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Floral dimorphism in plant populations with combined versus separate sexes

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• *Background and Aims* Dimorphism among floral traits can evolve through variation in selection intensity between female and male performance, especially when sex functions are separated between flowers on a plant (monoecy), or between individuals (dioecy). In animal-pollinated species, male floral traits are predicted to be larger because competition for pollinators should favour larger displays. Floral dimorphism may be greater in dioecious than monoecious populations because of trade-offs between female and male function and opportunities for selfing in hermaphrodites.
• *Methods* These predictions were tested by surveying flower size, total flowers per inflorescence and daily display size in the insect-pollinated *Sagittaria latifolia* (Alismataceae). This species is useful for comparative analysis because populations are mostly either monoecious or dioecious. We examined floral dimorphism in 13 monoecious and 16 dioecious populations in eastern North America.
• *Key Results* Male flowers were significantly larger than female flowers in monoecious and dioecious populations, but there was no evidence for greater flower size dimorphism in dioecious populations despite their larger flower sizes overall. Although inflorescences in both dioecious and monoecious populations produced more male flowers, daily floral displays were significantly larger for female than male function due to more synchronous female flower opening. Daily floral display dimorphism was significantly greater in dioecious populations, due to greater female daily floral displays. There was a positive relationship between mean flower size and total flowers per inflorescence for both sexes in dioecious populations, but no relationship for either sex function in monoecious populations. Flower size dimorphism was positively correlated with the frequencies of females in dioecious populations.
• *Conclusions* The increased size and number of male flowers and protracted male floral displays in *S. latifolia* are probably shaped by sexual selection for more effective pollen dispersal.

Key words: Sexual dimorphism, flower size, daily floral display, sexual selection, sex ratios, monoecy, dioecy, *Sagittaria latifolia*.

INTRODUCTION

In animal-pollinated plants with unisexual flowers, the evolution of dimorphism between female and male floral traits is often interpreted as the consequence of different intensities of selection on female versus male function (Willson, 1979; Bell, 1985; Delph, 1996; Geber, 1999; Ashman, 2000; Delph and Ashman, 2006). Pollinator-meditated selection should result in larger male floral displays because male outcrossed siring success may be limited by access to mates, resulting in competition for pollinator visitation. In contrast, female function is more likely to be limited by resources than mating opportunities because of the requirements for fruit and seed maturation ('Bateman's principle' – Bateman, 1948; Arnold, 1994). However, sexual selection may also extend the duration over which male anthesis occurs, thus increasing the number and variety of mating partners (Lloyd and Yates, 1982; Lloyd, 1984). Indeed, male function in plants is commonly associated with flowering schedules and floral mechanisms that serve to restrict the amount of pollen presented each day (Harder and Thomson, 1989; Thomson, 2006). Thus, inflorescence displays probably reflect a selective compromise between the benefits of pollinator attraction and the costs associated with inefficient pollen dispersal and geitonogamous pollination.

Studies investigating flower and inflorescence size dimorphism in species with unisexual flowers often report larger flowers and more conspicuous floral displays associated with male versus female function (reviewed in Lloyd and Webb, 1977; Delph *et al.*, 1996; Eckhart, 1999; Costich and Meagher, 2001), although exceptions to this pattern do occur, particularly in tropical ecosystems (Delph *et al.*, 1996; Humeau *et al.*, 2003). Most attention on the dimorphism of floral traits has focused on dioecious species, with less attention to differences in female and male flowers and floral displays of hermaphroditic individuals in monoecious populations. As a result, the pattern and magnitude of floral and inflorescence dimorphism have rarely been explicitly contrasted between these two sexual systems (but see Costich and Meagher, 2001; Humeau *et al.*, 2003). The nature of selection on floral dimorphism may differ depending on whether the sex functions are combined or on separate individuals, and thus comparisons between monoecious and dioecious populations are likely to be instructive.

Several factors have the potential to influence floral display sizes and patterns of floral dimorphism in dioecious

populations compared with monoecious populations. First, gender specialization and reproductive compensation lead to the prediction that floral display sizes should be larger in dioecious than monoecious populations (see table 1 in Costich and Meagher, 2001). Trade-offs between female and male allocation in hermaphrodite plants do not exist in unisexual individuals, potentially allowing for greater gender specialization of flower size and floral display in dioecious populations (Charnov, 1982; Geber, 1999; Costich and Meagher, 2001; Humeau *et al.*, 2003). Second, the evolution of dioecy is predicted to be accompanied by selection for reduced recombination between loci controlling female and male phenotypes (Charlesworth, 1991, 2002; Nicolas *et al.*, 2005), and as a result sex-linked traits in females and males develop in association with the evolution of gender specialization, a process that is not possible for female and male flowers of monoecious plants. Finally, plants in dioecious populations are obligately outcrossing whereas those in monoecious populations may, if self-compatible, have the opportunity to self-fertilize because of hermaphroditic sex expression. Inbreeding in monoecious populations should reduce the intensity of sexual selection, thus decreasing the potential for the evolution of floral dimorphism.

Correlations among sexually dimorphic floral traits (e.g. flower size, flower number, daily floral display) are likely to have both genetic and environmental components and could potentially differ between sexual systems depending on the nature of sex-specific selection. Positive or null correlations between floral traits are expected when resources are abundant, while negative relationships could be indicative of trade-offs when resources are limited (Fenster and Carr, 1997; Sugiyama and Bazzaz, 1998). Theoretical models of the evolution of floral display generally assume a negative relationship between flower size and number (Morgan, 1993; Sakai, 1993; Harder and Barrett, 1996), although empirical investigations have provided mixed results (reviewed in Worley and Barrett, 2000; Sargent *et al.*, 2007). Investigating correlations among dimorphic floral traits may provide insights into how selection shapes patterns of dimorphism in dioecious and monoecious populations.

Flower sex ratios in populations also have the potential to influence the magnitude of dimorphism in floral traits (Ashman and Diefenderfer, 2001; Delph and Ashman, 2006). In dioecious populations, the number of flowering female and male ramets and their respective display sizes will affect mating opportunities, as does the ratio of male to female flowers in monoecious populations. For example, in populations with female-biased floral sex ratios positive selection might be expected to increase female attractiveness to pollinators, decreasing the likelihood of pollen limitation. In contrast, in male-biased populations there should be intense male–male competition for access to female mates and selection for male attractiveness to improve the ability to outcompete other males for pollinator visitation. To our knowledge, the relationships between floral sex ratios and patterns of floral dimorphism have not been investigated in natural populations of monoecious and dioecious plants.

Here, we compare variation in the dimorphism between female and male flowers and inflorescences in populations of the insect-pollinated, clonal, wetland species *Sagittaria latifolia* (Alismataceae) (Fig. 1). This species is of particular value for investigating the consequences of combined versus separate sexes on floral dimorphism because populations are commonly either monoecious or dioecious (Wooten, 1971; Dorken *et al.*, 2002). This intraspecific variation in sexual systems enables explicit comparisons of floral dimorphism between groups that, although exhibiting some ecological and genetic differentiation (see below), are fully interfertile and share more similar evolutionary histories than would two biological species. We addressed the following main questions: (1) What are the patterns of variation in flower size, the total number of flowers per inflorescence and the number of flowers displayed daily for the three sex phenotypes (females, males and hermaphrodites) within and among monoecious and dioecious populations? We predicted larger flower sizes overall in dioecious populations and a greater degree of dimorphism for these traits in dioecious than monoecious populations. (2) What is the relationship between the sex ratio of dioecious populations and the degree of dimorphism in floral and inflorescence traits? We predicted a negative relationship between female frequency and the magnitude of dimorphism because of more intense male–male competition when females are infrequent. (3) What are the relationships between flower size and flower number per inflorescence in monoecious and dioecious populations? If negative relationships between flower size and number occur, this could limit the extent to which dimorphism in floral display can evolve. Throughout we refer to differences between female and male flowers in monoecious and dioecious populations as 'floral dimorphism'. We reserve the term 'sexual dimorphism' for dioecious populations in which the different sex functions (female and male) occur on separate individuals.

MATERIALS AND METHODS

Plant species

Sagittaria latifolia is an emergent aquatic that grows in diverse wetland habitats in North America and propagates by both sexual and clonal reproduction. Monoecious and dioecious populations are fully cross-compatible (Dorken and Barrett, 2004*a*), producing fertile offspring, and often occur in close geographical proximity. However, populations of the two sexual systems remain ecologically segregated over much of their geographical range as a result of contrasting life-history traits (Dorken *et al.*, 2002; Dorken and Barrett, 2003, 2004*b*). Monoecious populations occur more commonly in disturbed ephemeral habitats (e.g. ditches, ponds), whereas dioecious populations are found mostly in permanent wetlands and freshwater marshes, particularly those associated with large rivers and lakes (Dorken and Barrett, 2003). In southern Ontario, Canada, where most of our study was conducted, populations of the two sexual systems differ in ramet size and flowering time, reflecting differences in the productivity of habitats and degree of plant competition.

Ramets in monoecious populations of *S. latifolia* are significantly smaller and flower earlier in the season (mid June to August), whereas dioecious ramets are larger and flower in late July to September (see Dorken and Barrett, 2003,

FIG. 1. Inflorescences in monoecious and dioecious populations of *Sagittaria latifolia* from southern Ontario, Canada. (A) Hermaphrodite inflorescence from a monoecious population showing both female (basal) and male flowers. Note this situation is unusual as alternate sex functions usually do not overlap within an inflorescence, as discussed in the Methods. (B) Female inflorescence from a dioecious population. Note the smaller flowers than in the male inflorescence but the large daily display size. (C) Male inflorescence from a dioecious population with large flowers and small daily floral display.

fig. 2). Ramets produce several inflorescences in a growing season comprising white, unisexual, 1-d, nectar-producing flowers that are visited by a wide spectrum of pollinators including bees, flies, beetles, butterflies and wasps (Muenchow and Delesalle, 1994; Vamosi *et al.*, 2006; Glaettli and Barrett, 2008). Flowers are arranged in whorls of three and open sequentially from the bottom to the top of the inflorescence. In monoecious populations, female flowers occur on basal nodes of the inflorescence and male flowers on upper nodes (Fig. 1A). It is infrequent for female and male sex function to overlap within an inflorescence; thus, monoecious plants are protogynous at the inflorescence level. However, estimates of mating patterns in monoecious populations using allozyme markers indicate considerable selfing ($s = 0{\cdot}41$, $n = 6$ populations; Dorken *et al.*, 2002) as a result of inter-ramet geitonogamy.

Glasshouse common garden studies of numerous monoecious and dioecious populations of *S. latifolia* over the past decade have established that differences in life-history traits, including flower size and inflorescence dimorphism, between the sexual systems have a significant heritable component and are retained from year to year (Dorken *et al.*, 2002; Dorken and Barrett, 2004*a*; S. C. H. Barrett unpubl. observ.). Indeed, in a glasshouse study of phenotypic plasticity, Dorken and Barrett (2004*b*) demonstrated significantly larger flowers in dioecious ($n = 5$ populations) than monoecious populations ($n = 7$ populations), and that manipulated variation in nutrient availability had relatively little influence on this flower size variation.

Field survey of floral dimorphism

To investigate dimorphism of female and male floral traits in *S. latifolia*, we sampled 16 dioecious and 13 monoecious populations during the summers of 2005 and 2006. The vast majority of populations (25 of 29) were sampled in southern Ontario with a few in Quebec (three) and upper New York State, USA (one). The localities of populations are provided in the Appendix. In each population we sampled inflorescences randomly at 2-m intervals or more in an effort to avoid including ramets from the same clone. For each inflorescence, we recorded the total number of flowers and their sex function (pistillate or staminate; hereafter female or male flowers) and the daily display size (number of flowers in anthesis). In *S. latifolia* the sex function of buds and senesced flowers are easily determined, enabling measurement of the total number of flowers per inflorescence. Throughout we refer to female and male sex function in the context of the phenotypic gender of flowers and not the fitness achieved through each sex function. The mean ($\pm$ s.e.) number of flowering ramets sampled per population was $52{\cdot}9 \pm 4{\cdot}7$ and $62{\cdot}4 \pm 4{\cdot}1$ for dioecious and monoecious populations, respectively. Using digital callipers, we measured the widest diameter (to the nearest 0·01mm) of two to four flowers per flowering ramet and recorded their sex function and position within the inflorescence. We measured a mean ($\pm$ s.e.) of 127 ($\pm 9{\cdot}1$) female and 160 ($\pm 10{\cdot}9$) male flowers in monoecious populations and 115 ($\pm 13{\cdot}0$) female and 159 ($\pm 19{\cdot}3$) male flowers in dioecious populations. We also measured leaf mid-vein length (hereafter LML) of the subtending leaf to the inflorescence (to the nearest 0·5 cm) to control for plant size effects on floral and inflorescence traits. This measurement is positively correlated with flowering ramet height in *S. latifolia* (Sarkissian *et al.*, 2001).

To quantify differences in floral and inflorescence traits between female and male function, we estimated floral dimorphism for each trait and population as the ratio of the average male trait to female trait. Thus, values of 1·0 indicate that female and male traits are equal and values greater or less than 1·0 occur when either male or female traits are larger, respectively.

Comparing components of floral display

To investigate differences in total number of flowers per inflorescence between the three sex phenotypes (i.e. females, hermaphrodites and males) we used a nested analysis of covariance with the following descriptor variables: sexual system, population nested within sexual system, and LML as a covariate to account for any allometric relationships between ramet size and components of floral display. *F*-values reported are from this analysis. For this and all

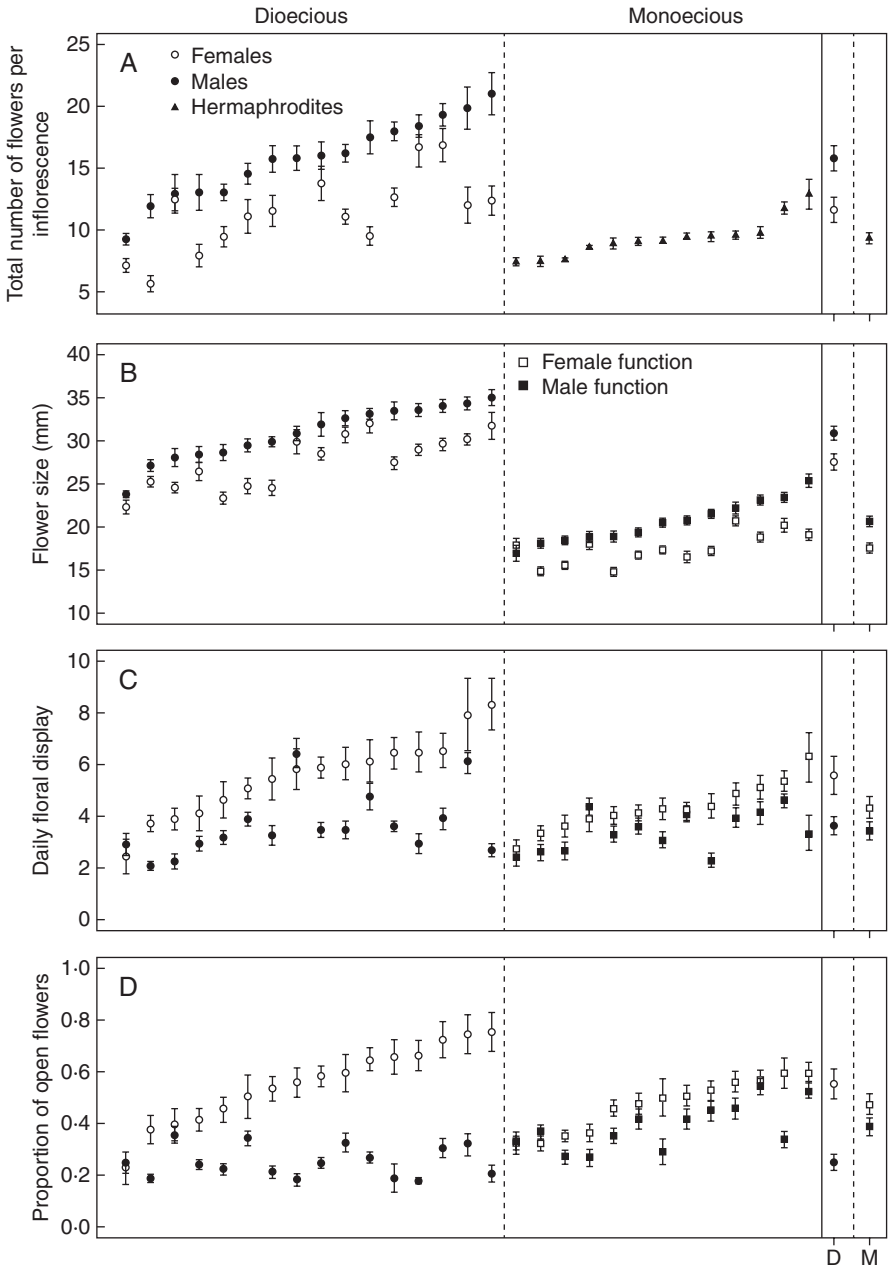

FIG. 2. Features of floral display in 16 dioecious and 13 monoecious populations of *Sagittaria latifolia*. (A) Total number of flowers per inflorescence, (B) flower size (mm), (C) daily floral display and (D) proportion of open flowers per day. All values are means and (± s.e.); grand means (± s.e.) for the dioecious (D) and monoecious (M) populations are indicated on the right-hand side of each figure. Females (A–D), males (A–D), hermaphrodites (A), female function (B–D) and male function (B–D) are as indicated in the key.

subsequent statistical analysis we used the statistical package R (version 2·8·1; R Development Core Team, 2008). Sexual system was a fixed factor whereas population was random. Because no transformation allowed us to meet test assumptions for total number of flowers per inflorescence, we performed randomization tests (1000 permutations) on mean squares (Manly, 1997) to estimate *P*-values. If fewer than 5 % of the permutations resulted in a larger mean square than the one obtained with the observed data, we considered the tested effect to be significant. We conducted randomizations as follows: we tested (1) the sexual system effect by randomizing entire populations among sexual systems, (2) the effect of LML by performing permutations within populations, (3) the population effect by randomizing individuals among populations within each sexual system, (4) the sexual system × LML interaction by performing permutations of entire populations between sexual systems combined with the permutation of LML within populations, and (5) population × LML by a randomization of data across the entire dataset.

To detect differences in the number of flowers per inflorescence between females and males within dioecious populations we also performed an analysis of covariance. We

considered populations (random), sex (fixed) and LML, the last of these as a covariate. Because no transformation of the data allowed us to meet the test's assumptions, we performed randomization tests as described previously and adapted the pattern of permutation for each tested effect.

We used a similar nested analysis of covariance to test for differences in flower size and daily floral display size. We added sex function as a fixed factor to detect differences between females and males in dioecious populations, and female and male sex function in monoecious populations. Again we performed randomization tests and accommodated our permutation patterns for each effect, as transformation of the data did not meet the test's assumptions. We calculated the percentage difference in average flower size between monoecious and dioecious populations by taking the difference between average dioecious and monoecious flower sizes and dividing by the average monoecious flower size.

Relationships among components of floral display and population sex ratios

We investigated the effect of population sex ratio (proportion of flowering female ramets in dioecious populations) and population floral sex ratio (proportion of female flowers in monoecious populations) on floral dimorphism in flower size and daily floral display. Similarly, we investigated the effect of population sex ratio on sexual dimorphism in total number of flowers per inflorescence for dioecious populations. We used regression to test for the significance of the slopes for each sexual system separately and confirmed that residuals were normally distributed.

To investigate relationships between flower size and number, we performed correlations between flower size and the total number of flowers per inflorescence divided by LML for each sexual morph within dioecious populations, and for female and male sex function within monoecious populations. Because of the large number of correlations performed, we used the R package 'qvalue' to account for the false discovery rate and to estimate the overall proportion of true null hypotheses (Storey, 2002; Verhoeven *et al.*, 2005). We also explored the relationships between flower size and flower number divided by LML for each sex function among populations for both sexual systems. Similarly, we tested whether daily display size was related to total inflorescence size both within and among populations.

RESULTS

Differences in inflorescence size among females, males and hermaphrodites and correlations with ramet size

Flowering ramets in monoecious populations produced significantly fewer flowers per inflorescence than female and male ramets in dioecious populations (Table 1, Fig. 2A). Within dioecious populations, male ramets produced 47 % more flowers per inflorescence than female ramets ($F_{1,884} = 238{\cdot}4$, $P < 0{\cdot}001$; Fig. 2A). Similarly, inflorescences in monoecious populations produced 42 % more male flowers than female flowers. However, within both sexual systems there was considerable variation among populations in the total number of flowers per inflorescence and in the number of flowers of each sex function (Table 1, Fig. 2A).

TABLE 1. *Nested analysis of covariance for the total number of flowers per inflorescence in monoecious and dioecious populations of* Sagittaria latifolia

Source of variation	d.f.	MS	*P*
Sexual system	1	9786	***
Leaf mid-vein length (LML)	1	10 066	***
Population (sexual system)	27	230	***
Sexual system × LML	1	3331	***
Population (sexual system) × LML	27	36	
Residuals	1634	18	

We treated sexual system (fixed) and population within sexual system (random) as qualitative variables and leaf mid-vein length (LML) as a covariate to control for ramet size.

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

LML, a proxy for ramet size, was positively related to inflorescence size among all populations, but the slope of this relationship differed among the three sex phenotypes. Indeed, we detected a significant sexual system × LML interaction when comparing sexual systems (Table 1). The slope of the regression between LML and total number of flowers per inflorescence was larger for dioecious ($\dot{\beta} = 1{\cdot}27$) than monoecious populations ($\dot{\beta} = 0{\cdot}85$). We also detected a significant sex function × LML interaction within dioecious populations ($F_{1,884} = 15{\cdot}9$, $P < 0{\cdot}01$) with a greater slope in males ($\dot{\beta} = 1{\cdot}47$) than females ($\dot{\beta} = 0{\cdot}96$).

Size dimorphism between female and male flowers

As predicted, mean flower size differed significantly between monoecious and dioecious sexual systems. Flowers

TABLE 2. *Nested analysis of covariance for daily floral display and flower size in monoecious and dioecious populations of* Sagittaria latifolia

	Daily floral display			Flower size	
Source of variation	d.f.	MS	*P*	MS	*P*
Sexual system	1	81·1		44486·00	***
Leaf mid-vein length (LML)	1	1285·0	***	11 001·00	***
Sexual function	1	828·3	***	5039·00	***
Population (sexual system)	27	36·7	***	263·00	***
Sexual system × LML	1	22·2		140·00	
Sexual system × sexual function	1	149·5	***	1·00	
LML× sexual function	1	97·7	***	0·44	
Population (sexual system) × LML	27	15·2	**	35·00	
Population (sexual system) × sexual function	27	15·7	***	26·00	
Sexual system × LML × sexual function	1	12·5		0·06	
Population (sexual system) × LML × sexual function	27	8·3		15·00	
Residuals	1634	5·4		12·00	

We treated sexual system (fixed), populations within sexual systems (random) and sex function (fixed) as qualitative descriptive variables, and used leaf mid-vein length (LML) as a covariate to control for ramet size.

* $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

(female and male combined) in dioecious populations were on average 53 % larger than those in monoecious populations (Table 2, Fig. 2B). Size dimorphism between female and male flowers was evident within both monoecious and dioecious populations with male flowers on average 15 % larger than female flowers, regardless of sexual system (Table 2, Fig. 2B). Accordingly, there was no significant difference between sexual systems in the magnitude of flower size dimorphism [Table 2; non-significant sexual system × sex function interaction; mean (± s.e.) dioecious populations = 1·12 ± 0·02; monoecious populations = 1·18 ± 0·03]. Flower size differed significantly among populations for both monoecious and dioecious sexual systems (Table 2, Fig. 2B), and for both sexual systems LML was positively correlated with flower size (Table 2). We found a negative correlation between flower position within an inflorescence and flower size weighted by the size of the ramet (i.e. LML; $r = -0{\cdot}66$, d.f. $= 24$, $P < 0{\cdot}001$), reflecting the trend that the size of flowers decreases from the bottom to the top of inflorescences for both sex functions and sexual systems.

Dimorphism in female and male daily floral display size

Dimorphism between female and male daily floral display size was evident for both sexual systems. There were more open flowers each day for female function than male function in both unisexual and hermaphroditic plants (Table 2, Fig. 2C, D). However, in contrast to flower size dimorphism, the magnitude of daily floral display dimorphism varied between monoecious and dioecious sexual systems (Table 2; significant sexual system × sex function interaction). The number of male flowers in anthesis on a given day was similar for both male and hermaphroditic plants, whereas for female function monoecious populations displayed on average 24 % more open flowers in female than male function and in dioecious populations this value was 54 % (Fig. 2C, D). Mean (± s.e.) dimorphism (i.e. ratio of male to female flowers) in daily floral display was therefore 0·68 ± 0·05 for dioecious populations and 0·80 ± 0·04 for monoecious populations.

We detected significant variation in the number of open flowers per inflorescence among populations for both sexual systems (Table 2, Fig. 2C). Similarly, the magnitude of dimorphism in daily floral display differed significantly among populations for both female and male function (population × sex function interaction; Table 2, Fig. 2C). Finally, LML was positively correlated with daily display size and correlation coefficients differed significantly between sex functions, as well as among populations within each sexual system (Table 2).

Relationships between dimorphism in floral traits and sex ratios

Among dioecious populations there was a positive relationship between the proportion of flowering ramets that were female and both the degree of dimorphism in flower size ($R^2 = 0{\cdot}28$, $\beta = 0{\cdot}23$, d.f. $= 14$, $P < 0{\cdot}05$; Fig. 3A) and the total number of flowers per inflorescence ($R^2 = 0{\cdot}24$, $\beta = 1{\cdot}0$, d.f. $= 14$, $P = 0{\cdot}053$; Fig. 3B). In contrast, within monoecious populations the proportion of female flowers within a population correlated negatively with dimorphism in daily floral display ($R^2 = 0{\cdot}59$, $\beta = -1{\cdot}06$, d.f. $= 11$, $P < 0{\cdot}01$). Dimorphism in flower size and daily floral display were positively correlated in monoecious ($r = 0{\cdot}56$, d.f. $= 11$, $P < 0{\cdot}05$) but not in dioecious populations.

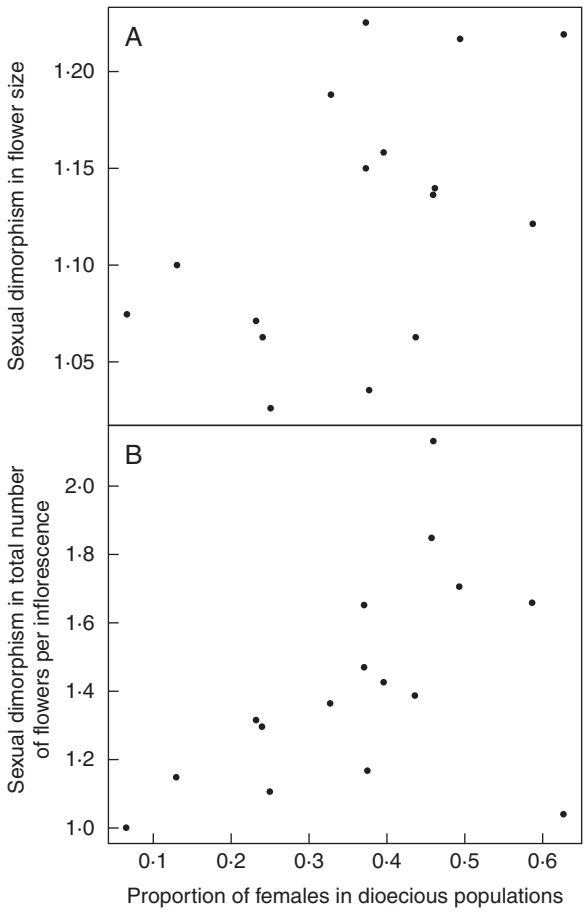

FIG. 3. Relationship between female frequency and (A) flower size dimorphism ($R^2 = 0{\cdot}28$, $\beta = 0{\cdot}23$, $P < 0{\cdot}05$), and (B) dimorphism in total number of flowers per inflorescence ($R^2 = 0{\cdot}24$, $\beta = 1{\cdot}0$, $P = 0{\cdot}053$) among 16 dioecious populations of *Sagittaria latifolia*.

Correlations between flower size and number

We investigated correlations between flower size and the total number of flowers per inflorescence/LML within each population for each sex function (i.e. 32 and 26 correlations for dioecious and monoecious populations, respectively). For dioecious populations, 47 % of the correlations were negative and 53 % positive; overall, 68 % of these correlations were estimated to be significant. In contrast, all but one correlation in monoecious populations was negative, and 72 % of these negative correlations were estimated to be significant. No differences in the direction or proportion of significant correlations were detected between sex functions. We also used population means to test for correlations between flower size and flower number/LML among dioecious populations and detected no significant relationship between mean total number of flowers per inflorescence scaled by ramet size and mean flower size for both females ($r = 0{\cdot}13$, d.f. $= 14$, $P =$

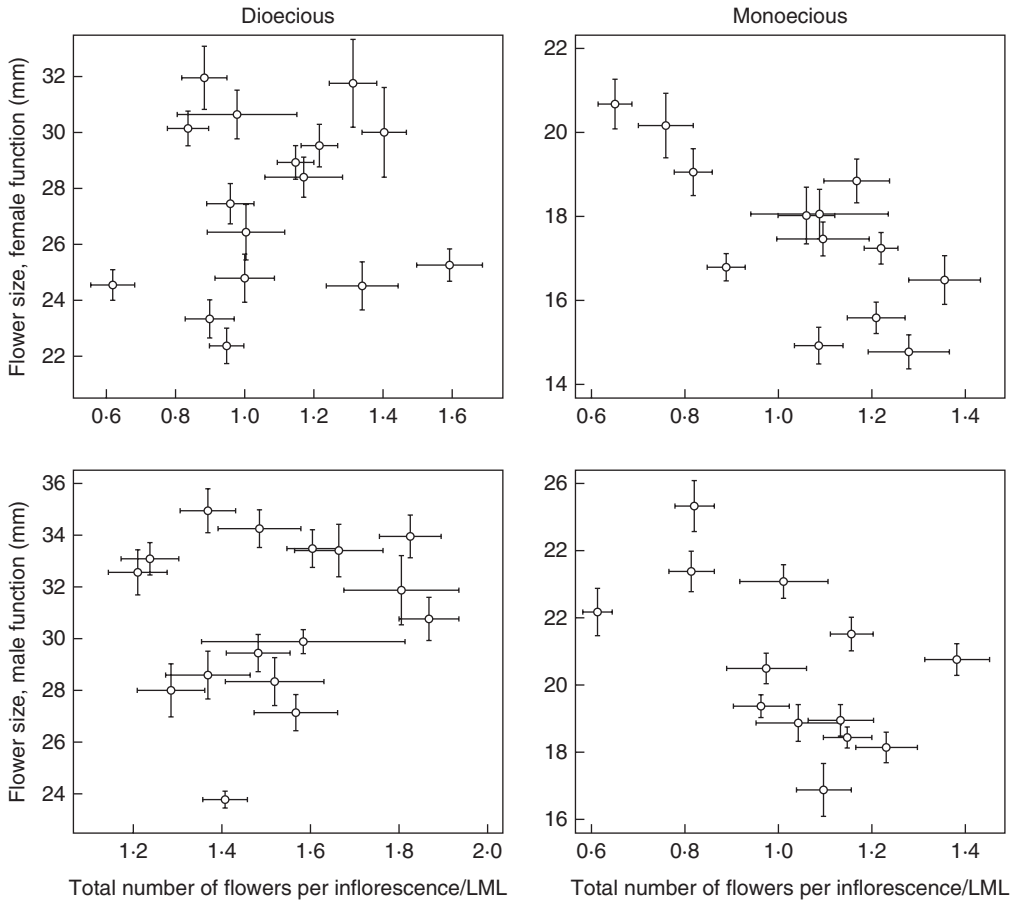

FIG. 4. Relationship between mean (± s.e.) total number of flowers per inflorescence/leaf mid-vein length (LML) and mean (± s.e.) flower size for female (upper graphs) and male (lower graphs) function in 16 dioecious (left) and 13 monoecious (right) populations of *Sagittaria latifolia*.

0·63; Fig. 4) and males ($r = 0{\cdot}21$, d.f. = 14, $P = 0{\cdot}43$; Fig. 4). In contrast, among monoecious populations there were significant negative relationships between flower size and flower number per LML for both female ($r = -0{\cdot}73$, d.f. = 11, $P = 0{\cdot}004$; Fig. 4) and male function ($r = -0{\cdot}73$, d.f. = 11, $P = 0{\cdot}04$; Fig. 4).

Relationship between daily and total floral display

We estimated that 95 % of the correlations between daily and total floral display were significant for female function and 74 % for male function, all of which were positive relationships. Ninety-five per cent were estimated to be significant for monoecious populations and 76 % for dioecious populations. Using population means, we found positive correlations between the mean total number of flowers per inflorescence and the mean daily floral display for each sex function. In dioecious populations, this correlation was significant for males ($r = 0{\cdot}52$, d.f. = 14, $P < 0{\cdot}05$; Fig. 5) and marginally significant for females ($r = 0{\cdot}47$, d.f. = 14, $P = 0{\cdot}07$; Fig. 5). In monoecious populations, correlations between daily floral display and total number of flowers per inflorescence were highly significant for female function ($r = 0{\cdot}86$, d.f. = 11, $P < 0{\cdot}001$; Fig. 5) but non-significant for male function ($r = 0{\cdot}23$, d.f. = 11, $P = 0{\cdot}45$; Fig. 5).

DISCUSSION

In this study, we compared floral and inflorescence traits between monoecious and dioecious populations of *Sagittaria latifolia*, a clonal aquatic plant. We found that hermaphrodites from monoecious populations had fewer and smaller flowers per inflorescence than individuals from dioecious populations. We also detected dimorphism between female and male components for all traits that we investigated; male flowers were larger than female flowers, although the magnitude of flower size dimorphism was similar between monoecious and dioecious populations. Significantly, daily floral display was larger for female than male function for both sexual systems because female flowers open more synchronously than male flowers. Contrary to our predictions, we detected positive relationships between female frequency and dimorphism in flower size and total flowers per inflorescence in dioecious populations. Our discussion focuses on the potential ecological and evolutionary causes and consequences of these results in the context of pollinator behaviour, mating patterns and sexual selection.

Variation in flower size dimorphism

Flower size dimorphism involving larger male than female flowers is commonly observed in plants with unisexual

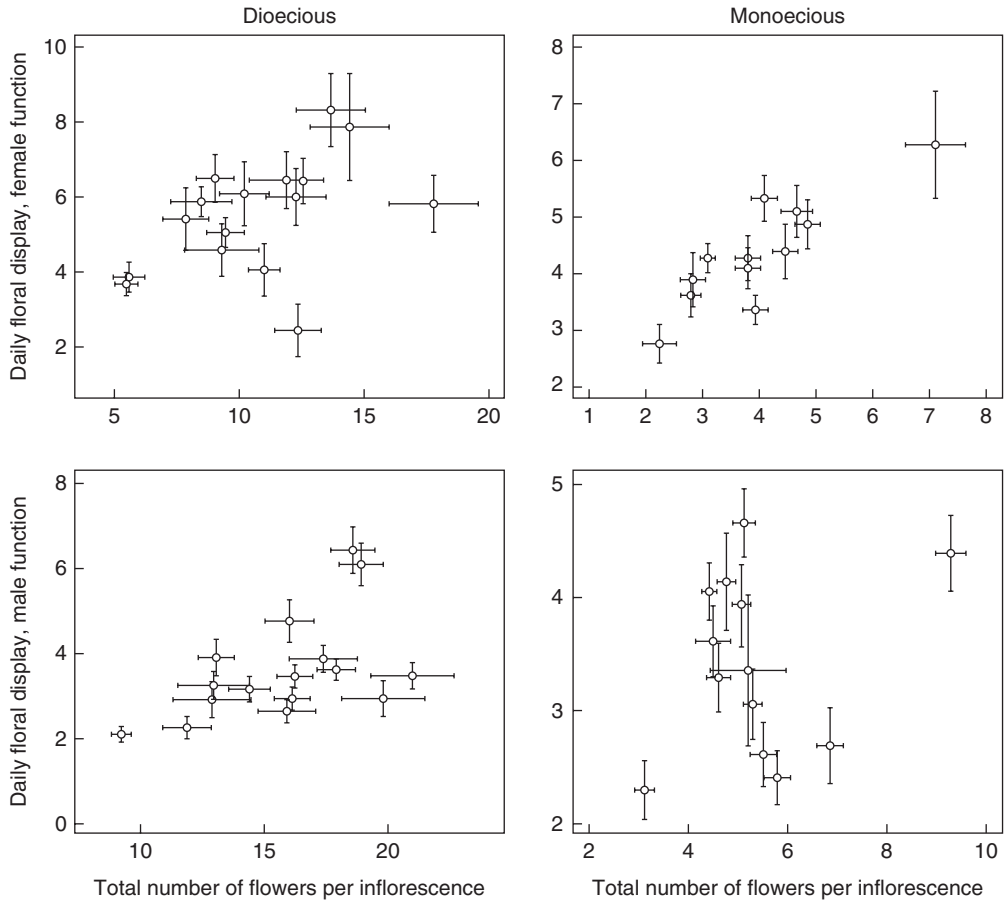

FIG. 5. Relationship between mean (± s.e.) total number of flowers per inflorescence and mean (± s.e.) daily floral display for female (upper graphs) and male (lower graphs) function in 16 dioecious (left) and 13 monoecious (right) populations of *Sagittaria latifolia*.

flowers, especially in animal-pollinated plants of the temperate zone (reviewed in Delph *et al.*, 1996; Eckhart, 1999). Our results corroborate earlier studies of *S. latifolia* reporting larger male than female flowers based on a restricted sampling of populations (Muenchow and Delesalle, 1994; Sarkissian *et al.*, 2001; Glaettli and Barrett, 2008). However, contrary to our predictions we found no evidence that the magnitude of flower size dimorphism was greater in dioecious than monoecious populations. Costich and Meagher (2001) also failed to show differences in the degree of flower size dimorphism in common glasshouse comparisons of monoecious and dioecious populations of *Ecballium elaterium* despite overall larger flower sizes in the latter. There are several potential explanations for similar amounts of relative flower size dimorphism in populations of the two sexual systems.

Pollinator observations of *S. latifolia* indicate that both monoecious and dioecious populations are visited by similar guilds of generalist pollinators, including bees, flies, beetles, butterflies and wasps (Muenchow and Delesalle, 1994; Vamosi *et al.*, 2006; Glaettli and Barrett, 2008). This may limit opportunities for pollinator-mediated differences in the intensity of sex-specific selection between the sexual systems. It has also been suggested that the degree of flower size dimorphism between females and males in animal-pollinated plants may be constrained if pollinators preferentially visit the larger-flowered sex, resulting in restricted pollen transfer to females (Vamosi and Otto, 2002). However, manipulation of flower size in experimental arrays of *S. latifolia* indicated that visitation to female flowers was relatively constant irrespective of flower size whereas increased male flower size was positively related to rates of visitation (Glaettli and Barrett, 2008). Thus, pollinator behaviour seems unlikely to limit the evolution of flower size dimorphism.

We observed a substantial amount of phenotypic variation in the degree of flower size dimorphism among populations of both sexual systems. For example, although in all 16 dioecious populations male flowers were on average larger than female flowers, in some populations this difference was negligible whereas in others male flowers (e.g. CMP2-ON see Appendix) were up to 33 % larger than female flowers (Fig. 2B). If a component of this variation in flower size is heritable, sex-specific selection imposed by pollinator preferences could adjust patterns of dimorphism within populations depending on the composition of the local pollinator fauna. Although previous common garden glasshouse studies have demonstrated that differences in flower size between the sexual systems have a genetic component (Dorken and Barrett, 2004*b*), more detailed common garden experiments that examine variation in dimorphism and measure the

heritability of floral traits would be useful for determining the genetic basis of floral dimorphism (Meagher, 1994, 1999; Ashman, 1999; Costich and Meagher, 2001). Also, studies of phenotypic selection on floral traits imposed by different pollinators (for example reviewed in Herrera *et al.*, 2006; Harder and Johnson, 2009) could be profitably undertaken to investigate the ecological and evolutionary mechanisms maintaining the striking variation in flower size dimorphism in *S. latifolia*.

Flower size dimorphism may also be influenced, in part, by the protective function of perianth parts with the minimum size determined by the size required to cover female and male reproductive organs (Delph *et al.*, 1996). According to this hypothesis overall flower size differences may be influenced by the relative size of female and male reproductive organs. To assess this possibility, we conducted a preliminary study to examine the relationship between flower size and the diameter of the carpel dome (to the nearest mm) and the number of stamens for female and male flowers, respectively. Plants from three monoecious and four dioecious populations were grown under uniform glasshouse conditions. We performed nested analysis of covariance for both carpel dome size and number of stamens per flower with LML and flower size as covariates; *P*-values were estimated by randomization of mean squares, as described for earlier analyses. This analysis demonstrated that the patterns of flower size variation were similar to that observed in the field. Mean ($\pm$ s.e.) number of stamens per male flower was larger in dioecious ($29{\cdot}4 \pm 5{\cdot}5$) than monoecious individuals ($20{\cdot}8 \pm 5{\cdot}1$; $F_{1,5} = 51{\cdot}4$, $P < 0{\cdot}001$) and mean ($\pm$ s.e.) carpel dome diameter of female flowers was larger in dioecious ($6{\cdot}0 \pm 0{\cdot}9$ mm) than monoecious individuals ($4{\cdot}9 \pm 0{\cdot}7$ mm; $F_{1,5} = 41{\cdot}4$, $P < 0{\cdot}01$). Consistent with the protection hypothesis, we detected a strong relationship between flower size and the size of reproductive organs for male ($F_{1,225} = 93{\cdot}1$, $P < 0{\cdot}001$) and female function ($F_{1,103} = 5{\cdot}19$, $P < 0{\cdot}001$), regardless of the sexual system. Thus, the protective role of the perianth appears to contribute to the observed patterns of flower size dimorphism in *S. latifolia*.

A final explanation that could account for the similarity in relative flower size dimorphism between monoecious and dioecious populations of *S. latifolia* concerns the mating patterns and fitness of selfed offspring in monoecious populations. We had predicted that because primary selfing rates in monoecious populations of *S. latifolia* are substantial, averaging around 40 %, this could reduce the intensity of sexual selection and limit the extent of flower size dimorphism compared with dioecious populations, where all offspring are outcrossed. However, marker-based estimates of inbreeding depression in monoecious populations indicate that few if any selfed seed survive to maturity (Dorken *et al.*, 2002). If most flowering plants in monoecious populations are outcrossed, it is possible that selection intensities on flower size dimorphism may be equivalent to those in dioecious populations, resulting in similar degrees of relative dimorphism.

Inflorescence size and daily floral display

Inflorescence size and flowering schedules are important components of floral display in animal-pollinated plants, influencing pollen dispersal and mating patterns. We detected sexual dimorphism for total number of flowers per inflorescence in dioecious populations, and dimorphism between the number of female and male flowers produced by inflorescences in monoecious populations. In both cases significantly more male flowers were produced than female flowers. This pattern is commonly observed in species with unisexual flowers and is consistent with the widely held expectation that male function is less costly per flower than female function and that selection to increase mating opportunities should favour larger male floral displays (Lloyd and Webb, 1977; Willson, 1979; Lloyd, 1984; Delph, 1996; Eckhart, 1999; Costich and Meagher, 2001). However, the functionally significant aspect of floral display is not the total number of flowers produced by an inflorescence but rather the number of flowers in anthesis each day and daily display size will depend on the nature of flowering schedules. Our observations in *S. latifolia* indicate that contrasting flowering schedules between female and male function play an important role in determining differences in daily display size.

In both monoecious and dioecious populations of *S. latifolia*, daily floral displays were generally larger for female than for male function. This pattern has been previously reported in several *Sagittaria* species, including *S. latifolia* (e.g. Muenchow and Delesalle, 1994; Sarkissian *et al.*, 2001; Huang *et al.*, 2006). Unlike flower size dimorphism, differences in daily floral display dimorphism were considerably larger in dioecious than monoecious populations. This difference occurs because female daily display size is significantly larger in dioecious than monoecious populations. On average, females in dioecious populations had 11·28 flowers open on a given day versus only 4·21 female-functioning flowers in monoecious populations, whereas male display sizes are equivalent between the two sexual systems. Female flowers open more synchronously, whereas the period of male flower anthesis is more gradual with male inflorescence function lasting much longer. This can be illustrated for dioecious populations by dividing the total number of flowers per inflorescence by the number in anthesis per day. Female function lasts on average 1·8 d whereas male function is completed in 3·9 d. This difference suggests that contrasting sex-specific selective forces have shaped male and female daily floral displays. Larger female than male daily display sizes may function to compensate for their smaller flower sizes and because they also lack pollen rewards. In contrast, the smaller daily floral display size and extended flowering associated with male function probably provides more effective pollen dispersal and greater mating opportunities (Thomson and Barrett, 1981; Lloyd and Yates, 1982; Lloyd, 1984).

Relationship between flower size and number

Our motivation for investigating the relationship between flower size and number was because of the potential for trade-offs between these components of display to influence the evolution of floral dimorphism (Meagher, 1994, 1999; Schemske and Ågren, 1995). For example, with a limited resource pool selection for increased male flower number could prevent increases in flower size, thus reducing opportunities for the evolution of flower size dimorphism. Evidence for such trade-offs is mixed (reviewed in Worley and Barrett,

2000; Sargent *et al.*, 2007) and in our study we found the relationship between flower size and number depended on sexual system. We detected no consistent relationship between these traits in females and males both within and among dioecious populations, whereas within and among monoecious populations flower size and number were negatively related, potentially indicating the possibility of trade-offs, although if these do occur they do not appear to influence the magnitude of flower size dimorphism.

It would be premature to conclude that variation in genetic correlations is responsible for the pattern we have observed in *S. latifolia* populations. Our studies were entirely phenotypic in nature and there are a variety of other factors that can influence the existence of trade-offs between flower size and number (reviewed in Worley and Barrett, 2000). However, the apparent differences that we revealed in the relationships between these two components of floral display between dioecious and monoecious populations suggest that further in-depth studies would be profitable. Selection experiments in *S. latifolia* would be useful to explore the extent to which flower size and number in plants with combined versus separate sexes are subject to genetic constraints.

Relationship between sex ratio and floral dimorphism

Variation in sex ratios in gender-dimorphic populations may alter the context for the evolution of floral traits and the expression of sexual dimorphism (Ashman and Diefenderfer, 2001; Delph and Ashman, 2006). However, to our knowledge there have been no extensive surveys of the relationship between sex ratios and sexual dimorphism in dioecious plant populations. Phenotypic sex ratios varied substantially among the dioecious populations of *S. latifolia*, with females ranging in frequency from 0·09 to 0·79. In populations with strongly female-biased sex ratios, decreased male–male competition for mating opportunities should result in positive selection for increased female flower size/number. This would relieve pollen limitation and could lead to a reduction in overall sexual dimorphism (see Ashman and Diefenderfer, 2001; Vamosi and Otto, 2002). However, we obtained results that were opposite to this prediction for both flower size and flower number dimorphism (Fig. 3A, B). Populations with more female-biased sex ratios exhibited greater sexual dimorphism than populations with male-biased sex ratios. This result is inconsistent with predictions based on sex-specific selection and at this stage is difficult to explain. Various other factors influence ramet sex ratios in populations of *S. latifolia*, including non-equilibrium conditions, a common feature of clonal populations, and geographical gradients in local resources potentially influencing female versus male flowering (Barrett *et al.*, 2010; S. B. Yakimowski and S. C. H. Barrett, unpubl. data). Further studies are required to determine the mechanism underlying the patterns we detected between phenotypic sex ratios and sexual dimorphism.

In conclusion, we have documented floral dimorphism for a range of flower and inflorescence traits in populations of *S. latifolia* with combined versus separate sexes. It is likely that the patterns of floral dimorphism we report are associated with differences in the selective optima for female and male function associated with Bateman's principle, although other factors including the protective function of perianth parts may also be involved. Several theories also predict that gender specialization and reproductive compensation should lead to larger flowers and inflorescences in dioecious than monoecious populations (summarized in Costich and Meagher, 2001). Our results are consistent with this expectation but we cannot rule out that some of these differences arise as allometric consequences of the life-history correlates of monoecy and dioecy owing to differences in the vegetative size of plants of the two sexual systems (Dorken and Barrett, 2003, 2004*b*). Large-scale common garden studies would be useful to investigate allometric relationships between vegetative and reproductive traits in *S. latifolia*.

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APPENDIX

List of *Sagittaria latifolia* populations sampled. The province or state of each population follows the hyphen for each ID: NY, New York; ON, Ontario; QB, Quebec. Sexual system is identified as monoecious (M) or dioecious (D). Ramet-level sex ratios are reported as proportion of female ramets, and flower-level sex ratios as proportion of female flowers. We also report the mean flower size and daily floral display for female and male function with standard error for each. The number of individuals (n) for which female and male flowers were measured accompanies flower size means and is the same for number of individuals counted for daily floral display. Dimorphism of flower size and daily floral display dimorphism were calculated as the mean male trait divided by mean female trait (values >1 indicate male trait larger than female trait, values <1 indicate female trait larger than male trait).

Population ID	Sexual system	Latitude (°N)	Longitude (°W)	Proportion ramets female	Proportion flowers female	Mean female flower size (mm) (± s.e.)	n	Mean male flower size (mm) (± s.e.)	n	Mean flower size dimorphism	Mean female daily floral display (± s.e.)	Mean male daily floral display (± s.e.)	Mean daily floral display dimorphism
ACT-ON	D	44·55	77·32	0·37	0·26	23·34 (0·68)	16	28·59 (0·92)	27	1·23	5·44 (0·81)	3·26 (0·34)	0·60
CGM-ON	D	45·33	77·59	0·46	0·31	24·56 (0·55)	17	27·99 (1·04)	20	1·14	3·88 (0·40)	2·25 (0·27)	0·58
FHR-ON	D	45·60	77·32	0·13	0·23	31·77 (1·57)	17	34·94 (0·86)	37	1·10	5·82 (0·77)	6·43 (0·56)	1·10
HMR-ON	D	42·00	82·51	0·46	0·34	30·15 (0·61)	53	34·26 (0·72)	63	1·14	5·06 (0·40)	3·89 (0·26)	0·77
DFF-ON	D	44·05	79·48	0·24	0·32	22·38 (0·62)	48	23·78 (0·32)	53	1·06	3·71 (0·28)	2·08 (0·14)	0·56
KGM-ON	D	44·29	76·44	0·25	0·25	30·00 (1·61)	16	30·77 (0·83)	28	1·03	7·88 (1·43)	6·11 (0·45)	0·78
LNR-QC	D	46·04	73·18	0·44	0·35	30·65 (0·86)	18	32·56 (0·86)	21	1·06	6·50 (0·67)	3·90 (0·44)	0·60
LYD-ON	D	43·84	78·97	0·37	0·29	29·54 (0·76)	20	33·97 (0·81)	34	1·15	4·10 (0·67)	2·94 (0·27)	0·72
PBN-QC	D	45·59	75·17	0·39	0·31	28·93 (0·60)	30	33·49 (0·71)	46	1·16	6·43 (0·59)	3·61 (0·21)	0·56
PTC-QC	D	45·46	76·30	0·23	0·32	26·45 (1·00)	16	28·33 (0·92)	27	1·07	4·63 (0·70)	3·19 (0·27)	0·69
RNW-ON	D	45·49	76·75	0·33	0·34	24·79 (0·87)	18	29·44 (0·72)	31	1·19	6·11 (0·84)	4·74 (0·54)	0·78
RRM-ON	D	42·29	82·48	0·37	0·35	31·95 (1·13)	32	33·10 (0·63)	54	1·04	8·31 (1·00)	2·67 (0·24)	0·32
SPT-ON	D	42·30	82·53	0·49	0·38	27·46 (0·72)	28	33·41 (1·01)	29	1·22	6·04 (0·63)	3·48 (0·32)	0·58
TR2-ON	D	45·62	77·40	0·07	0·25	25·28 (0·57)	27	27·15 (0·68)	34	1·07	5·89 (0·40)	3·47 (0·31)	0·59
UBC-ON	D	44·82	79·61	0·59	0·46	28·42 (0·71)	24	31·87 (1·36)	17	1·12	6·46 (0·78)	2·94 (0·37)	0·46
WLD-NY	D	42·42	77·09	0·63	0·63	24·52 (0·85)	20	29·88 (0·48)	12	1·22	2·45 (0·68)	2·92 (0·42)	1·19
CMP2-ON	M	44·30	77·83	0·00	0·43	19·07 (0·55)	33	25·32 (0·74)	36	1·33	4·12 (0·31)	3·61 (0·31)	0·88
COC-ON	M	44·14	77·79	0·00	0·28	14·77 (0·40)	20	18·95 (0·48)	30	1·28	2·75 (0·35)	2·40 (0·24)	0·87
CTP-ON	M	43·55	80·24	0·00	0·48	15·58 (0·37)	36	18·45 (0·30)	38	1·18	5·11 (0·45)	4·13 (0·43)	0·81
EMC-ON	M	44·39	78·57	0·00	0·42	18·84 (0·52)	20	23·09 (0·51)	28	1·23	3·35 (0·26)	2·61 (0·30)	0·78
GLR-ON	M	44·20	77·87	0·00	0·41	16·79 (0·32)	35	19·38 (0·34)	39	1·15	4·29 (0·25)	4·05 (0·26)	0·95
HLM-ON	M	44·20	79·53	0·00	0·29	18·02 (0·69)	16	16·88 (0·78)	22	0·94	3·63 (0·41)	2·68 (0·34)	0·74
HUR-ON	M	43·90	80·05	0·00	0·42	20·16 (0·77)	29	23·38 (0·60)	47	1·16	5·34 (0·39)	4·66 (0·31)	0·87
LAU-ON	M	43·95	80·17	0·00	0·44	17·47 (0·42)	35	20·49 (0·45)	41	1·17	4·03 (0·34)	3·29 (0·30)	0·82
MMT2-ON	M	44·10	80·19	0·00	0·63	20·68 (0·58)	23	22·18 (0·70)	28	1·07	4·39 (0·47)	2·29 (0·27)	0·52
MPL-ON	M	43·86	79·53	0·00	0·20	16·49 (0·58)	28	20·77 (0·45)	44	1·26	3·89 (0·48)	4·39 (0·35)	1·13
NBY-ON	M	46·28	79·45	0·02	0·58	18·07 (0·58)	22	18·87 (0·55)	20	1·04	6·27 (0·94)	3·35 (0·67)	0·53
OUS-ON	M	44·30	78·04	0·00	0·40	17·25 (0·37)	25	21·53 (0·50)	36	1·25	4·28 (0·39)	3·06 (0·32)	0·71
TDL-ON	M	45·88	80·11	0·00	0·47	14·92 (0·44)	37	18·15 (0·45)	43	1·22	4·86 (0·43)	3·93 (0·37)	0·81