

Pollinator responses to variation in floral display and flower size in dioecious *Sagittaria latifolia* (Alismataceae)

Mélanie Glaettli^{1,2} and Spencer C. H. Barrett¹

¹Department of Ecology and Evolutionary Biology, 25 Willcocks Street, University of Toronto, Toronto, Canada, M5S 3B2; ²Present address: Department of Biology, Institute of Plant Sciences, Altenbergrain 21, University of Bern, 3013 Bern, Switzerland

Summary

Author for correspondence:

Mélanie Glaettli

Tel: +4131 631 4927

Fax: +4131 631 4942

Email: melanie.glaettli@ips.unibe.ch

Received: 22 February 2008

Accepted: 5 May 2008

- In animal-pollinated plants with unisexual flowers, sexual dimorphism in floral traits may be the consequence of pollinator-mediated selection. Experimental investigations of the effects of variation in flower size and floral display on pollinator visitation can provide insights into the evolution of floral dimorphism in dioecious plants.
- Here, we investigated pollinator responses to experimental arrays of dioecious *Sagittaria latifolia* in which we manipulated floral display and flower size. We also examined whether there were changes in pollinator visitation with increasing dimorphism in flower size.
- In *S. latifolia*, males have larger flowers and smaller floral displays than females. Visitation by pollinators, mainly flies and bees, was more frequent for male than for female inflorescences and increased with increasing flower size, regardless of sex. The number of insect visits per flower decreased with increasing floral display in males but remained constant in females. Greater sexual dimorphism in flower size increased visits to male inflorescences but had no influence on the number of visits to female inflorescences.
- These results suggest that larger flower sizes would be advantageous to both females and males, and no evidence was found that females suffer from increased flower-size dimorphism. Small daily floral displays may benefit males by allowing extended flowering periods and greater opportunities for effective pollen dispersal.

Key words: floral display, flower size, pollinators, *Sagittaria latifolia*, sexual dimorphism.

New Phytologist (2008) doi: 10.1111/j.1469-8137.2008.02532.x

© The Authors (2008). Journal compilation © *New Phytologist* (2008)

Introduction

In animal-pollinated plants, reproductive success depends on the ability to attract pollinators to flowers. In dioecious species, pollinator attraction is of particular significance because individuals cannot engage in self-fertilization, and female and male plants differ in floral characteristics expected to influence pollinator preferences. Differing intensities of selection on floral traits between the sexes are thought to play a central role in the evolution of sexual dimorphism in dioecious species (Willson, 1979; Eckhart, 1999; Geber, 1999). Studies of species with gender dimorphism have generally found a positive response of pollinators to increasing flower size or

floral display, and more intense selection for larger flowers and larger floral displays in males compared with females (Bell, 1985; Vaughton & Ramsey, 1998; Ashman, 2000). However, some species have either larger female flowers or larger female floral displays (Delph *et al.*, 1996; Eckhart, 1999), and patterns of sex-specific selection in dioecious plants are not well understood.

The extent to which the degree of sexual dimorphism in floral traits can evolve may be limited by the discriminatory abilities of pollinators. As females in dioecious populations can be pollen limited (House, 1992; Knight *et al.*, 2005; Voigt *et al.*, 2005), sexual dimorphism could be reduced through positive selection on female floral traits if increased

attractiveness to pollinators reduces the intensity of pollen limitation (Johnson *et al.*, 1995; Ashman *et al.*, 2000). By contrast, large differences between female and male individuals in attractiveness to pollinators may cause pollinators to forage mostly on the showier sex. This could result in reduced pollen dispersal to females, with implications for the persistence of gender-dimorphic populations (Bawa, 1980; Vamosi & Otto, 2002; Vamosi *et al.*, 2006). To evaluate this idea, it is necessary to experimentally investigate whether an increase in the magnitude of floral dimorphism results in an increased bias in pollinator visitation between the sexes and greater pollen limitation.

The relations between pollinator attraction and display size have been investigated in *Sagittaria* (Alismataceae), a genus of emergent aquatics with monoecious and dioecious sexual systems (Muenchow & Delesalle, 1994; Huang *et al.*, 2006; Vamosi *et al.*, 2006). Using experimental arrays of monoecious *Sagittaria trifolia*, Huang *et al.* (2006) found a decelerating relation between increasing display size and pollinator visitation during male function, whereas during female function the proportion of visits increased linearly. Similarly, Muenchow & Delesalle (1994) found that the visitation rates to male flowers of dioecious *Sagittaria latifolia* and monoecious *Sagittaria australis* exhibited a positive but sharply decelerating response to increased display size. These results are not in accord with theoretical expectations based on sex allocation theory (Charnov, 1982; Lloyd, 1984). For example, Bawa (1980) predicted that in gender-dimorphic populations there should be a disproportionate increase in pollinator visitation to males with the largest display sizes, a result not found in *S. latifolia* (Muenchow & Delesalle, 1994). Further studies in *Sagittaria* investigating pollinator responses to both floral display and flower size and their consequences for male and female function are warranted and may provide insights into the evolution of floral dimorphism.

Here, we investigate the effects of variation in floral display and flower size on the foraging behaviour and types of pollinators visiting dioecious *Sagittaria latifolia*. We first determined the extent of sexual dimorphism in flower size and floral display in a large dioecious population that was the focus of our investigation. We then used experimental arrays set up within the population to examine the response of pollinators to patches in which we manipulated either floral display size or flower size. In arrays with equivalent floral displays, we manipulated flower size to explore the effects of variation in sexual dimorphism on pollinator attraction. Our experiments therefore addressed the following main questions.

- Does variation in floral display and flower size influence pollinator visitation and are the responses dependent on the sex of inflorescences? We predicted that males would be visited more frequently than females and that increasing flower and floral display size would increase pollinator visits but with differing sex-specific responses.
- Does the magnitude of sexual dimorphism influence pollinator preferences for females and males? In arrays with greater

flower-size dimorphism, we predicted an increase in visitation to males and a decrease in visitation to females.

Finally, in our experiments we also identified the types of pollinators visiting flowers to determine whether they differed in their responses to the sexual morphs of *S. latifolia*.

Materials and Methods

Study species and site

Sagittaria latifolia Willd. is a clonal aquatic plant that occupies diverse wetland habitats in North America. Two sexual systems occur in this species (Wooten, 1971; Dorken *et al.*, 2002). Monoecious and dioecious populations can often occur in close geographic proximity, but they are usually ecologically segregated because of their different life-history traits (Dorken & Barrett, 2003). In southern Ontario, Canada, where our study was conducted, plants flower between July and mid-September and produce several inflorescences with white, unisexual, 1-d flowers that produce nectar. These are arranged in whorls of three and open sequentially from the bottom to the top of the inflorescence. Female inflorescences flower over shorter periods than male inflorescences (Muenchow & Delesalle, 1992). Flowers of *Sagittaria* species are visited by a wide spectrum of generalist pollinators including bees, flies, beetles, butterflies and wasps (Muenchow & Delesalle, 1994; Vamosi *et al.*, 2006).

All studies described below were conducted in a large dioecious population of *S. latifolia* occurring in a roadside canal near Leamington, Essex County, southern Ontario (42°0'N, 85°51'W). The proportion of flowering female inflorescences in the population was 0.48.

Sexual dimorphism in floral traits

To examine sexual dimorphism we sampled female and male inflorescences in 2005 ($n = 27$ and 31, respectively) and 2006 ($n = 27$ and 32). We made a special effort to sample different clones within the population by sampling throughout the population. We counted the number of open flowers per inflorescence and using digital callipers measured (to the nearest 0.01 mm) the widest diameter of two to four flowers, recording their position within an inflorescence.

We performed analyses of variance (ANOVAs) using the statistical package R (version 2.5.0; R Development Core Team, 2007) to assess differences in floral display and flower size between sexes and sampling years. We considered year as random and sex as a fixed effect and square-root transformed floral display to meet assumptions of the analysis. To explore variation in flower size we used a similar ANOVA and additionally accounted for flower position within the inflorescence. Because no transformation allowed us to meet the assumptions of the test for flower size, we performed randomization tests (1000 permutations) on mean squares (Manly, 1997). If < 5% of the

permutations resulted in a larger mean square than that obtained with the observed data, we considered the tested effect significant. We conducted randomizations as follows: we tested the year effect by randomizing the data within sex and flower position; the sex effect by permutation of the data within year and flower position; the effect of flower position by randomizing the data within year and sex; the year \times sex interaction by permutation within flower position; the year \times flower position interaction by randomizing data within sex; the sex \times flower position interaction by permutation within year; and the three-way interaction, year \times sex \times flower position, by randomizing the data across the entire data set.

Experimental arrays

We observed the response of pollinators to six female and six male inflorescences in experimental arrays that varied in either floral display or flower size during August–September 2005 and 2006. We placed styrofoam floating platforms at the edge of the population with four adjacent water-filled plastic buckets (diameter 25 cm) arranged in a square (60 \times 60 cm) each containing three inflorescences. For each experimental array, we randomly sampled inflorescences from the population, manipulated flower size or floral display and randomized the positions of the 12 inflorescences among the four buckets. We allowed pollinators to visit each array for 30 min and then started recording visitation to the experimental array for 1 h (floral display) or 45 min (flower size). We recorded the identity of pollinators and their visitation to individual inflorescences. To ensure high pollinator visitation rates, we conducted experiments between 10:00 and 16:00 h on fine days without rain.

Floral display To determine the effects of floral display size and sex on the behaviour of pollinators, we manipulated the daily floral display in 17 arrays in 2006. Each array contained three different floral display sizes: three, six and nine flowers per inflorescence, with two inflorescences per sex for each inflorescence size. To control for flower size, we calculated mean flower size for each inflorescence. We used analysis of covariance (ANCOVA) to assess the effects of array, sex, floral display size, flower size, and insect type, grouped as bees, flies, wasps and 'others' (i.e. beetles and butterflies, which collectively accounted for only ~3% of the total visits), on the total number of visits per inflorescence and on the mean number of pollinator visits per flower for each inflorescence and each observation interval.

Transformation of visitation data did not meet the assumptions of the ANCOVA; we therefore performed a randomization test on mean squares and adapted the permutation patterns for each tested effect, as described previously (see 'Sexual dimorphism in floral traits').

Flower size To investigate pollinator responses to variation in flower size, we set up 14 arrays with equal floral display sizes

(i.e. a whorl of three flowers) during 2005 and 2006. In 2005 we used 10 arrays of unmanipulated flowers and in 2006 we used four additional arrays with manipulated flowers representing the extremes of flower size variation we observed in the population. For flower-manipulated arrays, we cut the edge of each male and female flower with scissors so that all females within an array had either equal or smaller flower sizes than males. We calculated a value for sexual dimorphism in each array by dividing male by female flower size. Thus, sexual dimorphism is 1 when the means of female and male flower sizes are equal, between 0 and 1 when male flowers are smaller than female flowers, and > 1 when males are larger than females. Our values of flower size dimorphism varied between 0.99 and 1.37.

We performed an ANCOVA for the total number of insect visits to inflorescences. We considered year (or treatment: manipulated vs unmanipulated), array nested within year, sex and insect type (i.e. bees, flies, wasps and others: beetles, butterflies) as qualitative variables and flower size as a quantitative variable. The year effect was significant because there were more visits to arrays in 2006 than in 2005. However, we removed the year effect from the analysis because it did not interact with any other variable and removal or inclusion did not change the results of our analysis. As in previous analyses, transformation of data did not meet the assumptions of the ANCOVA, so we performed randomization tests on mean squares and adapted the permutation patterns for each tested effect as described previously (see 'Sexual dimorphism in floral traits').

We performed an ANCOVA to investigate the effects of variation in flower size, sex and sexual dimorphism on the total number of insect visits to inflorescences. Flower size and sexual dimorphism were quantitative variables and sex was a qualitative variable. We accounted for flower size variation in our model to remove the potential confounding effects between flower size and sexual dimorphism. To meet the assumptions of the ANCOVA we square-root transformed the response variable.

Results

Sexual dimorphism in floral traits

We detected sexual dimorphism of daily display size and flower size in the population of *S. latifolia*. Floral display size was significantly affected by the sex of plants ($F_{1,133} = 14.09$, $P < 0.001$), with more open flowers per inflorescence for females (5.0 ± 0.4 (mean \pm SE)) than for males (3.7 ± 0.2). Daily display size was similar in the two years ($F_{1,133} = 0.47$, $P = 0.49$) and the magnitude of the difference between the sexes was not significant (year \times sex interaction; $F_{1,133} = 0.49$, $P = 0.48$). Flower size was significantly different between the sexes ($F_{1,191} = 43.23$, $P < 0.001$), with female flowers (30.29 ± 0.60 mm) being smaller than male flowers (34.32 ± 0.74 mm). However, the degree of flower size dimorphism varied

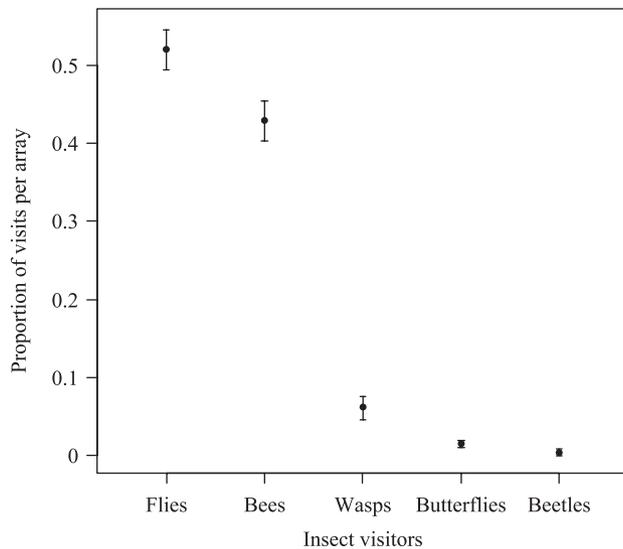


Fig. 1 The insect visitors to flowers of dioecious *Sagittaria latifolia* arrays during 2005–2006. The values presented are the mean (\pm SE) percentage of total visits to arrays among the five insect groups. The spectrum of visitors was similar in the two years. The following taxa were identified among the five insect groups: bees, *Apis mellifera* and *Halictus* sp.; flies, *Allograpta* sp., *Criorhina* sp., *Eristalis* sp., *Heliophilus* sp. and *Linnaemya* sp.; wasps, *Euodynerus* sp.; beetles, *Chauliognathus pennsylvanicus*, *Coleomegilla maculata* and *Diabrotica undecimpunctata*; butterflies, *Ancyloxypha numitor*.

between years. In 2005, male flowers were only 2% larger than female flowers, whereas in 2006 they were 24% larger. This difference was attributable to the size of female flowers, which were significantly smaller in 2006. Thus, there was a significant effect of the year of sampling ($F_{1,191} = 22.48$, $P < 0.05$), and a significant year \times sex interaction ($F_{1,191} = 21.68$, $P < 0.001$).

The smaller flower size of females sampled in 2006 was not the result of a phenological sampling bias associated with position effects within inflorescences. Although flower size decreases from basal to distal positions within the inflorescence ($F_{7,191} = 5.06$, $P < 0.01$), the positions of female flowers that we sampled were similar in the two years (Wilcoxon signed rank test; $W = 600.5$, $P = 0.82$). The difference in flower size between females and males is more likely to have been associated either with seasonal differences in the growing conditions and the productivity of the habitat, or with differences between years in maternal investment to seed provisioning.

Experimental manipulation of floral display

We observed 7518 insect visits during 17 h of observation to arrays in which plants varied in floral display size. Flies and bees were by far the most frequent visitors, accounting for 49 and 42% of the total visits to the arrays, respectively. The remaining 9% of visits were from wasps, beetles and butterflies (Fig. 1). All visitor groups served as pollinators of *S. latifolia*, because we commonly observed contact with the sex organs of female and male flowers.

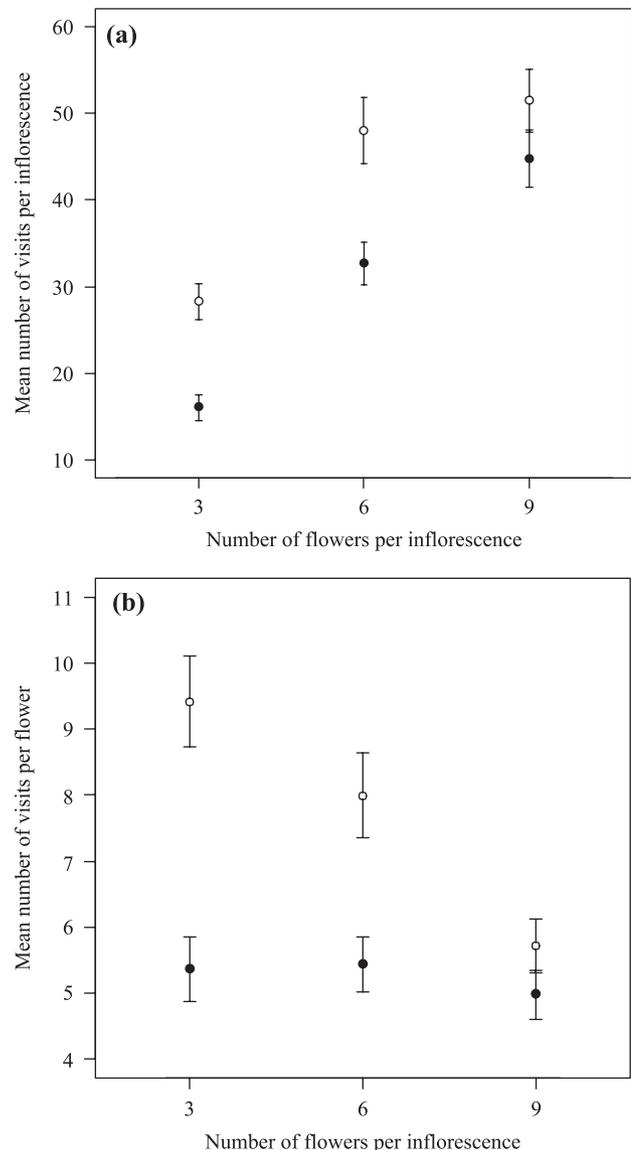


Fig. 2 The effects of variation in daily floral display size in experimental arrays of dioecious *Sagittaria latifolia*: (a) the mean (\pm SE) number of insect visits per inflorescence; (b) the mean (\pm SE) number of insect visits per flower in female (closed symbols) and male (open symbols) inflorescences.

Pollinators responded strongly to the floral display and sex of inflorescences. Insects were more likely to visit male inflorescences with large floral displays (Fig. 2a, Table 1). However, the mean number of visits per flower decreased significantly with increasing display size. By contrast, floral display size did not affect the mean number of visits per flower in females, as indicated by the significant sex \times floral display interaction (Fig. 2b, Table 1). Figure 3 illustrates the increase in mean number of insect visits per flower with increasing flower size for both female and male flowers.

The type of insect visiting flowers was influenced by both flower size and display size (Table 1). There was a significant

Table 1 Analysis of covariance for the total number of insect visits per inflorescence and the mean number of insect visits per flower in *Sagittaria latifolia* arrays with experimental manipulation of daily floral display

Source of variation	d.f.	Total number of visits per inflorescence	Mean number of visits per flower
		MS	MS
Array	16	162	4.7*
Insect type	3	7041***	215.2***
Sex	1	946***	43.8***
Flower size	1	959*	27.8†
Floral display	1	3181***	21.5***
Insect type × sex	3	181*	7.8***
Insect type × flower size	3	219**	7.0†
Insect type × floral display	3	608*	3.2
Sex × floral display	1	19	11.1**
Residuals	1395	46	1.5

† $P < 0.1$; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
d.f., degrees of freedom; MS, mean squares.

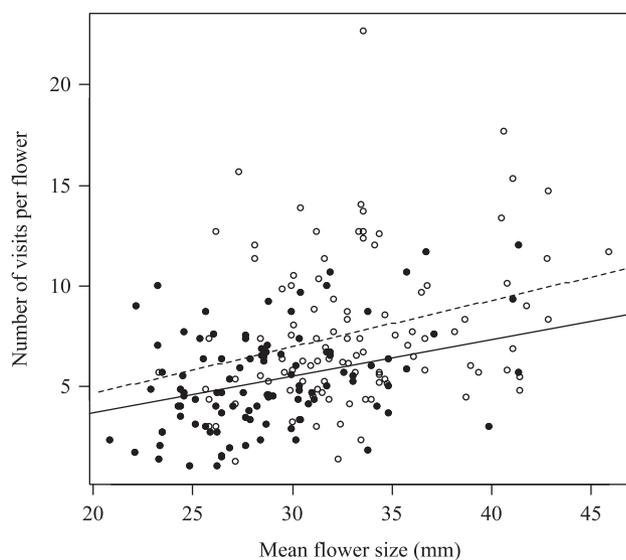


Fig. 3 The effect of variation in flower size in experimental arrays of dioecious *Sagittaria latifolia* on the mean number of insect visits per flower for female (closed symbols, solid line) and male (open symbols, dashed line) inflorescences.

insect type × floral display interaction for the total number of visits to inflorescences. Visits of bees and flies responded positively to increasing inflorescence size, whereas those of the other classes of insects (beetles, butterflies and wasps) did not (Fig. 4). There was a significant insect type × sex interaction for both response variables (Table 1). Bees and flies visited 44% more male inflorescences than female inflorescences, whereas wasps, beetles and butterflies did not discriminate between the sexes. There was also a significant insect type × flower size interaction for the total number of visits per inflorescence because bees and flies responded to increasing flower size whereas wasps, beetles and butterflies did not. The interaction

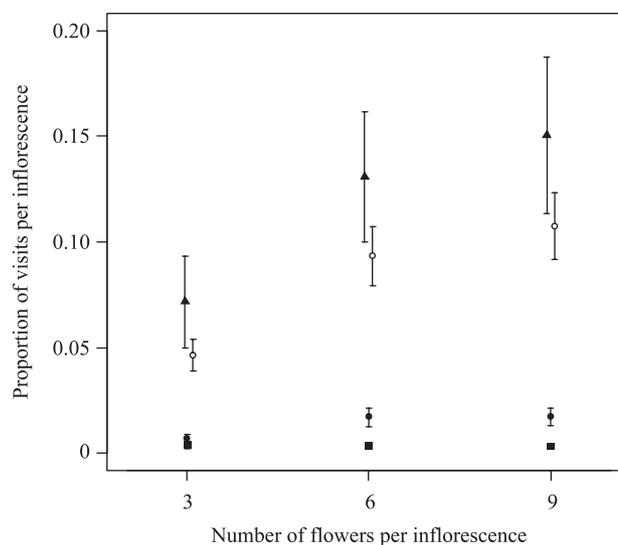


Fig. 4 The effects of floral display and insect group on the mean (\pm SE) proportion of insect visits per inflorescence to dioecious *Sagittaria latifolia* arrays. The insect groups are bees (triangles), flies (open circles), wasps (closed circles) and beetles and butterflies (squares).

between insect type and flower size for the mean number of visits per flower was also marginally significant (Table 1).

Experimental manipulation of flower size

We observed 2093 insect visits during 10.5 h of observations to arrays that varied in flower size but not display size. In common with the preceding experiment, larger flower sizes significantly increased the total number of visits to inflorescences ($F_{1,336} = 39.04$, $P < 0.001$), with males attracting 53% more insect visitors than females ($F_{1,336} = 19.09$, $P < 0.001$). The number of visits to inflorescences was also significantly

Table 2 Analysis of covariance for the square-root transformed total number of visits per inflorescence and the influence of flower size, sex and sexual dimorphism in experimental arrays of *Sagittaria latifolia*

Source of variation	Total number of visits per inflorescence	
	d.f.	MS
Flower size	1	26.1*
Sex	1	16.3***
Sexual dimorphism	1	1.9
Sex × sexual dimorphism	1	3.9**
Residuals	164	0.8

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

Floral display was held constant and flower size was manipulated. Sexual dimorphism was calculated as the ratio of male to female flower size within each array. d.f., degrees of freedom; MS, mean squares.

influenced by insect type ($F_{2,336} = 285.69$, $P < 0.001$) because most visits were by flies (56%) and bees (43%), and only a few visits were by beetles and butterflies (1%; Fig. 1). There was a significant insect type × flower size interaction ($F_{2,336} = 10.63$, $P < 0.05$). Unlike bees and flies, beetles and butterflies did not respond to increasing flower size. The total number of visits per inflorescence was significantly influenced by array ($F_{13,336} = 6.93$, $P < 0.05$) and its interaction with insect type ($F_{26,336} = 7.57$, $P < 0.001$). This was largely a result of the variation in daily and seasonal abundance of pollinator types in our experiment.

We examined whether the total number of visits to inflorescences was affected by variation in the degree of sexual dimorphism (i.e. the ratio of male to female flower size within arrays) and the sex of inflorescences (Table 2). As previously detected, males received more visits than females. After accounting for the effect of flower size, the total number of visits per inflorescence was affected by the sex × sexual dimorphism interaction: the difference in total number of visits between females and males increased with greater degrees of flower size dimorphism (Table 2, Fig. 5). The increasing discrepancy between male and female visits was the result of an increased preference of pollinators for male inflorescences, with the number of visits to females remaining constant, regardless of the amount of sexual dimorphism in the array (Fig. 5).

To perform our experiments we used cut inflorescences. This could potentially alter the nectar production and/or the fragrance of flowers and thus the foraging behaviour of pollinators while arrays were monitored. To test for such an effect we recorded the 'mid-time' of four different arrays and used an analysis of variance to test for differences between the two time periods and sexes in the total number of insect visits per inflorescence. Although 21% fewer pollinator visits occurred in the second half hour than in the first half hour ($F_{1,12} = 10.3$; $P < 0.01$), this decrease in pollinator visits was

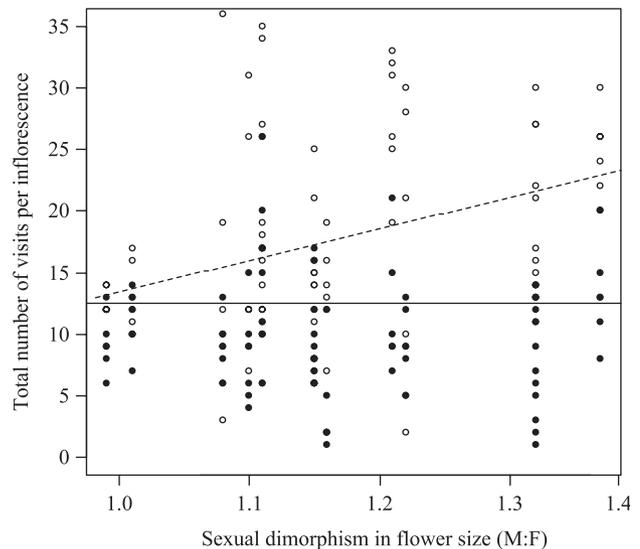


Fig. 5 The effects of sexual dimorphism in flower size on the total number of visits per inflorescence for male (open symbols, dashed line) and female (closed symbols, solid line) plants of *Sagittaria latifolia*. Sexual dimorphism was calculated as the ratio of male to female flower size within each array.

not significantly different between males and females ($F_{1,12} = 0.002$; $P = 0.96$). Similarly, the pollinator visitation frequency was significantly affected by floral display size ($F_{2,18} = 4.5$; $P < 0.05$). When floral display was equal to or larger than six open flowers, the decrease in the number of visits between the first and the second half hours was 58% larger than the decrease between these periods with three open flowers. However, this pattern was again similar for the two sexes (floral display × sex interaction: $F_{2,18} = 2.0$; $P = 0.16$). The lower number of visits in the second half of the array experiments was probably the consequence of a depletion of pollen and nectar as the arrays were running. However, as the effects were similar between the sexes it is unlikely to have influenced our comparisons.

Discussion

We detected sexual dimorphism in floral display and flower size in a dioecious population of *S. latifolia*. Our study corroborates earlier investigations of sexual dimorphism in dioecious *S. latifolia* (Sarkissian *et al.*, 2001; Vamosi *et al.*, 2006) and monoecious *S. australis* (Muenchow & Delesalle, 1994) and *S. trifolia* (Huang *et al.*, 2006). Male flowers are larger than female flowers, a pattern commonly observed in dioecious species (Eckhart, 1999), particularly in species from temperate ecosystems (Delph *et al.*, 1996). However, in *Sagittaria* species daily display sizes of male (dioecious) or male-functioning (monoecious) inflorescences are generally smaller compared with those of female or female-functioning inflorescences (Sarkissian *et al.*, 2001; Huang *et al.*, 2006).

Using experimental arrays, we demonstrated that the foraging behaviour of pollinators was influenced by flower size, daily floral display and whether inflorescences were female or male. Moreover, the various insect groups that visited our arrays responded differently to these attributes. The predominant pollinators, flies and bees, visited male inflorescences more frequently than female inflorescences, whereas other insect groups showed no such preference. Increasing flower size and floral display size was generally associated with greater visitation, regardless of sex. However, the mean number of visits per flower decreased with increasing daily floral display for male inflorescences but remained constant for female inflorescences.

The amount of pollinator visitation to plants is often considered a reasonable proxy for reproductive success via pollen removal (Huang *et al.*, 2006) and seed set (Ashman & Diefenderfer, 2001). If this is true for our arrays of *S. latifolia* it would suggest that increased flower size might benefit both sexes, whereas selection for smaller daily floral displays and extended flowering periods would benefit males. Our results also demonstrate that increasing flower size dimorphism decreased the proportion of female versus male insect visits without affecting the total number of pollinator visits to females. Thus, increasing sexual dimorphism in flower size probably increases male fitness without affecting female fertility.

Pollinator type preferences

Generalist pollinators commonly visit the relatively small, unshowy flowers that typically occur in dioecious species (Bawa, 1980; Charlesworth, 1993; Vamasi *et al.*, 2003; but see Renner & Feil, 1993). We observed a wide range of insects visiting the flowers of *S. latifolia* and mediating pollination. Among the five groups that we observed, bees and flies were the most common visitors. These two groups responded similarly to variation in floral display and flower size and showed preferences for male over female inflorescences. Ashman *et al.* (2000) also found that bees and flies showed similar preferences for polleniferous flowers while foraging on gynodioecious *Fragaria virginiana* and, in dioecious *Wurmbea dioica*, bees favoured males over females whereas flies did not differentiate between sexes (Vaughton & Ramsey, 1998). We were not able to detect differences in the preferences of wasps, beetles and butterflies in our arrays, probably because of the relatively low visitation frequencies of these insect groups in our experiments (Fig. 1). In gender-dimorphic species pollinated by diverse generalist pollinators, the differences among insect groups in response to floral traits may allow plants to evolve sexual dimorphism while limiting any potential negative effects on fertility.

Pollinator response to floral display size variation

Larger floral displays usually result in increased pollinator visitation rates, but the proportion of flowers that are visited

within an inflorescence often decreases (Fritz & Nilsson, 1996; Harder & Barrett, 1996; Mitchell *et al.*, 2004; Ohashi & Yahara, 2002; Kudo & Harder, 2005; Huang *et al.*, 2006). We found an increase in the total number of visits to large inflorescences for both sexes of *S. latifolia* (Fig. 2a). This resulted in either a decrease or a constant mean number of visits per flower for males and females, respectively (Fig. 2b). The studies of Muenchow & Delesalle (1994) and Huang *et al.* (2006) on *S. latifolia* and *S. trifolia*, respectively, also reported a positive but decelerating response of pollinators to male floral display size. Collectively, these results suggest that in *Sagittaria* species there may be stronger fitness consequences to variation in daily display size during male function than female function.

A glasshouse comparison of the flowering phenology of plants collected from our study population indicated that the duration of flowering of male inflorescences was significantly longer than that of female inflorescences (male: 5.1 ± 0.3 d, $n = 17$; female: 2.2 ± 0.2 d, $n = 9$; $F_{1,24} = 27.91$, $P < 0.001$). In *S. latifolia* the more synchronous flowering in females results in larger daily floral displays and may serve to compensate for the smaller flower size and reduced floral rewards (e.g. nectar only in females versus nectar and pollen in males). By contrast, the more protracted flowering of inflorescences in male plants should result in a greater number of individual insect visits during their lifetime (Thomson & Barrett, 1981; Lloyd & Yates, 1982; Lloyd, 1984; Thomson, 2006). The staggered anthesis times of male flowers within inflorescences might compensate for the decelerating response of pollinators to increased floral display and would be consistent with intrasexual selection on male function to increase the proficiency of pollen dispersal.

Pollinator response to flower size variation

We investigated whether differences in flower size between females and males influence the foraging patterns of pollinators. Our experiment was motivated by the suggestion that if pollinators visited larger male flowers disproportionately this could result in fewer pollinator transitions to females and the possibility of pollen limitation of maternal fertility (Bawa, 1980; Vamasi & Otto, 2002; Vamasi *et al.*, 2006). Although several studies have demonstrated that flowering sex ratios can influence the degree of pollen limitation of females in gender-dimorphic species (McCauley & Taylor, 1997; Ashman & Diefenderfer, 2001) we are not aware of studies that have previously investigated whether variation in flower size dimorphism influences pollinator visitation (although see Vamasi *et al.*, 2006).

In our study, differences in pollinator visitation between females and males increased with increasing flower size dimorphism (Table 2). However, this change in preference was the consequence of an increase in the number of visits to males only (Fig. 5). Thus, in our study of *S. latifolia* there was

no evidence that female fertility is likely to suffer as a result of pronounced sexual dimorphism in flower size. Indeed, a preliminary investigation of pollen limitation within our study population showed no evidence of pollen limitation. For each of 24 female inflorescences, we supplemented one flower with pollen from three different donors and marked the adjacent open-pollinated flowers of the same whorl. Within each flower, an average of 71% of the 525 ± 20 potential fruits per flower developed. Although pollen-supplemented flowers had on average 8% more fruits than open-pollinated flowers, pollen limitation was only marginally significant (paired *t*-test, $t = 1.98$, d.f. = 23, $P = 0.06$). Pollen limitation may be relatively infrequent in *S. latifolia* because of high visitation rates by its generalist pollinators, as expected in species with short floral longevities (Ashman & Schoen, 1994).

The larger size of male compared with female flowers in species with unisexual flowers is usually interpreted as the outcome of sexual selection on male function, although other explanations have also been proposed (reviewed in Bell, 1985; Delph *et al.*, 1996; Eckhart, 1999). The greater attractiveness of larger male flowers should attract more pollinators and increase mating opportunities, as predicted by Bateman's principle (Bateman, 1948; Arnold, 1994). In dioecious *W. dioica*, Vaughton & Ramsey (1998) reported that larger flower size in both sexes increased visitation rates but had no influence on seed set in females. They argued that selection to increase flower size in *W. dioica* has probably occurred through male rather than female function. We demonstrated that larger flowers in arrays of *S. latifolia* were more attractive to pollinators, regardless of sex. But, with greater size dimorphism of flowers, visitation increased to male inflorescences, probably promoting greater pollen dispersal and male fertility. However, in our study we did not directly investigate the mating consequences of increased visitation to male inflorescences. Estimates of pollen removal and deposition coupled with measurements of fertility and mating would be necessary to fully evaluate the fitness consequences of the observed inequalities in visitation patterns of pollinators to the sexes of *S. latifolia*. As yet this has not been attempted for a dioecious species.

Acknowledgements

We thank Sarah B. Yakimowski for field assistance and for comments on the manuscript, Julio Rivera for help with insect identification and Mark van Kleunen as well as two anonymous reviewers for comments on the manuscript. Financial support was provided by the Swiss National Science Foundation (grant no. PBLAA-109434), by the Association des affaires socio-culturelles de l'Université de Lausanne to MG and by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada and funding through a Canada Research Chair to SCHB.

References

- Arnold SJ. 1994. Bateman's principle and the measurement of sexual selection in plants and animals. *American Naturalist* **144**: S126–S149.
- Ashman T-L. 2000. Pollinator selectivity and its implications for the evolution of dioecy and sexual dimorphism. *Ecology* **81**: 2577–2591.
- Ashman T-L, Diefenderfer C. 2001. Sex ratio represents a unique context for selection on attractive traits: consequences for the evolution of sexual dimorphism. *American Naturalist* **157**: 334–347.
- Ashman T-L, Schoen DJ. 1994. How long should flowers live? *Nature* **371**: 788–791.
- Ashman T-L, Swetz J, Shivitz S. 2000. Understanding the basis of pollinator selectivity in sexually dimorphic *Fragaria virginiana*. *Oikos* **90**: 347–356.
- Bateman AJ. 1948. Intrasexual selection in *Drosophila*. *Heredity* **2**: 349–369.
- Bawa KS. 1980. Evolution of dioecy in flowering plants. *Annual Review of Ecology and Systematics* **11**: 15–39.
- Bell G. 1985. On the function of flowers. *Proceeding of the Royal Society of London B* **224**: 223–265.
- Charlesworth D. 1993. Why are unisexual flowers associated with wind pollination and unspecialized pollinators? *American Naturalist* **141**: 481–490.
- Charnov EL. 1982. *The theory of sex allocation*. Princeton, NJ, USA: Princeton University Press.
- Delph LF, Galloway LF, Stanton ML. 1996. Sexual dimorphism in flower size. *American Naturalist* **148**: 299–320.
- Dorken ME, Barrett SCH. 2003. Life-history differentiation and the maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **57**: 1973–1988.
- Dorken ME, Friedman J, Barrett SCH. 2002. The evolution and maintenance of monoecy and dioecy in *Sagittaria latifolia* (Alismataceae). *Evolution* **56**: 31–41.
- Eckhart VM. 1999. Sexual dimorphism in flowers and inflorescences. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*, Berlin, Germany: Springer-Verlag, 123–48.
- Fritz A-L, Nillson LA. 1996. Reproductive success and gender variation in deceit-pollinated orchids. In: Lloyd DG, Barrett SCH, eds. *Floral biology: studies on floral evolution in animal pollinated plants*, New York, NY, USA: Chapman & Hall, 319–338.
- Geber MA. 1999. Theories of the evolution of sexual dimorphism. In: Geber MA, Dawson TE, Delph LF, eds. *Gender and sexual dimorphism in flowering plants*, Berlin, Germany: Springer-Verlag, 97–122.
- Harder LD, Barrett SCH. 1996. Pollen dispersal and mating patterns in animal-pollinated plants. In: Lloyd DG, Barrett SCH, eds. *Floral biology: studies on floral evolution in animal pollinated plants*. New York, NY, USA: Chapman & Hall, 149–190.
- House SM. 1992. Population-density and fruit-set in three dioecious tree species in Australian tropical rain-forest. *Journal of Ecology* **80**: 57–69.
- Huang SQ, Tang LL, Sun JF, Lu Y. 2006. Pollinator response to female and male floral display in a monoecious species and its implications for the evolution of floral dimorphism. *New Phytologist* **171**: 417–424.
- Johnson SG, Delph LF, Elderkin CL. 1995. The effect of petal-size manipulation on pollen removal, seed set and insect-visitor behaviour in *Campanula americana*. *Oecologia* **102**: 174–179.
- Knight TM, Steets JA, Vamasi JC, Mazer SJ, Burd M, Campbell DR, Dudash MR, Johnston MO, Mitchell RJ, Ashman T-L. 2005. Pollen limitation of plant reproduction: pattern and process. *Annual Review of Ecology, Evolution, and Systematics* **36**: 467–497.
- Kudo G, Harder LD. 2005. Floral and inflorescence effects on variation in pollen removal and seed production among six legume species. *Functional Ecology* **19**: 245–254.
- Lloyd DG. 1984. Gender allocation in outcrossing cosexual plants. In: Dirzo R, Sarukhan J, eds. *Perspectives on plant population ecology*. Sunderland, MA, USA: Sinauer Associates Inc., 277–300.

- Lloyd DG, Yates JM. 1982. Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). *Evolution* 36: 903–913.
- Manly BFJ. 1997. *Randomization, bootstrap and Monte Carlo methods in biology*. London, UK: Chapman & Hall.
- McCauley DE, Taylor DR. 1997. Local population structure and sex ratio: evolution in gynodioecious plants. *American Naturalist* 150: 406–419.
- Mitchell RJ, Karron JD, Holmquist KG, Bell JM. 2004. The influence of *Mimulus ringens* floral display size on pollinator visitation patterns. *Functional Ecology* 18: 116–124.
- Muenchow G, Delesalle V. 1992. Patterns of weevil herbivory on male, monoecious and female inflorescences of *Sagittaria latifolia*. *American Midland Naturalist* 127: 355–367.
- Muenchow G, Delesalle V. 1994. Pollinator response to male floral display size in two *Sagittaria* species (Alismataceae). *American Journal of Botany* 81: 568–573.
- Ohashi K, Yahara T. 2002. Visit larger displays but probe proportionally few flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. *Functional Ecology* 16: 492–503.
- R Development Core Team. 2007. *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. <http://www.R-project.org>.
- Renner SS, Feil JP. 1993. Pollinators of tropical dioecious angiosperms. *American Journal of Botany* 80: 1100–1107.
- Sarkissian TS, Barrett SCH, Harder LD. 2001. Gender variation in *Sagittaria latifolia* (Alismataceae): is size all that matters? *Ecology* 82: 360–373.
- Thomson JD. 2006. Tactics for male reproductive success in plants: contrasting insights of sex allocation theory and pollen presentation theory. *Integrative and Comparative Biology* 46: 390–397.
- Thomson JD, Barrett SCH. 1981. Temporal variation of gender in *Aralia hispida* (Araliaceae). *Evolution* 35: 1094–1107.
- Vamosi JC, Otto SP. 2002. When looks can kill: the evolution of sexually dimorphic floral display and the extinction of dioecious plants. *Proceedings of the Royal Society of London B* 269: 1187–1194.
- Vamosi JC, Otto SP, Barrett SCH. 2003. Phylogenetic analysis of the ecological correlates of dioecy in angiosperms. *Journal of Evolutionary Biology* 16: 1006–1018.
- Vamosi JC, Vamosi SM, Barrett SCH. 2006. Sex in advertising: dioecy alters the net benefits of attractiveness in *Sagittaria latifolia* (Alismataceae). *Proceedings of the Royal Society of London B* 273: 2401–2407.
- Vaughton G, Ramsey M. 1998. Floral display, pollinator visitation and reproductive success in the dioecious perennial herb *Wurmbea dioica* (Liliaceae). *Oecologia* 115: 93–101.
- Voigt FA, Jung S, Farwig N, Böhning-Gaese K. 2005. Low fruit set in a dioecious tree: pollination ecology of *Commiphora harveyi* in South Africa. *Journal of Tropical Ecology* 21: 179–188.
- Willson MF. 1979. Sexual selection in plants. *American Naturalist* 113: 777–790.
- Wooten JW. 1971. The monoecious and dioecious conditions in *Sagittaria latifolia* (Alismataceae) L. *Evolution* 25: 549–553.