ANNALS OF
BOTANY
Founded 1887

The relative importance of architecture and resource competition in allocation to pollen and ovule number within inflorescences of *Hosta ventricosa* varies with the resource pools

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• *Background and Aims* Allocation of resources to floral traits often declines distally within inflorescences in flowering plants. Architecture and resource competition have been proposed as underlying mechanisms. The aim of the present study is to assess the relative importance of resource competition and architectural effects in pollen and ovule production on racemes of *Hosta ventricosa*, an apomictic perennial herb.
• *Methods* Combinations of two defoliation treatments (intact and defoliated) and two fruit-set treatments (no-fruit and fruit) were created, and the roles of architecture and resource competition at each resource level were assessed.
• *Key Results* Pollen and ovule number per flower increased after defoliation, but pollen to ovule ratio per flower did not change. Pollen, ovules and the pollen to ovule ratio per flower declined distally on racemes at each resource level. In the intact treatment, fruit development of early flowers did not affect either pollen or ovule number of late flowers. In the defoliated treatment, fruit development of early flowers reduced both pollen and ovule numbers of late flowers due to over-compensation caused by defoliation. Late flowers on defoliated fruit racemes produced less pollen than intact fruit racemes but the same number of ovules; therefore, the reduction in pollen number was not caused by over-compensation. In addition, the fruit-set rate of early flowers during flowering was higher in intact racemes than in defoliated racemes.
• *Conclusions* In flowering plants, the relative importance of architecture and resource competition in allocation to pollen and ovules may vary with the resource pools or the overall resource availability of maternal plants.

Key words: Apomixis, architectural effects, *Hosta ventricosa*, ovule number, pollen number, pollen to ovule ratio, resource competition, resource pools, within inflorescences.

INTRODUCTION

Resource allocation is central to the life history of plants. In the past three decades, many studies have documented variation in investment to flowers and fruits within inflorescences of flowering plants (reviewed by Ashman and Hitchens, 2000; Diggle, 2003). Commonly, the sizes of individual floral organs, pollen and ovule number, and female reproductive success decline from basal to distal positions, although the opposite pattern occasionally occurs (Ashman and Hitchens, 2000; Diggle, 2003). As determining the sources of variation in floral morphology and function is important for the interpretations of floral morphogenesis and theories of sexual diversification of flowers within inflorescences in plants (Ashman and Hitchens, 2000; Barrett, 2002; Diggle, 2003; Ortiz *et al.*, 2009; Brookes and Burd, 2010), the ubiquity of basal-to-distal declines in size or production of reproductive structures has led to a search for general underlying mechanisms.

One mechanism that may explain variation in reproductive traits within inflorescences is resource competition. The resource competition hypothesis assumes that resources from the maternal plant for developing flowers and/or fruits are limited, and that some flowers and/or their subsequent fruits on their inflorescences have an advantage over others in garnering resources (Stephenson, 1981; Lee, 1988; Thomson, 1989; Diggle, 1995). For acropetally flowering inflorescences, for example, basal flowers, which are often produced first, may have both spatial and temporal advantages in sequestering photosynthetic products from leaves, or mineral nutrients from underground roots, and are therefore larger. The resource competition hypothesis is indicated by increases in allocation to distal structures when basal structures are removed (Diggle, 1995, 1997). There may also be positional variation in reproductive traits that is inherent to inflorescence architecture. Architectural effects are indicated if declining investment in distal structures still occurs when maturation of basal fruits is prevented (Diggle, 1995, 1997) or when resources are adequate (Ashman and Hitchens, 2000; Pritchard and Edwards, 2005; Brookes and Burd, 2010).

Experimental manipulations to date suggest that the relative importance of resource competition and architectural effects varies greatly among species. Architecture is primarily responsible for variation in reproductive traits within inflorescences

or plants in some studies (Diggle, 1995; Ashman and Hitchens, 2000; Wolfe and Denton, 2001; Pritchard and Edwards, 2005; Buide, 2008; Zhao *et al.*, 2008), while resource limitation alone or in combination with architecture has been demonstrated in others (Ashman, 1992; Wolfe, 1992; Medrano *et al.*, 2000; Vallius, 2000; Guitián *et al.*, 2001; Kliber and Eckert, 2004; Brookes and Burd, 2010). The question remains why the relative importance of resource competition and architectural effects varies among species.

The relative importance of architecture and resource competition may also vary among reproductive traits within a single species (Wolfe, 1992; Diggle, 1997; Ortiz *et al.*, 2009). For instance, Wolfe (1992) showed, by creating two pollination treatments, that a seasonal decline in inflorescence size and seed weight was more severe for heavily pollinated plants compared with incompletely pollinated plants, but the decline in flower size through time was identical in both pollination treatments. This phenomenon can be explained in three ways: (1) different traits may be determined at different times during a reproductive episode (Wolfe, 1992); (2) selection may favour preserving a given level of allocation to some traits at the cost of others under resource-deficient conditions (Ashman, 1992; Wolfe, 1992); and/or (3) selection may favour canalization of some traits (Ashman, 1992).

Fruit development need not impose a cost on floral traits, because there are many mechanisms which may reduce the fruiting cost (Obeso, 2002). For instance, reproductive costs may be weak and, thus, undetectable when investment in reproduction is low or resources are abundant (Horvitz and Schemske, 1988). It is expected that reproductive costs will be most apparent when resource availability is low or when plants are stressed (Tuomi *et al.*, 1983; Reznick, 1985). Therefore, although the absence of a fruiting cost of basal early flowers on distal late flowers could be explained by architecture, it may also indicate that the maternal plant had plentiful resources during certain periods of offspring development.

In the present study, two levels of overall resource availability were created for reproduction in *Hosta ventricosa* (Hostaceae), an apomictic hermaphrodite with acropetally blooming racemes, by experimental defoliation before flowering. First intra-inflorescence variation in pollen and ovule number was assessed under each resource availability (defoliated and intact). Secondly, the fruit-set effects of basal and middle flowers were evaluated on pollen and ovule number of distal flowers (Diggle, 1997). Pollen and ovule number in hermaphroditic species are assumed to compete for the same resource pool, and intra-inflorescence variation in pollen and ovules has been the focus of many studies, both theoretical (Brunet and Charlesworth, 1995; Ishii and Sakai, 2002; Mazer *et al.*, 2009) and empirical (Delesalle *et al.*, 2008). Dissecting the causes of variation in intra-inflorescence allocation to pollen and ovules could enhance our understanding of the diverse patterns of floral sex allocation (Ashman and Hitchens, 2000).

MATERIALS AND METHODS

Study species

Hosta ventricosa (Salisb.) Stearn (Hostaceae), a natural tetraploid, can reproduce via seeds and propagate vegetatively via rhizomes. Seeds are produced by pseudogamous apomixis (Schmid, 1991). It is a perennial hermaphrodite which grows in a variety of habitats in China, including forest floors and edges, roadsides and open areas. Individual plants of *H. ventricosa* produce several ramets and form small-sized clone patches. Each ramet produces a single stem, which bears seveal leaves at the base. Leaves emerge in early April and seem to mature in mid-May. Bolting of flowering stems starts in mid-June. Flowering occurs from late June to mid-July. Flowers are produced on a raceme inflorescence and open sequentially from basal to distal positions (acropetal blooming). Commonly, only one or two flowers open on the same day within an inflorescence. Longevity of individual flowers is 1 d. This species is adichogamous and self-compatible, but pollinator visitation is necessary for pollination (G. Cao, unpubl. res.). Flowers produce nectar and have six tepals, six stamens of similar size, and a gynoecium consisting of three carpels. Major pollinators are bumblebees (*Bombus trifasciatus*, *B. bicoloratus*, *B. flavescens*). Fruits mature at the end of September. *Hosta ventricosa* is widely distributed in Bifengxia National Nature Reserve, Yaan, Sichuan Province (30°04′N, 102°59′E), south-west China. The experiment was carried out in a relatively large population near the forest edge.

Data collection and experimental manipulations

Soon after the bolting of the flowering stem in mid-June, 2009, 160 racemes were selected randomly, numbered with plastic tags, and divided into four groups of 40 racemes. Then, combinations of two defoliation treatments (intact and defoliated) and two fruit-set treatments (no-fruit and fruit) were created. Meanwhile, all leaves of each raceme were cut off at the base in the two defoliated groups; therefore resources available for reproduction in these groups came mainly from storage organs. Flower buds on each raceme were assigned to one of three relative positions: basal, middle and distal. In the two fruit groups, basal and middle flowers of each raceme were hand-pollinated and all distal flower buds sampled just before their opening. Pollen was collected from neighbouring plants. Hand-pollinated flowers were exposed to open pollination thereafter. The fates of all basal and middle flowers on each raceme were recorded in the two fruit groups each day until all distal flower buds were sampled. In the two no-fruit groups, all floral buds on each raceme were sampled. The pistil removed from the bud was placed into a tube and preserved in 70 % alcohol. All anthers in a flower were collected and placed in a tube. During the period of data collection, 1 raceme in the defoliated no-fruit group was destroyed, and the final sample size was 39 for this group.

To assess whether defoliation decreases the resources available for reproduction, we calculated fruit-set rates of basal and middle flowers on each raceme in the two fruit groups. Fruit-set rate was expressed as the production of fruits or ovaries attached to the raceme when all distal flower buds were sampled, divided by the flower number of basal and middle positions.

Anthers were dried at room temperature. After all anthers dehisced, 5 mL of 3 : 1 glycerol : lactic acid were added into

the tube. The suspension was stirred with a vortex mixer for 60 s. Eight replicate 0·02-mL subsamples were placed on a haemocytometer and counted under a dissecting microscope; the average of eight counts per subsample was used to calculate total pollen number per flower. Ovules in each flower were counted under a dissecting microscope.

Statistical analyses

Intra-inflorescence variation in pollen number, ovule number and pollen to ovule ratio per flower were analysed with Linear Mixed Models repeated-measures ANOVAs with type III sums of squares. In the model, flower position (basal, middle and distal) was the repeated effect, defoliation (defoliated and intact) and its interaction with flower position were fixed effects, and the individual raceme was the random effect. Non-significant interactions were dropped from the final model using backwards elimination. To evaluate fruit-set effects of basal and middle flowers on pollen and ovule number of distal flowers, a two-way analysis of variance was performed. In this analysis, defoliation, fruit set and their interaction were specified as fixed effects. To normalize pollen and ovule number per flower, the Box–Cox power transformation $Y' = Y^{\lambda}$ (Sokal and Rohlf, 1981) in MINITAB 14 was used. The optimum values of λ were 1·6 for transformation of pollen number per flower, and 2·0 for transformation of ovule number per flower. Values for graphical representation were backtransformed.

The Mann–Whitney U test was used to assess the effect of defoliation on fruit-set rates of basal and middle flowers. All statistical analyses were conducted using the program SPSS Statistics 17·0.

RESULTS

The overall effects of defoliation and floral sex allocation on the inflorescence

Both defoliation and flower position significantly affected pollen number per flower (Table 1), but a significant interaction between them was not found ($F = 0.719$, $P = 0.490$), indicating that variation in the pollen number of racemes was similar for intact and defoliated treatments. Pollen number per flower declined from basal to distal positions along the raceme, and defoliation significantly increased pollen number per flower (Fig. 1A).

Similarly, both defoliation and flower position significantly affected ovule number per flower (Table 1), but a significant interaction term was not found ($F = 1.089$, $P = 0.341$), indicating that variation in ovules on racemes was similar for intact and defoliated treatments. Ovule number per flower declined from basal to distal positions along the raceme, and defoliation significantly increased ovule number per flower (Fig. 1B).

Flower position significantly affected the pollen to ovule ratio per flower, but defoliation did not (Table 1), and a significant interaction term was not found ($F = 0.745$, $P = 0.477$). The pollen to ovule ratio per flower declined from basal to distal positions along the raceme (Fig. 1C).

The relative importance of resource competition and architectural effects in intra-inflorescence variation

Mean ($\pm$ s.e.) flower number in intact no-fruit, intact fruit, defoliated no-fruit and defoliated fruit racemes was $26·8 \pm 0·5$, $27·2 \pm 0·6$, $28·0 \pm 0·6$ and $27·7 \pm 0·7$, respectively. Neither defoliation nor fruit set, nor their interaction affected flower number (all $P > 0.13$, two-way ANOVA).

Fruit set of basal and middle flowers significantly affected the pollen number of distal flowers, but defoliation did not, and a significant interaction between them was detected (Table 2). This is because fruit set had little effect on the pollen number of intact racemes, whereas it markedly reduced the pollen number of defoliated racemes, below even that of intact fruit racemes (Fig. 2A).

Both defoliation and fruit set significantly affected the ovule number of distal flowers, and a significant interaction between them was detected (Table 2). This is because fruit set did not significantly affect the ovule number of intact racemes, whereas it markedly reduced the ovule number of defoliated racemes, and there is little difference in ovule number between defoliated and intact fruit racemes (Fig. 2B).

The effect of defoliation on fruit-set rates of basal and middle flowers

Fruit-set rates of basal and middle flowers on intact and defoliated racemes when all distal flower buds were sampled were $98·8 \pm 0·4$ % and $93·7 \pm 0·6$ % (mean $\pm$ s.e.),

TABLE 1. *Results of Linear Mixed Models with type III sums of squares testing whether differences in pollen number, ovule number and pollen to ovule ratio per flower in* H. ventricosa *are affected by treatment and flower position*

Dependent variable	Source	Numerator d.f.	Denominator d.f.	F	P
Pollen number per flower*	Intercept	1	83·885	12162·143	<0·001
	Defoliation	1	75·445	8·451	<0·01
	Flower position	2	95·383	465·076	<0·001
Ovule number per flower*	Intercept	1	84·232	9108·425	<0·001
	Defoliation	1	75·715	11·180	<0·001
	Flower position	2	86·085	148·994	<0·001
Pollen to ovule ratio per flower	Intercept	1	69·311	33836·842	<0·001
	Defoliation	1	72·386	0·070	0·791
	Flower position	2	113·181	267·906	<0·001

The values for denominator degrees of freedom were obtained by a Satterthwaite approximation and the linear mixed models in SPSS was used.

* Pollen and ovule number per flower were transformed to the power of 1·6 and 2, respectively, to conform to normality.

respectively. There was a highly significant difference between them ($Z = -6.103$, $P < 0.001$).

DISCUSSION

Defoliation significantly increased pollen and ovule production per flower. Previous studies have reported over-compensation in various reproductive characters, such as flower number
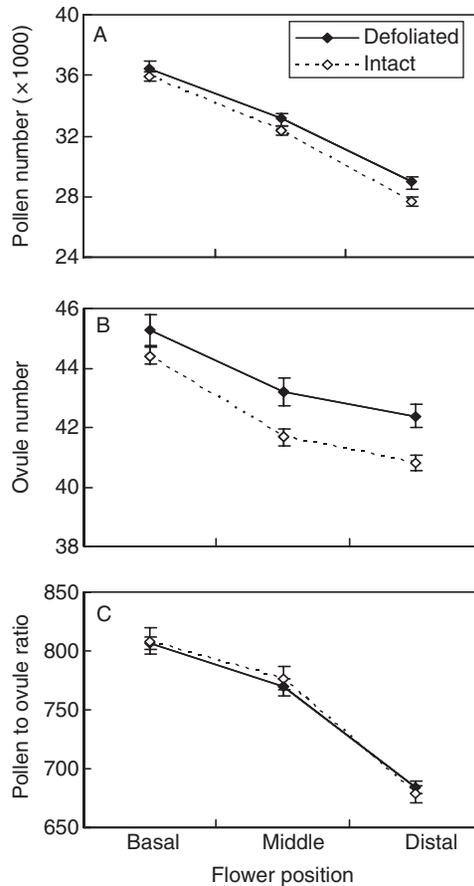

FIG. 1. Intra-inflorescence variation in pollen number, ovule number and pollen to ovule ratio per flower (means ± s.e.) of intact and defoliated racemes of *Hosta ventricosa*.

(e.g. Paige and Whitham, 1987), fruit number (e.g. Paige and Whitham, 1987), nectar production per flower (e.g. Fang *et al.*, 2006) and seed production per fruit (e.g. Marshall *et al.*, 2005), after herbivory or artificial defoliation. The underlying mechanism of reproductive over-compensation is still controversial (Tiffin, 2000). Two putative common mechanisms, increased photosynthetic activity and utilization of stored reserves, have been proposed (Strauss and Agrawal, 1999; Tiffin, 2000). In the present study, all leaves on defoliated racemes were clipped, and defoliated racemes did not compensate for this loss by producing new leaves during the flowering period. Individual plants of *H. ventricosa* produce several ramets and form small-sized clonal patches, such that leaves on each ramet are partly shadowed by those on nearby ramets of the same plant. It is therefore possible that clipping leaves on one ramet could increase the photosynthetic capability of leaves on nearby intact ramets (Thomson *et al.*, 2003). This explanation seems unlikely here, since flowers on intact ramets produced fewer pollen grains and ovules than those on defoliated ramets. *Hosta ventricosa* is a species with a perennial rhizome, from which leaves and flowering stems grow. Therefore, the increased resources allocated to pollen and ovules on defoliated ramets may come from their own underground rhizomes (Briske *et al.*, 1996), or/and nearby ramets of the same plant through underground ramet connections.

Over-compensation in pollen production per flower after defoliation cannot increase male fitness of *H. ventricosa*, as proposed for some taxa (Strauss *et al.*, 1996; Paige *et al.*, 2001), because it is an apomictic system. Over-compensation of ovules did not increase female fitness either, as defoliated racemes produced an average of 9.36 ± 0.60 (s.e.) seeds per flower, whereas intact racemes produced 13.29 ± 0.54 seeds per flower ($F_{1,77} = 23.938$, $P = 0.000$; both $n = 40$). On the contrary, over-compensation in pollen and ovule production after defoliation in *H. ventricosa* used the resources which otherwise might have been used for seed maturation, or for future growth and reproduction. Over-compensation thus may reduce lifetime fitness (Ackerman and Montalvo, 1990).

Pollen and ovule number declined along the inflorescence of *H. ventricosa*, as was the typical pattern of intra-inflorescence variation in floral and fruit traits (Diggle, 1995, 2003), including closely related *H. rectifolia* (Cao *et al.*, 2007). As compared with basal positions, pollen and ovule number at distal

TABLE 2. *Results of two-way ANOVA with type III sums of squares testing whether differences in pollen and ovule number per flower at distal positions of* H. ventricosa *are affected by defoliation and fruit set*

Dependent variable	Source	d.f.	MS	F	P
Pollen number per flower*	Intercept	1	2.563×10^{16}	12465.601	<0.001
	Defoliation	1	6.841×10^{11}	0.333	0.565
	Fruit set	1	6.200×10^{13}	30.156	<0.001
	Defoliation × fruit set	1	2.819×10^{13}	13.711	<0.001
	Error	155	2.056×10^{12}		
Ovule number per flower*	Intercept	1	4.629×10^{8}	17934.833	<0.001
	Defoliation	1	158274.409	6.132	<0.05
	Fruit set	1	127380.354	4.935	<0.05
	Defoliation × fruit set	1	202693.947	7.853	<0.01
	Error	155	25812.121		

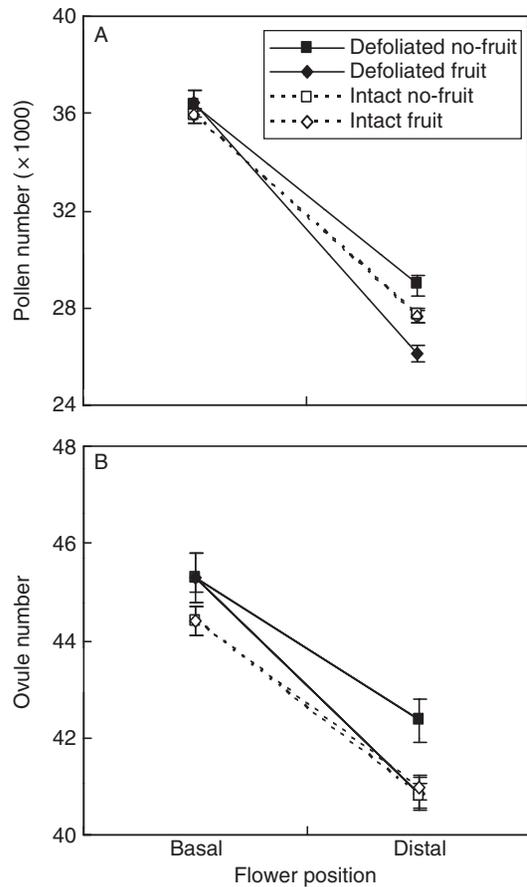* Pollen and ovule number per flower were transformed to the power of 1.6 and 2, respectively, to conform to normality.

FIG. 2. Variation in pollen and ovule number per flower between basal and distal positions (means ± s.e.) of fruit and no-fruit racemes in intact and defoliated treatments of *Hosta ventricosa*. Note that lines denoting variation in pollen number on intact fruit and no-fruit racemes almost overlap.

positions were reduced by 23 % and 8 %, respectively. Architectural effects were therefore trait-specific (Diggle, 1995, 2003). Defoliation did not change the overall pattern of intra-inflorescence variation in pollen and ovules. Few other studies have evaluated the effects of resource pools on intra-inflorescence variation in floral traits. Complete defoliation of *Aquilegia canadensis* (Kliber and Eckert, 2004) and *Platanthera bifolia* (Vallius and Salonen, 2006) before flowering did not alter the overall size or positional variation of individual floral organs. These results and those of the present study suggest that stored resources in these species were sufficient to support the size or production of floral traits.

When reproduction was supported by both reserved resources and current foliar photosynthesis, *H. ventricosa* did not develop fruits at the cost of either pollen or ovules. However, we cannot rule out the possibility that fruits were developed at the cost of other floral traits (Ashman, 1992; Diggle, 1995, 1997). For example, Ashman (1992) found that biomass allocated to sepals, androecium and petals declined in pollinated plants faster than in unpollinated plants, but allocation to pollen and ovules did not. Resource competition between developing fruits at basal and middle positions and distal flowers on the same raceme in *H. ventricosa* may be weak, since the delay between

pollination of basal and middle flowers, and anthesis of distal flowers of the raceme was only 12 d (standard deviation = 2, range = 7–15, *n* = 198), while fruits matured at the end of September. Alternatively, ramets may have plentiful resources available for fruit and flower development during flowering, since fruit-set rates of basal and middle flowers were very high and no fruit aborted in most of the 40 intact fruit racemes when distal flowers were mature.

Defoliation reduced the fruit-set rate of basal and middle flowers, indicating that current foliar photosynthesis contributed to fruit development, and/or overall resource availability was not sufficient to support the development of both fruits and flowers during flowering. Using only reserved resources, maturing fruits caused reductions in both pollen and ovule number. However, the response to fruit development differed between the two traits. Due to fruit set of basal and middle flowers, pollen and ovule numbers of distal flowers were reduced by 10 % and 4 %, respectively. In addition, distal flowers on defoliated fruit racemes produced 5 % less pollen than intact fruit racemes and the same number of ovules. Therefore, *H. ventricosa* allocated resources to maturing fruits at the cost of both pollen and ovules due to over-compensation caused by defoliation, and at the cost of pollen only in the absence of over-compensation.

Architectural patterns of intra-inflorescence variation in pollen and ovules have been viewed as the result of natural selection and thus may reflect adaptations to differences in mating opportunities and/or resource conditions among flowers at different positions (Brunet and Charlesworth, 1995; Mazer and Dawson, 2001). In many hermaphroditic species, ovule number per flower generally declines from early to late flowers, whereas pollen number per flower often remains constant or declines to a lesser degree (Brunet, 1996; Vogler *et al.*, 1999; Kudo *et al.*, 2001; Mazer and Dawson, 2001; García, 2003; Hiraga and Sakai, 2007; Zhao *et al.*, 2008; Zeng *et al.*, 2009). Male-biased sex allocation in late flowers may be associated with protandry (Brunet and Charlesworth, 1995) or low resource status of individual flowers (Mazer and Dawson, 2001). Temporal increases in female function in late flowers of protogynous *Aquilegia yabeana* (Huang *et al.*, 2004) and *Helleborus foetidus* (Guitián, 2006) are consistent with the predicted sex-allocation pattern for plants with protogynous flowers (Brunet and Charlesworth, 1995). However, in addition to *H. ventricosa* (the present study), female-biased sex allocation in late flowers has been found in another adichogamous plant *Aquilegia canadensis* (Kliber and Eckert, 2004). Both results could not be explained by the mating environment theory (Brunet and Charlesworth, 1995). In some studies (Ashman *et al.*, 2001; Ishii and Sakai, 2002; Cao *et al.*, 2007; Delesalle *et al.*, 2008; Mazer *et al.*, 2009), temporal change in floral sex allocation was estimated from one early flower and one late flower. In these studies, the fruit set of flowers was not prevented, and thus the effects of early fruits on pollen or ovule production of late flowers were unknown. The models of Brunet and Charlesworth (1995) were not designed to predict how sex allocation per flower should change over time when resources are depleted by early fruits. Fruit development of early flowers has been shown to increase the ovule number of late flowers in *Silene acutifolia*

(Buide, 2008) and decrease pollen number in *Emex spinosa* (Ortiz *et al.*, 2009). These two studies and the present one suggest that future theoretical and empirical studies of temporal sex allocation should take the fates of early flowers into account.

In flowering plants, the resources available to reproduction may come from stored organs and/or current foliar photosynthesis. Empirical studies suggest that the resource budget for current reproduction varies greatly among species. For instance, in comparisons of defoliation effects on seed output (Obeso, 1993), 44 % of herbaceous species did not change seed production after defoliation. This is because some species did not use current photosynthetic products by the leaves but rather used reserved resources for current reproduction (e.g. Lapointe, 1998). The present study demonstrates that all else being equal, the relative importance of resource competition and architecture in allocation to floral traits within inflorescences may depend upon the resource pools for reproduction or overall resource availability of maternal plants.

ACKNOWLEDGEMENTS

LITERATURE CITED

Ackerman JD, Montalvo AM. 1990. Short- and long-term limitations to fruit production in a tropical orchid. *Ecology* **71**: 263–272.

Ashman T-L. 1992. Indirect costs of seed production within and between seasons in a gynodioecious species. *Oecologia* **92**: 266–272.

Ashman T-L, Hitchens MS. 2000. Dissecting the causes of variation in intra-inflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *American Journal of Botany* **87**: 197–204.

Ashman T-L, Pacyna J, Diefenderfer C et al. 2001. Size-dependent sex allocation in a gynodioecious wild strawberry: the effects of sex morph and inflorescence architecture. *International Journal of Plant Sciences* **162**: 327–324.

Barrett SCH. 2002. The evolution of plant sexual diversity. *Nature Reviews Genetics* **3**: 274–284.

Briske DD, Boutton TW, Wang Z. 1996. Contribution of flexible allocation priorities to herbivory tolerance in C₄ perennial grasses: an evaluation with ¹³C labeling. *Oecologia* **105**: 151–159.

Brookes RH, Burd M. 2010. Reproductive investment within inflorescences of *Stylidium armeria* varies with the strength of early resource commitment. *Annals of Botany* **105**: 697–705.

Brunet J. 1996. Male reproductive success and variation in fruit and seed set in *Aquilegia caerulea* (Ranunculaceae). *Ecology* **77**: 2458–2471.

Brunet J, Charlesworth D. 1995. Floral sex allocation in sequentially blooming plants. *Evolution* **49**: 70–79.

Buide ML. 2008. Disentangling the causes of intrainflorescence variation in floral traits and fecundity in the hermaphrodite *Silene acutifolia*. *American Journal of Botany* **95**: 490–497.

Cao GX, Kudo G, Ida TY. 2007. Floral sex allocation in a hermpahrodite herb with 1-day flowers, *Hosta rectifolia* (Liliaceae). *Plant Species Biology* **22**: 191–196.

Delesalle VA, Mazer SJ, Paz H. 2008. Temporal variation in the pollen:ovule ratios of *Clarkia* (Onagraceae) taxa with contrasting mating systems: field populations. *Journal of Evolutionary Biology* **21**: 10–23.

Diggle PK. 1995. Architectural effects and the interpretation of patterns of fruit and seed development. *Annual Review of Ecology and Systematics* **26**: 531–552.

Diggle PK. 1997. Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. *International Journal of Plant Sciences* **158** (Suppl.): 99–107.

Diggle PK. 2003. Architectural effects on floral form and function: a review. In: Stuessy T, Horandl E, Mayer V. eds. *Deep morphology: toward a renaissance of morphology in plant systematics*. Koeltz: Konigstein.

Fang XW, Yun JL, Wang G et al. 2006. Fruit production of shrub, *Caragana korshinskii*, following above-ground partial shoot removal: mechanisms underlying compensation. *Plant Ecology* **187**: 213–225.

García MB. 2003. Sex allocation in a long-lived monocarpic plant. *Plant Biology* **5**: 203–209.

Guitián P. 2006. Phenotypic gender variation within inflorescences of the protogynous species *Helleborus foetidus* (Ranunculaceae). *Plant Systematics and Evolution* **258**: 137–146.

Guitián J, Guitián P, Medrano M. 2001. Causes of fruit set variation in *Polygonatum odoratum* (Liliaceae). *Plant Biology* **3**: 637–641.

Hiraga T, Sakai S. 2007. The effects of inflorescence size and flower position on mass and temporal sex allocation in *Lobelia sessiliflora*. *Plant Ecology* **188**: 205–214.

Horvitz CC, Schemske DW. 1988. Demographic cost of reproduction in a Neotropical herb: an experimental field study. *Ecology* **69**: 1741–1745.

Huang SQ, Tang LL, Yu Q et al. 2004. Temporal floral sex allocation in protogynous *Aquilegia yabeana* contrasts with protandrous species: support for the mating environment hypothesis. *Evolution* **58**: 1131–1134.

Ishii HS, Sakai S. 2002. Temporal variation in floral display size and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae). *American Journal of Botany* **89**: 441–446.

Kliber A, Eckert CG. 2004. Sequential decline in allocation among flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology* **85**: 1675–1687.

Kudo G, Maeda T, Narita K. 2001. Variation in floral sex allocation and reproductive success within inflorescences of *Corydalis ambigua* (Fumariaceae): pollination efficiency or resource limitation? *Journal of Ecology* **89**: 48–56.

Lapointe L. 1998. Fruit development in *Trillium*. *Plant Physiology* **117**: 183–188.

Lee TD. 1988. Patterns of fruit and seed production. In: Lovett Doust J, Lovett Doust L. eds. *Plant reproductive ecology: patterns and strategies*. New York, NY: Oxford University Press, 179–202.

Marshall DL, Abrahamson NJ, Avritt JJ et al. 2005. Differences in plastic responses to defoliation due to variation in the timing of treatments for two species of *Sesbania* (Fabaceae). *Annals of Botany* **95**: 1049–1058.

Mazer SJ, Dawson KA. 2001. Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): ontogenetic and among-plant variation. *American Journal of Botany* **88**: 819–831.

Mazer SJ, Dudley LS, Delesalle VA et al. 2009. Stability of pollen–ovule ratios in pollinator-dependent versus autogamous *Clarkia* sister taxa: testing evolutionary predictions. *New Phytologist* **183**: 630–648.

Medrano M, Guitian P, Guitian J. 2000. Patterns of fruit and seed set within inflorescences of *Pancratium maritimum* (Amaryllidaceae): nonuniform pollination, resource limitation, or architectural effects? *American Journal of Botany* **87**: 493–501.

Obeso JR. 1993. Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? *Functional Ecology* **7**: 150–155.

Obesco JR. 2002. The costs of reproduction in plants. *New Phytologist* **155**: 321–348.

Ortiz PL, Berjano R, Talavera M et al. 2009. The role of resources and architecture in modeling floral variability for the monoecious amphicarpic *Emex spinosa* (Polygonaceae). *American Journal of Botany* **96**: 2062–2073.

Paige KN, Whitham TG. 1987. Overcompensation in response to mammalian herbivory: the advantage of being eaten. *American Naturalist* **129**: 407–416.

Paige KN, Williams B, Hickox T. 2001. Overcompensation through the paternal component of fitness in *Ipomopsis arizonica*. *Oecologia* **128**: 72–76.

Pritchard K, Edwards W. 2005. Architectural constraint in fruit production of *Crotalaria spectabilis* (Fabaceae). *Plant Species Biology* **20**: 41–46.

Reznick D. 1985. Cost of reproduction: an evaluation of the empirical evidence. *Oikos* **44**: 257–267.

Schmid WG. 1991. *The genus* Hosta. London and Portland: Timber Press.

Sokal RR, Rohlf FJ. 1981. *Biometry: the principles and practice of statistics in biological research*, 2nd edn). San Francisco, CA: W. H. Freeman and Co.

Stephenson AG. 1981. Flower and fruit abortion: proximate causes and ultimate function. *Annual Review of Ecology and Systematics* **12**: 253–279.

Strauss SY, Agrawal AA. 1999. The ecology and evolution of plant tolerance to herbivory. *Trends in Ecology and Evolution* **14**: 179–185.

Strauss SY, Conner JK, Rush SL. 1996. Foliar herbivory affects floral characters and plant attractiveness: implications for male and female plant fitness. *American Naturalist* **147**: 1098–1107.

Thomson JD. 1989. Deployment of ovules and pollen among flowers within inflorescences. *Evolutionary Trends in Plants* **3**: 65–68.

Thomson VP, Cunningham SA, Ball MC *et al*. 2003. Compensation for herbivory by *Cucumis sativus* through increased photosynthetic capacity and efficiency. *Oecologia* **134**: 167–175.

Tiffin P. 2000. Mechanisms of tolerance to herbivore damage: what do we know? *Evolutionary Ecology* **14**: 523–536.

Tuomi J, Hakala T, Haukioja E. 1983. Alternative concepts of reproductive effort, costs of reproduction, and selection in life-history evolution. *American Zoologist* **23**: 25–34.

Vallius E. 2000. Position-dependent reproductive success of flowers in *Dactylorhiza maculata* (Orchidaceae). *Functional Ecology* **14**: 573–579.

Vallius E, Salonen V. 2006. Allocation to reproduction following experimental defoliation in *Platanthera bifolia* (Orchidaceae). *Plant Ecology* **183**: 291–304.

Vogler DW, Peretz S, Stephenson AG. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in *Campanula rapunculoides* (Campanulaceae). *American Journal of Botany* **86**: 482–494.

Wolfe LM. 1992. Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae)? Developmental constraints vs. resource limitation. *American Journal of Botany* **79**: 1286–1290.

Wolfe LM, Denton W. 2001. Morphological constraints on fruit size in *Linaria canadensis*. *International Journal of Plant Sciences* **162**: 335–342.

Zeng YF, Bai WN, Zhou Y *et al*. 2009. Variation in floral sex allocation and reproductive success in sequentially flowering inflorescence of *Corydalis remota* var. *lineariloba* (Fumariaceae). *Journal of Integrative Plant Biology* **51**: 299–307.

Zhao ZG, Meng JL, Fang BL *et al*. 2008. Reproductive patterns within racemes in protandrous *Aconitum gymnandrum* (Ranunculaceae): potential mechanism and among-family variation. *Plant Systematics and Evolution* **273**: 247–256.