Journal of Ecology 2004
92, 32–44

Phenotypic plasticity of vegetative and reproductive traits in monoecious and dioecious populations of *Sagittaria latifolia* (Alismataceae): a clonal aquatic plant

MARCEL E. DORKEN and SPENCER C. H. BARRETT
Department of Botany, University of Toronto, Toronto, Ontario, Canada M5S 3B2

Summary

1 The phenotypic plasticity of vegetative traits is a characteristic feature of aquatic plants, promoting survival and growth in the heterogeneous environments typical of wetlands. Less is known about plastic responses of life-history and reproductive traits, particularly patterns of sex allocation.

2 We investigated the plasticity of vegetative and reproductive traits in *Sagittaria latifolia*, a clonal aquatic plant whose populations are either monoecious or dioecious. Plants of the two sexual systems exhibit divergent life-history characters associated with the disturbed vs. competitive habitats in which monoecious and dioecious populations occur, respectively. We evaluated the prediction that populations of the two sexual systems would have different patterns of phenotypic plasticity because of the contrasting habitats in which they occur.

3 We grew four clonal replicates of 10 genotypes from seven monoecious and five dioecious populations (total = 480 plants) in two fertilizer treatments under glasshouse conditions and measured components of life history, leaf and flower morphology, and sex allocation.

4 The two sexual systems displayed divergent patterns of plasticity for four life-history traits but only flowering time and ramet production showed the expected pattern of greater plasticity in monoecious populations, and the reverse was true for flower production. Fertilization had opposite effects in the two sexual systems for corm production (increased in monoecious populations) and time to flowering (delayed in dioecious populations).

5 Leaf size generally increased due to the addition of fertilizer; however, this increase was substantially greater in dioecious populations. Larger leaf size in dioecious populations was associated with more convex leaves and greater surface area, potentially increasing light capture in the shaded and more competitive habitats in which these populations occur.

6 We found significant plasticity for female sex allocation in monoecious populations, with more female flowers at higher nutrient levels. However, the majority of populations had a significant genetic component to variation in sex allocation and/or significant genotype × environment interactions. These patterns are consistent with monoecy representing a flexible reproductive strategy for regulating mating opportunities in heterogeneous habitats.

Key-words: leaf-shape variation, life-history traits, monoecy and dioecy, phenotypic plasticity, sex allocation

Journal of Ecology (2004) **92**, 32–44

Introduction

Phenotypic plasticity is the ability of a genotype to modify its growth and development in response to changes in the environment. In plants, the well-developed plasticity of many traits is usually interpreted as an adaptive response to environmental heterogeneity as a consequence of immobility and modular growth. Although studies of phenotypic plasticity have a long history in plant ecology (reviewed in Bradshaw 1965; Schlichting 1986; Scheiner 1993; Schlichting &

Correspondence: S. C. H. Barrett (tel. +1 416 9784151; fax +1 416 9785878; e-mail barrett@botany.utoronto.ca).

Pigliucci 1998; Pigliucci 2001), the extent to which patterns of plasticity differ among traits, life histories and habitats, and the adaptive basis of this variation are largely unresolved questions. Comparative studies of populations or closely related species differing in ecology provide one approach to understanding the functional basis of phenotypic plasticity in plants.

Aquatic plants have played an important role in the development of ideas on phenotypic plasticity. Many aquatics occur in habitats characterized by strong environmental gradients and often display dramatic responses in their vegetative traits to changing water levels. Indeed the ecological and evolutionary significance of leaf-shape variation has attracted particular attention and represents a classic example of developmental plasticity (Arber 1920; Schmalhausen 1949; Sculthorpe 1967; Cook & Johnson 1968; Cook 1968). In the widely distributed Eurasian aquatic, *Sagittaria sagittifolia*, three forms of specialized leaves are produced (submerged, floating and aerial leaves) in response to variation in water depth. Moreover, this variation in leaf morphology responds to experimental manipulation of resource availability (Sculthorpe 1967). Such plastic responses in vegetative structures are thought to promote survival and propagation in the heterogeneous environments typical of many wetland habitats (Wells & Pigliucci 2000; Santamaría 2002). Much less is known about the plasticity of reproductive traits in aquatic plants.

Aquatic plants display a remarkable range of reproductive strategies, including diverse sexual systems and means of clonal propagation (reviewed in Barrett *et al*. 1993). Because reproductive strategies affect the response of populations to environmental heterogeneity (Ronce & Olivieri 1997; Barrett & Pannell 1999; Heilbuth *et al*. 2001; Crowley & McLetchie 2002), variation in reproductive traits will influence the ability of populations to colonize and persist in different types of aquatic habitats. For example, dioecious (with separate and female and male plants) and self-compatible hermaphroditic populations have different capacities to colonize disturbed habitats subject to regular population turnover (i.e. Baker's Law; Baker 1955; Pannell & Barrett 1998). This is because a minimum of two plants is required for a dioecious population to establish, whereas only one plant may be required to initiate an hermaphroditic population. Accordingly, reproductive assurance among plants from hermaphroditic populations is expected to promote colonization and survival in ephemeral habitats (Pannell 1997). In contrast, dioecious populations are likely to be suited to stable habitats where mate availability is more certain (and see Vamosi & Otto 2002). Because both vegetative and reproductive traits affect the ability of populations to tolerate environmental heterogeneity, integrated studies of these traits in aquatic species are necessary to determine how their patterns of plasticity influence the particular wetland habitats that populations occupy.

Here we investigate the phenotypic plasticity of vegetative and reproductive traits in the widely distributed North American emergent aquatic, *Sagittaria latifolia* Willd. (Alismataceae). This species is well suited to studies of phenotypic plasticity because it possesses striking variability within and among populations in both vegetative structures, particularly leaf shape (Smith 1894; Bogin 1955; Sculthorpe 1967) and patterns of gender variation (Wooten 1971; Sarkissian *et al*. 2001; Dorken & Barrett 2003a). Populations of *S. latifolia* are either self-compatible and monoecious (i.e. plants are hermaphroditic with unisexual flowers) or dioecious occurring in wetland habitats where disturbance and competition, respectively, are more important (Dorken & Barrett 2003a). Specifically, monoecious populations typically grow in ephemeral habitats, such as roadside ditches, farm ponds and stream and lake edges exposed to frequent disturbance. As a result they experience significantly higher rates of population extirpation than dioecious populations (Dorken & Barrett 2003a). In contrast, dioecious populations inhabit large wetlands and the river systems that drain into them. In these more stable and competitive habitats, clones of *S. latifolia* are subject to shading from taller interspecific neighbours, the most common of which is *Typha latifolia* (Dorken & Barrett 2003a). These ecological associations are consistent with expectations arising from the success of monoecious vs. dioecious populations under disturbance and competition, respectively.

The occupation of contrasting habitats by monoecious and dioecious populations of *S. latifolia* is associated with divergent life-history strategies, as revealed through common garden and transplant studies (Dorken & Barrett 2003a). These differences include earlier flowering and greater investment in sexual and asexual propagation in monoecious populations, compared with delayed flowering and increased investment in growth and perennation in dioecious populations. Because of these contrasting life-history strategies, we predicted that populations of the two sexual systems would respond to variation in environmental conditions in different ways (see Grime *et al*. 1986; Grime & Mackey 2002). Specifically, we predicted that, in response to low resource conditions, monoecious populations would accelerate the timing of reproduction, thus increasing the probability that offspring are produced. In contrast, we predicted that under similar conditions, dioecious populations would increase allocation to clonal propagation, facilitating the location of resource-rich zones of the habitat. Overall, we expected that vegetative and reproductive traits in monoecious populations would exhibit more plasticity than those in dioecious populations because populations belonging to this sexual system commonly experience less predictable growing conditions.

We investigated phenotypic plasticity in *S. latifolia* by growing clonally replicated genotypes from 12 populations in contrasting fertilizer treatments. We chose resource manipulation for evaluating phenotypic responses because our previous work indicated substantial variation in productivity of the habitats occupied by the two sexual systems (Dorken & Barrett 2003a). Moreover, gender variation in monoecious populations is strongly associated with plant size (Sarkissian *et al*. 2001), and is therefore likely to be related to the resource status of plants. Our study had three main objectives: (i) to evaluate the prediction that the more intense disturbance typical of habitats occupied by monoecious populations is associated with higher levels of phenotypic plasticity compared to dioecious populations; (ii) to investigate the plasticity of leaf shape, and to determine whether leaf size responds more to fertilizer addition in dioecious than monoecious populations because of the importance of light capture in shaded environments; and (iii) to evaluate the relative importance of genetic and environmental factors in governing gender variation in monoecious populations. Here we were interested in determining if there was evidence for a significant genetic component to variation in sex allocation because of its importance for models of the evolution of dioecy from monoecy, a transition that has apparently occurred within *S. latifolia*.

Materials and methods

STUDY SPECIES

Plants of *S. latifolia* grow as a rosette of emergent leaves and propagate clonally via the production of ramets during the growing season and corms towards the end of the growing season. Ramets may be either vegetative or reproductive. Both clonal ramets and corms are produced at the terminal ends of axillary stolons. Corms are the sole perennating structures, although seed production is an important means of propagation, particularly in monoecious populations, where seedlings are commonly observed. Throughout this study, we define 'plant' as a single ramet or shoot, excluding clonal ramets produced by the plant via stolons during the growing season. In southern Ontario, where populations sampled in this study originated, *S. latifolia* flowers between July and September, producing racemes with three unisexual flowers at each node.

PLASTICITY OF VEGETATIVE AND REPRODUCTIVE TRAITS

To investigate patterns of phenotypic plasticity of vegetative and reproductive traits between monoecious and dioecious populations, we grew replicates of clonally propagated corms from the two sexual systems under two fertilizer treatments. In May 1999, we collected plants from seven monoecious and five dioecious populations from southern Ontario. To limit repeated sampling of the same clones, we collected plants separated by at least 2 m. We reduced the influence of previous environmental conditions by growing these plants under uniform glasshouse conditions for an entire growing season before starting our experiment. We considered each plant grown in 1999 as a different genotype. To confirm this, we evaluated each plant's multilocus genotype using 13 allozyme loci (see Dorken *et al*. 2002). We estimated the minimum proportion of unique clonal genotypes sampled to be 0.93 ± 0.08 in dioecious populations, and 0.78 ± 0.07 in monoecious populations. At the end of the growing season, the four largest corms from 10 plants per population were collected and stored in a cold room at 5 °C for 5 months.

In mid-April 2000, we weighed these corms (4 replicates × 10 genotypes × 12 populations = 480 plants), planted them into 7.6 cm pots and grew them under uniform glasshouse conditions. Once corms had sprouted, all plants received one dose of fertilizer; 3 weeks later we transferred them to 15.2 cm pots after measuring the height of each plant from the soil surface to the tip of the tallest leaf. We placed each pot into a separate 5 L bucket and assigned two ramets from each clonal genotype to each of two fertilizer treatments: no fertilization following transplantation, or weekly fertilization with 200 mL of a 10% fertilizer solution (Plant-Prod® 20 : 20 : 20 all-purpose fertilizer, Brampton, Canada). We then arranged the buckets into randomized blocks, with the glasshouse benches serving as blocks. We monitored water levels daily to maintain fully saturated soils in each bucket.

For each plant, we recorded the date of first flowering for every inflorescence and counted the number of female and male flowers. We also estimated the size of up to three female and/or male flowers on each plant by measuring the width of the flower at its widest point using digital callipers. We standardized these floral measurements by using the flowers at the lowest node of the inflorescence at which flowers of that sex occurred. Following peak flowering, we measured plant height as above and the number of ramets produced by each plant. The fertilizer treatment was continued until plants senesced completely in early October coincident with senescence in local populations. We then counted and weighed all corms produced by each plant.

We evaluated interactions between fertilizer treatment and sexual system using analysis of covariance (ANCOVA). We examined variation among populations by including population, nested within sexual system, as a random effect in our analyses, and used log-likelihood ratio tests (Sokal & Rohlf 1995) calculated using the restricted maximum likelihood estimates provided by JMP (v. 4.0.4, SAS Institute 2000) for evaluating significance of random effects. We also evaluated block and gender effects and interactions between block and sexual system. Block effects and their interactions were not significant, and were removed from the analyses presented below. Gender effects (i.e. differences between females and males in dioecious populations) were evaluated by including gender, nested within sexual system as a factor in our analyses. These effects were included to take into account differences between females and males in allocation to vegetative and reproductive traits that may arise from different costs of reproduction between the sexes. We controlled for differences in initial shoot height among plants by using this as the covariate for final plant height. There was no difference in initial shoot height between monoecious and dioecious populations (mean ± SE for monoecious populations = 14.8 ± 0.3 cm, dioecious populations = 14.6 ± 0.3 cm, one-way ANOVA of population means: $F_{1,10} = 0.15$, $P = 0.71$). For our analyses of corm mass and corm production, we controlled for differences among plants by using the total mass of corms produced as the covariate following Dorken & Barrett (2003a). To meet ANOVA assumptions, we $\log_{10}$-transformed flower production and average corm mass.

PLASTICITY OF LEAF SHAPE

To investigate the plasticity of leaf shape, we collected leaves from each plant used in the preceding experiment and recorded their outlines using a video camera and the program MorphoSys (Meacham & Duncan 1991). Two standardized collections of leaves were made per plant: those produced prior to flowering (vegetative plants) and those produced after flowering had commenced (reproductive plants). We sampled and pressed the leaves for each collection over the course of a single day. We manually designated four landmarks per leaf on each outline (Fig. 1). We then used MorphoSys to calculate the perimeter and area of each leaf, and to find the Euclidean coordinates of the leaf landmarks (modified from McLellan & Endler 1998; McLellan 2000). Because *S. latifolia* has bilaterally symmetrical leaves, these landmarks are sufficient to describe leaf-shape variation. To reduce the influence of size variation on the position of landmarks among leaves, we scaled and rotated each leaf such that landmark 2 was located at $(x, y) = (0, 0)$ and landmark 4 was at (1, 0), leaving only landmarks 1 and 3 free to vary. We used principal components analysis to evaluate leaf-shape variation due to differences in the positions of landmarks 1 and 3 among leaves.

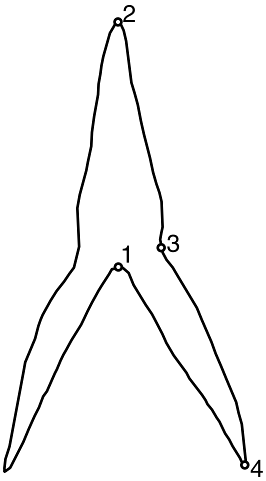

Fig. 1 Placement of landmarks on leaves of *Sagittaria latifolia* used in our analysis of leaf shape. Point 1 was placed in the outline at the junction of the petiole and the lamina; points 2 and 4 were placed at the blade and lobe apexes, respectively; and point 3 was placed by eye at the point of inflection between the distal portion of the blade and the lobe.

We evaluated the contributions of sexual system, treatment and the date of collection on variation in leaf size and shape using nested analysis of variance. As described above, we included population as a random effect in our analyses, and sexual system, treatment, collection date and gender as fixed effects. We evaluated four components of leaf morphology. First, we examined variation in overall leaf size using leaf area as the response variable in our analyses. We then analysed three measures of leaf shape using the scores for PC1, PC2 and the inverse of the dissection index (Kincaid & Schneider 1983). This index describes the deviation of a shape from that of a perfect circle (leaves with values approaching 1 approximate the shape of a perfect circle, whereas those with values approaching zero correspond to a shape with an infinite perimeter).

PLASTICITY OF SEX ALLOCATION IN MONOECIOUS POPULATIONS

We evaluated the plasticity of sex allocation among genotypes using analysis of variance. Here, we were explicitly interested in partitioning the genotypic and environmental contributions to variation in fitness through female sex function in monoecious populations. Standard measures of gender are expressed as the proportional allocation to female sex function (Lloyd 1980). Accordingly, we used the proportion of female flowers produced per plant as our response variable. Because we found significant heterogeneity among the monoecious populations in the response of flower production to the fertilizer treatment, we analysed variation in female allocation separately for each population. We considered fertilizer treatment as a fixed effect, and genotype and the interaction between genotype and treatment as random effects. As above, we used log-likelihood ratio tests to evaluate significance for random effects. Finally, we calculated the broad-sense heritability of genotypic and phenotypically plastic contributions to variation in sex allocation. We calculated variance components using the restricted maximum-likelihood routine in JMP and used these to estimate the proportion of variance explained by these components using the method described by van Kleunen *et al.* (2002).

Results

PLASTICITY IN MONOECIOUS VS. DIOECIOUS POPULATIONS

Monoecious and dioecious populations of *S. latifolia* exhibited different patterns of phenotypic plasticity (i.e. a significant sexual system × treatment interaction) for four of the six life-history traits measured (flowering time and ramet, flower and corm production, Table 1). For ramet production and days to flowering, the response of monoecious populations to fertilization was significantly greater than for dioecious populations, consistent with our prediction of greater plasticity for monoecious populations (one-way ANOVA of population means for the magnitude of the response: ramet production, $F_{1,10} = 14.2$, $P < 0.01$; days to flowering, $F_{1,10} = 104.6$, $P < 0.0001$; see Fig. 2). On average, plants from monoecious populations produced 55% more ramets in the high vs. low fertilizer treatment, compared to 36% for dioecious populations (Fig. 2b). Monoecious populations flowered on average 8.5 days earlier in the high vs. low fertilizer treatment whereas in dioecious populations this trend was reversed (Fig. 2c).

However, the majority of traits examined showed no evidence that the response of monoecious populations to fertilization was greater in magnitude than for dioecious populations. Plant height and corm mass, showed no difference in the plastic responses of the two sexual systems (Table 1, Fig. 2a,f). Although, for corm production the responses were similar in magnitude, they were in opposite directions (Fig. 2e), with corm production in monoecious populations increasing by 38% in response to fertilizer application, but decreasing by 37% in dioecious populations. Finally, flower production responded in a manner opposite to our prediction, with dioecious populations producing 160% more flowers in response to the addition of fertilizer, but monoecious populations only producing 8% more (one-way ANOVA: $F_{1,10} = 149.3$, $P < 0.0001$; means are in Fig. 2d).

For all traits examined, with the exception of days to flowering, we detected significant differences between the sexual systems that were evident regardless of fertilizer treatments (Table 1). Plants from dioecious populations had lower investment in ramet, flower and corm production, but were taller, with larger flowers and greater investment in corm mass compared to monoecious populations under both low and high fertilizer conditions (Fig. 2).

Gender-specific plastic responses

Our analyses revealed substantial differences in the plastic responses of reproductive traits between females and males. For flower production, we found a significant gender × treatment interaction (Table 1), with fertilized females producing 4.9 times more flowers compared to only 2.8 times for males. In the high fertilizer treatment, a similar percentage of females (91%) and males (98%) flowered, but with low fertilizer fewer females (78%) flowered than males (96%), resulting in significant heterogeneity in the likelihood of flowering between females and males in the low vs. high fertilizer treatments (*G*-test: $G = 9.8$, $P = 0.002$). For vegetative traits, we found no evidence that females and

Table 1 Analysis of covariance of life-history traits for seven monoecious and five dioecious populations of *Sagittaria latifolia* grown in contrasting fertilizer treatments. Initial plant height was used as the covariate for analyses of plant height, ramet production, days to flowering and flower production. For our analyses of corm production and average corm mass we used total corm mass as the covariate. Tests of significance for terms including population, a random effect, were carried out using log-likelihood ratio tests

	Plant height	Ramet production	Days to flowering	Flower production	Corm production	Average corm mass	Female flower width	Male flower width
Sexual system	$F_{1,10} = 8.0$*	$F_{1,10} = 8.9$*	$F_{1,10} = 0.5$	$F_{1,10} = 10.5$**	$F_{1,10} = 12.4$**	$F_{1,10} = 9.1$*	$F_{1,10} = 22.9$***	$F_{1,10} = 30.6$***
Treatment	$F_{1,10} = 28.1$***	$F_{1,10} = 46.9$***	$F_{1,10} = 1.5$	$F_{1,10} = 17.3$**	$F_{1,10} = 0.1$	$F_{1,10} = 6.6$*	$F_{1,10} = 3.1$	$F_{1,10} = 0.1$
Sexual system × treatment	$F_{1,10} = 1.7$	$F_{1,10} = 6.4$*	$F_{1,10} = 6.7$*	$F_{1,10} = 15.5$**	$F_{1,10} = 12.5$**	$F_{1,10} = 0.4$	$F_{1,10} = 3.1$	$F_{1,10} = 1.8$
Population [sexual system]	$\chi^2 = 3.4$*	$\chi^2 = 6.4$**	$\chi^2 = 0.0$	$\chi^2 = 8.4$**	$\chi^2 = 1.3$	$\chi^2 = 1.3$	$\chi^2 = 1.5$	$\chi^2 = 2.7$*
Gender [sexual system]	$F_{1,446} = 2.9$	$F_{1,448} = 0.0$	$F_{1,414} = 0.8$	$F_{1,448} = 26.9$***	$F_{1,453} = 3.1$	$F_{1,453} = 0.6$	–	–
Treatment × population [sexual system]	$\chi^2 = 5.1$*	$\chi^2 = 0.5$	$\chi^2 = 0.6$	$\chi^2 = 0.4$	$\chi^2 = 25.0$*	$\chi^2 = 2.2$	$\chi^2 = 0.4$	$\chi^2 = 1.9$
Treatment × gender [sexual system]	$F_{1,446} = 0.5$	$F_{1,448} = 0.3$	$F_{1,414} = 1.1$	$F_{1,448} = 2.0$	$F_{1,453} = 1.4$	$F_{1,453} = 2.6$	–	–
Covariate	$F_{1,446} = 3.8$*	$F_{1,448} = 6.4$*	$F_{1,414} = 3.3$	$F_{1,448} = 83.5$***	$F_{1,453} = 588.1$***	$F_{1,453} = 290.6$***	$F_{1,265} = 47.1$***	$F_{1,245} = 16.3$***

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

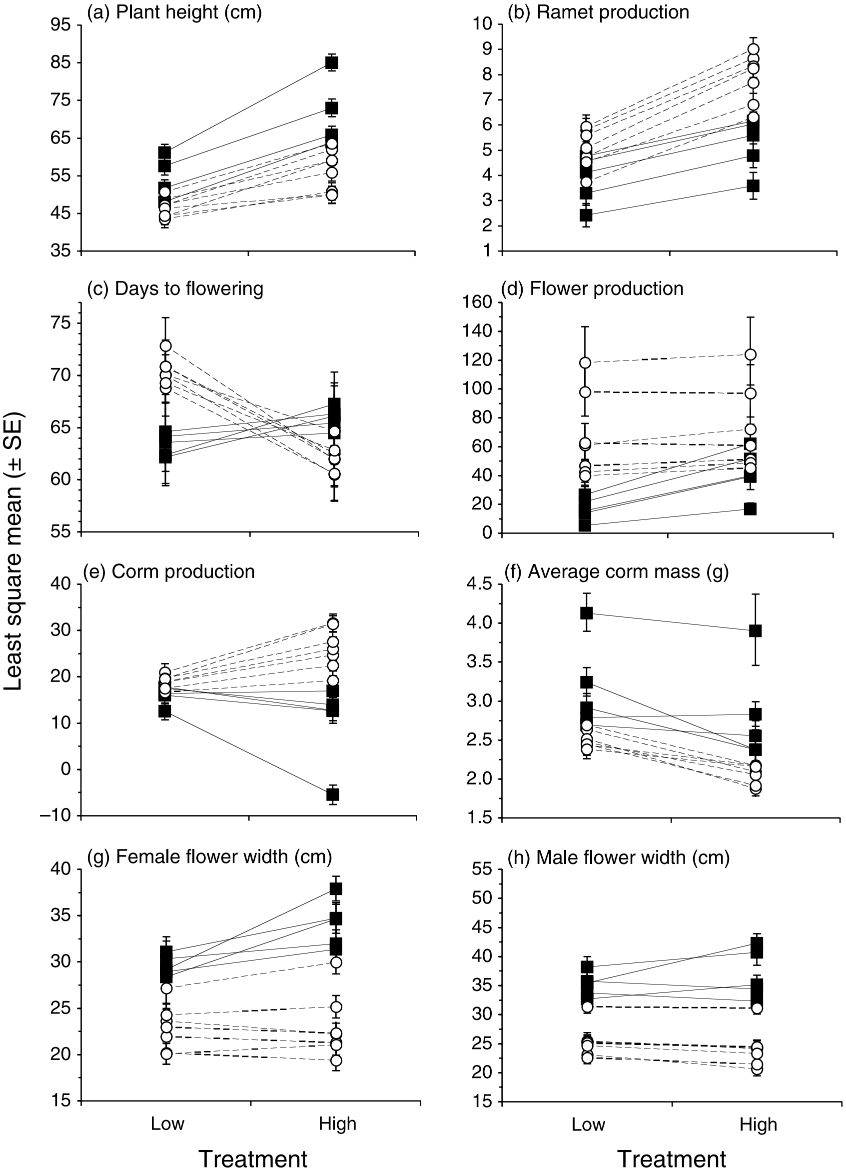

Fig. 2 Phenotypic plasticity for components of growth and reproduction among seven monoecious (○) and five dioecious (■) populations of *Sagittaria latifolia* grown in contrasting fertilizer treatments. Values shown are least-square means (± SE).

males responded differently to the fertilizer treatments (Table 1).

LEAF-SHAPE PLASTICITY

Leaf size and shape in *S. latifolia* was influenced by developmental stage (i.e. collection), sexual system and resource availability (Table 2). Leaf area generally increased from the low to high fertilizer treatment and from the first collection to the second for both sexual systems (Fig. 3a). However, the increase in leaf area from low to high fertilizer conditions was substantially greater for dioecious populations in the second leaf collection, resulting in a significant three-way interaction between the main effects.

Principal components analysis of leaf landmarks also revealed substantial variation in leaf shape due to differences between the sexual systems, treatments and collection dates. Scores along the first axis, explaining 48% of total variation in leaf shape, decreased from the first to the second collection, and from high to low fertilizer treatments (Fig. 3b). Variation in PC1 was largely driven by differences in the position of the leaf inflection point with wider leaves, and leaves with an inflection point towards the base of the leaf, having smaller values for PC1 (Fig. 4). This interpretation is generally supported by a strong, negative correlation between PC1 and the inverse of the dissection index (correlation of population means for each treatment and collection combination: $r = -0.85$, $n = 48$, $P < 0.0001$).

Leaf shape, as described by variation in scores from the second axis differed significantly between the two sexual systems (Table 2, Fig. 3c). This axis explained 40% of the variation in leaf shape in the experiment. Differences in PC2 between the sexual systems

Table 2 Analysis of variance for measures of leaf morphology from seven monoecious and five dioecious populations of *Sagittaria latifolia* grown under high vs. low fertilizer conditions. Tests of significance for terms including population, a random effect, were carried out using log-likelihood ratio tests

	Area	PC1	PC2	Inverse dissection index
Sexual system	$F_{1,10} = 3.3$	$F_{1,10} = 0.1$	$F_{1,10} = 9.0$*	$F_{1,10} = 0.0$
Treatment	$F_{1,10} = 61.7$***	$F_{1,10} = 228.5$***	$F_{1,10} = 0.0$	$F_{1,10} = 309.4$***
Sexual system × treatment	$F_{1,10} = 5.0$*	$F_{1,10} = 0.7$	$F_{1,10} = 0.2$	$F_{1,10} = 36.8$***
Collection	$F_{1,821} = 185.5$***	$F_{1,823} = 502.9$***	$F_{1,823} = 1.6$	$F_{1,821} = 673.1$***
Sexual system × collection	$F_{1,821} = 6.3$*	$F_{1,823} = 5.1$*	$F_{1,823} = 4.1$*	$F_{1,821} = 19.6$***
Treatment × collection	$F_{1,821} = 67.7$***	$F_{1,823} = 36.5$***	$F_{1,823} = 8.4$*	$F_{1,821} = 16.2$***
Sexual system × collection × treatment	$F_{1,821} = 14.9$***	$F_{1,823} = 0.0$	$F_{1,823} = 2.6$	$F_{1,821} = 4.7$*
Population [sexual system]	$\chi^2 = 2.4$	$\chi^2 = 17.0$***	$\chi^2 = 2.1$	$\chi^2 = 12.4$***
Gender [sexual system]	$F_{1,821} = 0.3$	$F_{1,823} = 0.2$	$F_{1,823} = 2.9$	$F_{1,821} = 3.9$*
Treatment × population [sexual system]	$\chi^2 = 102.4$***	$\chi^2 = 0.1$	$\chi^2 = 12.4$***	$\chi^2 = 0.3$
Treatment × gender [sexual system]	$F_{1,821} = 1.0$	$F_{1,823} = 0.1$	$F_{1,823} = 0.2$	$F_{1,821} = 0.3$

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

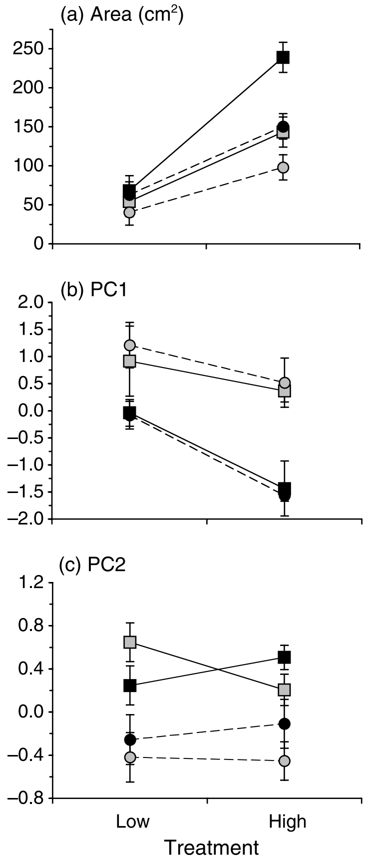

Fig. 3 Variation in leaf size and shape for monoecious (circles) and dioecious (squares) populations of *Sagittaria latifolia* collected before flowering (shaded symbols) and following peak flowering (closed symbols) grown in contrasting fertilizer treatments. Values shown are sexual system least-square means (± SE).

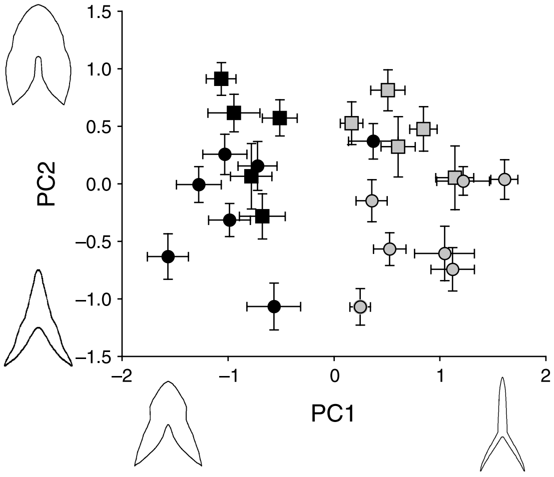

Fig. 4 Bivariate plot of the first two principal components showing variation in the coordinates of leaf landmarks for seven monoecious populations (circles) and five dioecious populations (squares) of *Sagittaria latifolia* collected before flowering (shaded symbols) and after peak flowering (closed symbols). PC1 and PC2 explained 48% and 40% of the variation in landmark location, respectively.

described variation in the position of the two landmarks in the plane perpendicular to the main axis of the leaf, and generally indicated whether the outer contour of the leaf was convex or concave (Fig. 4). On average, dioecious populations had significantly higher scores for PC2, and therefore more convex leaves than monoecious populations (Fig. 3c).

GENDER PLASTICITY IN MONOECIOUS POPULATIONS

In monoecious populations, relative allocation to female flower production increased significantly, as expected (average proportional increase 41%) from low to high fertilizer treatments (Fig. 5, Table 3). However, we also detected substantial variation among genotypes in sex allocation in both low and high fertilizer treatments (Fig. 5). In four populations (M1, M4, M6 and M7) there were significant differences in allocation to female flowers among genotypes. In addition, in populations M5 and M6, there was substantial variation among genotypes in their response to the fertilizer treatments, resulting in significant genotype × treatment interactions. In contrast, all genotypes in M2 and M3 exhibited similar increases in allocation to female flower production from low to high fertilizer conditions (Table 3, Fig. 5). Finally, the fertilizer treatments also influenced the sex

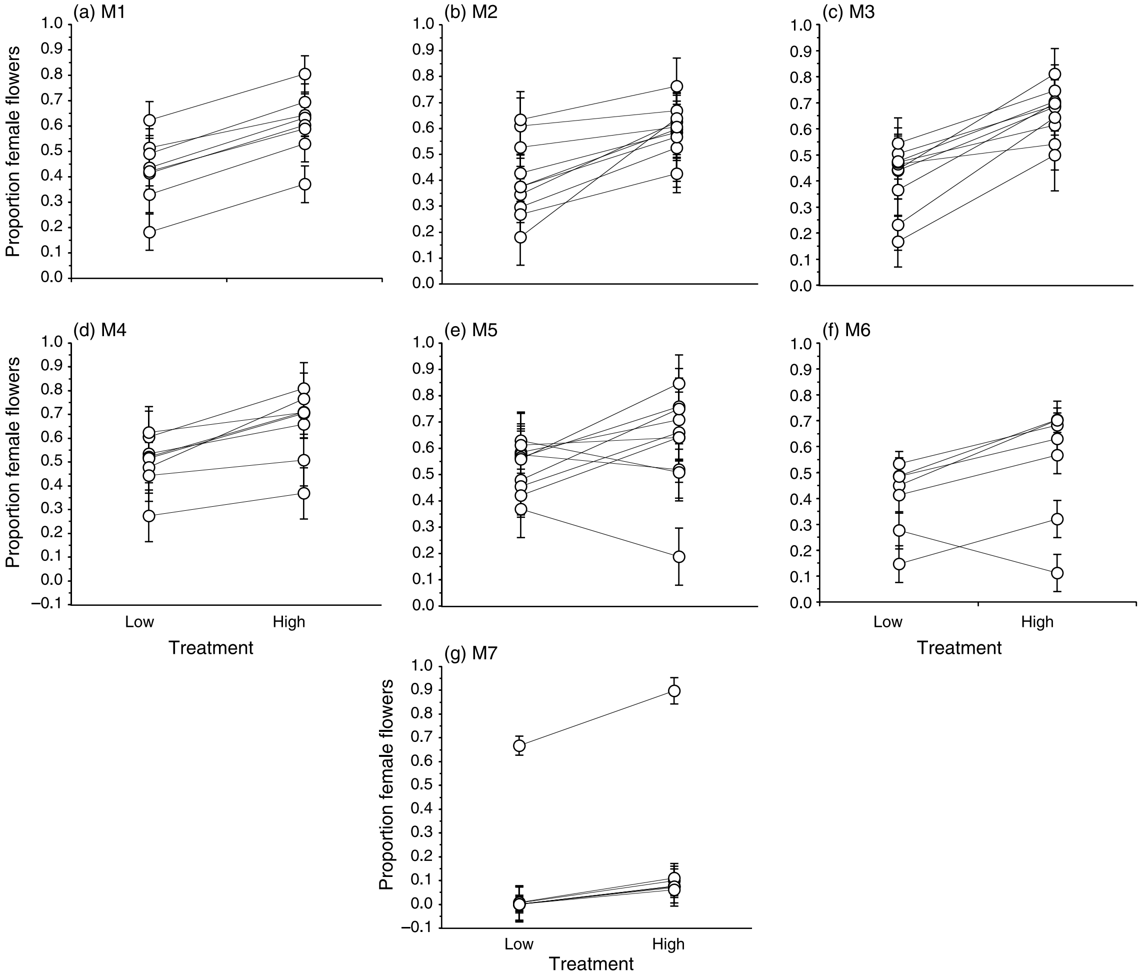

Fig. 5 Phenotypic plasticity for allocation to female flower production among seven monoecious populations of *Sagittaria latifolia* grown in contrasting fertilizer treatments. Values shown are least-square means (± SE) from two-way analysis of variance of genotype and treatment effects.

Table 3 Analysis of variance for allocation to female flower production in seven monoecious populations of *Sagittaria latifolia* grown under high vs. low fertilizer conditions. Tests of significance for the random effects genotype and genotype × treatment were carried out using log-likelihood ratio tests

Population	Genotype	Treatment	Genotype × treatment
M1	$\chi^2 = 2.8$*	$F_{1,7} = 11.5$*	$\chi^2 = 0.3$
M2	$\chi^2 = 2.0$	$F_{1,9} = 10.6$**	$\chi^2 = 2.1$
M3	$\chi^2 = 0.3$	$F_{1,9} = 32.6$***	$\chi^2 = 1.2$
M4	$\chi^2 = 3.8$*	$F_{1,7} = 14.1$**	$\chi^2 = 0.6$
M5	$\chi^2 = 2.6$	$F_{1,9} = 6.0$*	$\chi^2 = 5.9$**
M6	$\chi^2 = 3.4$*	$F_{1,6} = 4.4$	$\chi^2 = 6.9$**
M7	$\chi^2 = 15.8$***	$F_{1,7} = 76.7$***	$\chi^2 = 0.1$

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

expression of entire inflorescences, with a total of 57 out of 1326 inflorescences produced that were completely female. Significantly, 53 of these occurred in the high fertilizer treatment, compared to only four in the low fertilizer treatment (*G*-test: $G = 36.2$, $P < 0.0001$).

We found substantial broad-sense heritability for sex allocation within the populations for which we detected significant genotypic effects using analysis of variance (H^2: M1 = 0.51; M4 = 0.37; M6 = 0.34; M7 = 0.84). Broad-sense heritabilities for plasticity in sex allocation were much lower than for sex allocation alone; however, for the two populations with significant genotype × treatment interactions (M5 and M6), 25% (i.e. $H^2 = 0.25$) and 29% of this variation, respectively, had a genetic component.

Discussion

Our study revealed striking differences in the phenotypic plasticity of vegetative and reproductive traits between monoecious and dioecious populations of *S. latifolia*. To our knowledge, this represents the first demonstration of intraspecific variation in plasticity associated with the sexual systems of plants. Because monoecious and dioecious populations of *S. latifolia* commonly occur in close geographical proximity (Dorken *et al*. 2002) and are fully inter-fertile (Dorken & Barrett 2003a), the observed differences have implications for the ecology of reproductive isolation. Although we found evidence that the plasticity of life-history traits is greater in monoecious populations in only two traits, our study supports the view that monoecy represents a flexible reproductive strategy for the adjustment of allocation to female vs. male sex function in response to environmental heterogeneity.

PHENOTYPIC PLASTICITY OF LIFE-HISTORY TRAITS

Comparative analysis of phenotypic plasticity between closely related groups provides a useful tool for investigating the role of plasticity in governing species' distributions across ecological gradients (Schlichting 1986). In spite of this, such comparisons for plants are uncommon (but see Schlichting & Levin 1984; Pigliucci *et al*. 1997; Sultan 2001). We found no evidence that populations of *S. latifolia* from ephemeral aquatic habitats displayed greater overall phenotypic plasticity compared to populations from permanent wetlands. However, our comparisons, despite being limited to only two experimental conditions, did reveal contrasting patterns of phenotypic plasticity between monoecious and dioecious populations for flowering time, and the production of flowers, ramets and corms. Because each of these life-history traits is directly involved in propagation and/or survival, differences in their plastic responses should be important in regulating the distribution of plants across gradients of competition and disturbance.

Ecological theories for the expression of phenotypic plasticity in response to variable environmental conditions predict that plants from competitive environments should increase allocation to clonal propagation to escape poor environmental conditions (Grime *et al*. 1986; Grime & Mackey 2002). We measured two components of clonal propagation and found significant differences in their responses between the sexual systems that were consistent with this prediction. First, as a proportion of the number of ramets produced under high fertilizer conditions, dioecious populations maintained greater investment in ramet production, compared to monoecious populations, under low fertilizer conditions (Fig. 2b). Second, holding variation in total investment in corm mass constant, dioecious populations had greater allocation to corm production in the low compared to the high fertilizer treatment (Fig. 2e). In dioecious populations, the greater response of corm vs. ramet production to reduced nutrient availability may be related to differences in the dispersal of these structures, and the ability to escape reduced resource availability. Clonal ramets remain rooted close to the parent plant (Dorken & Barrett 2003a) and are unlikely to be dispersed large distances by water. In contrast, corms are produced at the end of ephemeral stolons and can detach from the parent plant and float to other sites. This ability to locate resource rich areas is likely to be favoured in the competitive wetlands in which dioecious populations occur.

Grime *et al*. (1986) have also suggested that differences in the plastic responses of flowering time should occur between plants from contrasting habitats. Specifically, they propose that in unfavourable environments, plants from disturbed habitats would be expected to reproduce earlier than plants from competitive habitats to ensure reproduction. Contrary to these predictions, we found that populations of *S. latifolia* from more disturbed habitats had delayed flowering when grown in low nutrient conditions (Fig. 2c), while those from competitive habitats either had accelerated flowering times, or did not flower. Under field conditions monoecious populations flower significantly earlier than dioecious populations, and have greater total investment in flower production (Dorken & Barrett 2003a). If there are trade-offs between flowering time and flower production, then delayed flowering in monoecious populations should result in increased investment in total flower production. In contrast, plants from dioecious populations generally have greater investment in plant size, delayed flowering and lower flower production compared to monoecious populations (Dorken & Barrett 2003a). In our experiment, plants from these populations responded in different ways to reduced resource availability. On average, these plants flowered earlier than those in the high fertilizer treatment, while 14% did not flower before senescence. It is unclear why these distinct responses occurred in dioecious populations. However, it is significant that most (86%) of the non-flowering clones were female. This result implicates gender-specific differences in response to reduced nutrient availability, presumably because of differences in the costs of reproduction between the sexes (reviewed in Delph 1999).

Divergent patterns of phenotypic plasticity in life-history traits between monoecious and dioecious populations have implications for the maintenance of the two sexual systems in areas of sympatry. In spite of the close geographical proximity between monoecious and dioecious populations in southern Ontario, gene flow between the sexual systems is restricted (Dorken *et al*. 2002). Reproductive isolation between monoecious and dioecious populations appears to be largely governed by differences in habitat occupancy because of the contrasting suites of life-history traits associated with the two sexual systems (Dorken & Barrett 2003a). Divergent patterns of phenotypic plasticity for particular life-history traits are also likely to reinforce reproductive isolation between monoecious and dioecious populations. For example, monoecious populations produced a larger number of smaller corms in response to increased resource availability (Fig. 2e,f). However, because there is a positive relation between corm size and emergence success in *S. latifolia* (Garbisch & McIninch 1994), the production of many small corms is unlikely to promote survival in the competitive environments in which dioecious populations occur (and see Grace 1993). Similarly, several of the plastic responses of dioecious populations may be maladaptive in the less productive and disturbed habitats occupied by monoecious populations. For example, in the low fertilizer treatment flowering was more strongly inhibited in dioecious populations. Reduced flowering and seed production are likely to limit opportunities for the colonization and persistence of plants in disturbed habitats where seeds are more important than vegetative structures for regeneration following changes in environmental conditions.

PLASTICITY IN LEAF SIZE AND SHAPE

Leaf shape in aquatic plants is determined by complex interactions between developmental, genetic and environmental factors, particularly water depth and nutrient regime (Bruni *et al.* 1995; Wells & Pigliucci 2000). In common with many aquatic plants, *Sagittaria* species commonly show a developmental series of leaf morphologies (i.e. heteroblasty) from submerged, juvenile leaves to emergent, adult leaves (reviewed in Sculthorpe 1967). In addition, the shape of adult leaves may often respond in a plastic manner to changes in environmental conditions (Wells & Pigliucci 2000). In our study we were interested in comparing the plastic changes of ‘vegetative’ and ‘reproductive’ leaves in monoecious and dioecious populations. Not unexpectedly, our investigation of variation along the first axis of the principal component analysis (PC1) revealed developmental changes in leaf width relative to leaf length, with younger emergent leaves exhibiting different shapes from older leaves (Fig. 4). More importantly, we found substantial plastic changes in PC1 in response to increased resource availability. Indeed, visual inspection of the leaves indicates that this variation in leaf shape, originally used to characterize taxonomic forms of *S. latifolia* (Smith 1894), is more simply explained by variation in resource availability. In contrast, PC2, which described the extent to which leaves are convex vs. concave, showed little plastic response and, instead, the primary source of variation was the sexual system of populations from which the leaves were sampled.

Plastic changes in leaf size will affect the ability of plants to capture light and therefore compete with neighbours (Westoby 1998; Belaguer *et al.* 2001). Not unexpectedly, plants of both sexual systems displayed phenotypic plasticity for leaf area by producing larger leaves when grown in higher resource conditions. However, plants from dioecious populations responded by producing leaves that were 54% larger in size than monoecious populations under these conditions. This striking difference in leaf-size plasticity between the sexual systems appears to be governed, in part, by non-plastic differences in leaf shape, with plants from monoecious populations apparently constrained to produce leaves that are more concave, and thus smaller in surface area than dioecious populations. The ability of plants from more competitive habitats to respond to heterogeneity in resource availability, particularly in terms of increased leaf area, is predicted by Westoby’s (1998) leaf-height-seed (LHS) plant strategy scheme. According to this hypothesis, the ability to respond to opportunities for rapid growth by increasing leaf size is beneficial in more competitive environments. In contrast, reduced plant height is favoured in disturbed habitats, where the reallocation of resources to clonal propagation should allow plants to escape local disturbance. Hence, the contrasting patterns of leaf-size plasticity in monoecious and dioecious population of *S. latifolia* seem likely to reflect adaptive differences associated with the contrasting habitats they occupy.

PLASTICITY IN FLOWER NUMBER AND SIZE

Modular organisms typically respond to increased resources by increasing the number or the size of structures. Plants commonly increase the number of structures while keeping their sizes relatively constant (e.g. seed number vs. seed size; Harper 1977). Consistent with these findings, plants of *S. latifolia* responded to regular fertilizer treatment by increasing the number of flowers they produced, but not their size (and see Cresswell *et al.* 2001). Interestingly, the main source of variation in flower size among plants in our experiment was the sexual system to which populations belonged. Plants from dioecious populations had both larger female and male flowers than the unisexual flowers of monoecious populations. This pattern is the opposite of that generally found in comparative surveys of the association between flower size and sexual system in flowering plants (reviewed in Sakai & Weller 1999), where dioecious species usually have smaller flowers in comparison with their cosexual ancestors (Vamosi *et al.* 2003). This result therefore raises the question of why in *S. latifolia* the usual pattern of association between flower size and sexual system is reversed.

Plants from dioecious populations are larger than those from monoecious populations (Dorken & Barrett 2003a; Fig. 2a). There is a strong correlation between plant and flower size (correlation between plant height and flower diameter using population means from both fertilizer treatments: female flowers: $r = 0.72$, $n = 24$, $P < 0.0001$; male flowers: $r = 0.73$, $n = 24$, $P < 0.0001$), implying that this allometric relationship may explain most of the difference in flower size between the two sexual systems. However, differences in the relative importance of pollinator attraction and pollen dispersal between monoecious and dioecious populations are also likely to be important in governing patterns of flower size between the sexual systems.

Monoecy is not the typical form of cosexuality in flowering plants and most transitions from cosexuality to dioecy involving reduced flower size have probably occurred from perfect-flowered ancestors (and see Weiblen *et al*. 2000). In contrast to perfect-flowered populations, pollen vectors are an absolute requirement for pollen dispersal from flower to flower in both monoecious and dioecious populations of *S. latifolia*. Larger flower size in dioecious populations of *S. latifolia* may therefore indicate that pollinator attraction and cross-pollination is more important than in monoecious populations. Because male plants achieve reproductive success only as paternal parents, competition among plants for access to ovules should lead to selection for larger flower size in males compared to females in dioecious populations (Ashman & Diefenderfer 2001). However, the magnitude of sexual dimorphism is likely to be constrained by pollinator preferences for larger female flowers (i.e. those that are similar in size to male flowers; Schemske & Ågren 1995), or genetic correlations between female and male flower size (Meagher 1992). As a result, such constraints may indirectly lead to larger flower size overall in dioecious compared to monoecious populations.

GENDER PLASTICITY AND THE EVOLUTION OF SEXUAL STRATEGIES

Gender plasticity is limited in dioecious species because of strong genetic control of sex determination (Grant 1999). Although sex inconstancy (Lloyd & Bawa 1984) or sex switching (Freeman *et al*. 1980) can provide opportunities for environmental modification of gender (e.g. Barrett *et al*. 1999), most individuals are usually either female or male (Rottenberg 1998). In contrast, striking variation in gender expression within individuals (e.g. *Arisaema triphyllum*; Bierzychudek 1982) and populations (e.g. *Acer grandidentatum*; Barker *et al*. 1982) is a characteristic feature of monoecious species, with the influence of environmental factors commonly implicated (Freeman *et al*. 1981). Gender plasticity is clearly evident within and among monoecious populations of *S. latifolia*, where allocation to female vs. male flower number is strongly size-dependent (Sarkissian *et al*. 2001). In our experiment, we observed inflorescences with the entire range of gender expression (i.e. with male flowers only, with varying proportions of female and male flowers, and strictly female inflorescences), depending on their size. Because this variation directly influences reproductive success through its effect on mating opportunities, determining the relative contribution of genetic vs. environmentally induced gender variation is necessary for understanding the ecology and evolution of sexual strategies in plants.

We examined the simultaneous contributions of genetic and environmental factors to gender variation in *S. latifolia* by manipulating resource availability among replicated clones from monoecious and dioecious populations. Although the addition of fertilizer resulted in the increased production of female flowers in both monoecious and dioecious populations, there were differences between sexual systems in the extent to which gender responded to resource availability. In dioecious populations, where sex is largely determined by the simple Mendelian segregation of alleles (Dorken & Barrett 2004), we found no evidence for sex switching. Instead, environmental effects on gender were largely limited to differences between treatments in flowering and flower production in females (e.g. females were 5.5 times less likely to flower than males in the low fertilizer treatment). As a result, within dioecious populations the distribution of gender phenotypes can be expected to vary across gradients of resource availability, but the gender of unisexual clones is unlikely to be modified by local environmental conditions.

In monoecious populations, the addition of resources had a strong positive effect on allocation to female flower production, a result consistent with the occurrence of size-dependent sex expression in *S. latifolia* (Sarkissian *et al*. 2001). However, the magnitude of the response to the fertilizer treatment on sex allocation was striking. In the high fertilizer treatment, 53 inflorescences produced only female flowers. Because flower production should often be resource limited, greater allocation to female flowers under high resource availability is likely to result in increased reproductive success if plants are not pollen limited (Campbell & Halama 1993). Because monoecious populations of *S. latifolia* generally have strongly male-biased floral sex ratios (Sarkissian *et al*. 2001), 'female' inflorescences of plants growing under favourable conditions are unlikely to experience pollen limitation. The ability of plants from monoecious populations to adjust the proportion of female vs. male flowers on each inflorescence in response to local conditions represents a flexible allocation strategy for coping with environmental heterogeneity (and see Dorken & Barrett 2003b).

We also detected a significant genetic component to gender variation in four monoecious populations of *S. latifolia*, and significant genotype by environment interactions for allocation to female flower production in two populations. Given the important role that environmental factors play in governing gender variation in monoecious species (Freeman *et al*. 1981; Bierzychudek 1982; Delesalle 1992; Sarkissian *et al*. 2001; Dorken & Barrett 2003b), these results are significant, in part because the causes of gender variation establish opportunities for the evolution of sexual systems. The presence of heritable variation in gender within monoecious populations is a necessary prerequisite for the evolution of dioecy from monoecy (Charlesworth & Charlesworth 1978; Charlesworth 2002), a transition that seems likely to have occurred in *S. latifolia*. Future studies are required to determine whether the pervasive gender plasticity in *S. latifolia* obscures the genetic influences on selection for sexual dimorphism, thus constraining the evolution of dioecy, or, alternatively, whether phenotypic plasticity may have actually aided the transition between sexual systems by facilitating the invasion of genetic modifiers of gender when environmental conditions lead to skewed sex ratios.

Acknowledgements

We thank Tim Dickinson from the Center for Biodiversity and Conservation Biology at the ROM and especially Laura Clarke for their help in measuring and analysing leaf shape, Sara Wagner and Dominic Halas for their assistance in the glasshouse, Bill Cole, Bruce Hall and Andrew Petrie for help in setting up and maintaining the experiment, Peter Klinkhamer and two anonymous referees for constructive comments on a previous version of the manuscript, and the Natural Sciences and Engineering Research Council (Canada) for a postgraduate scholarship to M.E.D. and a research grant to S.C.H.B.

References

Arber, A. (1920) *Water Plants: A Study of Aquatic Angiosperms*. Cambridge University Press, Cambridge.

Ashman, T.-L. & Diefenderfer, C. (2001) Sex-ratio represents a unique context for selection on attractive traits: consequences for the evolution of sexual dimorphism. *American Naturalist*, **157**, 334–347.

Baker, H.G. (1955) Self-compatibility and establishment after 'long-distance' dispersal. *Evolution*, **9**, 347–348.

Barker, P., Freeman, D.C. & Harper, K.T. (1982) Variation in the breeding system of *Acer grandidentatum*. *Forest Science*, **28**, 563–572.

Barrett, S.C.H., Case, A.L. & Peters, G.B. (1999) Gender modification and resource allocation in subdioecious *Wurmbea dioica* (Colchicaceae). *Journal of Ecology*, **87**, 123–137.

Barrett, S.C.H., Eckert, C.G. & Husband, B.C. (1993) Evolutionary processes in aquatic plants. *Aquatic Botany*, **44**, 105–145.

Barrett, S.C.H. & Pannell, J.R. (1999) Metapopulation dynamics and mating-system evolution in plants. *Molecular Systematics and Plant Evolution* (eds P. Hollingsworth, R. Bateman & R. Gornall), pp. 74–100. Taylor & Francis, London.

Belaguer, L., Martínez-Ferri, E., Valladares, F., Pérez-Corona, M.E., Baquedano, F.J., Castillo, F.J. & Manrique, E. (2001) Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology*, **15**, 124–135.

Bierzychudek, P. (1982) The demography of jack-in-the-pulpit, a forest pernnial that changes sex. *Ecological Monographs*, **52**, 335–351.

Bogin, C. (1955) Revision of the genus *Sagittaria* (Alismataceae). *Memoirs of the New York Botanical Garden*, **9**, 179–233.

Bradshaw, A.D. (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances in Genetics*, **13**, 115–155.

Bruni, N.C., Young, J.P. & Dengler, N.G. (1995) Leaf developmental plasticity of *Ranunculus flabellaris* in response to terrestrial and submerged environments. *Canadian Journal of Botany*, **74**, 823–837.

Campbell, D.R. & Halama, K.J. (1993) Resource and pollen limitations to life-time seed production in a natural plant population. *Ecology*, **74**, 1043–1051.

Charlesworth, D. (2002) Plant sex determination and sex chromosomes. *Heredity*, **88**, 94–101.

Charlesworth, D. & Charlesworth, B. (1978) Population genetics of partial male-sterility and the evolution of monoecy and dioecy. *Heredity*, **41**, 137–153.

Cook, C.D.K. (1968) Phenotypic plasticity with particular reference to three amphibious plant species. *Modern Methods in Plant Taxonomy* (ed. V.H. Heywood), pp. 97–111. Academic Press, London.

Cook, S.A. & Johnson, M.P. (1968) Adaptation to heterogeneous environments. I. Variation in heterophylly in *Ranunculus flammula* L. *Evolution*, **22**, 496–516.

Cresswell, J.E., Hagen, C. & Woolnough, J.M. (2001) Attributes of individual flowers of *Brassica napus* L. are affected by defoliation but not by intra-specific competition. *Annals of Botany*, **88**, 111–117.

Crowley, P.H. & McLetchie, D.N. (2002) Trade-offs and spatial life-history strategies in classical metapopulations. *American Naturalist*, **159**, 190–208.

Delesalle, V.A. (1992) Architecture and gender allocation: floral sex expression along branches of the monoecious cucurbit, *Apodanthera undulata*. *International Journal of Plant Science*, **153**, 108–116.

Delph, L.F. (1999) Sexual dimorphism in life history. *Gender and Sexual Dimorphism in Flowering Plants* (eds M.A. Geber, T.E. Dawson & L.F. Delph), pp. 149–173. Springer-Verlag, Berlin.

Dorken, M.E. & Barrett, S.C.H. (2003a) Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution*, **57**, 1973–1988.

Dorken, M.E. & Barrett, S.C.H. (2003b) Gender plasticity in *Sagittaria sagittifolia* (Alismataceae), a monoecious species. *Plant Systematics and Evolution*, **237**, 99–106.

Dorken, M.E. & Barrett, S.C.H. (2004) Sex determination and the evolution of dioecy from monoecy in *Sagittaria latifolia* (Alismataceae). *Proceedings of the Royal Society of London B*, in press.

Dorken, M.E., Friedman, J. & Barrett, S.C.H. (2002) The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution*, **56**, 31–41.

Freeman, D.C., Harper, K.T. & Charnov, E.L. (1980) Sex change in plants – old and new observations and new hypotheses. *Oecologia*, **47**, 222–232.

Freeman, D.C., McArthur, E.D., Harper, K.T. & Blauer, A.C. (1981) Influence of environment on the floral sex-ratio of monoecious plants. *Evolution*, **35**, 194–197.

Garbisch, E. & McIninch, S. (1994) The establishment of *Sagittaria latifolia* from large and small tubers as a function of water depth. *Wetland Journal*, **6**, 19–21.

Grace, J.B. (1993) The adaptive significance of clonal reproduction in angiosperms: an aquatic perspective. *Aquatic Botany*, **44**, 159–180.

Grant, S.R. (1999) Genetics of gender dimorphism in higher plants. *Gender and Sexual Dimorphism in Flowering Plants* (eds M.A. Geber, T.E. Dawson & L.F. Delph), pp. 247–274. Springer-Verlag, Berlin.

Grime, J.P., Crick, J.C. & Rincon, J.E. (1986) The ecological significance of plasticity. *Plasticity in Plants* (eds D.H. Jennings & A. J. Trewavas), pp. 5–29. Company of Biologists, Cambridge University Press, Cambridge, UK.

Grime, J.P. & Mackey, J.M.L. (2002) The role of plasticity in resource capture by plants. *Evolutionary Ecology*, **16**, 299–307.

Harper, J.L. (1977) *Population Biology of Plants*. Academic Press, London.

Heilbuth, J.C., Ilves, K.L. & Otto, S.P. (2001) The consequences of dioecy for seed dispersal: modeling the seed-shadow handicap. *Evolution*, **55**, 880–888.

Kincaid, D.T. & Schneider, R.B. (1983) Quantification of leaf-shape with a microcomputer and Fourier-transform. *Canadian Journal of Botany*, **61**, 2333–2342.

van Kleunen, M., Fischer, M. & Schmid, B. (2002) Experimental life-history evolution: selection on the allocation to sexual reproduction and its plasticity in a clonal plant. *Evolution*, **56**, 2168–2177.

Lloyd, D.G. (1980) Sexual strategies in plants. III. A quantitative method for describing the gender of plants. *New Zealand Journal of Botany*, **18**, 103–108.

Lloyd, D.G. & Bawa, K.S. (1984) Modification of the gender of seed plants in varying conditions. *Evolutionary Biology*, **17**, 255–338.

McLellan, T. (2000) Geographic variation and plasticity of leaf shape and size in *Begonia dregei* and *B. homonyma* (Begoniaceae) *Botanical Journal of the Linnean Society*, **132**, 79–95.

McLellan, T. & Endler, J.A. (1998) The relative success of some methods for measuring and describing the shape of complex objects. *Systematic Biology*, **47**, 264–281.

Meacham, C.A. & Duncan, T. (1991) *MorphoSys*. Regents of the University of California, Berkeley, California.

Meagher, T.R. (1992) The quantitative genetics of sexual dimorphism in *Silene latifolia* (Caryophyllaceae). I. Genetic variation. *Evolution*, **46**, 445–457.

Pannell, J. (1997) The maintenance of gynodioecy and androdioecy in a metapopulation. *Evolution*, **51**, 10–20.

Pannell, J.R. & Barrett, S.C.H. (1998) Baker's Law revisited: reproductive assurance in a metapopulation. *Evolution*, **52**, 657–668.

Pigliucci, M. (2001) *Phenotypic Plasticity: Beyond Nature and Nurture*. Johns Hopkins University Press, Baltimore, Maryland.

Pigliucci, M., Diiorio, P. & Schlichting, C.D. (1997) Phenotypic plasticity of growth trajectories in two species of *Lobelia* in response to nutrient availability. *Journal of Ecology*, **85**, 265–276.

Ronce, O. & Olivieri, I. (1997) Evolution of reproductive effort in a metapopulation with local extinctions and ecological succession. *American Naturalist*, **150**, 220–249.

Rottenberg, A. (1998) Sex ratio and gender stability in the dioecious plants of Israel. *Botanical Journal of the Linnean Society*, **128**, 137–148.

Sakai, A.K. & Weller, S.G. (1999) Gender and sexual dimorphism in flowering plants: a review of terminology, biogeographic patterns, ecological correlates, and phylogenetic approaches. *Gender and Sexual Dimorphism in Flowering Plants* (eds M.A. Geber, T.E. Dawson & L.F. Delph), pp. 1–31. Springer-Verlag, Berlin.

Santamaría, L. (2002) Why are most aquatic plants widely distributed? Dispersal, clonal growth and small-scale heterogeneity in a stressful environment. *Acta Oecologia*, **23**, 137–154.

Sarkissian, T.S., Barrett, S.C.H. & Harder, L.D. (2001) Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology*, **82**, 360–373.

SAS Institute (2000) *JMP Statistics and Graphics Guide*, Version 4.0. SAS Institute Inc, Cary, North Carolina.

Scheiner, S.M. (1993) Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, **24**, 35–68.

Schemske, D.W. & Ågren, J. (1995) Deceit pollination and selection on female flower size in *Begonia involucrata*: an experimental approach. *Evolution*, **49**, 207–214.

Schlichting, C.D. (1986) The evolution of phenotypic plasticity in plants. *Annual Review of Ecology and Systematics*, **17**, 667–693.

Schlichting, C.D. & Levin, D.A. (1984) Phenotypic plasticity of annual *Phlox*: tests of some hypotheses. *American Journal of Botany*, **71**, 252–260.

Schlichting, C.D. & Pigliucci, M. (1998) *Phenotypic Evolution: A Reaction Norm Perspective*. Sinauer, Sunderland, Massachusetts.

Schmalhausen, I.I. (1949) *Factors of Evolution: The Theory of Stabilizing Selection*. University of Chicago Press, Chicago, Illinois.

Sculthorpe, C.D. (1967) *The Biology of Aquatic Vascular Plants*. Edward Arnold, London.

Smith, J.G. (1894) A revision of the North American species of *Sagittaria* and *Lophotocarpus*. *Annual Report of the Missouri Botanic Garden*, **6**, 27–64.

Sokal, R.R. & Rohlf, F.J. (1995) *Biometry*, 3rd edn. Freeman, New York, New York.

Sultan, S.E. (2001) Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology*, **82**, 328–343.

Vamosi, J.C. & Otto, S.P. (2002) When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. *Proceedings of the Royal Society of London B*, **269**, 1187–1194.

Vamosi, J.C., Otto, S.P. & Barrett, S.C.H. (2003) Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *Journal of Evolutionary Biology*, **16**, 1006–1018.

Weiblen, G.D., Oyama, R.K. & Donoghue, M.J. (2000) Phylogenetic analysis of dioecy in monocotyledons. *American Naturalist*, **155**, 46–58.

Wells, C.L. & Pigliucci, M. (2000) Adaptive phenotypic plasticity: the case of heterophylly in aquatic plants. *Perspectives in Plant Ecology, Evolution and Systematics*, **3**, 1–18.

Westoby, M. (1998) A leaf-height-seed (LHS) plant ecology strategy scheme. *Plant and Soil*, **199**, 213–227.

Wooten, J.W. (1971) The monoecious and dioecious conditions in *Sagittaria latifolia* L. (*Alismataceae*). *Evolution*, **25**, 549–553.

Received 5 June 2003
revision accepted 11 November 2003