Ecology, 82(2), 2001, pp. 360–373

GENDER VARIATION IN *SAGITTARIA LATIFOLIA* (ALISMATACEAE): IS SIZE ALL THAT MATTERS?

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Abstract. Gender in flowering plants is governed by a complex interplay of genetic and environmental factors. The perennial aquatic herb *Sagittaria latifolia* displays remarkable variation within and among populations in gender expression, from monoecious populations composed of plants with differing numbers of female and male flowers to completely dioecious populations with separate female and male plants. Here we examine the role of environmental factors in modifying gender expression in these two sexual systems by investigating the relation between ramet size and flower number in three sex phenotypes (hermaphrodite, female, and male). In particular, we assess the extent to which hermaphroditic plants have the option of altering the number of female and male flowers to accommodate their specific environmental circumstances. We sampled 12 populations from diverse wetland habitats in southern Ontario, Canada, estimated their phenotypic gender, and examined the relations between ramet size and a range of vegetative and floral traits. On average, plants from dioecious and monoecious populations did not differ in leaf length, a correlate of ramet size. However, in monoecious populations with significant numbers of both male and hermaphroditic ramets, hermaphrodites were larger and produced more flowers than males. This contrasting pattern was also observed when plants were grown under glasshouse conditions. In monoecious populations, variation in ramet size did not affect the production of male flowers, whereas female flower production varied positively with plant size. These relations enabled statistical prediction of the dependence of gender on plant size and the frequency distribution of gender within monoecious populations. These relations also imply that the male phenotype can be determined environmentally, whereas the female phenotype cannot. The size dependence of floral sex ratios in monoecious populations provides novel insights into the likely evolutionary pathway by which dioecy has evolved from monoecy in *Sagittaria.*

Key words: aquatic plant; dioecious; hermaphrodite; monoecious; phenotypic gender; plant gender; plant size; reproductive effort; Sagittaria latifolia; *sexual systems.*

INTRODUCTION

Gender represents an individual's relative genetic contributions as a female and/or a male parent of adults in the next generation (Lloyd 1979). Within angiosperms, gender varies considerably, ranging from dioecious species, in which individuals are usually either exclusively female or male (e.g., Bawa 1980, Rottenberg 1998, but see Freeman et al. 1980), through subdioecious and gynodioecious species, in which males often exhibit sex inconstancy whereas females generally do not (Delph and Lloyd 1991, Wolfe and Shmida 1997, Barrett et al. 1999), to hermaphroditic (cosexual) species, in which gender varies continuously in individuals (Primack and Lloyd 1980, Thomson and Barrett 1981, Klinkhamer et al. 1997). Such variation can arise from phenotypic plasticity, often associated with differences in plant size or age (Lloyd and Bawa 1984, Freeman et al. 1997), or from genetic determination of sex phenotypes (Westergaard 1958, Meagher 1988, Charlesworth 1999).

Size-dependent variation in gender should often be part of an adaptive life history when reproductive investment increases with plant size and contributions as female and male parents involve different costs (Ghiselin 1969, Lloyd and Bawa 1984, Charnov and Bull 1985). In general, female reproduction bears greater expense than male, because of the added costs of fruiting both in direct expenditure on current fruit and indirect consequences for future survival and reproduction (Charnov 1982, Policansky 1982, Lloyd and Bawa 1984, Pickering and Ash 1993). Because large plants bear these costs more readily than small plants, relative allocation to female function commonly increases with plant size (Freeman et al. 1980, Bierzychudek 1984, Schlessman 1988, Maki 1993, Klinkhamer et al. 1997, Kudo and Maeda 1998, Wright and Barrett 1999). Large size can also be detrimental for male success because of local mate competition and increased geitonogamy (Lloyd and Bawa 1984, Klinkhamer and de Jong 1997). Hence, large plants will often perform better as females and worse as males and so should benefit

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by being relatively more female than small plants. Such gender plasticity should be particularly common in monoecious plants, because the production of separate female and male flowers enables greater freedom to respond to specific environmental circumstances (Fox 1993, Renner and Ricklefs 1995, Méndez 1998).

In addition to causing gender variation, contrasting patterns of female and male flower production by genetically hermaphroditic plants could create distinct sex phenotypes within a population. For example, phenotypic males could occur if small reproductive plants cannot afford to produce costly female flowers (e.g., Schlessman 1988, Barrett 1992). Alternatively, if production of male flowers declines with increasing plant size, very large plants may produce no male flowers and so function solely as females (e.g., Policansky 1982, Lloyd and Bawa 1984). Consequently, depending on the size variation within a population and its influences on female and male flower production, a population could include small male plants, intermediate sized hermaphroditic plants and large female plants. In addition, variation among populations in the relative frequencies of distinct sex phenotypes could arise solely because environmental differences in resource abundance create different size distributions.

In contrast to pure size dependence, genetic determination of sex phenotype should be associated with broadly overlapping size distributions for female and male plants. In such plants, the general increase in absolute reproductive investment that accompanies increased plant size should change flower production similarly for both sexes. This relation of female and male flower production to plant size differs qualitatively from that expected from size-dependent determination of gender. Hence, patterns of female and male flower production should provide clues concerning both sex-dependent reproductive policies and the determination of sex phenotypes. Clearly, this approach cannot identify the proximate controls of gender for a specific plant; however, it should distinguish the relative importance of genetic and environmental factors in governing gender variation.

The causes of gender variation within and between populations also establish the opportunity for the evolution of sexual systems. For example, Renner and Ricklefs (1995) found that dioecy occurs most commonly in angiosperm clades in which cosexual taxa produce separate female and male flowers. Despite the apparent prevalence of this shift in sexual system (but see Weiblen et al. 2000), most evolutionary studies of dioecy have instead focused on the transition from cosexual species with hermaphroditic flowers via the gynodioecy pathway (reviewed in Lloyd 1976, Charlesworth 1999). As a consequence, little is known about the ecological basis for the evolution of dioecy from monoecy, including the role that size-dependent gender plasticity may play in influencing this shift in sexual system.

Ecological and evolutionary analysis of gender plasticity must consider absolute measures of female and male effort and success, because relative measures of gender confound relative sex allocation with variation in total reproductive investment (see Venable 1992). Consider the implications of size-dependent production of female and male flowers for Lloyd's (1980*a*; see also Lloyd and Bawa 1984) widely accepted measure of phenotypic gender (G_i). This index depicts the standardized phenotypic femaleness of plant i in a population as

$$G_i = o_\mathrm{i}/(o_\mathrm{i} + p_\mathrm{i}E) \tag{1}$$

where o_i is the number of ovule-bearing flowers, p_i is the number of polleniferous flowers, and E is the ratio of ovule-bearing to polleniferous flowers in the population as a whole:

$$E = \sum o_\mathrm{i} \Big/ \sum p_\mathrm{i}. \tag{2}$$

G_i ranges from 0 for plants that produce only pollen to 1 for plants that produce only ovules. When all plants invest equivalently in reproduction, plants that expend more effort on female function must be less male in both their absolute and relative contributions of gametes, and G_i accurately exposes this tradeoff. However, as Fig. 1 illustrates, this correspondence between absolute and relative female or male effort need not exist when reproductive effort varies with plant age or size. For example, large plants in Fig. 1c produce less pollen than small plants and so are more female in both a relative and absolute sense. In contrast, large plants in Fig. 1b produce more female and male flowers than small plants and so should contribute more genes through both sex roles. Nevertheless, G_i characterizes large plants in both cases as being the most female (Fig. 1d). Clearly, to interpret large plants in Fig. 1b as being less male than small plants (as implied by G_i) misrepresents their functional role in the population. Hence, analysis of size dependence requires decomposition of gender into the separate sex roles and consideration of their respective sources and patterns of variation.

In this paper, we interpret the relative contribution of size dependence to gender expression by the perennial, emergent-aquatic herb *Sagittaria latifolia* Willd. (Alismataceae). This species exhibits wide intraspecific variation in gender, and hence provides a useful model system for studying the ecological and genetic factors responsible for patterns of gender variation. Populations of this plant differ in the proportions of three sex phenotypes (hermaphrodites, females, and males), resulting in a continuum from monoecious to dioecious populations. A survey of 41 *S. latifolia* populations in southern Ontario, Canada, revealed an association between sexual system and habitat characteristics (M. E. Dorken and S. C. H. Barrett, *unpublished data*). This variation may partly reflect gender plasticity in response to growth conditions, perhaps involving plant

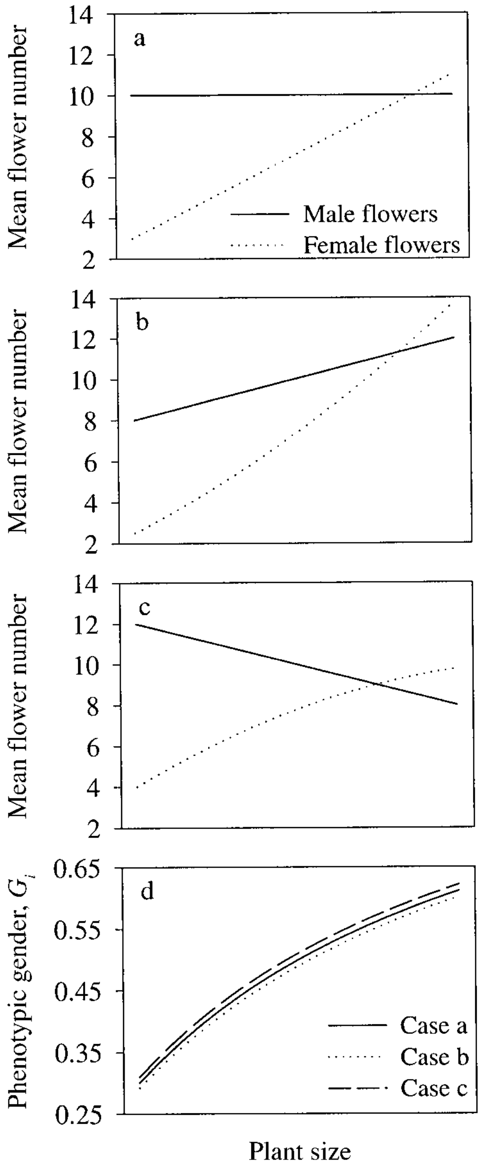

FIG. 1. Three contrasting patterns of size-dependent production of female and male flowers by hermaphroditic plants (panels a, b, and c) that result in the same relation of phenotypic gender to plant size (panel d).

size. However, previous characterizations of populations as monoecious, polygamomonoecious, subdioecious, and dioecious (Smith 1894, Radford et al. 1968, Wooten 1971, Delesalle and Muenchow 1992) imply genetic determination of sex phenotypes. These contrasting perspectives highlight the importance of identifying the relative contribution of environmental influences on gender expression in the three sex phenotypes (also see Muenchow 1998).

Our study investigates size-dependent patterns of female and male flower production in 12 *S. latifolia* populations from diverse wetland habitats. We first describe patterns of gender expression within and among populations and contrast size-related traits for populations with different sexual systems. To assess the association of a plant's sex phenotype with its resource status, we then quantify the relations between female and male flower production to plant size. We were particularly interested in contrasting these relations for monoecious and dioecious populations (defined as populations comprised mainly of hermaphroditic and unisexual plants, respectively) and determining whether variation in plant size affected the incidence of male plants in monoecious populations (see Muenchow 1998), and hermaphroditic plants in dioecious populations. To illustrate the influence of plant size on phenotypic gender within monoecious populations we provide the first demonstration that the size dependence of flower production can be used to predict both gender variation among individuals and its frequency distribution in natural populations. Finally, we consider the relevance of size-dependent gender variation to alternate courses for the evolution of dioecy in *S. latifolia.*

METHODS

Study species

Sagittaria latifolia occupies shallow water along lake and stream margins, marshes, swamps, ponds, and roadside ditches. Plants grow clonally, producing both vegetative and reproductive shoots (ramets). In southern Ontario, Canada, where we conducted this study, plants usually begin flowering in July, producing racemes with three flowers at each node. Within an inflorescence, the single-day flowers open sequentially from bottom to top. In the populations we sampled, virtually all flowers were either staminate or pistillate. Although hermaphroditic flowers occur, we observed only a few at our study sites. Hermaphroditic plants always produce female flowers basally and male flowers distally, which, coupled with the anthesis pattern, creates protogynous inflorescences. Diverse insects visit *S. latifolia* flowers, including flies, bees, beetles, and wasps (Muenchow and Delesalle 1994).

Data collection

During August 1994 and July and August 1995, we sampled 12 populations of *S. latifolia* in southern Ontario (Table 1). Populations sampled during 1994 were clustered within ~10 km of the Queen's University Biological Station near Kingston and, except for population 6, occupied relatively stable, permanent wetlands. The populations sampled during 1995 were located along a 300-km transect north of Toronto and, except for population 10, occurred in ephemeral, recently colonized habitats. We estimated population size as the total number of ramets, both flowering and nonflowering.

In all populations, we sampled randomly chosen ramets separated by a minimum of 1 m to limit repeated sampling of genets. For each plant we measured the

TABLE 1. General information on the 12 populations of *Sagittaria latifolia* sampled in southern Ontario, Canada.

Population	Locality	Latitude (°N)/ Longitude (°W)	Estimated population size (individuals)	Habitat	Sampling date	N
1	Dorset	45°10′/78°50′	60	roadside ditch	1 August 1995	25
2	Carnarvon	45°04′/78°43′	380	wet meadow	1 August 1995	40
3	Gooderham	44°47′/78°22′	250	roadside marsh	31 July 1995	32
4	Humber Bay	43°38′/79°27′	300	man-made marsh	26 July 1995	40
5	Westwood	44°18′/78°03′	140	rock-bottom stream	1 August 1995	22
6	Davis Lock	44°34′/76°17′	125	roadside ditch	19 August 1994	46
7	Cow Island	44°34′/76°19′	200	permanent marsh	18 August 1994	50
8	Hart Lake	44°32′/76°20′	200	lake margin	18 August 1994	46
9	Pothole Lake	44°32′/76°21′	300	lake margin	19 August 1994	47
10	Heart Lake	43°44′/79°47′	1000	permanent marsh	24 August 1995	44
11	Chaffey's Locks	44°35′/76°18′	100	permanent marsh	19 August 1994	57
12	Lake Opinicon	44°32′/76°22′	500	swamp	18 August 1994	54

total number and sexes of flowers on the inflorescence, the diameter of one fully open flower on the lowest inflorescence whorl, and the midvein length of the basal leaf with the longest petiole. For the six populations sampled during July and August 1995 (1, 2, 3, 4, 5, and 10) we also measured plant (ramet) height above the soil surface. Because midvein length correlates strongly with plant height ($r^2 = 0.768$, $F_{1\ 196} = 234.95$, slope $= 0.774$, $P < 0.001$) we use midvein length as a measure of ramet size for most analyses. We quantified gender for individual plants in each population using Lloyd's measure of phenotypic gender (Eqs. 1 and 2).

To examine further whether sex expression in monoecious populations varies with plant resource status (i.e., size), we monitored flower production through the flowering period (23 July–25 August 1995) for plants maintained in a glasshouse. We excavated 35 plantlets at the two- or three-leaf stage from population 5 approximately 1 mo before flowering and grew them in pots in water-filled trays. On the first day that each plant flowered, we measured plant height, leaf length, and the total number of flowers per inflorescence to assess the status of plants at the onset of flowering. Each day during flowering, we recorded the number of open flowers and their sexes. At the end of flowering all plants were classified as either male or hermaphrodite (no plants produced only female flowers).

Statistical analyses

Analyses of ramet size, flower production, and flower size involved general linear models (Neter et al. 1990) as implemented by SAS/STAT (SAS Institute 1990, 1997) in either the GLM procedure (analyses without repeated measures) or the MIXED procedure (repeated measures analyses). For comparisons between populations we treated population as a fixed effect, so that our interpretations apply only to the populations sampled. We ln-transformed dependent variables when necessary to assure normality and homoscedasticity of residuals. In addition, for analyses of size-dependent production of female and male flowers we ln-transformed the covariate representing ramet size (leaf midvein length) to straighten the relation between dependent and independent variables.

Production of female and male flowers by hermaphroditic plants in monoecious populations requires repeated measurement of the same subject. To account for the lack of independence that can accompany such repeated measures we used the MIXED procedure of SAS/STAT (SAS Institute 1997) to characterize the covariance structure of flower production within plants by restricted maximum likelihood (see Table 2). This analysis selected between pooled or separate (unstructured) variance models for each population based on Akaike's Information Criterion (see Littell et al. 1996).

The relations between female and male flower production and plant size in monoecious populations enabled us to predict each plant's gender (G_i). If size is an important determinant of gender variation within populations, the observed and predicted frequency distributions of G_i should be similar. In particular, we used the regression relations of female and male flower production to leaf midvein length (Table 2) to predict flower production for each plant (the number of female flowers was set at 0 if the predicted number was negative). We then calculated each plant's predicted gender using Eq. 1 and calculated the ratio of female to male flowers (Eq. 2) from the sum of the predicted numbers of female and male flowers in the population. With these gender predictions, we constructed the cumulative frequency distribution of G_i for each population and compared it to the observed distribution with a Kolmogorov-Smirnov two-sample test (Sokal and Rohlf 1995).

RESULTS

Gender variation among populations

The relative frequencies of the three sex phenotypes varied considerably among populations (Fig. 2): female frequencies varied from 0 to 0.477, whereas male fre-

TABLE 2. Significance tests and partial regression coefficients (with 95% CI) for general linear models comparing flower production by the three sex phenotypes (hermaphrodite, male, or female) in 12 populations of *Sagittaria latifolia*, with leaf midvein length (log-transformed) as a covariate.

Population	Sex F (df)	Midvein F (df)	Sex × Midvein F (df)	Female		Male	
				Intercept	Slope	Intercept	Slope
Hermaphroditic plants							
1	96.76 (1,48)***	⋯	⋯	3.24 ± 0.38	0	5.84 ± 0.38	0
2	28.75 (1,75)***	22.33 (1,75)***	20.40 (1,75)***	−8.26 ± 3.84	6.46 ± 1.97	6.32 ± 3.83	0.15 ± 1.97
3	23.73 (1,54)***	0.57 (1,54)	10.27 (1,54)*	−1.97 ± 3.53	2.88 ± 2.17	9.47 ± 3.12	−1.78 ± 1.95
4	25.03 (1,67)***	18.53 (1,67)***	7.25 (1,67)*	−10.40 ± 6.48	6.14 ± 2.86	9.25 ± 4.42	1.41 ± 2.03
5	21.45 (1,14)*	⋯	⋯	2.50 ± 0.60	0	5.59 ± 1.09	0
6	56.78 (1,40)***	28.74 (1,39)***	⋯	−6.39 ± 3.99	4.43 ± 1.67	−0.22 ± 4.25	4.43 ± 1.67
7	2.69 (1,12)	⋯	⋯	5.64 ± 1.04	0	5.64 ± 1.04	0
8	2.85 (1,15)	6.80 (1,15)*	7.75 (1,15)*	4.87 ± 3.76	−0.81 ± 1.97	−6.36 ± 12.87	8.86 ± 6.72
Unisexual plants							
7	0.34 (1,40)	16.05 (1,40)***	⋯	−5.78 ± 6.91	7.33 ± 3.70	−5.78 ± 6.91	7.33 ± 3.70
8	5.30 (1,26)*	24.38 (1,26)***	⋯	−25.01 ± 16.55	17.58 ± 7.32	−19.58 ± 15.66	17.58 ± 7.32
9	41.96 (1,43)***	25.93 (1,43)***	⋯	−3.39 ± 5.24	6.62 ± 2.62	2.08 ± 5.08	6.62 ± 2.62
10	29.63 (1,40)***	28.30 (1,40)***	⋯	−26.24 ± 13.09	14.55 ± 5.53	−20.91 ± 12.25	14.55 ± 5.53
11	8.15 (1,53)*	28.28 (1,53)***	6.82 (1,53)*	−25.29 ± 15.51	16.58 ± 7.66	−1.27 ± 6.66	5.66 ± 3.43
12	16.79 (1,51)***	24.42 (1,51)***	⋯	−3.53 ± 5.41	6.71 ± 2.73	−0.46 ± 5.16	6.71 ± 2.73

Notes: The analyses for hermaphroditic plants treated floral sex as a repeated measure. F values are not provided for terms that were nonsignificant and removed from the model. A common partial regression coefficient was estimated for female and male flower production in populations with a nonsignificant interaction between floral sex and midvein length (see Fig. 5).

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

quencies ranged from 0 to 0.719. Population 1 consisted entirely of hermaphrodites, whereas this phenotype was absent in populations 11 and 12. The range of population gender patterns includes two contrasting types of populations. One extreme includes populations comprised mainly of hermaphrodites in relatively ephemeral, recently colonized habitats (1, 2, 3, 4, 5, 6; hereafter referred to as ‘‘monoecious populations:’’ Table 1). The other extreme involves populations comprised almost exclusively of females and males in relatively stable, permanent habitats (9, 10, 11, 12; hereafter referred to as ‘‘dioecious populations:’’ Table 1). Populations 7 and 8 link these extremes, as they contain appreciable numbers of all three sex phenotypes. These ‘‘mixed populations’’ occupied permanent aquatic habitats similar to those of dioecious populations (Table 1).

Size relations among sex phenotypes

Comparisons between sexual systems.—On average, ramets of the dominant sex phenotypes in populations with contrasting sexual systems had leaves of equivalent size (hermaphrodites in monoecious populations, mean ± 1 SE = 7.40 ± 0.15 cm; unisexual ramets in dioecious populations, mean ± 1 SE = 7.44 ± 0.14 cm; $F_{1, 367} = 0.04$, $P > 0.5$, $n = 377$). Hence, contrasting sexual systems do not arise simply because of dissimilar growth conditions in different habitats. However, leaf size varied significantly among populations within sexual systems ($F_{8, 367} = 30.02$, $P < 0.001$).

Despite the similar sizes of hermaphroditic ramets in monoecious populations and unisexual ramets in dioecious populations, phenotypic males from monoecious populations were significantly smaller than those in dioecious and mixed populations (Fig. 3). This smaller stature involved leaf size, flower production, and flower size. In contrast, male ramets from mixed and dioecious populations did not differ significantly for any of these characters.

Comparisons between hermaphrodites and males.—Within monoecious and mixed populations, phenotypically male ramets were generally smaller than hermaphrodites (Table 3 and Fig. 4, natural populations). Overall, male ramets from the three monoecious populations sampled during 1995 were significantly shorter than hermaphroditic ramets (gender effect; Table 3) and this difference did not vary significantly among populations (gender × population interaction). Leaf length, flower number, and flower size exhibited more complex relations, as the differences between male and hermaphroditic ramets varied between populations. Averaged over all five populations, hermaphroditic ramets produced larger leaves and more flowers than phenotypic males (gender effect), but these phenotypes differed significantly within only populations 4 and 7 (gender × population interaction; Table 3 and Fig. 4). In general, male flower size did not differ between phenotypes, except within population 4 where hermaphroditic ramets produced significantly larger flowers than males (gender × population interaction; Table 3 and Fig. 4).

Despite little difference between the sex phenotypes for wild-grown plants from population 5, male and hermaphroditic ramets from this population grown in the glasshouse differed significantly (Fig. 4). In particular, individuals that produced only male flowers on the first

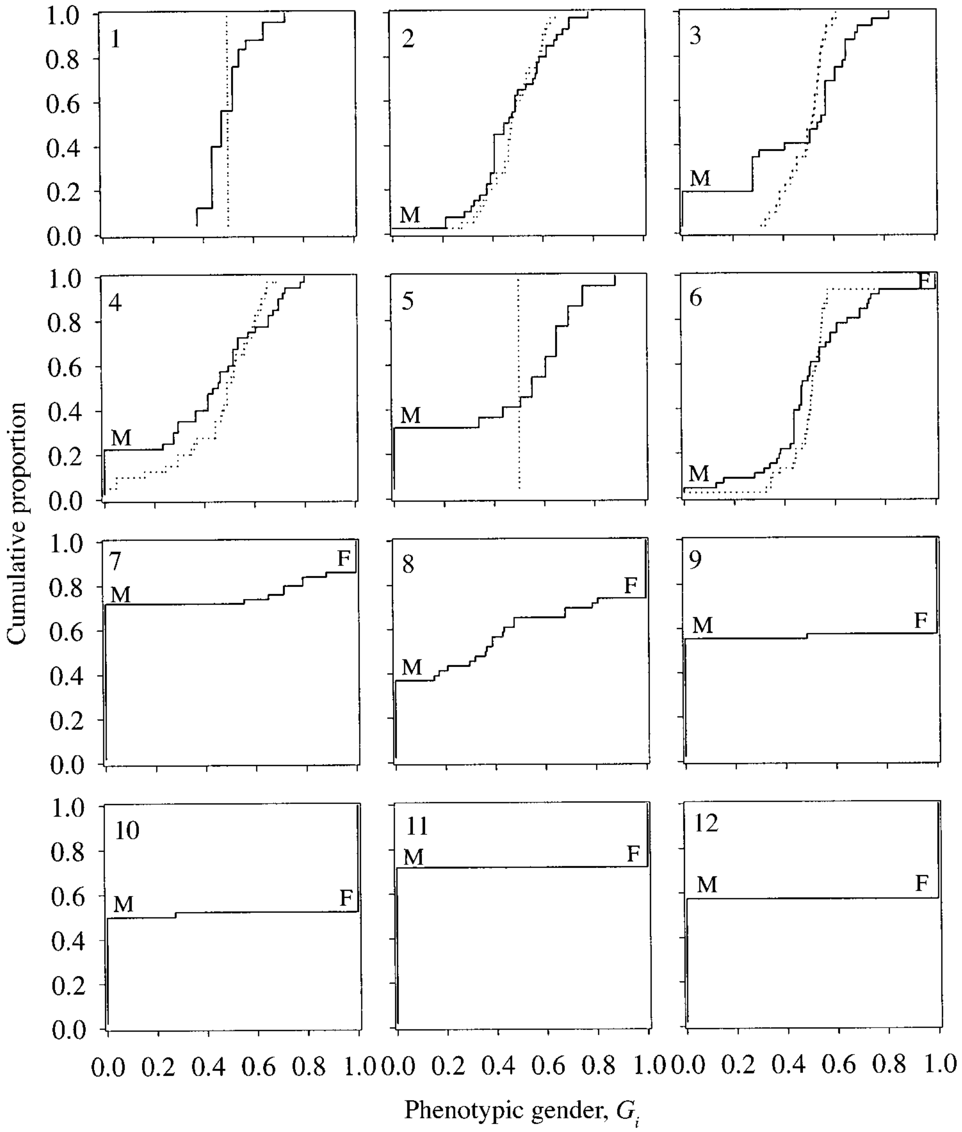

FIG. 2. Variation in phenotypic gender in 12 populations of *Sagittaria latifolia* sampled in southern Ontario, Canada. The solid lines represent the observed cumulative frequency distribution of standardized phenotypic femaleness (G_i) for plants sampled from each population ($G_i = 0$ for pure males, and $G_i = 1$ for pure females). Within each population, the height of the vertical line at $G_i = 0$ from the bottom of the panel to the point "M" represents the frequency of male ramets; the height of the vertical line from the point "F" to the top of the panel at $G_i = 1$ represents the frequency of female ramets; hermaphroditic ramets occur between these two points. The dotted lines represent the predicted cumulative frequency distribution of gender in monoecious populations based on the relations of male and female flower production to midvein length (see Table 4 and Fig. 5). Data for population 2 are from Barrett et al. (2000).

inflorescence were significantly shorter ($F_{1,33} = 6.99$, $P < 0.025$), had smaller leaves ($F_{1,33} = 6.02$, $P < 0.025$), and fewer flowers per inflorescence ($F_{1,33} = 7.35$, $P < 0.025$) than individuals that produced both female and male flowers. Of the 12 ramets producing only male flowers on their first inflorescence, seven produced subsequent inflorescences, of which six produced inflorescences with both female and male flowers. These results indicate that differences between sex phenotypes evident in a common environment can be obscured in natural populations by the influence of environmental heterogeneity on plant development.

The plants grown in the glasshouse provide unequivocal evidence of the labile nature of sex expression in hermaphroditic individuals. Of these 35 plants, 26 produced one or more additional inflorescences. Among the plants producing multiple inflorescences, nine changed sex phenotype, with five switching from male to hermaphrodite, three changing from hermaphrodite to male, and one switching from male to hermaphrodite

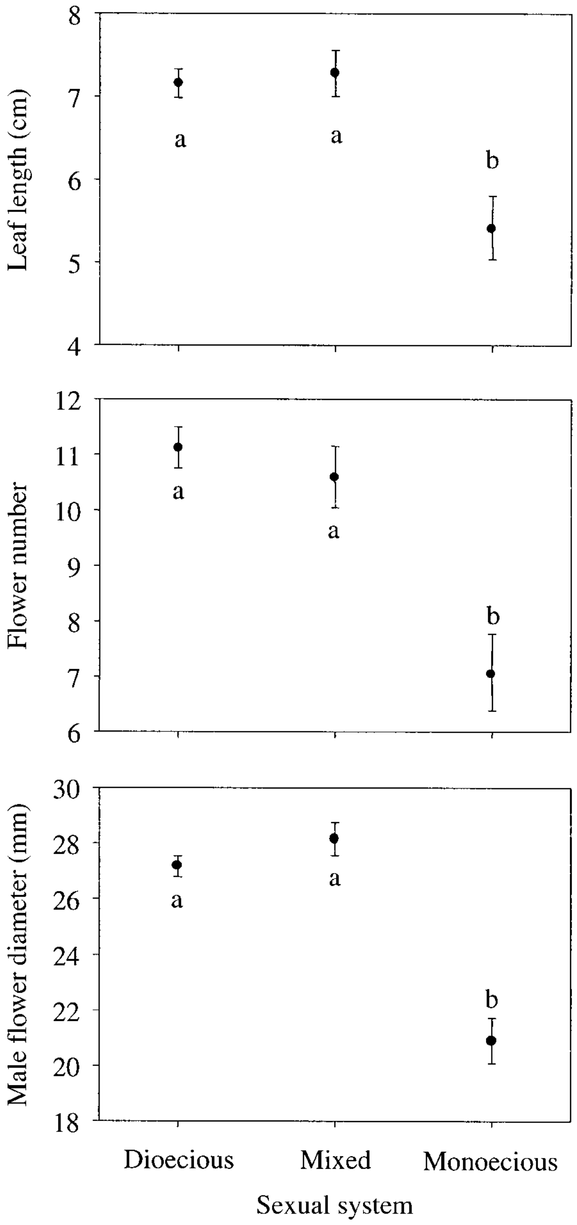

FIG. 3. Comparison of phenotypically male plants in dioecious, mixed, and monoecious populations of *Sagittaria latifolia* with respect to mean (± 1 SE) leaf length, flower number, and male flower size. All traits varied significantly among sexual systems ("sex") and population ("pop") nested within sexual system. (For leaf length, "sex" $F_{2,187} = 7.53$, $P < 0.05$; "pop" $F_{8,187} = 5.50$, $P < 0.001$. For flower number, "sex" $F_{2,187} = 9.58$, $P < 0.001$; "pop" $F_{8,187} = 10.83$, $P < 0.001$. For male flower size, "sex" $F_{2,182} = 22.88$, $P < 0.001$; "pop" $F_{8,182} = 11.22$, $P < 0.001$.) Means with different letters differ significantly, based on Tukey's multiple comparisons ($\alpha = 0.05$).

to male. In addition, two plants that produced a series of hermaphroditic inflorescences on their first ramet subsequently produced a second ramet with a single male inflorescence. Only one plant that produced male inflorescences did not also produce a hermaphroditic inflorescence.

Comparisons between females and males.—In contrast to the difference between sex phenotypes in monoecious populations, female and male ramets in dioecious (9, 10, 11, and 12) and mixed populations (7 and 8) did not differ in size, although they differed in floral traits (Table 4). The lack of vegetative differences between female and male ramets occurred despite considerable variation among populations in mean leaf length. Overall, female ramets produced significantly smaller flowers (mean diameter ± 1 SE: 23.8 ± 0.53 mm, $n = 94$) than male ramets (27.4 mm ± 0.37, $n = 168$), even though they produced fewer flowers per inflorescence (females, mean = 8.5, lower SE = 0.38, upper SE = 0.40, $n = 99$; males, mean = 10.4, lower SE = 0.34, upper SE = 0.35, $n = 173$). This difference suggests either greater reproductive effort by male ramets, or that male flowers cost less than female flowers. Despite producing fewer flowers, female ramets displayed a larger proportion of their flowers at once than male plants (females, mean = 0.69, lower SE = 0.035, upper SE = 0.033, $n = 99$; males, mean = 0.23, lower SE = 0.012, upper SE = 0.013, $n = 173$). This difference in daily floral display occurred because male ramets stagger the opening of flowers on an inflorescence, thereby prolonging their mean flowering period for 4.1 d compared to 1.8 d for female ramets (Kruskal-Wallis test, $\chi^2_1 = 35.42$, $P < 0.001$). The floral differences between female and male ramets did not vary among populations (i.e., no gender × population interaction), even though populations differed significantly with respect to flower size, number, and daily display (Table 4).

Size-dependent allocation to female and male flowers

Monoecious populations.—In four of the six populations (2, 3, 4, and 6), allocation to female flowers increased with ramet size. In contrast, in five of these populations (1, 2, 3, 4, and 5) allocation to male flowers varied independently of size, whereas in population 6 male flower production increased with size (Table 2 and Fig. 5). Combined analysis of the six monoecious populations (with the sex of the flower and population as main effects and ln[midvein length] as the covariate) detected a significant interaction between population, sex, and midvein length ($F_{5\ 192} = 5.23$, $P < 0.001$). However, this interaction became nonsignificant when we excluded population 6 from the analysis ($F_{4,136} = 0.83$, $P > 0.5$), indicating that ramet size affected the joint relations of female and male flower production similarly in the remaining five populations. For populations 1, 2, 3, 4, and 5 overall, female flower production increased with ramet size (female partial regression coefficient, $b = 3.77$, $P < 0.001$), whereas male flower production did not vary with ramet size ($b = -0.77$, $P > 0.05$: sex × midvein interaction, $F_{1,143} = 31.55$, $P < 0.001$).

TABLE 3. *F* values for two-factor analyses of variance of the effects of gender (male or hermaphrodite) and population on plant height, leaf length, number of flowers per inflorescence, and male flower size in populations 3, 4, 5, 7, and 8 of *Sagittaria latifolia*.

Source	Plant height ($n = 94$)	Leaf length ($n = 171$)	Flower number ($n = 171$)	Flower size ($n = 150$)
Gender	19.99 (1, 88)***	14.15 (1, 161)***	7.03 (1, 161)**	0.69 (1, 140)
Population	44.55 (2, 88)***	14.09 (4, 161)***	21.47 (4, 161)***	14.99 (4, 140)***
Gender × Population	2.13 (2, 88)	7.47 (4, 161)***	5.22 (4, 161)***	4.89 (4, 140)***

Notes: All response variables, except male flower size, were log-transformed for analysis. See Fig. 4 for a posteriori contrasts for the Gender × Population interactions. Degrees of freedom are shown in parentheses.

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

We predicted the phenotypic gender for ramets in monoecious populations (Fig. 6) from the regressions of female and male flower production on midvein length (Table 2) and Eqs. 1 and 2. For four of the six monoecious populations (2, 3, 4, and 6) the femaleness of ramets increased nonlinearly with increasing ramet size, as measured by midvein length. In populations 4 and 6, small plants were predicted to produce only male flowers, in general agreement with the observations. In contrast, in population 3 the incidence of males seemed unrelated to ramet size as no males were predicted within the size range observed (also see Fig. 5). In populations 1 and 5 all ramets were predicted to have $G_i = 0.5$ because female and male flower production did not vary significantly with ramet size in these populations.

The relations of flower production to midvein length also provided reasonable predictions of population-level gender variation in monoecious populations (compare solid and dotted lines in Fig. 2). The observed and predicted cumulative frequency distributions for G_i did not differ significantly for population 2 (two-sample Kolmogorov-Smirnov statistic, $D = 0.225$, $P > 0.05$), population 4 ($D = 0.225$, $P > 0.05$), and population 6 ($D = 0.283$, $P > 0.05$). The significant lack of fit for population 3 ($D = 0.375$, $P > 0.02$) largely reflects the lack of association between producing only male flowers and ramet size. In populations 1 ($D = 0.56$, $P < 0.001$) and 5 ($D = 0.591$, $P < 0.001$) all gender values were again predicted to be 0.5.

Dioecious populations.—Flower production by both female and male ramets in dioecious populations (9, 10, 11, and 12) increased with ramet size (Table 2 and Fig. 5). The male intercept always exceeded the female intercept, suggesting a higher cost of producing female flowers. For most dioecious populations, flower production increased similarly with ramet size for female and male plants. However, in population 11 flower production increased faster for female plants than for male plants. Coincidentally, population 11 was the only dioecious population which deviated from a 1:1 population sex ratio ($\chi^2_1 = 10.964$, $P < 0.05$), being male biased (see Fig. 2).

Mixed populations.—Hermaphroditic ramets in populations 7 and 8 showed a different pattern of allocation to female and male flowers with size than populations 1–5 (Table 2 and Fig. 5). In population 8, female flower production did not vary with ramet size, whereas male flower production increased with ramet size. Neither female nor male flower production varied with ramet size in population 7. Relations for unisexual plants were similar to those observed for dioecious populations. However, in population 7, the costs of producing the two types of flowers did not differ (i.e., no sex-specific difference in intercepts).

DISCUSSION

Among sexually dimorphic plant species, *Sagittaria latifolia* exhibits unusual diversity in gender expression. Our results indicate that this diversity arises from two distinct sexual systems that differ in the effect of environmental factors on gender. In particular, monoecious populations include ramets whose gender depends on their size, whereas ramets in dioecious populations express a specific sex phenotype regardless of their size. This contrast implicates the joint action of genetic and environmental factors in governing the range of gender variation in this species. We now consider the nature of size dependency, assess the extent to which the three sex phenotypes respond to environmental influences, and explore the implications of size dependence for the evolution of sexual systems in plants.

Effects of size on gender

Plants in monoecious populations of *S. latifolia* do not maintain either a fixed sex phenotype or stable gender. Almost all plants in these populations produce male flowers, but the number of these flowers does not vary with ramet size (except in population 6, see Fig. 5). Most plants also produce female flowers; however, the plants grown in the glasshouse indicate that production of female flowers varies between ramets of the same genet. In the larger sample of plants from natural populations' production of female flowers generally increased with ramet size (Fig. 5). As a result, phenotypically male plants tend to be small (Fig. 4) and the relative femaleness of hermaphroditic plants generally increases with ramet size (Fig. 6).

The contrasting relations of female and male flower

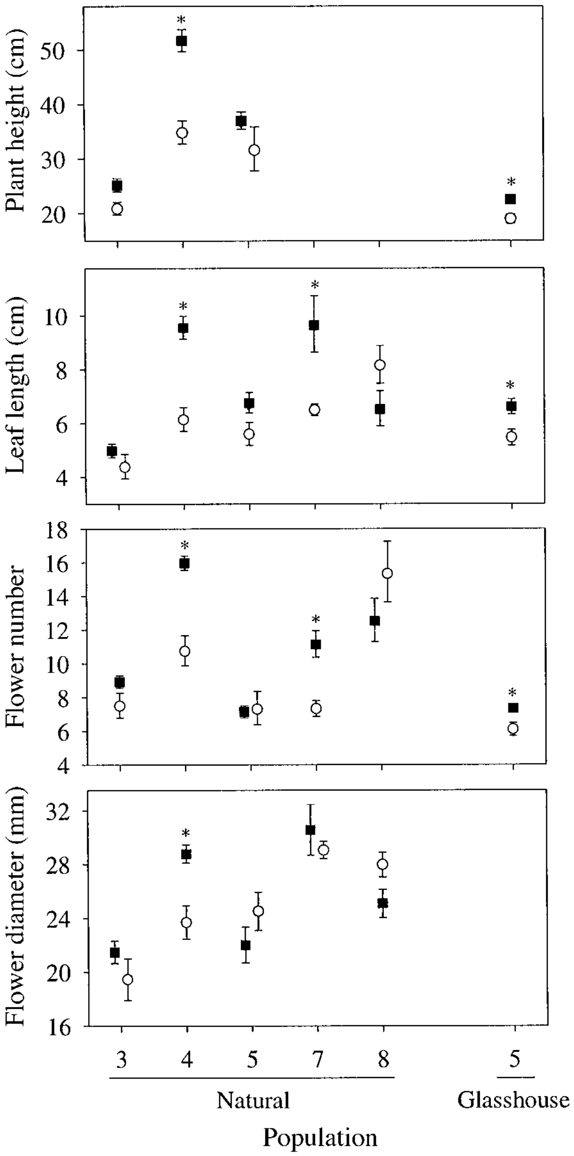

FIG. 4. Comparison of hermaphroditic (solid squares) and male (open circles) ramets from five natural populations and one glasshouse population of *Sagittaria latifolia* with respect to mean (± 1 SE) plant height, leaf length, flower number, and male flower size. Asterisks indicate significant differences between phenotypes within a population as determined by Dunn-Sidák multiple comparisons (Ury 1976). See Table 2 for statistical details.

production to the size of hermaphroditic plants indicate that complex gender dynamics (Fig. 6) arise from relatively simple changes in floral investment (Fig. 5). Flower production seems to involve two size thresholds: a lower threshold for production of male flowers, and a higher threshold for production of female flowers. The enhanced production of female flowers with increasing ramet size indicates roughly fixed, or increasing, relative investment in female function. In contrast, production of male flowers does not vary with ramet size, so that large plants expend proportionately less on male flowers than small flowering plants.

Why should the effect of plant size on flower production differ for female and male flowers in hermaphroditic plants? If the observed relations are advantageous, then large plants must not benefit from producing more male flowers. Two factors could diminish the value of additional male flowers. First, large ramets have more resources for both sexual reproduction and clonal propagation and so are more likely to have daughter ramets than small plants. As a result, large plants would experience greater within-genet pollen transfer. Because *S. latifolia* suffers significant inbreeding depression (Delesalle and Muenchow 1992), such self-pollination bears large genetic costs. In addition, inter-ramet geitonogamy could also reduce pollen export to other plants, creating a mating cost (see Harder and Barrett 1995, Klinkhamer et al. 1997). Second, because small hermaphroditic plants in monoecious populations produce few female flowers, these populations contain many more male flowers than female flowers (Fig. 5). As a result, plants likely compete more intensively as males, for access to ovules, than as females. Such a differential in reproductive opportunities places a premium on investing additional resources in female flowers, rather than male flowers, as plants grow.

In contrast to hermaphroditic plants, flower production by male plants in dioecious populations increased with ramet size, generally paralleling the pattern seen in female plants (Fig. 5). Because male plants in these populations could not produce female flowers, the additional resources available to large plants must be spent on extra male flowers or diverted to vegetative growth. In contrast to hermaphroditic plants, producing more male flowers bears neither a mating nor a genetic cost for male plants, so that this option is likely beneficial. The contrasting relations between size and female and male flower production for monoecious and dioecious populations expose dissimilar options for allocating reproductive resources by individuals with combined vs. separate sexes.

Genetic and environmental components of gender

The preceding discussion relies on the assumption of genetic determination of gender in dioecious populations, whereas male phenotypes in monoecious populations represent gender plasticity of small hermaphroditic plants. For the dioecious populations, several lines of evidence support the canalized nature of sex expression. First, in these populations, female and male plants did not differ significantly in size, casting doubt on the occurrence of size-dependent sex reversal. Second, ramet sex ratios in three of four dioecious populations did not differ significantly from unity, an unexpected pattern if gender varied with size. Third, unisexual plants from dioecious populations grown under glasshouse conditions have not changed sex over many

TABLE 4. *F* values for two-factor analyses of variance of the effects of gender (male or female) and population on leaf length (log-transformed), flower size, flower number (log-transformed), and the proportion of flowers open (logit-transformed) in populations 7, 8, 9, 10, 11, and 12 of *Sagittaria latifolia*.

Source	Leaf length ($n = 272$)	Flower size ($n = 262$)	Flower number ($n = 272$)	Proportion of flowers open ($n = 272$)
Gender	3.27 (1, 260)	72.89 (1, 250)***	22.43 (1, 260)***	192.85 (1, 260)***
Population	16.28 (5, 260)***	30.13 (5, 250)***	11.79 (5, 260)***	13.11 (5, 260)***
Gender × Population	0.72 (5, 260)	1.37 (5, 250)	1.37 (5, 260)	2.06 (5, 260)

Note: Degrees of freedom are shown in parentheses.
*** $P < 0.001$.

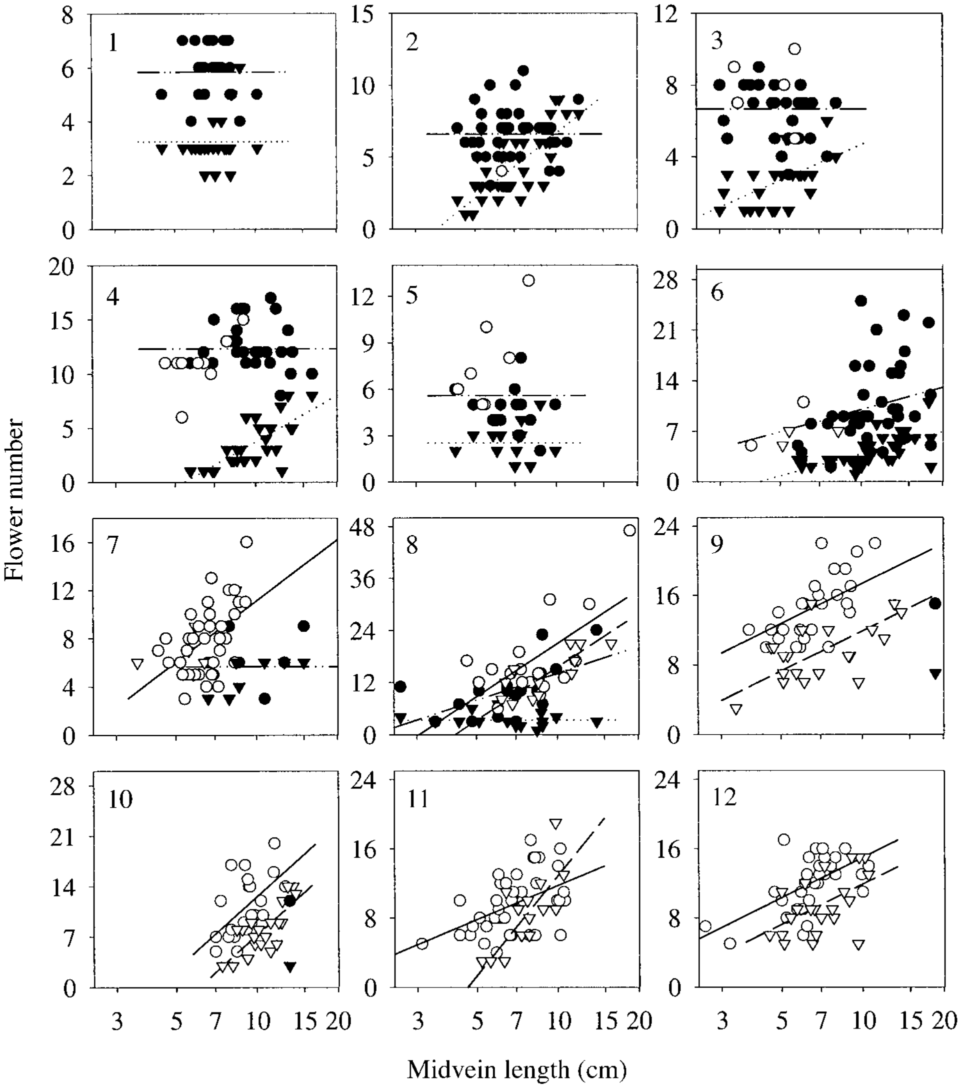

FIG. 5. The relations of female and male flower production to the size of plants sampled in 12 populations of *Sagittaria latifolia.* Open symbols represent observed flower production by female (open triangles) and male ramets (open circles), and the dashed and solid lines, respectively, illustrate the corresponding regression predictions based on ln(midvein length). Closed symbols represent observed production of female (solid triangles) and male flowers (solid circles) by hermaphroditic plants, with predicted production depicted by dotted and dashed–dotted lines, respectively. In population 7, the relation between ramet size and male and female flower production in hermaphroditic ramets is the same as that for unisexual plants. See Table 4 for statistical details. Data for population 2 are from Barrett et al. (2000).

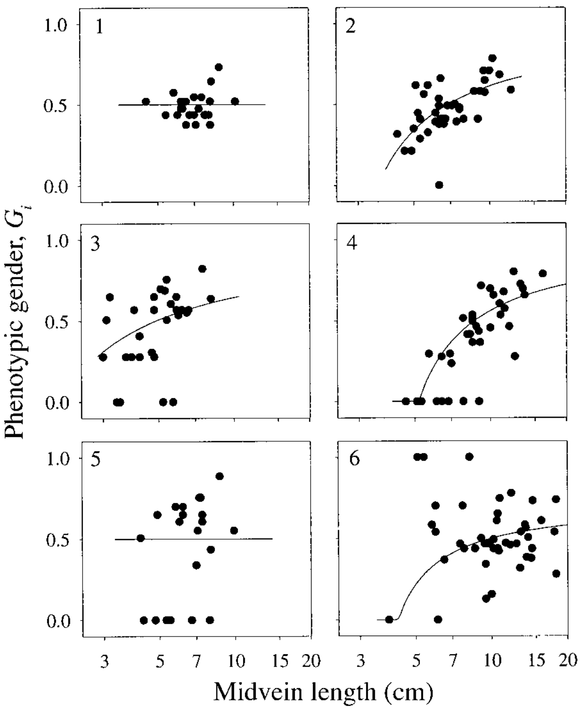

FIG. 6. The observed (scatter plot) and predicted (solid line) relations between a hermaphroditic plant's phenotypic gender (G_i) and the midvein length of its longest leaf for monoecious populations of *Sagittaria latifolia.* Predicted gender is based on the general linear models presented in Table 4 and Eqs. 1 and 2. Data for population 2 are from Barrett et al. (2000).

years (S. C. H. Barrett, *personal observations*). Finally, the sex phenotypes in dioecious populations differed in a number of secondary sexual traits (flower size, flower number, and the proportion of flowers open) that commonly distinguish females and males (Lloyd and Webb 1977, Delph 1996). Such differences likely reflect genetically based strategies that promote maternal vs. paternal fitness in the sexual morphs of dioecious populations.

One monoecious population (6) and both mixed populations (7 and 8) contained some purely female individuals. In contrast to the production of male phenotypes in these populations, the relations of female and male flower production to ramet size argue against environmental determination of purely female plants. In particular, because production of male flowers does not decline with increased ramet size, hermaphroditic plants cannot grow into a purely female phenotype. Furthermore, some of the smallest plants in populations 6 and 7 produced only female flowers (Fig. 5). These size relations implicate genetic, rather than size-dependent, determination of purely female plants in monoecious populations. The presence of genetic females in monoecious populations could arise by dispersal from dioecious populations, because both sexual systems occur in close proximity in southern Ontario. Alternatively, females may originate in situ through the spread of male sterility genes. Regardless of their origin, if females reach appreciable frequencies in monoecious populations the sexual system of such populations would best be described as gynodioecious.

Although we demonstrated that hermaphroditic plants can be phenotypically male when they are small, monoecious populations could also include genetic males. For the related monoecious species, *S. lancifolia,* Muenchow (1998) demonstrated genetic control of maleness and criticized the ''widely held preconception that apparent males in an otherwise monoecious population are just 'small' or 'young' cosexuals'' (p. 520). The incidence of some relatively large males in the monoecious populations that we sampled suggests that these populations may contain a mixture of genetic males and hermaphrodites that were phenotypically male at the time of sampling. This possibility points to the complex interplay of genetics and environmental factors in governing gender variation in *S. latifolia.* If the monoecious populations of *S. latifolia* that we examined contain significant numbers of genetic males, then they would be better described as subandrodioecious, as Muenchow (1998) has done for populations of the related *S. lancifolia.* However, as we discuss below, this designation for *S. latifolia* may be inappropriate.

Aquatic plants are notorious for their extensive dispersal (Sculthorpe 1967), so populations containing all three sex phenotypes are not unexpected. We interpret the mixed populations, 7 and 8, as dioecious populations that have been invaded by hermaphrodites from monoecious populations. Hermaphroditic plants may be more likely to colonize new environments than unisexual plants because of the reproductive assurance provided by possession of two sex functions. Despite possessing unisexual flowers, isolated hermaphroditic plants from monoecious populations can set seed through pollinator-mediated intra-inflorescence geitonogamy (S. C. H. Barrett, *unpublished data*). In southern Ontario, monoecious populations are distributed more widely and often occur in ephemeral aquatic environments (M. E. Dorken and S. C. H. Barrett, *unpublished data*). In contrast, the mixed populations we investigated occupied relatively stable wetland environments characteristic of dioecious populations, further supporting our interpretation of their origin.

Implications of size dependence for the evolution of sexual systems

Intraspecific variation in sexual systems, such as we observed for *Sagittaria latifolia,* has been reported in only a few taxa, including *Mercurialis* (Durand 1963), *Leptinella* (formerly *Cotula*; Lloyd 1972), *Ecballium* (Costich 1995), and *Elatostema* (Lahav-Ginott and Cronk 1993). However, unlike *S. latifolia,* monoecious and dioecious populations of these species rarely occur in the same geographical area with clear ecological differentiation. Hence, the variation in *S. latifolia* provides a unique opportunity for analyzing the evolution and maintenance of sexual systems. Most of the ~20 *Sagittaria* species are monoecious, with dioecy restricted to just a few taxa (Bogin 1955; S. C. H. Barrett, *unpublished data*). This pattern suggests that dioecy evolved from monoecy, although identification of the evolutionary polarity of these sexual systems awaits explicit phylogenetic analysis. If dioecy is derived in *Sagittaria*, what does the occurrence of monoecy and dioecy within *S. latifolia* tell us about the evolution and maintenance of sexual systems within the genus?

Dioecy originates most commonly via either the gynodioecy or the monoecy–paradioecy pathways (Charlesworth and Charlesworth 1978*a*, Charlesworth and Charlesworth 1978*b*, Lloyd 1980*b*, Thomson and Brunet 1990, Webb 1999), with transitions via androdioecy occurring very rarely, if at all (Charlesworth 1984). These pathways differ critically with respect to the types of gender variation upon which selection acts. In the gynodioecy pathway, female variants spread in cosexual populations with selection then favoring male function in hermaphrodites through genetic modifiers that reduce female fertility. In contrast, in the monoecy–paradioecy pathway disruptive selection on gender variation within cosexual populations gradually increases gender specialization. Hence, in this pathway unisexual individuals arise as endpoints of the selective process, rather than as variants that initiate selective changes in hermaphroditic individuals. Which of these pathways likely accounts for the evolution of sexual dimorphism in *S. latifolia*?

We propose that the observed relations of female and male flower production to ramet size for hermaphroditic plants (Fig. 5) renders evolution via gynodioecy more likely than the monoecy–paradioecy pathway. The disruptive selection responsible for sexual specialization in the monoecy–paradioecy pathway requires genetic variation among plants in the relative production of female and male flowers. Such variation may exist in *S. latifolia*, but the extreme gender plasticity of monoecious individuals obscures the genetic influences on realized gender, greatly diminishing opportunities for disruptive selection. Evolution via androdioecy (see Muenchow 1998) also seems unlikely, because the limited production of female flowers by small plants creates a male-biased floral sex ratio within monoecious populations. In particular, the mean ratio of male to female flowers for the six monoecious populations that we studied (excluding female plants) exceeded that of the four dioecious populations by ~35%. In such an environment, a genetically male variant would suffer a considerable mating disadvantage because its presence aggravates an already male-biased sex ratio, precluding establishment of androdioecy.

In contrast, a genetic female in such a male-biased environment would benefit greatly, because she contributes through the under-represented sex role regardless of her size. As a consequence of the high reproductive value of ovules, gynodioecy should establish readily. Whether introduction and spread of female variants then facilitates subsequent establishment of genetic males depends on the relative success of resident hermaphroditic individuals in the presence of genetic females. According to this view, populations 6, 7, and 8 may represent transitional stages in the gynodioecious pathway. Whether this interpretation is correct is not clear. If true, it would be unusual because the gynodioecy pathway is not commonly found as an intermediate stage in the evolution of dioecy from monoecy (see Charlesworth and Charlesworth 1978*a* and Webb 1999 for further discussion of this point).

Our analysis of gender plasticity leads to different conclusions than would the common approach of inferring sexual strategies based on Lloyd's (1980*a*) gender measure, G_i. For example, contrast the interpretations arising from Figs. 5 and 6 for monoecious populations. Consideration of the relation of G_i to ramet size (Fig. 6) leads to the conclusion that large plants are more female than small plants. In contrast, the relations of female and male flower production to ramet size (Fig. 5) indicate that large hermaphroditic plants are more female, but no less male than small hermaphroditic plants. Such contrasting conclusions could lead to differing ecological and evolutionary interpretations of the causes and consequences of gender variation. In general, analyses that focus on relative investment in sex roles, including the standard theoretical approaches to sex allocation (Lloyd and Bawa 1984, Charnov and Bull 1985, Klinkhamer et al. 1997), can be misleading when plants differ in their total reproductive effort. This difficulty arises because arguments that emphasize relative allocation and contributions through either female or male function deflect attention from the fundamental dependence of reproductive success on the number of gametes contributed as a female or a male parent, or as both. Hence, a complete interpretation of genetic or environmental differences in resource status between plants requires a direct analysis of absolute investment in sex roles (see van Noordwijk and de Jong 1986, Venable 1992). This conclusion applies equally to species with unisexual flowers, such as *S. latifolia*, and to the more common condition, species with hermaphroditic flowers.

ACKNOWLEDGMENTS

We thank Jon Ågren, Patrik Dinnétz, and Colin Webb for comments on the manuscript, William Cole for technical advice, Nancy Frasier and Brendon Larson for field assistance, the Queen's University Biological Station for field accommodations, and the Natural Sciences and Engineering Research Council of Canada (NSERC) for funding this work through Research Grants to S. C. H. Barrett and L. D. Harder.

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