

## **Gender plasticity in *Sagittaria sagittifolia* (Alismataceae), a monoecious aquatic species**

**M. E. Dorken and S. C. H. Barrett**

Department of Botany, University of Toronto, Toronto, Ontario, Canada

Received September 16, 2002; accepted October 23, 2002

Published online: March 20, 2003

© Springer-Verlag 2003

**Abstract.** Aquatic plants are well known for their high degree of phenotypic plasticity in vegetative structures, particularly leaves. Less well understood is the extent to which their sexuality can be modified by environmental conditions. Here we investigate gender plasticity in the European clonal monoecious aquatic *Sagittaria sagittifolia* (Alismataceae) to determine how floral sex ratios may vary with plant size and inflorescence order. We sampled two populations from aquatic habitats in East Anglia, U.K. and measured a range of plant attributes including ramet size and the number of female and male flowers per inflorescence. The two populations exhibited similar patterns of phenotypic gender, despite contrasting patterns of total allocation to female and male flower number. Plants produced male-biased floral sex ratios but female flower number increased from the first to the second inflorescence whereas male flower number decreased. Size-dependent gender modification occurred in both populations, but the patterns of allocation to female flower production differed between the two populations. Our results are consistent with the view that monoecy is a sexual strategy that enables plants to adjust female and male allocation in response to changing environmental conditions.

**Key words:** Gender, monoecy, *Sagittaria sagittifolia*, arrowhead, Alismataceae, size-dependent gender modification.

Gender, an individual's relative contribution to the next generation as an ovule or pollen parent, varies widely within and among species of flowering plants. This variation is the product of numerous genetic and environmental factors that alone, or in combination, govern the distribution of sex phenotypes within populations (Lloyd and Bawa 1984). Differences among species in gender expression are determined primarily by genes regulating the production of unisexual versus hermaphroditic flowers, resulting in the wide array of sexual systems that characterize flowering plants (reviewed in Grant 1999, Barrett 2002). However, differences among individuals and populations within species may also be governed by interactions between genetic and environmental factors through their effects on variation in resource status among plants and investment in female and male components of reproduction, which typically involve different costs (Lloyd and Bawa 1984, Charnov 1982, Klinkhamer et al. 1997).

Most flowering plants produce hermaphroditic flowers, potentially constraining their ability to adjust the production of female and male gametes in response to changes in condition (but see Wright and Barrett 1999). However, in monoecious species the production of

unisexual flowers may facilitate variable allocation to each sex function through adjustments to the production of female versus male flowers (Lloyd 1972, Costich 1995, Delesalle 1992, Fox 1993, Sarkissian et al. 2001). Such plasticity in gender expression in monoecious species may be adaptive whenever differences in the cost of reproduction between female and male sex function leads to variation in fitness between plants of different resource status. Because female sex function is usually more costly than male function, large plants should be more female biased in comparison with smaller plants (Lloyd and Bawa 1984, Klinkhamer et al. 1997). For many species this appears to be the case (reviewed in Eckhart 1999), yet we still know relatively little about how gender varies among individuals within monoecious species, or the extent to which patterns of size-dependency may vary among populations. Variation in these patterns may be expected as a result of ecological differences causing different size distributions of plants to occur among populations. In addition, because plant size usually increases over the course of the growing season, size-dependent gender modification should also be apparent through changes in gender during the developmental sequence of inflorescence production.

Here we investigate gender variation in the European monoecious aquatic *Sagittaria sagittifolia*. In common with many clonal aquatic plants, this species is well known for its plasticity in vegetative characters and dramatic leaf-shape variation characterizes many populations (reviewed in Arber 1920, Sculthorpe 1967). However, little is known about the plasticity of reproductive traits, or the factors regulating gender expression. We were particularly interested in investigating *S. sagittifolia* because recent work on *S. latifolia* and *S. trifolia*, two widespread species that superficially resemble *S. sagittifolia* in both morphology and ecology, indicates striking variation in gender within and among populations (Sarkissian et al. 2001, Huang et al. 2002). For both of these species, this variation involves considerable reproductive plasticity,

including evidence within monoecious populations of size-dependent gender modification (Sarkissian et al. 2001, Huang et al. 2002). Here, we quantify variation in gender expression in two monoecious populations of *S. sagittifolia* from East Anglia, U.K. We were interested in addressing two primary questions: 1) Does gender vary between successive inflorescences produced during the growing season? 2) Is there evidence of size-dependent gender modification and does this differ between populations because of habitat related variation in the size distributions of plants?

### Materials and methods

*Sagittaria sagittifolia* L. is a stoloniferous aquatic perennial found in a variety of wetland habitats throughout Europe. Ramets grow as a rosette of leaves and propagate clonally via the production of corms towards the end of the growing season. Submerged plants may produce a combination of emergent, floating and submerged leaves. Throughout this study, our unit of investigation was a single reproductive ramet or shoot. In the U.K., *S. sagittifolia* flowers between August and September, producing racemes with three unisexual flowers at each node. As in all monoecious *Sagittaria* species, female flowers occur at the basal nodes of the inflorescence with male flowers at upper nodes.

In August 2000, we sampled two populations, Stokesby (ST: 52°38.8'N × 01°35.0'E) and Waveny Farm (WA: 52°28.3'N × 01°39.4'E), of *S. sagittifolia* located 20 km apart in the Norfolk Broads, East Anglia, U.K. In both populations, plants were growing in drainage ditches in water as deep as 1 m. However, in population WA plants were also growing at the margins of a downstream pond. In each population, we sampled 20 plants (flowering ramets), each spaced a minimum of 2 m apart to limit repeated sampling of the same genet. For each plant, we measured the height of the tallest leaf from the base of the plant and the total number of inflorescences produced. For each inflorescence we counted the number of female and male flowers and measured the mid-vein length of the leaf subtending the inflorescence. Previous research on the morphologically similar congener *S. latifolia* has shown that the length of the mid-vein of the largest leaf on a ramet is a good overall surrogate of ramet size (Sarkissian et al. 2001). In *S. sagittifolia* these two

parameters are also positively correlated ( $r=0.39$ ,  $n=73$ ,  $P=0.0007$ ).

**Gender variation in *S. sagittifolia*.** We calculated standardized phenotypic femaleness for each reproductive shoot following Lloyd (1980),  $G_i = d_i / (d_i + l_i E)$ , where  $d_i$  and  $l_i$  are the numbers of female and male flowers per plant for each plant sampled, respectively, and  $E = \sum d_i / \sum l_i$  per population. This approach yields a quantitative estimate of each plant's potential gametic contribution to the next generation via ovules and pollen. Using this calculation, plants receive scores ranging between 0 (phenotypically male) and 1 (phenotypically female). Plants with intermediate scores can potentially contribute as both ovule and pollen parents.

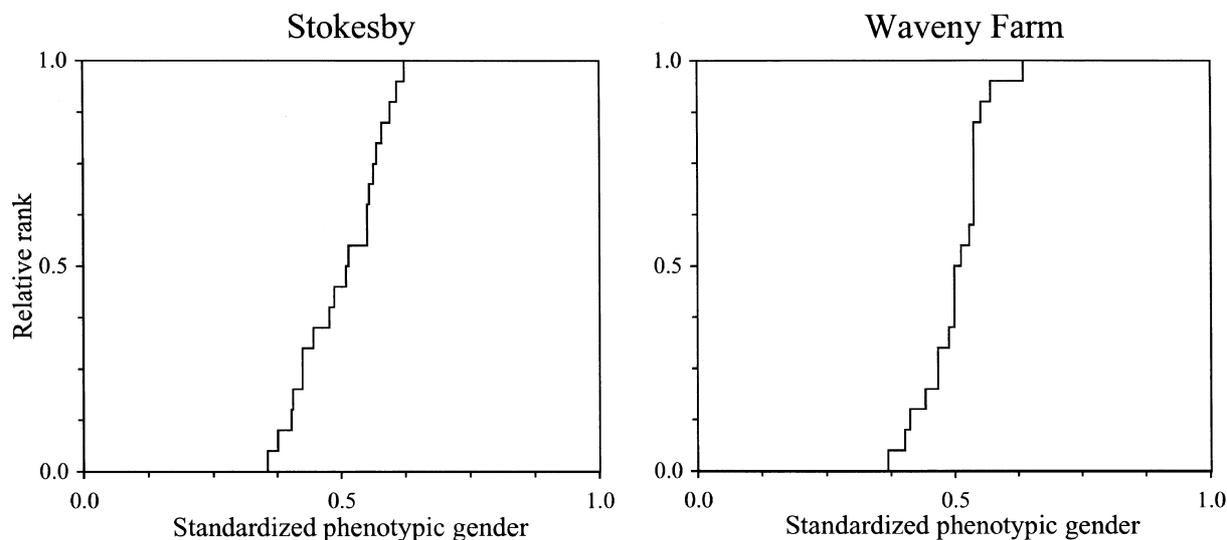
**Temporal changes in gender expression.** We investigated developmental changes in gender in the two populations by examining temporal variation in female and male flower production between successive inflorescences using two-way analysis of variance. In the populations, ramets produced a maximum of three (WA) and five (ST) inflorescences. To maintain sample sizes in each category, we restricted our analysis of temporal changes in flower production to the first two inflorescences produced by a ramet. We used *a posteriori* contrasts to investigate differences in flower production between inflorescences within populations.

**Size-dependent gender expression.** We examined patterns of size-dependent gender modification in two ways. First, we investigated the association

between plant size and female and male flower production at the inflorescence level using analysis of covariance. For this analysis we used mid-vein length (ln-transformed to assure normality and homoscedasticity of residuals) as the covariate and treated flower production as a repeated measure, following Sarkissian et al. (2001). Second, we investigated the interaction between total female and male flower production per ramet with our measure of overall plant size, the height of the tallest leaf, using analysis of covariance. For both analyses, we examined differences in female and male flower production between the two populations, the influence of plant size on flower production, and whether the relations between size and flower production were similar between the two populations. All analyses were conducted using JMP (version 4.0.4, SAS Institute 2000).

## Results

**Gender variation in *S. sagittifolia*.** Our analysis of the distribution of phenotypic gender in the two monoecious populations of *S. sagittifolia* revealed remarkably similar patterns of gender variation (Fig. 1). In ST, phenotypic gender values ranged between 0.36–0.62, and in WA gender ranged between 0.37–0.64. However, analysis of covariance revealed significant differences in total female flower production



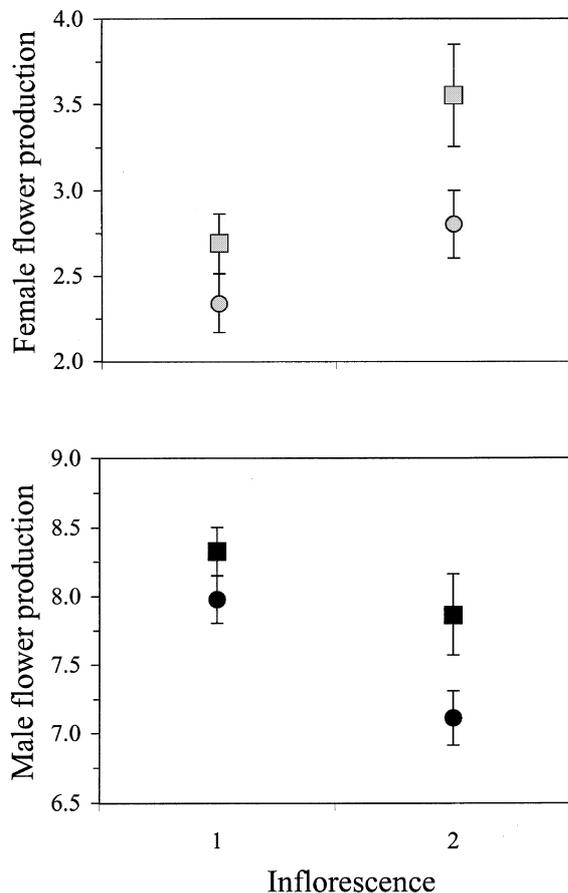
**Fig. 1.** Variation in phenotypic gender in two populations of *Sagittaria sagittifolia* sampled in East Anglia, U.K. Lines represent the cumulative frequency of standardized phenotypic femaleness ( $G_i$ )

**Table 1.** Analysis of covariance comparing the production of female and male flowers by plants from two populations (ST & WA) of *S. sagittifolia*, with plant height as a covariate. Degrees of freedom are indicated in parentheses

	Female		Male	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Population (Pop)	5.0 (1,36)	0.03	0.0 (1,36)	0.98
Plant height (Ht)	0.5 (1,36)	0.47	0.2 (1,36)	0.68
Pop × Ht	7.2 (1,36)	0.01	6.9 (1,36)	0.01

**Table 2.** Analysis of variance comparing female and male flower production between the first and second inflorescence in two populations (ST & WA) of *S. sagittifolia*. Degrees of freedom are indicated in parentheses

	Female		Male	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Population	0.3 (1,59)	0.57	17.8 (1,59)	<0.0001
Inflorescence	8.0 (1,59)	0.006	0.8 (1,59)	0.38
Pop × Infl	0.0 (1,59)	0.88	2.8 (1,59)	0.10



**Fig. 2.** Changes in female and male flower production from the first to the second inflorescence in two populations of *Sagittaria sagittifolia* (squares = ST; circles = WA)

between the two populations, with plants from ST producing more female flowers in comparison with WA (Table 1; ST:  $5.6 \pm 0.71$  female

flowers per plant; WA:  $3.4 \pm 0.65$ ). In contrast, there was no significant difference in the mean number of male flowers produced per plant between the two populations (Table 1; ST:  $12.1 \pm 2.0$ ; WA:  $12.2 \pm 1.8$ ). In common with many monoecious species, plants in both populations produced substantially more male flowers than female flowers. The proportion of male flowers was 0.68 ( $\pm 0.02$ ) in ST and 0.77 ( $\pm 0.01$ ) in WA.

**Temporal changes in gender expression.** Analysis of variance revealed significant differences in female and male flower production between the first and second inflorescences produced by ramets of *S. sagittifolia* (Table 2). The absence of any statistical interaction between the effects of population and inflorescence order indicates that developmental changes in female and male flower production were similar in the two populations studied. In both populations, the total number of female flowers increased from the first to the second inflorescence. In contrast, the number of male flowers decreased from the first to the second inflorescence (Fig. 2).

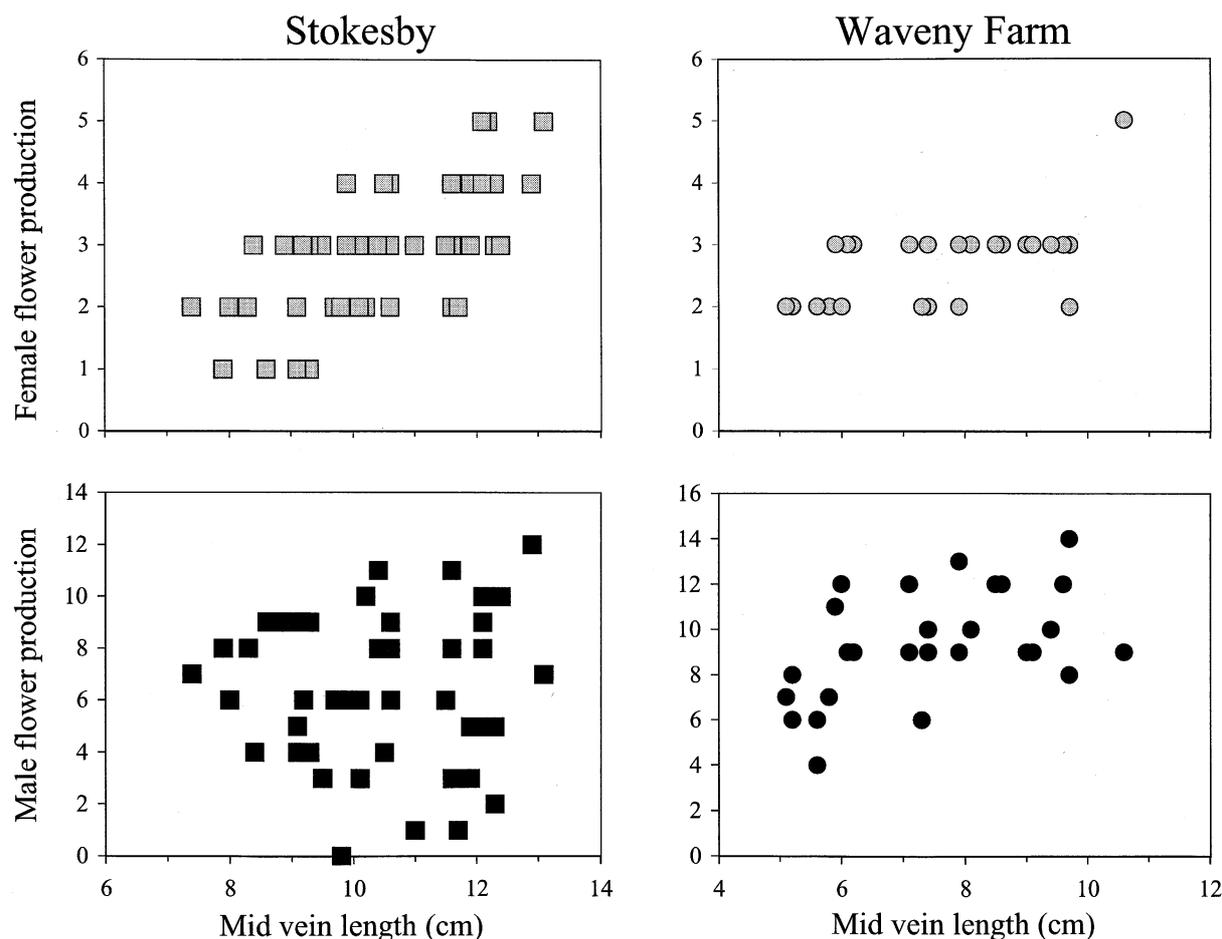
**Size-dependent gender expression.** We detected different patterns of size-dependent gender expression between the two populations of *S. sagittifolia*. These differences were apparent at both the inflorescence (Table 3) and whole plant levels (Table 1). At the inflorescence level, differences in size-dependency between the populations were determined by changes in female flower production with size.

For both populations, there was a positive correlation between mid-vein length and female flower production (Fig. 3; ST:  $r = 0.67$ ,  $n = 45$ ,  $P < 0.0001$ ; WA:  $r = 0.59$ ,  $n = 28$ ,  $P = 0.0009$ ; note for WA this correlation remains significant following the removal of the outlier:

$r = 0.49$ ,  $n = 27$ ,  $P = 0.009$ ), however, the slopes of these relations were significantly different (Table 3). We detected no difference between populations in the relation between male flower production and size. For male flower production there was a positive correlation with size in

**Table 3.** Analysis of covariance comparing female and male flower production per inflorescence between two populations (ST & WA) of *S. sagittifolia*, with mid-vein length as a covariate. Degrees of freedom are indicated in parentheses

	Female		Male	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Population (Pop)	9.7 (1,38)	0.003	22.4 (1,38)	< 0.0001
Mid-vein length (MVL)	44.3 (1,31)	< 0.0001	2.6 (1,31)	0.12
Pop × MVL	9.0 (1,31)	0.005	2.2 (1,31)	0.15



**Fig. 3.** Relations between plant size (blade mid-vein length) and female and male flower production for two populations of *Sagittaria sagittifolia*

WA ( $r = 0.48$ ,  $n = 28$ ,  $P = 0.009$ ). However, in ST this correlation was not significant ( $r = 0.02$ ,  $n = 45$ ,  $P = 0.88$ ), and there was no significant difference in slopes for the relations between size and male flower production between populations (Table 3).

At the whole plant level, the two populations had divergent patterns of size-dependent female and male flower production (Table 1). This was reflected in a significant interaction between population and plant size effects using analysis of covariance. These interactions were due to positive, but non-significant correlations between size and total female and male flower production in population ST (female:  $r = 0.38$ ,  $n = 20$ ,  $P = 0.10$ ; male:  $r = 0.39$ ,  $n = 20$ ,  $P = 0.09$ ), and negative correlations in WA (female:  $r = -0.47$ ,  $n = 20$ ,  $P = 0.04$ ; male:  $r = -0.43$ ,  $n = 20$ ,  $P = 0.06$ ).

## Discussion

Monoecy is a sexual strategy that enables plants to adjust female and male allocation in response to changing environmental and developmental conditions. This form of phenotypic plasticity occurs primarily through changes to floral sex ratios within and between inflorescences produced by individual ramets. Our study of monoecious *S. sagittifolia* demonstrates that in addition to the well developed phenotypic plasticity of vegetative structures, particularly leaf shape, this species displays considerable variation in floral sex ratios at the inflorescence, ramet and population levels. Here we consider some of the proximate factors governing these patterns of gender variation and discuss their significance for the evolution and maintenance of sexual systems in *Sagittaria*.

**Size-dependent gender expression.** Our results provide evidence for size-dependent gender modification in *S. sagittifolia*. Larger ramets produced more female flowers than smaller ramets and a similar pattern was evident for male flower production in population WA but not ST. Interestingly, the patterns of size-dependent gender modification differed between the two populations that we sampled.

This was evident by the different slopes in WA and ST for the relation between plant size and female flower production. Contrasting patterns of size-dependent sex expression could arise because of differences in genetic and/or environmental factors regulating gender. Without experimental studies it is not possible for us to address these alternatives directly, however, it is possible that both factors could play a role in governing the distribution of gender phenotypes in *S. sagittifolia*.

Although both populations occurred in broadly similar aquatic habitats, there were important differences in growth conditions within and between the populations. All plants in ST were restricted to growth in the deep water (up to 1 m) of a drainage ditch whereas plants in WA occurred in a range of water depths from deep water to near terrestrial conditions. These differences in water depth were reflected in contrasting patterns of plant size. Ramets at ST were, on average, taller than ramets at WA (one way analysis of variance  $F_{1,38} = 11.3$ ,  $P = 0.002$ ; mean ramet height ST =  $102.8 \pm 2.5$  cm; WA =  $87.9 \pm 3.7$  cm) and the amount of variation in ramet height was much greater at WA (C.V. = 18.8) than ST (C.V. 10.8). Larger ramet size at ST was associated with an increased production of female flowers in comparison with WA. However, smaller ramets at WA were advanced in their flowering phenology and had produced more flowers and inflorescences than the larger plants growing in deeper water. These observations indicate that variation in water depth plays a critical role in influencing plant size with effects on flowering phenology and floral sex ratios. Experimental studies of the influence of variation in water depth on gender expression in aquatic groups such as *Sagittaria* could be profitably undertaken to explore this problem further. To date, most studies on the influence of water depth on aquatics have focused on the morphological and physiological responses of vegetative structures (reviewed in Sculthorpe 1967, Crawford 1987).

Genetic factors may also play a role in generating variation among populations in

size-dependent gender expression. In the related *S. latifolia*, monoecious populations also display different patterns of size-dependent gender expression (see Fig. 5, Sarkissian et al. 2001). These differences persist when plants are grown under common environmental conditions in the glasshouse and resource supply is manipulated to produce plants of varying size (M.E. Dorken and S.C.H. Barrett, unpublished data), suggesting that genetic factors may also be involved in these differences in floral sex ratios. Similar experiments could reveal whether population differentiation in size-dependent sex allocation also occurs in *S. sagittifolia*.

**Evolution of gender strategies.** Studies of the variation and evolution of sexual systems in plant species commonly employ Lloyd's index of phenotypic gender to portray the patterns of gender variation within and among populations (e.g. Webb 1979, Barrett 1992, Wolfe and Shmida 1997, Vaughton and Ramsey 2002). In *S. sagittifolia*, our measurements of phenotypic gender revealed remarkably similar distribution patterns in the two populations (Fig. 1), perhaps leading to the conclusion that the two populations possessed near identical gender strategies. However, this similarity results from the use of an equivalence factor ( $E = \sum d_i / \sum l_i$ ) in the calculation of the index of phenotypic gender and does not reflect similar investment in female and male flower production in the two populations. On average, plants in ST produced significantly more female flowers and the same number of male flowers than plants in WA. As pointed out by Sarkissian et al. (2001, see their Fig. 1), ecological and evolutionary analysis of gender plasticity should consider absolute measures of female and male investment, because relative measures of gender such as Lloyd's index can confound relative sex allocation with variation in total reproductive investment.

*Sagittaria* is comprised of approximately 20–30 species, the majority of which are monoecious (Bogin 1955). However, several other gender strategies also occur within the genus, including andromonoecy in *S. guyan-*

*ensis* and *S. montevidensis* subsp. *calycina* (S.C.H. Barrett unpublished data), androdioecy in *S. lancifolia* (Muenchow 1998), and dioecy in *S. latifolia* (Smith 1894, Wooten 1971, Sarkissian et al. 2001, Dorken et al. 2002). In *S. latifolia*, monoecy is associated with the presence of highly variable gender expression within and among populations, and dioecious populations also occur (see Fig. 2, Sarkissian et al. 2001). A component of gender variation in monoecious populations of *S. latifolia* has a genetic basis (M. Dorken and S.C.H. Barrett unpublished data). The presence of heritable variation in gender may have facilitated the evolution of dioecy from monoecy in this species. In comparison with *S. latifolia*, gender variation in *S. sagittifolia*, and other species in the genus (M.E. Dorken and S.C.H. Barrett, unpublished data), appears to be more canalized. For example, in *S. sagittifolia*, despite considerable variation in the number of female and male flowers per inflorescence, no inflorescences possessed more than two whorls of female flowers, and all inflorescences produced at least one female flower. In contrast, inflorescences in monoecious populations of *S. latifolia* commonly produce in excess of two whorls of female flowers and small inflorescences frequently produce male flowers only (Sarkissian et al. 2001, M.E. Dorken and S.C.H. Barrett unpublished data). Limits to variation in gender may explain, in part, why dioecy has apparently not established elsewhere in the genus. The evolution of gender strategies is likely to be constrained by both the amount of variation in gender expression and by the interaction between the genetic and environmental factors governing gender phenotypes. In *S. sagittifolia*, different patterns of size-dependent gender modification implicate such an interaction between genetic and environmental factors.

We thank Peter Hollingsworth and Jonathan Silvertown for advice on locating populations of *S. sagittifolia*, Tony Davy for hospitality, John Pannell for help in the field, and the Oxfordshire

Constabulary for not pressing trespassing charges. This work was funded by an NSERC Research Grant to S. C. H. Barrett and a graduate scholarship to M. E. Dorken from NSERC.

## References

- Arber A. (1920) Water plants: a study of aquatic angiosperms. Cambridge University Press.
- Barrett S. C. H. (1992) Gender variation and the evolution of dioecy in *Wurmbea dioica* (Liliaceae). *J. Evol. Biol.* 5: 423–444.
- Barrett S. C. H. (2002) The evolution of plant sexual diversity. *Nature Rev. Genet.* 3: 274–284.
- Bogin C. (1955) Revision of the genus *Sagittaria* (Alismataceae). *Mem. NY Bot. Gard.* 9: 179–233.
- Charnov E. L. (1982) The theory of sex allocation. Princeton University Press, Princeton, U.S.A.
- Costich D. E. (1995) Gender specialization across a climatic gradient: experimental comparison of monoecious and dioecious *Ecballium*. *Ecology* 76: 1036–1050.
- Crawford R. M. M. (ed.) (1987) Plant life in aquatic and amphibious habitats. Special Publication of the British Ecological Society No. 5. Blackwell Scientific Publications, Oxford, U.K.
- Delesalle V. A. (1992) Architecture and gender allocation: floral sex expression along branches of the monoecious cucurbit, *Apodanthera undulata*. *Int. J. Plant Sci.* 153: 108–116.
- 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.
- Eckhart V. M. (1999) Sexual dimorphism in flowers and inflorescences. In: Geber M. A., Dawson T. E., Delph L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, pp. 123–148.
- Fox J. F. (1993) Size and sex allocation in monoecious woody plants. *Oecologia* 94: 110–113.
- Grant S. R. (1999) Genetics of gender dimorphism in higher plants. In: Geber M. A., Dawson T. E., Delph L. F. (eds.) Gender and sexual dimorphism in flowering plants. Springer, Berlin, pp. 247–274.
- Huang S., Sun S., Takahashi Y., Guo Y. (2002) Gender variation of sequential inflorescences in a monoecious plant *Sagittaria trifolia* (Alismataceae). *Ann. Bot.* 90: 613–622.
- Klinkhamer P. G. L., de Jong T. J., Metz H. (1997) Sex and size in cosexual plants. *Trends Ecol. Evol.* 12: 260–265.
- Lloyd D. G. (1972) Breeding systems in *Cotula* L. (Compositae, Anthemideae). II. Monoecious populations. *New Phytol.* 71: 1195–1202.
- Lloyd D. G. (1980) Sexual strategies in plants. III. A quantitative method for describing the gender of plants. *N. Zeal. J. Bot.* 18: 103–108.
- Lloyd D. G., Bawa K. S. (1984) Modification of the gender of seed plants in varying conditions. *Evol. Biol.* 17: 255–338.
- Muenchow G. E. (1998) Subandrodioecy and male fitness in *Sagittaria lancifolia* subsp. *lancifolia* (Alismataceae). *Amer. J. Bot.* 85: 513–520.
- 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 Inc. (2000) JMP® Statistics and graphics guide, Version 4.0 Edition. SAS Institute Inc., Cary NC, U.S.A.
- Sculthorpe C. D. (1967) The biology of aquatic vascular plants. Edward Arnold, London, U.K.
- Smith J. G. (1894) A revision of the North American species of *Sagittaria* and *Lophotocarpus*. *Ann. Rep. Missouri Bot. Gard.* 6: 27–64.
- Vaughton G., Ramsey M. (2002) Evidence of gynodioecy and sex ratio variation in *Wurmbea biglandulosa* (Colchicaceae). *Plant Syst. Evol.* 232: 167–179.
- Webb C. J. (1979) Breeding systems and the evolution of dioecy in New Zealand apioid Umbelliferae. *Evolution* 33: 662–672.
- Wolfe L. M., Shmida A. (1997) The ecology of sex expression in a gynodioecious Israeli desert shrub (*Ochradenus baccatus*). *Ecology* 78: 101–110.
- Wooten J. W. (1971) The monoecious and dioecious conditions in *Sagittaria latifolia* L. (Alismataceae). *Evolution* 25: 549–553.
- Wright S. I., Barrett S. C. H. (1999) Size-dependent gender modification in a hermaphroditic perennial herb. *Proc. R. Soc. Lond. B* 266: 225–232.

Address of the authors: Marcel E. Dorken (e-mail: dorken@botany.utoronto.ca), Spencer C. H. Barrett, Department of Botany, University of Toronto, Toronto, Ontario, M5S 3B2, Canada.