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Size-dependent sex allocation in a monoecious species *Sagittaria pygmaea* (Alismataceae)

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Sagittaria species have been reported to display remarkable variation in gender expression. Here, we investigated gender variation in *Sagittaria pygmaea*, the smallest sized monoecious species in the genus. We used the midvein length as an indicator of plant size and production of male and female flowers as an indicator of gender variation in a single inflorescence. We counted the total number of inflorescences to assess the effect of inflorescence variation on the gender variation pattern. Our results showed that variation in inflorescence number did not affect gender variation. Male flower production increased with increasing plant size, but female flower production did not. Plants of *S. pygmaea* might enhance their paternal reproductive success by increasing the number of male flowers with increasing plant size.

Key words: Alismataceae, monoecious, *Sagittaria pygmaea*, size-dependent sex allocation

Introduction

The gender of flowering plants varies widely within and among species (Lloyd & Bawa 1984). This variation is the product of a complex interplay of genetic and environmental factors (Lloyd & Bawa 1984, Sarkissian *et al*. 2001). Genes could regulate the production of unisexual versus hermaphroditic flowers and lead to a wide array of sexual systems (Dorken & Barrett 2003, and references therein).

Environmental factors play an important role in sex allocation mainly through their effect on resource status and investment in female and male components of reproduction among plants (Gilbert & Bolker 2003, Sultan 2003a, 2003b, Sultan 2005).

In general, when environmental conditions play a role in gender variation in cosexual organisms, sex allocation is considered to be associated with plant size (Policansky 1982, Klinkhamer *et al*. 1997, Barrett *et al*. 1999). According to several theoretical models concerned with size-dependent sex allocation, in a plant population the larger individuals should be more female than the smaller ones, because they have more resources to afford the greater expense and added costs of fruiting both in direct expenditure on current fruit and indirect consequences for future survival (Charnov 1982, Lloyd & Bawa 1984, Klinkhamer *et al.* 1997, Zhang 2006). In addition, the degree of local mate competition and increased geitonogamy may increase with size and can be detrimental for male success for large size plants (Lloyd & Bawa 1984, Klinkhamer *et al.* 1997). Hence, large plants will often perform better as females and worse as males, and so should benefit by being relatively more female than small plants (Sarkissian *et al.* 2001). Such gender plasticity should be particularly common in monoecious plants, because the production of separate female and male flowers enables greater freedom to respond to specific environmental circumstances (Sarkissian *et al.* 2001, and references therein).

Sagittaria is a worldwide genus comprising of approximately 30 species (Chen 1989). Most *Sagittaria* species are basically monoecious (Bogin 1955), but some species display remarkable variation in gender expression (Barrett *et al.* 2000, Huang *et al.* 2002, and references therein). *Sagittari*a *pygmaea* is an emergent or submersed aquatic annual in shallow waters along marshes, ponds, stream banks and rice fields in southern and southeastern Asia. The species is 7–15 cm high and has 3–5 sequential inflorescences; each inflorescence typically has only 0–2 female and 3–8 male flowers (Chen 1989). The species is dichogamous and the flowering periods of the sequential inflorescences do not overlap. Only one or two flowers on an inflorescence of *S. pygmaea* bloom in a day and the stigmas become non-receptive within eight hours after flowering (F. Liu unpubl. data). Due to the remarkable gender plasticity in *Sagittaria* species, several studies have been conducted on the sex allocation in the genus (Sarkissian *et al.* 2001, Huang *et al.* 2002). Most of those studies have focused on sex allocation patterns in which the female flower production varied widely with plant size while male flower production changed little or was unaffected (e.g. *S. latifolia*, Sarkissian *et al.* 2001; *S. trifolia*, Huang *et al.* 2002); however, our field observations on populations of *S. pygmaea* indicated that with increase in plant size the number of female flowers on an inflorescence changed little, while that of male flowers varied widely among different individuals in a population. The sex allocation patterns observed in *S. pygmaea* do not seem to conform to those reported in other *Sagittaria* species. The sex allocation patterns in *S. pygmaea* are still poorly understood.

We investigated the sex allocation patterns in *S. pygmaea* on four natural populations from southeast to southwest China. The plant size usually changes during the growing season and the inflorescence number may change with plant size throughout the flowering period. Observation of variation in inflorescence number in the field is often hampered by fluctuating parameters including environmental factors such as drought and flood, which may affect inflorescence production. These hindrances can be partly overcome by conducting the experiments on plants in cultivation. In our study to establish whether inflorescence number varies with plant size throughout the flowering period in *S. pygmaea* we included one cultivated population. In the study we addressed the following questions: (1) Is there any evidence of size-dependent sex allocation in *S. pygmaea*? (2) What is the adaptive significance of this size-dependent sex allocation pattern in the species?

Material and methods

Data collection

During July 2005 and July and August 2006, we investigated four different populations of *S. pygmaea* in southeastern and southwestern China (Table 1). In 2005, we collected 35 corms from Population ZJ and grew them in Wuhan Botanical Garden. In March 2006 ramets were developing and were transplanted one month later into 4 × 18-arranged 20-cm-deep pots placed 0.5 m apart.

In both natural and cultivated populations, for each plant we counted the number of male and female flowers on each inflorescence and measured the plant height and midvein length. For cultivated populations, we also calculated the

Table 1. General information on the four sampled natural populations and one cultivated population of *Sagittaria pygmaea* in China.

Population	Location	Latitude/longitude	Habitat	Sample size
ZJ	Zhijang, Hubei Province	30°20´/111°35´	Marsh	22
WYS	Wuyishan, Fujian Province	27°33´/117°51´	Paddy field	26
YS	Yanshan, Guangxi Province	25°01´/110°19´	Paddy field	9
PB	Pingba, Guizhou Province	26°25´/106°16´	Ditch	21
WH	Wuhan, Hubei Province	30°29´/114°19´	Cultivated	32

number of inflorescences and the total number of male and female flowers. We used the midvein length of the longest leaf rather than plant weight (Sarkissian *et al*. 2001) as a measure of plant size, because it was easy to measure the midvein length on natural populations and the midvein length strongly correlated with the plant height ($r = 0.809$, $F_{1,146} = 274.54$, $P < 0.001$) and the plant weight (the above ground dry mass) ($r = 0.845$, $F_{1,146} = 360.75$, $P < 0.001$).

Statistical analyses

We first performed an analysis of covariance (ANCOVA) on female and male flower production, with plant size as the covariate and population as a fixed effect using GLM procedure. Thereafter, we compared the relationship between male and female flower production and plant size using correlation analysis. All the analyses were performed using SAS/STAT (SAS Institute 1998).

Results

Each of the *S. pygmaea* individuals could produce several male flowers but almost all individuals (99%) in natural populations produced only one female flower (Figs. 1 and 2). ANCOVA showed that there was no significant difference in female flower production among all populations (Table 2). However, we found a significant difference in male flower production (Table 2). Correlation analysis of size-dependent sex allocation found a positive correlation between midvein length and male flower production in three of the four natural populations (Fig. 1, ZJ: $r = 0.4555$, $F_{1,21} = 5.24$, $P = 0.0331$; WYS: $r = 0.5808$, $F_{1,25} = 12.22$, $P = 0.0018$; YS: $r = 0.4558$, $F_{1,8} = 1.66$, $P = 0.2391$; PB: $r = 0.6239$, $F_{1,20} = 12.11$, $P = 0.0025$).

The number of flowers per inflorescence in the cultivated population displayed similar sex allocation patterns as those in the natural populations. The cultivated plants produced a total of 77 inflorescences and each inflorescence produced a single female flower. The number of male flowers was positively correlated to plant size in all inflorescences ($r = 0.3332$, $F_{1,76} = 9.36$, $P = 0.0031$). However, throughout the flowering period, we did not find any correlation between the midvein length (plant size) and the number of inflorescences on an individual (Fig. 2, $r = 0.0111$, $F_{1,31} = 0.00$, $P = 0.9512$).

Discussion

Several studies have investigated the relationship between gender variation and plant size in *Sagittaria*. *Sagittaria trifolia* and *S. latifolia* exhibited a positive correlation between the female flower

Table 2. Analyses of covariance (ANCOVA) of the differences on male and female flower production for four natural populations and one cultivated population of *Sagittaria pygmaea* with population as a fixed effect and the plant size (midvein length) as a covariate.

	Male flower		Female flower	
	F	*P*	*F*	*P*
Population	80.68	< 0.0001	3.04	0.0833
Midvein length	14.35	0.0002	1.04	0.3089
Population × midvein length	1.30	0.2555	1.61	0.2061

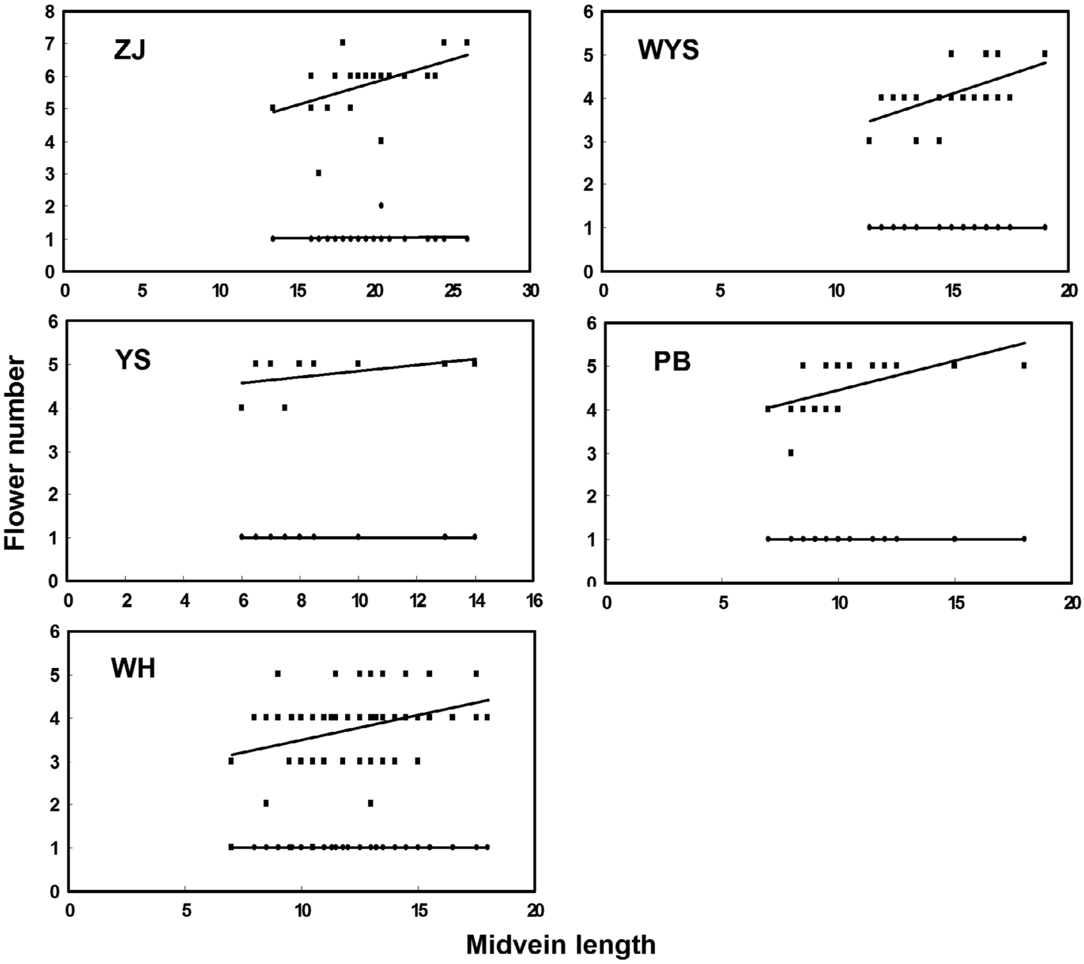

Fig. 1. The relationship between male and female flower production and plant size (midvein length, cm) per inflorescence among four natural populations and one cultivated population on *Sagittaria pygmaea* (squares = male flower production; circles = female flower production).

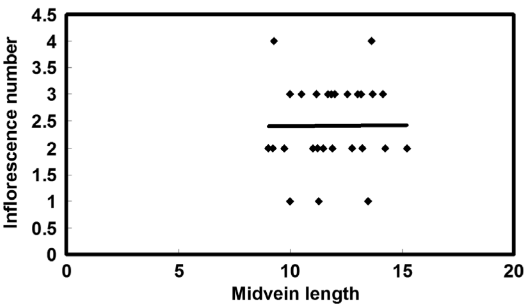

Fig. 2. Relationship between inflorescence number and plant size (midvein length, cm) for cultivated populations of *Sagittaria pygmaea*.

number and plant size, while the number of male flowers did not exhibit this correlation (Sarkissian *et al*. 2001, Huang *et al*. 2002). Our study of four natural populations of *S. pygmaea* occurring in different climatic zones in China revealed a different pattern of gender variation in this species, which has the smallest-sized individuals in the genus. In field observations we found that male flower production in *S. pygmaea* varied widely and was positively correlated with plant size (midvein length) in all the sampled populations except for the YS population (r = 0.4558, $F_{1,8}$ = 1.66, P = 0.2391). The lack of a positive correlation between male flower production and midvein length in YS population might be associated with the small sample size of only nine individuals occasioned by the few *S. pygmaea* individuals flowering in August of 2006. Female flower production among all the sampled populations did not change with plant size. This indicated that environmental variation (which impacts strongly on plant size) had an impact on male flower production but it did not appear to affect female flower production.

The increased male flower production and consistent female flower production with increasing plant size will lead to more males than females (phenotypically) with increase in plant size. This situation is common in wind-pollinated species, because larger plant size is advantageous for wind-mediated pollen dispersal and pollen could spread to a longer distance (Burd & Allen 1988). However for entomophilous species, increase in male flower production with increased plant size may lead to more competition for ovules by pollen from the same parent (local mate competition, Lloyd & Bawa 1984, Ishii 2004), and more male flowers may result in increased geitonogamy and reduce the pollen available for outcrossing (pollen discounting, Harder & Barrett 1995, de Jong 2000, Ishii 2004). *Sagittaria pygmaea* is an entomophilous species, which is self-compatible and has a facultative mating system (Wang & Chen 1999). The species is dichogamous and the blooming times of flowers in the sequential inflorescence do not overlap. The possibility of pollen discounting seems unlikely in this species. Furthermore, since the plant has only one or two flowers in bloom each day on each inflorescence and the stigma becomes non-receptive within only half a day, an increase in the number of male flowers with plant size would not cause an increase in local mate competition considering that there are only a few flowers in bloom at any one time. In fact, larger individuals of *S. pygmaea* should have more chance to attract pollinators and should be more successful in seed production than smaller ones.

Unlike in some other congeneric species including S. *trifolia* and *S. latifolia*, female flower production in *S. pygmaea* remains consistent with changes in plant size. It is conceivable that this consistency may be compensated for by variations in ovule and inflorescence characteristics with plant size. However we found out that biomass of *S. pygmaea* plants had little effect on ovule number ($r = 0.142$, $F_{1,31} = 0.62$, $P = 0.438$; F. Liu unpubl. data) furthermore, variation in inflorescence number had no impact on gender modification in this species. The consistent female production may be a mechanism for the plants to optimize sex allocation to increase reproductive success. Due to the small plant body of *S. pygmaea*, the total resources allocated to sex reproduction may be so limited that the plant may be unable to afford to produce more females; the plant could produce one female flower to ensure ovule production and more male flowers to increase reproductive success. Indeed, in our field surveys we rarely observed individuals with more than one female flower in natural populations.

In our analysis of *S. pygmaea*, maleness often increases with plant size while femaleness appears to be unaffected. Increase in functional maleness with increase in plant size indicates that the resource allocation to males is more plastic than to females. Thus, the plants may in fact benefit more from increasing male flower production to enhance pollinator attraction and their paternal success than from producing more female flowers to increase maternal reproductive success.

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