spergularia marina* (Mazer and Delasalle 1996; Mazer et al. 1999) that were not evident before selection. We also use pedigree information from the selection experiment to estimate heritabilities and additive genetic correlations between flower size and number. We compare these estimates with realized heritabilities and correlations based on responses to selection. Both when assessing selection responses and calculating genetic correlations, we account for phenotypic variation in two indices of resource status, leaf area of the flowering module and age at flowering. Thus, we statistically control for variation in resource status and experimentally increase variation in flower size and number.

We use perianth area as our index of investment per flower (hereafter referred to as ''flower size'') because in *E. paniculata* it is strongly correlated with dry mass and provides a visual indicator of reward levels to potential pollinators.

However, investment per flower also involves nectar and gamete production. Correlated responses of nectar, pollen, and ovules to selection on flower size may differ in magnitude or direction, depending on their relations with flower size and with each other. For example, petal size and nectar volume are thought to contribute disproportionately to male function in animal-pollinated species (cf. Bateman 1948) and, in *Raphanus sativus,* are more closely related to pollen than to ovule production (Stanton and Preston 1988; Stanton et al. 1991). In addition, trade-offs are expected between female and male allocation (Charlesworth and Charlesworth 1981; Charnov 1982). If flower size is more closely related to male than female investment, selection for larger perianths could actually decrease ovule production.

In this study, we address the following specific questions: (1) Do flower size and flower number exhibit significant heritable variation and respond to direct selection? (2) Are genetic correlations and correlated responses to selection consistent with a trade-off between flower size and number? If this trade-off constrains the evolution of floral display, selecting for large flowers should cause correlated decreases in flower number and vice versa. (3) How do nectar, pollen, and ovule production vary with flower size, and do they show significant correlated responses to selection on flower size? Trade-offs between flower size and number seem more likely if increased flower size corresponds to increases in other aspects of investment per flower.

Methods

Study Species

Eichhornia paniculata is an emergent aquatic native to the Neotropics, especially northeastern Brazil and the Caribbean. Plants in the field behave predominantly as annuals because they occupy ephemeral ponds or ditches. As a result, early flower production and seed set are important components of fitness. Plants typically grow in monospecific stands and are often even-aged due to synchronous germination following rain. The species is easily grown under greenhouse conditions and can be raised from seed to flowering in three to four months (see Barrett 1985; Barrett and Husband 1997 for details of the species' natural history).

When *E. paniculata* plants are growing vigorously, they produce new reproductive shoots every seven to 14 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 leaflike structure on the module (Richards and Barrett 1984). The compound inflorescence produces 10–100 flowers over seven to 18 days. Individual flowers last six to eight hours (Morgan and 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 pollinated by bumble-bees and most Brazilian populations are tristylous and largely outcrossing. The seed source for this experiment was population B181, a large outcrossing ($t = 0.84$) population near the city of Agrestina, Pernambuco state, northeastern Brazil.

The area of the large, leaflike bract (hereafter referred to as a ''leaf'') subtending each inflorescence is likely to provide an index of resource availability per inflorescence for two reasons. First, the leaf and inflorescence are part of the same module and should be similarly influenced by resource status during development. Second, the leaf likely supplies photosynthates to the inflorescence during anthesis, so that its area may provide an index of the module's resource status during flowering.

Data Collection and Selection Regime

Parental generation

Mature seed capsules were collected in 1994 from population B181. We planted seeds from 60 maternal families in the University of Toronto greenhouses on March 8, 1995. On April 24–26, we transplanted six plants from each maternal family into individual pots, for a total of 360 plants (60 maternal families $\times$ 6 plants/family). These plants were placed in a randomized block design in which the blocks were the water-filled trays that contained the plants.

The data used in this study were from the first inflorescence produced by each plant and included a measure of flower size, counts of both daily and total flower number, and two indices of module size or resource status. On the fourth day of flowering, we assessed flower size by measuring perianth width and length on three flowers. We multiplied each length and width measurement to obtain an index of perianth area, which corresponded well to the dry weight of individual flowers ($R^2 = 0.65$, $P < 0.001$, $N = 69$). Perianth areas were averaged to estimate mean flower size per inflorescence. We used the number of flowers open on the fourth day of flowering to indicate daily flower number because day 4 is generally the peak of daily flower production (Morgan and Barrett 1989). Genetic variation in flower number is most likely to be detectable on this day, rather than early or late in flowering when few flowers are open. The indices of module size were leaf area (see rationale under Study Species) and age at flowering. Day 1 was the day that the first plant flowered, and subsequent days were numbered consecutively until all plants had flowered. All plants increased more than twofold in height and width during the experiment. Thus, plants that flowered later accumulated more resources before flowering (cf. Dorn and Mitchell-Olds 1991; Mitchell-Olds 1996).

First and second generations of selection

Plants were selected for high daily flower number (N+), large flower size (S+), and small flower size (S−) or were used as controls (C). We selected on daily flower number rather than total number because daily number determines display size. Selection was based on the first inflorescence that each plant produced. First, 20 plants were chosen randomly for the control line; there were no significant differences in flower size or number between the control lines and the rest of the parents (flower size: $t_{23} = 0.70$, $P > 0.4$; flower number: $t_{21} = 0.43$, $P > 0.6$). From the remaining plants, we selected 40 individuals from approximately the top or bottom 20% of the flower size or number distribution for each direction of selection. Because inbreeding reduces flower number in *E. paniculata* (Barrett and Charlesworth

TABLE 2. Crossing design used in the selection experiment. Each selection line consisted of 20 plants. Plants could be used as male or female parents in a given cross, and the identity of mother and father were specified in the pedigree.

Plant	1	2	3	4	5	6	7	8	9	10
11	x	x								x
12	x	x	x							
13		x	x	x						
14			x	x	x					
15				x	x	x				
16					x	x	x			
17						x	x	x		
18							x	x	x	
19								x	x	x
20	x								x	x

1991), we did not select more than two individuals from the same maternal family. Selected plants were sampled with replacement so that 10 plants contributed to both S− and N+ lines and four plants contributed to both S+ and N+ lines. Selected plants were ranked according to floral traits and individuals from successive pairs of plants were randomly assigned to two replicate lines (20 parents per line). In both control and selected lines, each plant was crossed with three other arbitrarily chosen, unrelated plants from the same selection line (Table 2). This crossing design gave a potential of 30 families per selection line and ensured a large number of unrelated families in the next generation. Plants could be used either as male or female parents, and we recorded paternal and maternal identities for each cross. We ranked the 30 families according to mean floral phenotype of the parents. Five seedlings per family from each of the 20 top-ranked families were transplanted to individual pots and grown to flowering, for a total of 100 plants per selection line. Using the top-ranked families increased the strength of selection, and we considered the contribution of each parent to the next generation when calculating realized selection differentials (see Data Analysis, Response to Selection). The extra families provided insurance against germination failure or early mortality, although they were rarely used. The 20 plants from each line with the most extreme phenotypes were crossed in a similar design to produce the second generation. Crosses between known relatives (full or half sibs) were avoided.

Before choosing the parents for selection, we adjusted flower size and number for effects of leaf area and block, to allow us to select plants at the extremes of allocation more effectively. We adjusted floral traits for variation in leaf area as follows: adjusted trait = measured trait + b(population-mean leaf area − leaf area). Here, b refers to the partial regression coefficient relating variation in leaf area to the floral trait of interest (from mixed-model analyses, see Data Analysis). When we made the adjustment, we had not yet included age at flowering as a measure of resource status. However, adjusting for leaf area accounted for some variation in age at flowering because the two indices of resource allocation were positively correlated (see Results).

Growth conditions and data collected for the selected plants were similar to the parental generation. Seeds from the first generation were sown on July 2, 1996 and plants began flowering in mid-September. Seeds from the second generation were sown on April 10, 1997 and flowering started in early July. In these generations, we measured two, rather than three, flowers on each inflorescence. Measurements of individual flowers within an inflorescence were highly repeatable ($r \geq 0.88$) so that measuring only two flowers would have had negligible effects on accuracy (Falconer and Mackay 1996).

Correlates of flower size

To assess whether selection on perianth size is likely to alter within-flower allocation, we measured nectar and gamete production on approximately 25 small- and 25 large-flowered plants from the S− and S+ lines in the selection experiment. Some measurements required destructive sampling, so these traits were measured on one of several flowers from the same inflorescence that were open on the same day.

We selected plants with young (two- to five-day-old) inflorescences with at least four open flowers and collected pollen from the long-level anthers of one flower for pollen counts. All plants were either mid- or short-styled. A second flower was collected to use for microscopic measurements of pollen grain size as well as ovule size and number. The inflorescence with the remaining two flowers was enclosed in a plastic bag for four hours to minimize evaporation of nectar. We measured nectar volume per flower with 2-μl microcapillary tubes. We obtained pollen measurements from fresh, dry pollen and used material preserved in 70% ethanol for pollen counts and ovule measurements. We used a compound microscope (Zeiss Axioplan IS1988, Carl Zeiss, North York, ON, Canada) and a digital imaging program (Northern Exposure Image Analysis Software, Release 2.9x, Empix Imaging, Inc., Mississauga, ON, Canada) to measure the length and width of 20 pollen grains and 10 ovules from each flower. Ovules were counted under a dissecting microscope (Zeiss stereomicroscope SV8). Pollen counts were obtained using a Particle Data Elzone 282PC particle counter (methods in Harder 1990).

Data Analysis

Genetic parameters

Heritabilities and genetic correlations were estimated using the VCE REML package by Neumaier and 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, analysis of variance) . First, VCE estimates variance components using a restricted-maximum-likelihood approach (REML), which has the capacity to simultaneously use information from parent-offspring, full-sib, and half-sib relations. Second, the REML approach deals well with statistically unbalanced data and nontraditional crossing designs (Shaw 1987; Falconer and Mackay 1996; Lynch and Walsh 1998). Third, 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

correlations among, floral traits that are independent of genetic and environmental variation in module size.

We used all three generations to estimate narrow-sense heritabilities (h^2) and additive genetic correlations in the base population. The REML models factored out the fixed effect of block as well as random maternal effects (environmental and nonadditive genetic) as indicated by maternal identity, and included pedigree effects to estimate additive genetic variance components. The pedigree specified the heritage of individuals in all three generations. Because their heritage was fully described, use of selected plants did not bias estimates of genetic parameters. Blocks were the trays that plants were grown in, and plants from different generations were given different block designations so that block effects also accounted for environmental differences between generations. We assessed the significance of individual estimates within each analysis using one-sample *t*-tests (one-tailed for heritability estimates and two-tailed for genetic correlations). We calculated α-levels using the sequential Bonferroni technique (Rice 1989) because each analysis involved multiple tests of significance.

A correlation matrix was first calculated for all five traits, and then for the three floral traits with the two module size estimates (leaf area, age at flowering) included as covariates in the REML models. The first approach allowed us to assess genetic variation in each floral trait and the two size indices, along with 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.

Responses to selection

We analyzed responses of floral traits after two generations of selection with mixed models fitted using restricted maximum likelihood (PROC MIXED; SAS Institute 1997). Categorical factors in the analyses included direction of selection, block, line nested within each selection treatment (all fixed effects), and family within line (random effect). Covariates included leaf area and age at flowering in all analyses. All possible two- and three-way interactions involving covariates were included in the initial models and nonsignificant terms involving covariates were dropped using backward elimination ($\alpha = 0.05$). Daily and total flower number were square-root transformed prior to analysis to stabilize variances, but we report back-transformed means, which have asymmetric standard errors.

We calculated realized heritabilities, $h^2 = S/R$, based on cumulative responses, R, to two generations of selection. To obtain accurate selection differentials, S, we first calculated the mean values of parents in each selection line, weighted by their contribution to the next generation (Falconer and Mackay 1996). We then used the absolute difference between the weighted means of selected parents and the mean of the pool from which they were selected as the selection differential. Responses in each generation were the differences between control and selected lines. Standard errors, $SE(h^2)$, were estimated as SE_{response}/S (Falconer and Mackay 1996).

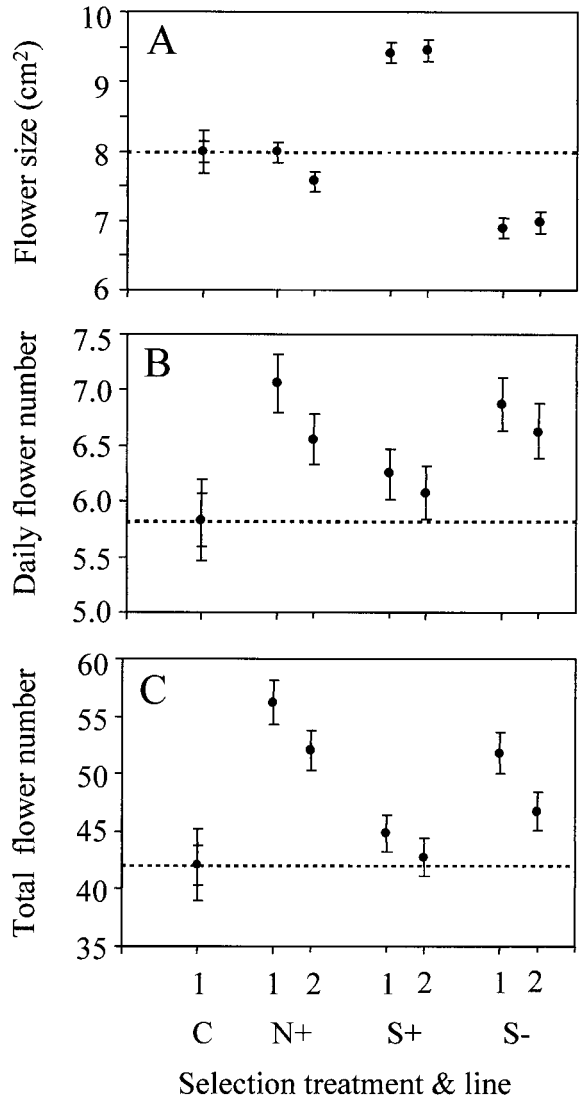

FIG. 1. Direct and correlated responses to selection for large flowers (S+), small flowers (S−), and high daily flower number (N+) in *Eichhornia paniculata.* Observed means and standard errors for (A) flower size, (B) daily flower number, and (C) total flower number are presented for the control line, C, and two replicate selection lines within each selection regime. Two standard errors are presented for the control line; the smaller represents measurement error and the larger also includes expected variation due to genetic drift. The dashed lines indicate trait means for the control line and provide a reference against which to compare selected lines. These data are from mixed-model analyses (Table 1) that included leaf area and age at flowering and are therefore adjusted for the effects of these indices of module size. See Methods for further details.

These standard errors do not include potential variation due to genetic drift, but variation among replicate lines gives an empirical indication of drift variation. Because the control line was not replicated, we added potential effects of drift to the standard error presented in Figure 1,

$$SE_{\text{control}} = s_P\sqrt{\frac{th^2}{N_e} + \frac{1}{M}}, \quad (1)$$

where s_P is the standard deviation of measurements on control

plants, t is the number of generations, N_e is the number of parents per generation, and M is the number of individuals measured (Hill 1972; Falconer and Mackay 1996).

We also calculated realized genetic correlations within each selection line as follows. First, we estimated the realized selection intensities on the trait under direct selection (trait 1), $i_1 = S_1/s_{P1}$, where S_{P1}^2 is the phenotypic variation in trait 1. Second, we calculated the genetic correlation, r_{12}, and its standard error, $SE(r_{12})$,

$$r_{12} = \frac{CR_2}{i_1 h_1 h_2 s_{P2}}, \quad \text{and} \tag{2}$$

$$SE(r_{12}) = (1 - r_{12}^2)\sqrt{\frac{SE(h_1^2)SE(h_2^2)}{h_1^2 h_2^2}}, \tag{3}$$

where CR_2 is the correlated response of trait_2 to selection on trait 1 (Falconer and Mackay 1996). We used estimates of realized heritability for flower size and daily flower number and the REML estimate of heritability for total flower number because we had no realized estimate. We present direct and correlated responses with data adjusted for variation in leaf area and age at flowering. Including leaf area and age in the analyses did not change general trends, but improved our ability to detect differences among selection lines by accounting for size-related variation in flower number. We therefore report adjusted means, that is, the least-squared means from analyses that account for variation in size indices as well as block effects (unadjusted means are given in the Appendix).

Correlates of flower size

We used *t*-tests to assess whether divergence in flower size between S+ and S− lines resulted in significant differences in nectar, pollen, or ovule production (PROC T-TEST; SAS Institute 1997). We converted pollen grain length and width measurements to volume using the formula:

$$\text{volume} = 4/3\pi(\text{width}/2)^2(\text{length}/2) \tag{4}$$

to account for the oval shape of the grains. We did not convert ovule measurements to volume because preservation made their original shape difficult to assess. Instead, we multiplied ovule length by ovule width to estimate ovule area. We estimated total allocation to pollen and ovules by multiplying the size and number of gametes produced. Finally, we examined relations among floral traits after removing the effect of flower size to test whether aspects of intrafloral allocation covaried independently of flower size (PROC REG; SAS Institute 1997). In this analysis, we used mean flower size for each plant (see below). Measures of flower size on flowers from the same plant were highly correlated ($r \geq 0.97$).

RESULTS

Heritabilities and Genetic Correlations

The REML analyses of resemblance between relatives indicated that all traits exhibited significant heritable variation (Table 3A). Almost half the variation in flower size was attributable to additive genetic variation, whereas additive genetic variation in daily flower number, leaf area, and age at flowering ranged from 10% to 17% of total phenotypic variation. Heritability of total flower number was intermediate at 23% (Table 3A). Genetic correlations between flower size and number were low but positive and did not support the occurrence of a genetic trade-off between flower size and number. Daily and total flower numbers were perfectly correlated. Genetic correlations between floral traits and the two size indices, leaf area and age at flowering, were positive and of moderate magnitude ($r = 0.25$–0.63, Table 3A), indicating that some of the genetic variation in floral traits was associated with genetic variation in module size.

To test the expectation that mutual dependence on module size resulted in the positive genetic correlations between flower size and number, we analyzed genetic relations between floral traits after accounting for phenotypic variation in leaf area and age at flowering. Reductions in additive genetic variance in daily number from 0.028 to 0.017 and in total number from 0.494 to 0.261, indicated that leaf area and age at flowering accounted for 40–50% of the genetic variation in these traits. Additive genetic variance in flower size changed from 0.788 to 0.696, a 10% reduction. Leaf area and age at flowering also accounted for similar proportions of residual variation, so that heritability estimates based on size-adjusted data differed little from those based on measured data (Table 3B). After accounting for leaf area and age at flowering, genetic correlations between flower size and

TABLE 3. Heritabilities (diagonal) and additive genetic correlations (above diagonal) for floral traits and indices of module size among greenhouse-grown *Eichhornia paniculata* plants. (A) Genetic parameters estimated on measured traits. (B) Genetic parameters estimated on traits after accounting for variation in leaf area and age at flowering. Genetic parameters were estimated with VCE version 4.2 and were based on three generations. Standard errors of the estimates are in parentheses, and numbers in boldface differ significantly from zero. The tablewide significance of all estimates was assessed using α-levels calculated according to the sequential Bonferroni technique (Rice 1989).

(A)

	Flower size	Daily flower number	Total flower number	Leaf area	Age at flowering
Flower size	**0.48** (0.012)	**0.15** (0.060)	**0.18** (0.063)	**0.25** (0.074)	**0.44** (0.064)
Daily flower number		**0.10** (0.029)	**1.00** (0.003)	**0.25** (0.065)	**0.42** (0.055)
Total flower number			**0.23** (0.034)	**0.62** (0.084)	**0.63** (0.072)
Leaf area				**0.14** (0.033)	**0.67** (0.126)
Age at flowering					**0.17** (0.033)

(B)

	Flower size	Daily flower number	Total flower number
Flower size	**0.49** (0.028)	−0.07 (0.092)	−0.07 (0.076)
Daily flower number		**0.08** (0.018)	**1.00** (0.000)
Total flower number			**0.21** (0.028)

TABLE 4. Mixed-model analyses of factors affecting floral traits in *Eichhornia paniculata* plants after two generations of selection ($N = 627$ plants). All analyses initially included the two size indices (leaf area, age at flowering) and all possible two-way interactions. Nonsignificant terms involving covariates were dropped using backward elimination ($\alpha = 0.05$), and only terms that were significant in at least one analysis are included in the table. Regression coefficients for significant covariates are in italics underneath the relevant *F*-statistic. The random family effects were tested with likelihood-ratio tests.

Effect	Flower size	Daily flower number	Total flower number
Block	$F_{61,531} = 1.51$**	$F_{61,544} = 1.08$	$F_{61,546} = 1.32$
Select	$F_{3,141} = 102.19$***	$F_{3,129} = 5.47$**	$F_{3,132} = 16.21$***
Line (select)	$F_{3,141} = 1.45$	$F_{3,130} = 0.94$	$F_{3,132} = 2.53$
Family (line select)	$G_{151} = 12.75$*	$G_{151} = 1.73$	$G_{151} = 1.28$
Leaf area	$F_{1,551} = 12.95$***	$F_{1,547} = 43.78$***	$F_{1,556} = 140.81$***
($b \pm s_b$)	*0.011 ± 0.003*	*0.020 ± 0.003*	*0.036 ± 0.003*
Age at flowering	$F_{1,556} = 12.42$***	$F_{1,552} = 6.25$*	$F_{1,548} = 73.05$***
($b \pm s_b$)	*0.015 ± 0.004*	*0.011 ± 0.004*	*0.035 ± 0.004*
Leaf area × age	—	$F_{1,548} = 4.40$*	—
($b \pm s_b$)	—	*−0.0001 ± 0.00008*	—

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

number were no longer positive, but they did not differ significantly from zero (Table 3B).

Responses to Selection

Artificial selection altered flower size, daily flower number, and total flower number in *E. paniculata* (Table 4). Responses to selection did not differ significantly between lines within each selection treatment (Table 4). Therefore, we do not often distinguish between them in our description below, although selection lines are presented separately in the tables and figures. Analyses including leaf area and age at flowering as covariates explained 15% more of the variation in daily and total flower number and about 2% more of the variation in flower size than analyses without these two indices of module size (*F*-tests for each covariate are given in Table 4). Flower size and number varied positively with both indices of module size (Table 4).

Flower size responded strongly to direct selection, but not to selection on flower number. Two generations of direct selection increased mean flower size by 1.4 cm^2 in S+ lines and decreased flower size by 1.1 cm^2 in S− lines (Fig. 1A). Overall, these responses corresponded to a realized $h^2 = 0.45$ for flower size, which was very close to our REML estimate of $h^2 = 0.48$ (Table 5). In contrast to direct responses, flower size did not decrease significantly in response to selection for high daily flower number, although mean flower size in one N+ line decreased by 0.43 cm^2 (Fig. 1A). However, the low heritability for daily flower number ($h^2 = 0.10$) indicates that a fairly strong genetic correlation between flower size and daily number would be required to generate a significant correlated response. For example, decreased flower size in N+, line 2 corresponded to a realized $r = -0.49$ (Table 6), but the decrease in flower size was only significant when N+, line 2 was considered in isolation from N+, line 1 ($t_{139} = 2.1$, $0.03 < P < 0.05$).

Daily flower number changed significantly in response to both direct selection and selection on flower size. The cumulative effects of two generations of selection increased daily flower number by one flower in N+ lines (Fig. 1B). Mean realized heritability for the two lines corresponded to an $h^2 = 0.16$, which did not differ significantly from the REML estimate of $h^2 = 0.10$ (Table 5). The twofold difference between heritability estimates for each line (Table 5) indicated that changes in daily flower number were probably affected by sampling error or genetic drift. In addition to the direct response, daily flower number increased by 0.75 flowers in lines selected for small flowers (Fig. 1B). These responses corresponded to a mean genetic correlation between

TABLE 5. Selection differentials (*S*), responses to selection (*R*) on flower size and daily flower number in *Eichhornia paniculata,* and realized heritabilities (h^2). Standard errors are in parentheses. The average realized h^2 for each selection treatment is compared to the REML estimate of heritability. Daily flower number was square-root transformed before analysis. Data in this table are based on trait means that were adjusted for variation in block, leaf area, and age at flowering. Estimates based on unadjusted means were qualitatively similar.

Trait	Selection, line	*S*	*R* (SE)	Realized h^2 (SE)	REML h^2 (SE)
Daily flower number	N+, line 1	1.21	0.243 (0.0700)	0.20 (0.058)	
	N+, line 2	1.30	0.147 (0.0678)	0.11 (0.052)	
	Mean N+	1.26	0.195 (0.0599)	0.16 (0.041)	0.10 (0.029)
Flower size (cm^2)	S+, line 1	2.97	1.42 (0.207)	0.48 (0.070)	
	S+, line 2	2.75	1.45 (0.215)	0.53 (0.078)	
	Mean S+	2.86	1.44 (0.184)	0.50 (0.064)	0.48 (0.012)
	S−, line 1	−2.83	−1.10 (0.210)	0.39 (0.074)	
	S−, line 2	−2.49	−1.02 (0.213)	0.41 (0.086)	
	Mean S−	−2.66	−1.06 (0.185)	0.40 (0.070)	0.48 (0.012)

TABLE 6. Genetic correlations (*r*) estimated from correlated responses to selection (*CR*) in *Eichhornia paniculata.* Standard errors are in parentheses, and correlations from selection treatments in which the mean correlated responses differed significantly from zero are in bold face. Note that flower size in N+, line 2 differs significantly from the control when considered in isolation from N+, line 1. Daily and total flower numbers were square-root transformed before calculations. Data in this table are based on trait means that were adjusted for variation in block, leaf area, and age at flowering. Estimates based on unadjusted means were qualitatively similar.

Selection, line	Flower size × daily number		Flower size × total number		Daily number × total number	
	CR (SE)	*r* (SE)	*CR* (SE)	*r* (SE)	*CR* (SE)	*r* (SE)
N+, 1	−0.01 (0.214)	−0.01 (0.177)	—	—	**1.01 (0.184)**	**1.53 (0.261)**
N+, 2	**−0.43 (0.208)**	**−0.49 (0.134)**	—	—	**0.73 (0.178)**	**0.96 (0.016)**
S+, 1	0.09 (0.068)	0.29 (0.163)	0.21 (0.177)	0.22 (0.124)	—	—
S+, 2	0.05 (0.071)	0.19 (0.170)	0.06 (0.185)	0.08 (0.129)	—	—
S−, 1	**0.21 (0.069)**	**−0.64 (0.103)**	**0.71 (0.181)**	**−0.68 (0.069)**	—	—
S−, 2	**0.16 (0.070)**	**−0.60 (0.113)**	**0.35 (0.181)**	**−0.38 (0.112)**	—	—

daily number and flower size of $r = -0.62$ (Table 6). In contrast, daily flower number showed no correlated decrease in response to selection for large flowers (Fig. 1B). The changes in S+ lines therefore did not support significant genetic correlations between daily number and flower size (Table 6). However, plants from S+ lines did produce significantly fewer flowers than S− plants ($t_{129} = 2.5$, $P < 0.02$). This difference corresponded to a genetic correlation of $r = -0.34$ (± 0.111).

All selection on total flower number was indirect. However, responses to selection closely paralleled those for daily flower number (Fig. 1C). Total flower number increased by 12 flowers in lines selected for high daily number (Fig. 1C). These differences corresponded to an average genetic correlation of $r = 1.24$ between daily and total flower number, which did not differ significantly from our REML estimate of $r = 1.00$ (Table 6). Responses by total number to selection on flower size also paralleled those for daily number. Total flower number in the S− lines increased by seven flowers relative to control lines (Fig. 1C). These increases resulted in an average realized correlation of $r = -0.53$ between flower size and total number (Table 6). In contrast, total flower number did not decrease in S+ lines (Fig. 1C) and did not indicate a significant realized genetic correlation. Total flower number in S+ lines was significantly lower than in S− lines (Fig. 1B; $t_{132} > 3.2$, $P < 0.002$). This difference corresponded to a genetic correlation of $r = -0.30$ (± 0.078) between flower size and total flower number.

TABLE 7. Comparisons of mean nectar, pollen, and ovule production between *Eichhornia paniculata* plants from S+ and S− selection treatments. These comparisons indicate whether divergence in flower size caused significant divergence in other floral traits.

Trait	*N*	S+ Mean (SE)	S− Mean (SE)	t_{diff} (H_0: S+ = S−)
Nectar volume (μl)	32	0.54 (0.068)	0.23 (0.048)	3.64***
Male gametophyte (mm^3) (total pollen volume)	42	0.26 (0.013)	0.21 (0.011)	2.51*
Pollen grain volume (μm^3)	42	28,351 (1033)	26,700 (700)	1.32
Pollen grain number	47	9097 (625)	7864 (376)	1.69†
Female gametophyte (mm^2) (total ovule size)	40	30.9 (1.41)	27.5 (1.28)	1.75†
Ovule size (mm^2)	40	0.30 (0.006)	0.28 (0.008)	2.10*
Ovule number	42	102 (3.5)	99 (3.9)	0.51

† $P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Correlates of Flower Size

Most aspects of investment per flower were higher in S+ than in S− plants (Table 7, Fig. 2). Divergence between S+ and S− lines in flower size caused a 2.3-fold difference in mean nectar production (Table 7, Fig. 2A). Differences in total pollen and ovule production were more modest (Fig. 2B, C), but were significant for total pollen volume and ovule size and marginally significant for pollen grain number and total ovule size (Table 7).

Among floral traits, only pollen grain size and number were significantly related. The negative relation was evident before adjustment for variation in flower size and became slightly stronger after adjustment (Fig. 3). Analysis of log-transformed data indicated that the proportional change in grain number in response to increased grain volume was slightly steeper than −1.0, which would indicate an isometric relation (proportional change ± SE = −1.17 ± 0.025). Elimination of the three large-flowered outliers with unusually large pollen grains would have resulted in an even steeper slope (Fig. 3). The large degree of overlap among large- and small-flowered plants indicates that the relation between pollen grain size and number is independent of variation in flower size (Fig. 3) because flower size and pollen size were unrelated (Table 7).

DISCUSSION

The idea that trade-offs between flower size and number influence floral evolution has intuitive appeal and is supported by natural-history observations of variation in floral display among animal-pollinated plants. Responses to artificial selection by *E. paniculata* gave limited support for genetically based trade-offs at the population level. Genetic correlations and responses to selection supported the presence of genes with positive and independent effects on both traits. Below, we first discuss the evidence that genetic variation in module size, or in relative allocation to flowering, causes positive genetic correlations between flower size and number. We then outline potential causes of asymmetrical correlated responses. We also consider the possibility that flower size and number vary independently. Finally, we discuss how the

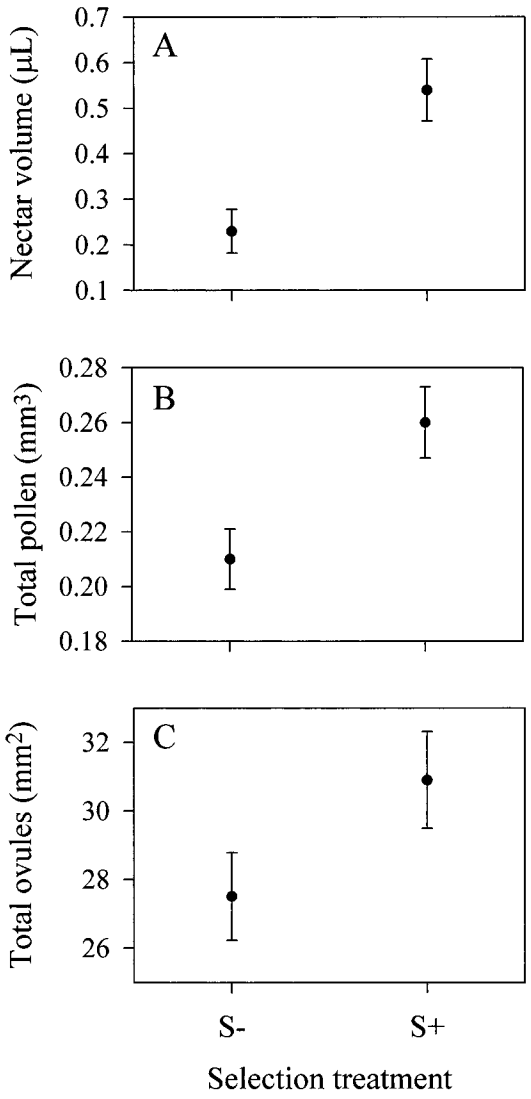

FIG. 2. Divergence in (A) nectar volume, (B) total pollen volume (pollen grain number × volume), and (C) total ovule area (ovule number × area) for *Eichhornia paniculata* plants from small-flowered (S−) and large-flowered (S+) selection lines. Analyses of divergence between selection treatments for all floral traits are in Table 7.

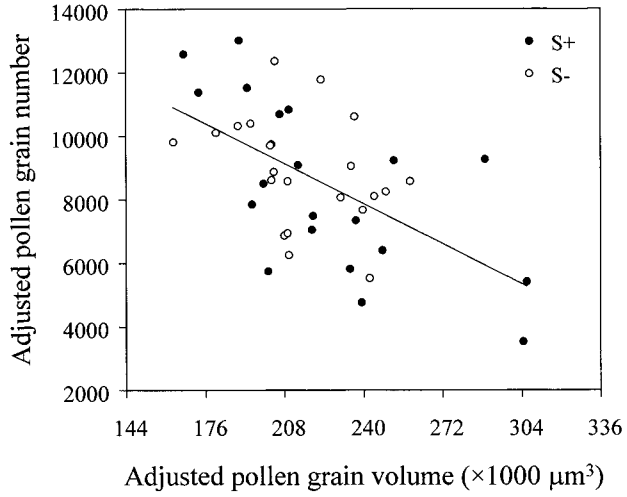

FIG. 3. Relation between pollen grain number and size in *Eichhornia paniculata* plants from small-flowered (S−) and large-flowered (S+) selection lines (grain number = 17,294 − 0.04 [grain volume], $R^2 = 0.33$, $P < 0.001$). Both pollen grain size and number are adjusted for variation in flower size.

changes in allocation to nectar, pollen, and ovule production that accompany changes in flower size may influence trade-offs between flower size and number.

Evidence for Trade-offs from Genetic Correlations

Two lines of evidence indicated that genetic variation in both flower size and number resulted from genetic variation in module size (resource status). First, all genetic correlations between size indices and floral traits were positive. Positive phenotypic correlations between plant size and flower production are well documented (e.g., Herrera 1991; Mitchell 1994), and positive genetic correlations have also been reported (Mazer 1989; Meagher 1992). Flower size may not necessarily depend on plant size, although positive phenotypic and genetic correlations between plant height and various floral measurements occur in *S. latifolia* (Meagher 1992) and *S. granulata* (Andersson 1996). Second, additive genetic variation in floral traits was lower after accounting for variation in leaf area and age at flowering. These reductions imply that genetic variation in floral traits resulted either directly from variation in leaf area and age at flowering or from other factors, such as resource status, that influence both size indices and floral traits.

Our results also suggested that genetic variation in module size caused positive genetic correlations between flower size and number. Two other studies reporting positive genetic correlations between floral traits found module size to be positively correlated with both flower size and number (Meagher 1992; Andersson 1996). Although controlling for module size removed the positive genetic correlations between flower size and number in *E. paniculata,* it did not support the occurrence of a trade-off by revealing significant negative correlations. One interpretation of this result is that flower size and number in *E. paniculata* vary independently, apart from the positive correlation introduced by mutual dependence on resource status. Alternatively, high variation in allocation to flowering may have masked the trade-off between flower size and number (cf. de Laguerie et al. 1991; de Jong 1993; see introduction). If the latter interpretation is true, creating divergence in flower size and number through artificial selection may reveal trade-offs between flower size and number. We imposed two generations of artificial selection on floral traits to test this possibility.

Evidence for Trade-offs from Selection Responses

Selection responses partially confirmed our REML estimates of heritabilities and genetic correlations. Direct responses to selection on flower size and number coincided well with all heritability estimates (Table 5) and confirmed that this *E. paniculata* population has the capacity to respond to selective pressures on flower size and number in the green-

house. Correlated responses of total flower number to selection on daily number also supported a correlation of 1.0 between these traits. However, support for trade-offs between flower size and number was mixed because the relevant correlated responses were asymmetrical (Table 6, Fig. 1). Of the six independent lines, the two S− lines clearly supported a trade-off, and reduced flower size in one of the N+ lines also indicated that linked or pleiotropic genes may have opposing effects on flower size and number. In contrast, genetic correlations on measured and size-adjusted traits, as well as responses in the remaining selection lines, supported the presence of genes with positive and independent effects on each trait.

It is possible that flower size and number may be influenced both by genes with opposing and with positive effects on each trait, even after accounting for variable resource status. Allocation to flowering is likely to be hierarchical, with allocation to reproduction versus vegetative growth preceding allocation to flower size versus number. Allocation hierarchies may cause positive genetic correlations between traits involved in trade-offs because genes controlling resource allocation near the base of hierarchies should have positive pleiotropic effects on all traits along a given pathway (van Noordwijk and de Jong 1986; Houle 1991; de Laguerie et al. 1991; de Jong 1993). As a result, short-term evolution in wild populations could involve simultaneous increases in flower size and number. For trade-offs to influence genetic correlations and short-term responses to selection, variation in allocation to flower size versus number must exceed variation in allocation to flowering (de Laguerie et al. 1991). This condition seems most likely to hold when differences in flower size and number are large. In support of this reasoning, trade-offs between flower size and number seem most evident among taxa with substantially different flower sizes (Sato and Yahara 1999; Worley et al. 2000) or after artificial selection (Meagher 1994).

Asymmetrical Correlated Responses

Why correlated responses to selection were asymmetrical is unclear, although such responses are common (reviewed by Villanueva and Kennedy 1992; Roff 1997), and they often result in conflicting evidence for trade-offs. For example, responses to selection for thermal tolerance in *Escherichia coli* indicated trade-offs between tolerance of low and high temperatures when selection was for cold tolerance, but not when selection was for heat tolerance (Mongold et al. 1996). Similar results have also followed selection on plant reproductive allocation. Selection for low ovule number in *S. marina* caused correlated increases in anther number, but selection for high ovule number caused neither direct nor correlated responses (Mazer et al. 1999). Similarly, in *S. latifolia*, flower number responded to selection on flower size, but not to direct selection (Meagher 1994).

Genetic drift is one important cause of asymmetric correlated responses to selection. Drift may increase both variability among replicate selection lines and the proportion of ''wrong-way'' responses (Gromko 1995; Lascoux 1997). The correspondence between independent lines within selection treatments suggests that drift was not high in our experiment. However, the possibility of drift remains, as is illustrated by the expected variance due to drift in the control line (Fig. 1). In addition, responses to selection are often erratic in the first few generations, with correlated responses more so (Falconer and Mackay 1996). Continued selection may have yielded stronger evidence for trade-offs.

Several conditions other than drift can cause asymmetric responses to selection (Falconer and Mackay 1996). Of these, inbreeding depression seems unlikely because our crosses were largely between unrelated plants. Also, all of the asymmetries we observed involved a lack of expected decreases in fitness-related traits, the opposite of what inbreeding depression would produce. Other explanations involve contrasting heritabilities in up- and down-selected lines due to scalar (Robertson 1977) or genetic asymmetry (Bohren et al. 1966). Scalar asymmetry occurs when the genetic contribution to phenotypic variation is higher at one phenotypic extreme than the other. Genetic asymmetry refers to initial allele frequencies that differ from those that contribute maximally to heritability. Interestingly, correlated traits are particularly sensitive to genetic asymmetry when some loci contribute positively and others negatively to the genetic covariance (Bohren et al. 1966). However, in short-term selection experiments, both asymmetries should cause nonlinear parent-offspring regressions (Falconer and Mackay 1996). We did not detect nonlinearity (A. C. Worley, unpubl. data) but the low heritability of daily number may have hindered detection.

Could Flower Size and Flower Number Vary Independently?

Several other empirical studies have not found strong evidence for a trade-off between flower size and number (Table 1), whereas trade-offs between seed size and number (reviewed by Roff 1992; Méndez 1997) and pollen grain size and number (Fig. 3; Vonhof and Harder 1995) are better supported. The equivocal results for flower size and number raise questions about the importance of trade-offs between these traits and whether flowers differ fundamentally from other repeated parts or products.

One important difference between flowers and other products, such as seeds or pollen, is that investment in flowers is not final because they can become fruit, containing seeds. The relevant trade-off could be between investment per reproductive propagule (flower + fruit) and propagule number. Some theoretical models considering floral evolution have assumed that flowering and fruiting draw from separate resource pools (e.g., Morgan 1993; Schoen and Ashman 1995; but see Ashman and Schoen 1997). Others have not distinguished between allocation to ovules versus seeds and have combined the two as female allocation (e.g., Sakai 1993, 1995), although ovule production does not necessarily indicate total female allocation. Indeed, a recent study supporting trade-offs between flower size and number in *Impatiens hypophylla* included seed production as a component of investment per flower (Sato and Yahara 1999).

Another possibility is that flower size and number do not directly compete for resources, but are involved in trade-offs with other traits. For instance, trade-offs between vegetative and reproductive allocation are well demonstrated (Snow and

Whigham 1989; Stearns 1992; Calvo 1993), and flower number sets an upper limit on reproductive allocation (Lloyd 1980). Also trade-offs between flowering and fruiting (Diggle 1993; Ashman and Schoen 1997) may decrease initiation of new flowers or investment per flower. Fruit production decreases the number (Morgan and Barrett 1989) and size (A. C. Worley, unpubl. data) of flowers subsequently produced by *E. paniculata.* In animal-pollinated species, selection for efficient pollination could determine flower size (Stebbins 1971; Conner and Via 1993; Mazer and Hultgård 1993), whereas flower number may reflect selection on reproductive investment or pollinator attraction. Thus, trade-offs between flower size and number may not play as universal a role in plant reproductive allocation as is often assumed. It may be worth relaxing the assumptions of trade-offs between flower size and number in theoretical models (e.g., Cohen and Dukas 1990; Morgan 1993; Venable 1996) to explore this possibility.

A final explanation for the apparent lack of a trade-off between flower size and number could be that a trade-off is only evident from lifetime flower production. *Eichhornia paniculata*'s occurrence in ephemeral habitats and its predominantly annual life history suggest that trade-offs that are not evident early in flowering would not be of great evolutionary importance. We counted inflorescence production over six weeks in the parental population (A. C. Worley, unpubl. ms.). Both phenotypic relations and correlations among maternal families supported a trade-off between inflorescence number and size (flower size $\times$ flower number), although estimates of broad-sense heritability for inflorescence size were nonsignificant.

Flower Size and Allocation to Nectar, Pollen and Ovules

Selection on flower size in *E. paniculata* caused correlated increases in nectar, pollen, and ovule production (Table 7, Fig. 2). Divergence in flower size was associated with greater changes in nectar than gamete production. Thus, selection for flowers with large perianths resulted in higher allocation to pollinator attraction (tepals and nectar) than to pollen or ovules. These results suggest that for natural selection to favor large perianths, fitness benefits caused by increased attractiveness should outweigh reductions in relative gamete production, especially if large-flowered plants also produce fewer flowers. However, selection favoring alternative combinations of floral characters may break down these associations (Stanton and Young 1994). The expectation that a close association between flower size and male function might cause changes in pollen production to exceed those in ovule production (see Introduction) was not strongly supported. However, greater divergence in flower size might be required to accurately assess relative changes in pollen and ovule production.

Pollen grain production was subject to a trade-off between size and number, at least at the phenotypic level. Few studies have examined pollen grain size and number within species, although strong negative relations occurred between these traits within *Raphanus sativus* (Stanton and Young 1994) and within and among 21 papilionaceous legumes (Vonhof and Harder 1995). The strength and magnitude of this trade-off contrast sharply with the relation between flower size and number. This difference may occur because, as mentioned earlier, investment per pollen grain is final and, unlike flowers, pollen grains perform a single function so that investment per grain may be a better index of performance. Interestingly, in *E. paniculata,* this trade-off was independent of changes in flower size because selection on flower size did not alter pollen grain size (Table 7).

Theoretical treatments of trade-offs between flower size and number are largely concerned with investment per flower, and they assume that plants with large flowers allocate proportionately more resources per flower to all floral parts. Our results confirm this assumption by demonstrating that plants of *E. paniculata* with large flowers generally invest more in pollen, ovules, and nectar per flower. These findings are encouraging because they suggest that discussing floral allocation in terms of flower size is a useful generalization. However, our results also illustrate the complexity of composite traits such as flower size, and indicate that changes in perianth size may not correspond to equivalent changes in all floral traits. Complex relations among different aspects of floral allocation may alter the strength and evolutionary consequences of trade-offs between flower number and investment per flower.

Acknowledgments

We thank D. Houle and S. Mazer for advice; B. Hill, J. Conner, and two anonymous reviewers for comments on an earlier version of the manuscript; E. Elle, T. Meagher, J. Gibson, G. Vandervoot, and M. Quinton for discussions about VCE; and L. Howes, T. Sarkissian, H. S. Yoon, S. Wright, D. Chung, L. Jesson, and B. Cole for help in the greenhouse. This research was funded by a Natural Science and Engineering Research Council research grant to SCHB and an Ontario Graduate Scholarship to ACW.

Literature Cited

Ågren, J., and D. W. Schemske. 1995. Sex allocation in the monoecious herb *Begonia semiovata.* Evolution 49:121–130.

Andersson, S. 1996. Floral variation in *Saxifraga granulata*: phenotypic selection, quantitative genetics and predicted responses to selection. Heredity 77:217–223.

Ashman, T.-L., and D. J. Schoen. 1997. The cost of floral longevity in *Clarkia tembloriensis*: an experimental investigation. Evol. Ecol. 11:289–300.

Barrett, S. C. H. 1985. Floral trimorphism and monomorphism in continental and island populations of *Eichhornia paniculata* (Spreng.) Solms. (Pontederiaceae). Biol. J. Linn. Soc. 25:41–60.

Barrett, S. C. H., and D. Charlesworth. 1991. Effects of a change in the level of inbreeding on the genetic load. Nature 352: 522–524.

Barrett, S. C. H., and B. C. Husband. 1997. Ecology and genetics of ephemeral plant populations: *Eichhornia paniculata* (Pontederiaceae) in Northeast Brazil. J. Heredity 88:277–284.

Bateman, A. J. 1948. Intrasexual selection in *Drosophila.* Heredity 2:349–368.

Bohren, B. B., W. G. Hill, and A. Robertson. 1966. Some observations on asymmetrical correlated responses to selection. Genet. Res., Cambridge 7:44–57.

Calvo, R. 1993. Evolutionary demography of orchids: intensity and frequency of pollination and the costs of fruiting. Ecology 74: 1043–1051.

Campbell, D. R. 1997. Genetic correlation between biomass allo-

cation to male and female functions in a natural population of *Ipomopsis aggregata*. Heredity 79:606–614.
Carroll, S. B., and L. F. Delph. 1996. The effects of gender and plant architecture on allocation to flowers in dioecious *Silene latifolia* (Caryophyllaceae). Int. J. Plant Sci. 157:493–500.
Charlesworth, D., and B. Charlesworth. 1981. Allocation of resources to male and female function in hermaphrodites. Biol. J. Linn. Soc. 15:57–74.
Charnov, E. 1982. The theory of sex allocation. Princeton Univ. Press, Princeton, NJ.
Cohen, D., and R. Dukas. 1990. The optimal number of female flowers and the fruits-to-flowers ratio in plants under pollen and resource limitation. Am. Nat. 135:218–241.
Conner, J. K., and S. Rush 1996. Effects of flower size and number on pollinator visitation to wild radish, *Raphanus raphanistrum*. Oecologia 105:509–516.
Conner, J., and S. Via. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. Evolution 47:704–711.
de Jong, G. 1993. Covariances between traits deriving from successive allocations of a resource. Funct. Ecol. 7:75–83.
de Jong, T. J., and P. G. L. Klinkhamer. 1994. Plant size and reproductive success through male and female function. J. Ecology 82:399–402.
de Jong, T. J., N. M. Waser, and P. G. L. Klinkhamer. 1993. Geitonogamy: the neglected side of selfing. Trends Ecol. Evol. 8: 321–325.
de Laguerie, P., I. Olivieri, A. Atlan, and P.-H. Gouyon. 1991. Analytic and simulation models predicting positive genetic correlations between traits linked by trade-offs. Evol. Ecol. 5: 361–369.
Delph, L. F. 1996. Flower size dimorphism in plants with unisexual flowers. Pp. 217–237 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York.
Diggle, P. K. 1993. Developmental plasticity, genetic variation, and the evolution of andromonoecy in *Solanum hirtum* (Solanaceae). Am. J. Bot. 80:967–973.
Dorn, L. A., and T. Mitchell-Olds. 1991. Genetics of *Brassica campestris*. 1. Genetic constraints on evolution of life-history characters. Evolution 45:371–379.
Elle, E. 1998. The quantitative genetics of sex allocation in the andromonoecious perennial, *Solanum carolinense* (L.). Heredity 80:481–488.
Falconer, D. S., and T. F. C. Mackay. 1996. Introduction to quantitative genetics. 4th ed. Longman Group Ltd., Harlow, U.K.
Fenster, C. B., and D. E. Carr. 1997. Genetics of sex allocation in *Mimulus* (Scrophulariaceae). J. Evol. Biol. 10:641–661.
Fishbein, M., and D. L. Venable. 1996. Evolution of inflorescence design: theory and data. Evolution 50:2165–2177.
Gromko, M. H. 1995. Unpredictability of correlated responses to selection: pleiotropy and sampling interact. Evolution 49: 685–693.
Harder, L. D. 1990. Pollen removal by bumblebees and its implications for pollen dispersal. Ecology 71:1110–1125.
Harder, L. D., and S. C. H. Barrett. 1995. Mating cost of large floral displays in hermaphrodite plants. Nature 373:512–515.
———. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. Pp. 140–190 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York.
Herrera, C. 1991. Dissecting factors responsible for individual variation in plant fecundity. Ecology 72:1436–1448.
Hill, W. G. 1972. Estimation of genetic change. I. General theory and design of control populations. Anim. Breed. Abstr. 40:1–15.
Houle, D. 1991. Genetic covariance of fitness correlates: what genetic correlations are made of and why it matters. Evolution 45: 630–648.
Lascoux, M. 1997. Unpredictability of correlated responses to selection: linkage and initial frequency also matter. Evolution 51: 1394–1400.
Lloyd, D. G. 1980. Sexual strategies in plants. I. An hypothesis of serial adjustment of maternal investment during one reproductive session. New Phytol. 86:69–79.
Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer, Sunderland, MA.
Mazer, S. J. 1989. Family mean correlations among fitness components in wild radish: controlling for maternal effects on seed weight. Can. J. Bot. 67:1890–1897.
Mazer, S. J., and V. A. Delasalle. 1996. Covariation among floral traits in *Spergularia marina* (Caryophyllaceae): geographic and temporal variation in phenotypic and among-family correlations. J. Evol. Biol. 9:993–1015.
Mazer, S. J., and U.-M. Hultgård 1993 Variation and covariation among floral traits within and among four species of northern European *Primula* (Primulaceae). Am. J. Bot. 80:474–485.
Mazer, S. J., V. A. Delasalle, and P. R. Neal. 1999. Responses of floral traits to selection on primary sexual investment in *Spergularia marina* (Caryophyllaceae): the battle between the sexes. Evolution 53:717–731.
Meagher, T. R. 1992. The quantitative genetics of sexual dimorphism in *Silene latifolia* (Caryophyllaceae). I. Genetic Variation. Evolution 46:445–457.
———. 1994. The quantitative genetics of sexual dimorphism in *Silene latifolia* (Caryophyllaceae). II. Response to sex-specific selection. Evolution 48:939–951.
Méndez, M. 1997. Sources of variation in seed mass in *Arum italicum*. Int. J. Plant Sci. 158:298–305.
Mitchell, R. J. 1994. Effects of floral traits, pollinator visitation, and plant size on *Ipomopsis aggregata* fruit production. Am. Nat. 143:870–889.
Mitchell-Olds, T. 1996. Genetic constraints on life-history evolution: quantitative-trait loci influencing growth and flowering in *Arabidopsis thaliana*. Evolution 50:140–145.
Mongold, J. A., A. F. Bennett, and R. E. Lenski. 1996. Evolutionary adaptation to temperature. IV. Adaptation of *Escherichia coli* at a niche boundary. Evolution 50:35–43.
Morgan, M. T. 1993. Fruit to flower ratios and trade-offs in size and number. Evol. Ecol. 7:219–232.
———. 1998. Female fertility per flower and trade-offs between size and number in *Claytonia virginica* (Portulaceae). Am. J. Bot. 85:1231–1236.
Morgan, M. T., and S. C. H. Barrett. 1989. Reproductive correlates of mating system variation in *Eichhornia paniculata* (Spreng.) Solms (Pontederiaceae). J. Evol. Biol. 2:183–203.
Neumaier, A., and E. Groeneveld. 1988. Restricted maximum likelihood estimates of covariances in sparse linear models. Gen. Sel. Evol. 30:3–26.
Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution 43:223–225.
Richards, J. H., and S. C. H. Barrett. 1984. The developmental basis of tristyly in *Eichhornia paniculata* (Pontederiaceae). Am. J. Bot. 71:1347–1363.
Robertson, A. 1977. The non-linearity of parent-offspring regression. Pp. 297–304 *in* E. Pollak, O. Kempthorne, and T. B. Bailey, eds. Proceedings of the international conferences on quantitative genetics. Iowa State Univ. Press, Ames.
Robertson, A. W., A. Diaz, and M. R. McNair. 1994. The quantitative genetics of floral characters in *Mimulus guttatus*. Heredity 72:300–311.
Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.
———. 1997. Evolutionary quantitative genetics. Chapman and Hall, New York.
SAS Institute. 1997. SAS/STAT software. Rel. 6.12. SAS Institute, Cary, NC.
Sakai, S. 1993. Allocation to attractive structures in animal-pollinated plants. Evolution 47:1711–1720.
———. 1995. Evolutionarily stable selfing rates of hermaphroditic plants in competing and delayed selfing modes with allocation to attractive structures. Evolution 49:557–564.
Sato, H., and T. Yahara. 1999. Trade-offs between flower number and investment to a flower in selfing and outcrossing varieties of *Impatiens hypophylla* (Balsaminaceae). Am. J. Bot. 86: 1699–1707.

Schemske, D. W., and J. Ågren. 1995. Deceit pollination and selection on female flower size in *Begonia involucrata*: an experimental approach. Evolution 49:207–214.
Schemske, D. W., J. Ågren, and J. Le Corff. 1996. Deceit pollination in the monoecious neotropical herb *Begonia oaxacana* (Begoniaceae). Pp. 292–318 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York.
Schoen, D. J., and T.-L. Ashman. 1995. The evolution of floral longevity: resource allocation to maintenance versus construction of repeated parts in modular organisms. Evolution 49: 131–139.
Schoen, D. J., and M. Dubuc. 1990. The evolution of inflorescence size and number: a gamete-packaging strategy in plants. Am. Nat. 135:841–857.
Shaw, R. G. 1987. Maximum-likelihood approaches applied to quantitative genetics of natural populations. Evolution 41: 812–826.
Snow, A. A., and D. F. Whigham. 1989. Costs of flower and fruit production in *Tipula discolor* (Orchidaceae). Ecology 70: 1286–1293.
Snow, A. A., T. P. Spira, R. Simpson, and R. A. Kilps. 1996. The ecology of geitonogamous pollination. Pp. 191–216 *in* D. G. Lloyd and S. C. H. Barrett, eds. Floral biology: studies on floral evolution in animal-pollinated plants. Chapman and Hall, New York.
Stanton, M. L., and R. E. Preston. 1988. Ecological consequences and phenotypic correlates of petal size variation in wild radish *Raphanus sativus* (Brassicaceae). Am. J. Bot. 75:528–539.
Stanton, M. L., and H. J. Young 1994. Selecting for floral character associations in wild radish, *Raphanus sativus* L. J. Evol. Biol. 7:271–285.
Stanton, M., H. J. Young, N. C. Ellstrand, and J. M. Clegg. 1991. Consequences of floral variation for male and female reproduction in experimental populations of wild radish, *Raphanus sativus* L. Evolution 45:268–280.
Stearns, S. C. 1992. The evolution of life-histories. Oxford Univ. Press, New York.
Stebbins, G. L. 1971. Adaptive radiation of reproductive characters in angiosperms. I. Pollination mechanisms. Annu. Rev. Ecol. Syst. 1:307–326.
van Noordwijk, A. J., and G. de Jong. 1986. Acquisition and allocation of resources: their influence on variation in life-history tactics. Am. Nat. 128:137–142.
Venable, D. L. 1996. Packaging and provisioning in plant reproduction. Philos. Trans. R. Soc., Lond. B Biol. Sci. 351: 1319–1329.
Villanueva, B., and B. W. Kennedy. 1992. Asymmetrical correlated responses to selection under an infinitesimal genetic model. Theor. Appl. Genet. 84:323–329.
Vonhof, M. J., and L. D. Harder. 1995. Size-number trade-offs and pollen production by papilionaceous legumes. Am. J. Bot. 82: 230–238.
Worley, A. C., A. M. Baker, J. D. Thompson, and S. C. H. Barrett. 2000. Floral display in *Narcissus*: variation in flower size and number at the species, population, and individual levels. Int. J. Plant. Sci. 161:69–79.

Corresponding Editor: J. Conner

Appendix

Means ± 1 SE for floral traits and size indices in greenhouse-grown *Eichhornia paniculata* plants. Means presented for daily and total flower number are from data that is square-root transformed.

Line and generation	Flower size	Daily flower number	Total flower number	Leaf area	Age at flowering
Base population (B181)	5.44 ± 0.061	2.16 ± 0.019	5.43 ± 0.044	23.6 ± 0.47	17.6 ± 0.30
Control					
Generation 1	9.20 ± 0.193	2.60 ± 0.081	7.59 ± 0.260	76.4 ± 3.01	40.4 ± 1.25
Generation 2	8.09 ± 0.156	2.48 ± 0.055	6.76 ± 0.163	54.2 ± 1.83	36.9 ± 1.50
Many flowers (N+, line 1)					
Generation 1	8.81 ± 0.191	2.42 ± 0.080	7.15 ± 0.257	73.5 ± 2.97	36.7 ± 1.24
Generation 2	8.04 ± 0.153	2.65 ± 0.053	7.61 ± 0.160	49.6 ± 1.79	36.5 ± 1.47
Many flowers (N+, line 2)					
Generation 1	8.47 ± 0.189	2.18 ± 0.079	6.28 ± 0.254	69.9 ± 2.93	32.8 ± 1.23
Generation 2	7.57 ± 0.143	2.59 ± 0.049	7.25 ± 0.149	52.0 ± 1.66	32.0 ± 1.37
Large flowers (S+, line 1)					
Generation 1	9.66 ± 0.194	2.41 ± 0.081	6.92 ± 0.262	71.7 ± 3.03	37.6 ± 1.26
Generation 2	9.40 ± 0.142	2.49 ± 0.050	6.66 ± 0.149	48.8 ± 1.66	33.1 ± 1.36
Large flowers (S+, line 2)					
Generation 1	10.07 ± 0.190	2.63 ± 0.079	7.89 ± 0.255	77.2 ± 2.94	40.7 ± 1.23
Generation 2	9.44 ± 0.155	2.47 ± 0.054	6.58 ± 0.162	50.6 ± 1.83	33.7 ± 1.48
Small flowers (S−, line 1)					
Generation 1	8.01 ± 0.191	2.41 ± 0.079	7.04 ± 0.258	76.6 ± 2.96	35.0 ± 1.23
Generation 2	6.82 ± 0.145	2.57 ± 0.051	7.01 ± 0.152	46.7 ± 1.71	31.2 ± 1.39
Small flowers (S−, line 2)					
Generation 1	8.32 ± 0.191	2.48 ± 0.080	7.31 ± 0.258	76.9 ± 2.96	37.7 ± 1.23
Generation 2	6.93 ± 0.150	2.56 ± 0.053	6.74 ± 0.157	50.0 ± 1.76	30.0 ± 1.44