Evolutionary Ecology Research, 2013, **15**: 903–917

An association between floral sex allocation and floral longevity in a non-sequentially blooming ladybell

Chang-Qiu Liu[1] and Shuang-Quan Huang[2]

[1]*Key Laboratory for Plant Diversity and Biogeography of East Asia,*
Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, China and
[2]*School of Life Sciences, Central China Normal University, Wuhan, China*

ABSTRACT

Background: Floral longevity has been assumed to reflect a balance between the fitness gain through increased pollination and the cost of flower maintenance.

Question: Is there variation in the amount of resources allocated to male and female functions within flowers? Does floral longevity vary with temporal variation in floral sex allocation?

Organism: *Adenophora jasionifolia*, a perennial species with flowers differing greatly in floral sex allocation.

Field site: Shangri-La Alpine Botanical Garden, Yunnan Province, southwest China.

Methods: Daily observations of flowers for floral longevity, counts of traits indicating resource allocation, bagging flowers to reveal the effect of pollinator visitation on floral longevity.

Results: Within a six-flowered inflorescence, the apical flower opened first followed by the middle flower, before two pairs of flowers in different spatial positions opened simultaneously. The patterns of floral sex allocation and floral longevity were strongly associated with the order of flower opening but not with the spatial arrangement of flowers within inflorescences. The shift to male-biased sex allocation in late-opening flowers corresponded with a decrease in floral longevity, and reduced female allocation.

Conclusion: There is a temporal decline of floral longevity and female sex allocation in *A. jasionifolia*. This positive relationship between floral longevity and floral sex allocation provides new insights into the evolution of floral longevity.

Keywords: *Adenophora jasionifolia*, Campanulaceae, dichogamy, floral longevity,
floral sex allocation, mating-environment hypothesis, pollen–ovule ratio,
pollen removal and receipt.

INTRODUCTION

Variation in floral longevity, the time over which a flower remains open and functional, affects the total number of pollinator visits and consequently the amount and quality of

---

Correspondence: S.-Q. Huang, School of Life Sciences, Central China Normal University, Wuhan 430079, China.
e-mail: sqhuang@whu.edu.cn *or* hsq@mail.ccnu.edu.cn

pollen removal and receipt (Primack, 1985; Ashman and Schoen, 1996; Harder and Johnson, 2005). Increased floral longevity may increase mating opportunities, but investing resources to increase floral longevity may come at the cost of plant maintenance and growth (Ashman and Schoen, 1994; Castro *et al.*, 2008). As plants often experience pollination failure because of inefficient pollen transfer (Harder and Routley, 2006), male reproductive success might be maximized by having flowers that have a relatively high investment in pollen also have a relatively long male phase (see Itagaki and Sakai, 2006), given that the length of the male phase could limit pollen removal and waste resources. In other words, floral longevity might correlate with investment and pollinator attraction within flowers. Experimental evidence in support of this hypothesis is scarce. Observations by Kerner von Marilaun in 1895 suggested that floral longevity was positively related to pollen production per flower (see Ashman and Schoen, 1996). A comparison of 110 species from a Costa Rican forest indicated that individual flower longevity was positively related to flower size and ovule number per flower (Stratton, 1989). Lastly, floral longevity was positively correlated with flower size and nectar reward in 13 bee-pollinated Labiatae species in Israel (Dafni, 1991).

Floral sex allocation, or the proportion of resources allocated to male and female functions within flowers, and floral longevity generally vary with flowering sequence. Early-opening flowers tend to allocate more resources to female function (Stephenson, 1981; Wyatt, 1982; Ashman and Hitchens, 2000; Medrano *et al.*, 2000; Mazer and Dawson, 2001; Diggle, 2003) and to remain open longer relative to later-opening flowers (Sargent and Roitberg, 2000; Ishii and Sakai, 2001; Itagaki and Sakai, 2006). For example, a survey of 14 liliaceous species found that ovule production commonly declined among sequentially blooming flowers but pollen production did not (Thomson, 1989). Such a temporal pattern of floral sex allocation has been attributed to variation in the mating environment (opportunity for pollen donation and/or receipt) experienced by individual flowers, i.e. the mating-environment hypothesis (Brunet and Charlesworth, 1995). For example, in protandrous species, flowers produced early may have a low ratio of available ovules to pollen competing for these ovules (Brunet, 1996; Ishii and Sakai, 2002; Huang *et al.*, 2004; Brookes and Jesson, 2010). The hypothesis predicts that protandry would select for a more female-biased allocation (more ovules) in early flowers with an increase to male-biased allocation (more pollen) in later blooming flowers.

It remains unclear whether temporal variation in floral sex allocation correlates with variation in floral longevity. Specifically, it is of interest whether variation in floral longevity covaries with the shift of floral allocation from female- to male-biased in protandrous species. Itagaki and Sakai (2006) found a positive relationship between floral sex allocation and floral longevity in the protandrous, two-flowered plant species, *Aquilegia buergeriana*, in which the second flower opens one week after the first. The first flower remains open a greater number of days, has a significantly longer male phase, and produces more pollen grains than the second flower (Itagaki and Sakai, 2006). These results suggest that the longer duration of the male phase could be related to the larger number of pollen grains in the first flowers. However, the male-biased allocation in the first flowers contrasts with the expectation of protandrous species, which have been shown frequently to exhibit relatively lower pollen production in earlier- than later-blooming flowers (Brunet, 1996; Vogler *et al.*, 1999; Mazer and Dawson, 2001; Hiraga and Sakai, 2007; Delesalle *et al.*, 2008; Zhao *et al.*, 2008).

To examine the relationship between floral sex allocation and floral longevity, we investigated the proportion of reproductive resources allocated to male and female functions within flowers in the six-flowered inflorescence of the alpine ladybell *Adenophora jasionifolia* (Campanulaceae). Floral sex allocation has frequently been reported to vary among flowers.

In inflorescences in which flowers mature either acropetally or basipetally [the normal situation (see review in Stephenson, 1981; Thomson, 1989)], it is difficult to separate the effect of position from the effect of the flowering sequence (Wesselingh and Arnold, 2003; Huang *et al.*, 2004; Buide, 2008). However, in *A. jasionifolia*, the time of flower opening and position on the inflorescence are largely decoupled. Flowering begins with the apical flower and then follows an unusual sequence: a relatively distal flower may open earlier or later than a basal flower. In particular, some flowers blooming simultaneously occupy different positions, while overall the flower positions can be divided into different temporal ranks that each contain one or two flower positions. This characteristic provides an ideal opportunity to disentangle temporal from positional effects. Additionally, flowers of *A. jasionifolia* are protandrous with secondary pollen presentation typical of many *Campanula* species, making male and female phases distinguishable. Thus, the great variation in sex allocation within inflorescences and distinct demarcation between male and female phases enabled us to investigate the relationship between sex allocation and floral longevity.

MATERIALS AND METHODS

Study species and site

Adenophora jasionifolia Franch. (Campanulaceae) is a perennial herb, endemic to southwest China. It grows in alpine meadows or at the edges of forests. Individual plants produce 1–4 unbranched stalks in late spring. Each stalk develops a terminal inflorescence in summer with 1–8 blue bell-shaped flowers (Fig. 1). The inflorescence is called a 'pseudoraceme', which is essentially a monochasium (Hong, 1983). The apical flower bud develops first but the developmental process of the inflorescence does not follow the basipetal pattern that is common in monochasia. Flowers of *A. jasionifolia* are strongly protandrous, and exhibit secondary pollen presentation like other Campanulaceae species (Nyman, 1993). The male phase lasts 1–3 days and is followed by a female phase of 2–4 days (see Results). Before flowers open, the five anthers release pollen onto pollen-collecting hairs of the style when the stigmatic lobes are still closed with no receptive surface exposed. At this point, the style functions as the pollen-presenting organ. In late anthesis (female phase), the stigmatic lobes open with the receptive surface exposed following the retraction of pollen-collecting hairs. Bumblebees are effective pollinators (Liu and Huang, 2013).

In late July to August 2008 and 2010, we studied a natural population with hundreds of individuals, 3200 m above sea level in Shangri-La Alpine Botanical Garden, Yunnan Province, southwest China (27°54′05″N, 99°38′17″E). In this study, we chose plants with six-flowered inflorescences (Fig. 1), which were the most common in the field.

Floral longevity

To document the order of flower opening in an inflorescence, we randomly tagged 46 (in 2008) and 51 (in 2001) inflorescences, each from a different plant, and recorded flowering status at 8:00 and 17:00 h every day. From top to bottom, we named the six flowers flower A (the apical flower), B, C, D, E, and F, respectively (Fig. 1). On each inflorescence, we assigned a number to each flower according to the temporal flowering sequence. We used the average value of these temporal series per spatial position to represent the sequential flowering of the inflorescences.

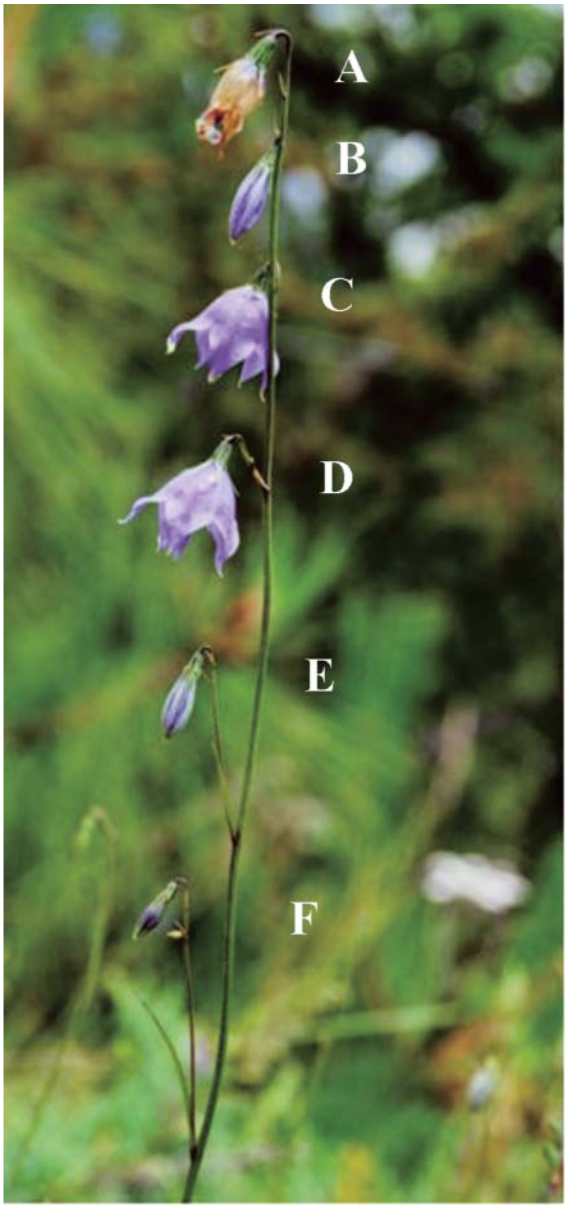

Fig. 1. An inflorescence of *Adenophora jasionifolia*. Flowers are labelled according to basipetal position. The apical flower is wilted. In this inflorescence, D flowered, followed by C.

To compare duration of male and female phases, the time of each flower opening on 30 (in 2008) and 50 (in 2010) inflorescences was monitored in detail twice per day. Reflexation of stigmatic lobes and exposure of receptive surfaces was considered to be the beginning of the female phase (see Evanhoe and Galloway, 2002). The beginning of corolla wilting was defined as the beginning of senescence. We defined floral longevity as the duration from flower opening to senescence, the male phase of the flower as the duration from flower opening to exposure of the stigma, and the female phase of the flower as the duration from exposure of the stigma to senescence.

To examine whether pollen removal or receipt affected floral longevity in *A. jasionifolia*, we bagged the apical flowers (flower A) with nylon nets to exclude pollinators. To demonstrate the effect of pollen removal on the duration of the male phase, we bagged 27 flowers before they opened in 2010 and used 27 open-pollinated flowers as controls. To determine the effect of pollen receipt on the duration of the female phase, we bagged 30 flowers that were at male phase according to morphology, but that already had their pollen removed from the styles by insects; 28 open-pollinated flowers were chosen as controls. To reduce the possible effects of weather and fluctuation in pollinator visitation, an equal number of flowers received the above treatments on a given day.

Floral allocation and pollinator attraction

To evaluate floral sex allocation, we collected the anthers of each flower on 22 (in 2008) and 48 (in 2010) six-flowered inflorescences. Young flowers are light blue and turn dark blue just before anther dehiscence and anthesis (Liu and Huang, 2013). This colour change permitted us to collect anthers and estimate pollen number before dehiscence with secondary pollen presentation. We longitudinally incised each corolla with a razor blade, and removed the anthers with forceps. All anthers from the same flower were fixed in standard FAA solution (formalin–acetic acid–70% ethanol in a ratio of 5:6:89 by volume) in one microcentrifuge tube. In the laboratory, the anthers were split and suspended, and pollen grains in ten drops of pollen solution (amounting to 20 mL) were counted under a microscope. To count ovules, we correspondingly collected the flowers from which anthers were removed for counting 3 weeks after the last flower wilted. Pollen and ovule production and pollen–ovule ratio were calculated for each flower position.

To investigate whether floral longevity was positively related to flower size in *A. jasionifolia*, we measured the length of the corolla tube to 0.1 mm, using calipers from each fully open, male-phase flower per inflorescence from those 28 randomly chosen inflorescences for which we measured floral longevity. Length of a corolla tube was the distance from the corolla base to the apex of the longest corolla lobe.

Statistical analyses

Repeated-measures analyses of variance (ANOVAs) followed by Bonferroni tests were used to compare floral sex allocation, flower size, and floral longevity, as well as the duration of male and female phase among flower positions. To detect whether the pattern of floral sex allocation and floral longevity among temporal flower positions differed between years, two-way ANOVAs were used. We used Pearson's correlation tests to examine the relationship between average floral sex allocation and floral longevity, as well as the duration of male or female phase of each flower position. The effect of insect visitation on the duration of the male and female phases was assessed with one-way ANOVAs. We analysed the data using SPSS 16.0.1.

RESULTS

Flowering sequence

Flowering sequence did not differ between the two years, as indicated by the lack of a significant interaction between year and flowering sequence in the two-way ANOVAs. Flower positions differed significantly in flowering sequence ($F_{5,45} = 220.66$, $P < 0.0001$ in 2008; $F_{5,50} = 261.07$, $P < 0.0001$ in 2010) (Fig. 2). Bonferroni tests further demonstrated that there were significant differences in flowering sequences for all flower positions, except between positions C and E, B and F, and D and E (the latter differing only in 2008). Based on flowering sequence, the six positions could be classified into four temporal ranks. Flower A was always the first to bloom. Flower D was the most likely flower to follow. Next came flower C and E with equal probability. Flowers B and F bloomed last (Fig. 2).

Pollen and ovule allocation, and flower size

Although pollen counts and the pollen–ovule ratio varied among flowers, the pattern of variation in these two variables among flowers did not change between years (no significant interactions were observed between position and year). Variation in floral allocation between flower positions was significant (Table 1; Fig. 2). Both pollen and ovule production declined from early to late flowers, but ovule production declined more abruptly. The earlier flowers opened, the more pollen they produced ($F_{5,21} = 72.62$, $P < 0.0001$ in 2008; $F_{5,47} = 36.54$, $P < 0.0001$ in 2010) (Fig. 2C, D), although flower D did not produce significantly more pollen than flower C or E (Bonferroni tests, $P > 0.05$ in both years). Two flowers within any one temporal rank (flowers B and F, flowers C and E) did not differ significantly in pollen production ($P > 0.05$ in both years; Fig. 2C, D).

The sequentially opening flowers differed significantly in ovule production ($F_{5,21} = 147.64$, $P < 0.0001$ in 2008; $F_{5,47} = 112.23$, $P < 0.0001$ in 2010) (Fig. 2E, F) and early flowers con-

Table 1. Results of two-way ANOVAs examining whether patterns of floral sex allocation and floral longevity differed among flower positions and between years

		Pollen production		Ovule production		Pollen–ovule ratio	
Variable	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Year	1	9.8	**0.002**	1.6	0.207	8.9	0.003
Flower position	5	10.2	**<0.0001**	50.7	**<0.0001**	18.5	**<0.0001**
Year × flower position	5	0.1	0.994	0.7	0.607	0.2	
Error	408						

		Male phase duration		Female phase duration		Total floral longevity	
Variable	d.f.	*F*	*P*	*F*	*P*	*F*	*P*
Year	1	200.3	**<0.0001**	5.7	**0.017**	170.8	**<0.0001**
Flower position	5	0.5	0.747	43.9	**<0.0001**	21.6	**<0.0001**
Year × flower position	5	0.5	0.812	0.1	0.983	0.3	0.905
Error	468						

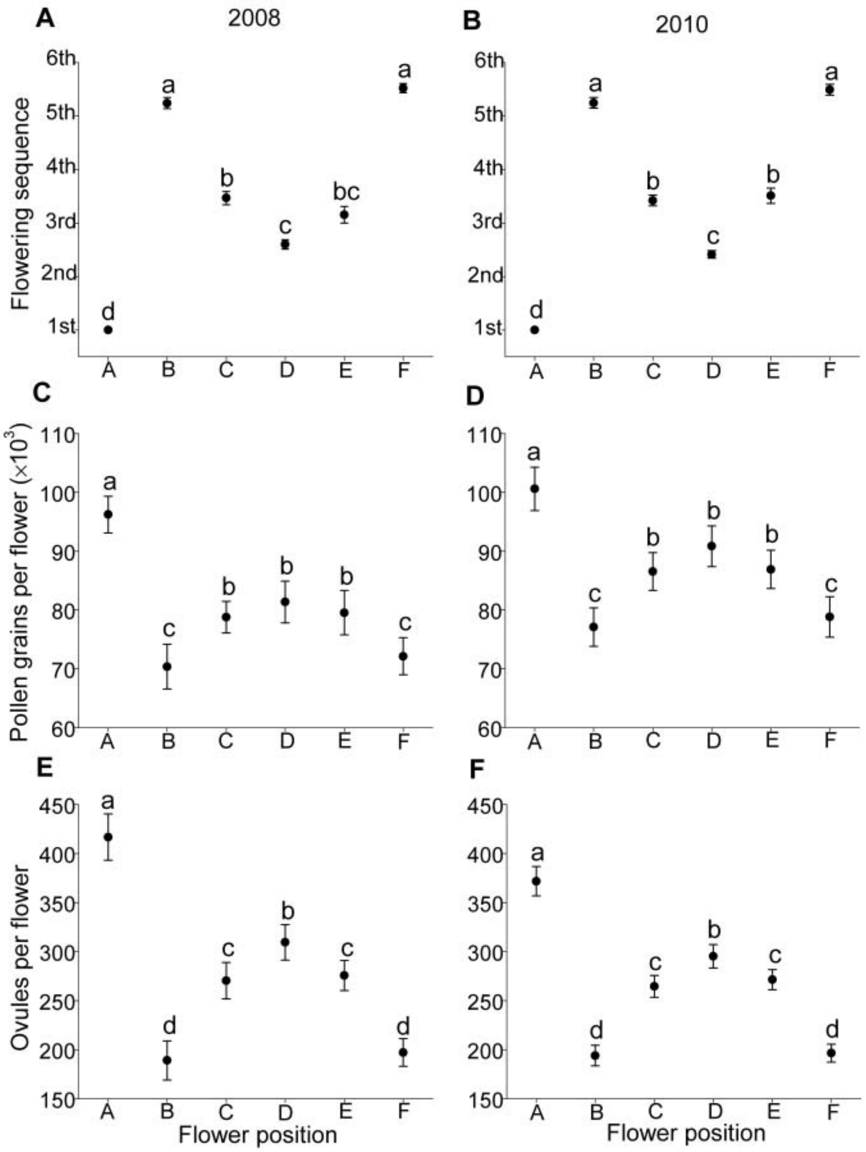

Fig. 2. Flowering sequence (A, B), variation in pollen (C, D), and ovule production (E, F) in the six-flowered inflorescence (mean ± 1 s.e.) in two years: 2008 and 2010. Positions sharing a superscript letter are not significantly different.

tained more ovules than later flowers (multiple comparisons, $P < 0.05$ in both years). Two flowers within any one temporal rank did not differ significantly in ovule production ($P > 0.05$ in both years).

The pollen–ovule ratio increased significantly from early to late flowers ($F_{5,21} = 34.07$, $P < 0.0001$ in 2008; $F_{5,47} = 29.21$, $P < 0.0001$ in 2010) (Fig. 3). Compared with late blooming

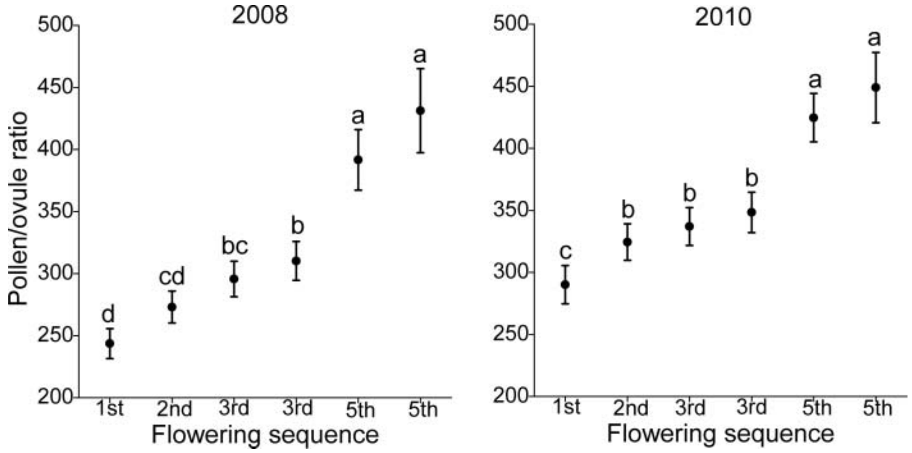

Fig. 3. Temporal decline in pollen–ovule ratio in sequentially blooming flowers (mean ± 1 S.E.) in two years: 2008 and 2010. Means sharing a superscript letter are not significantly different.

flowers, the apical flower produced the greatest number of pollen grains but had the lowest pollen–ovule ratio.

Like pollen and ovule production, corolla-tube length declined from early to late flowers ($F_{5,27} = 41.35$, $P < 0.0001$), except the length of flower D did not differ significantly from that of flower C or E ($P > 0.3$).

Floral longevity

The duration of the female phase and total floral longevity were significantly different between years and flower positions (except male phase duration), but there was no significant interaction (Table 1; Fig. 4). Total floral longevity varied among sequentially blooming flowers, with earlier flowers lasting longer than later flowers ($F_{5,29} = 9.06$, $P < 0.0001$ in 2008; $F_{5,49} = 20.36$, $P < 0.0001$ in 2010). While the duration of the female phase declined significantly between early and late flowers ($F_{5,29} = 22.96$, $P < 0.0001$ in 2008; $F_{5,49} = 39.02$, $P < 0.0001$ in 2010), the duration of the male phase did not vary significantly among flowers ($F_{5,29} = 0.30$, $P = 0.914$ in 2008; $F_{5,49} = 1.21$, $P = 0.303$ in 2010) (Fig. 4).

Effect of pollen removal and receipt on floral longevity

Compared with open-pollinated flowers, the duration of the male phase of flowers from which pollen was not removed was significantly prolonged ($F_{1,52} = 149.63$, $P < 0.0001$), while the duration of the female phase of flowers that did not receive pollen was not significantly affected ($F_{1,56} = 0.491$, $P = 0.486$) (Fig. 5), indicating that pollination only affected the male phase in this species.

Correlation between floral longevity and floral sex allocation

Floral longevity was significantly correlated with allocation to female and male functions in both study years. Given that the patterns were similar in 2008 and 2010, we only report

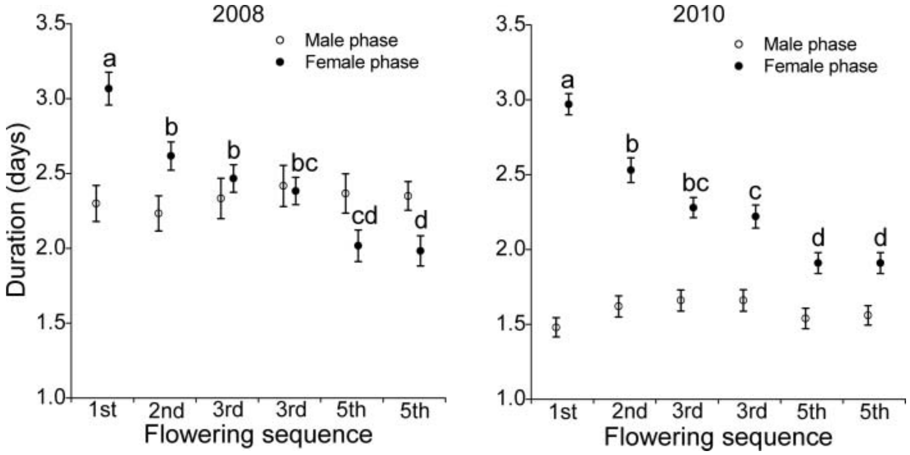

Fig. 4. Variation in the duration of female and male phases in sequentially blooming flowers (mean ± 1 S.E.) in two years: 2008 and 2010. Means sharing a superscript letter are not significantly different.

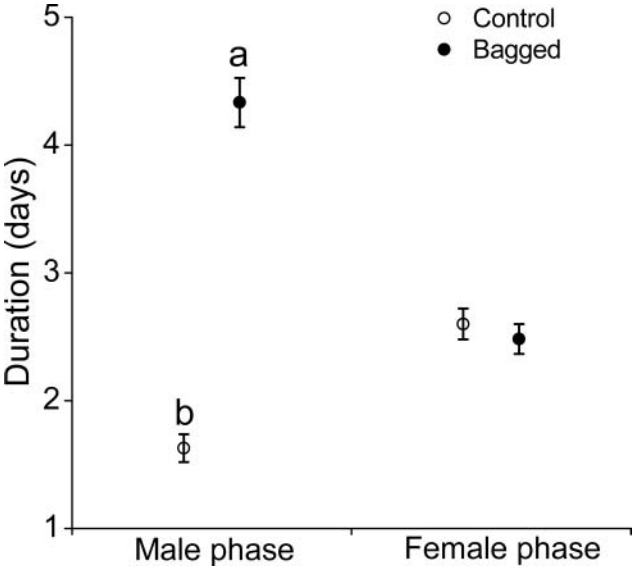

Fig. 5. Effects of pollen removal and receipt on the duration of male and female phases. Means with different letters are significantly different.

the results for 2008 (Fig. 6). Female-phase duration and floral longevity were both significantly correlated with average pollen production per flower ($r = 0.981$, $P = 0.0003$; $r = 0.986$, $P = 0.0005$), ovule production per flower ($r = 0.997$, $P = 0.001$; $r = 0.992$, $P < 0.0001$), and pollen–ovule ratio ($r = -0.939$, $P = 0.005$; $r = -0.932$, $P = 0.007$). However, neither trait correlated with male-phase duration (all $P > 0.3$). Corolla-tube length was significantly correlated with pollen production ($r = 0.950$, $P = 0.004$), ovule production per flower

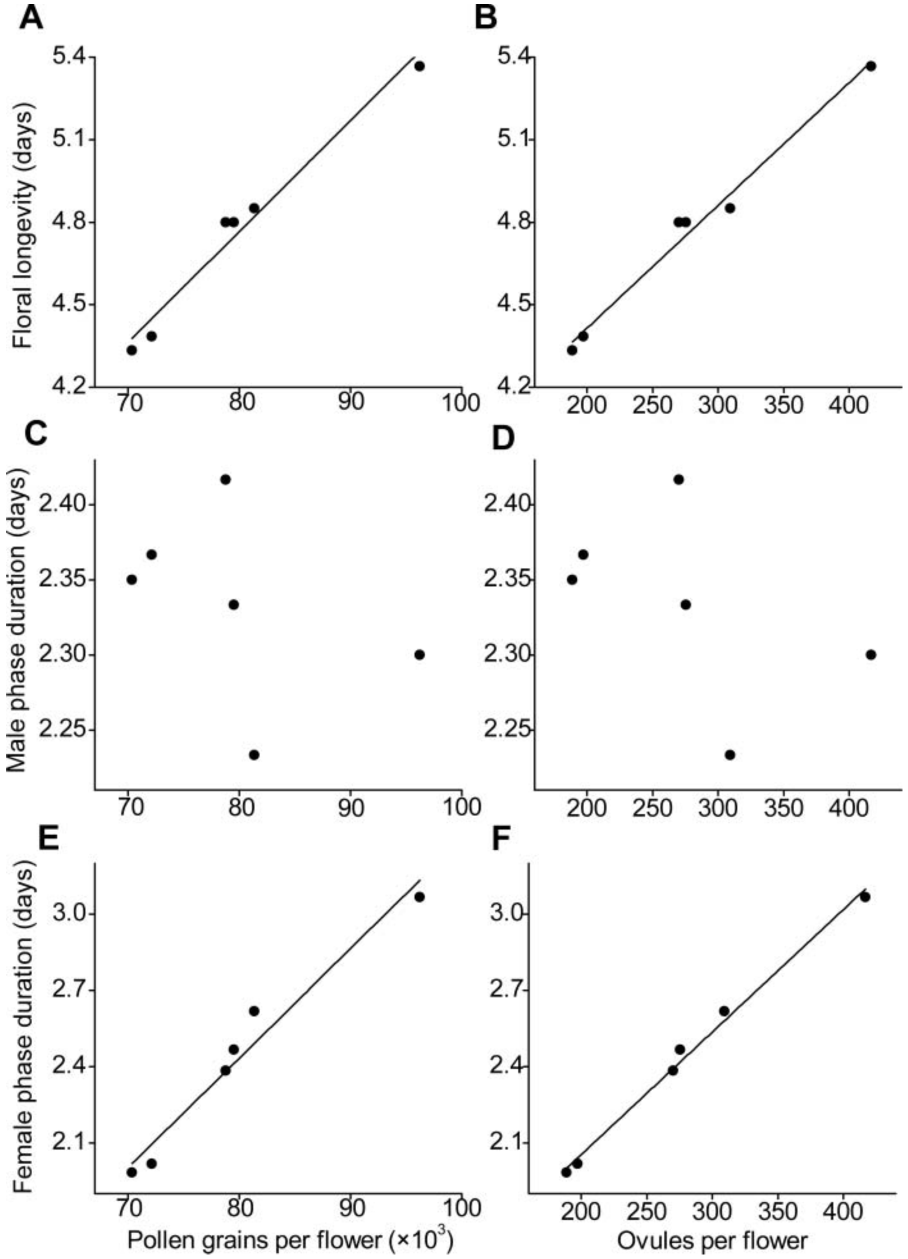

Fig. 6. Relationship between allocation to floral sex functions and floral longevity in 2008. Floral allocation was significantly positively correlated with floral longevity and female phase but not with the duration of the male phase.

($r = 0.975$, $P = 0.001$), female-phase duration ($r = 0.980$, $P = 0.001$), and total floral longevity ($r = 0.979$, $P = 0.001$), but not with male-phase duration ($P = 0.379$).

DISCUSSION

Flower-level investment into pollen and ovule production varied with the temporal sequence of flowering rather than the spatial position of flowers in inflorescences of *Adenophora jasionifolia*. Both pollen and ovule number declined with the sequence of blooming (early to late flowers) whereas the pollen–ovule ratio increased. This demonstrates temporal variation in floral sex allocation in a dichogamous species consistent with the predictions of the mating-environment hypothesis (Brunet and Charlesworth, 1995; Brunet, 1996). Duration of the female phase and overall floral longevity declined with flowering sequence, but the duration of the male phase did not. This correspondence of allocation of sex-specific resources with floral longevity may reflect adaptation to variation in the mating environment.

In particular, the flowers that simultaneously bloom in different positions (flowers B and F, flowers C and E) have similar flower size, floral allocation, and floral longevity. Additionally, if the temporal reduction in total floral allocation stems from resource competition between flowers (Stephenson, 1981), one might expect later-blooming flowers to be smaller than earlier-blooming flowers (Brunet, 1996). Pollen and ovule production in *A. jasionifolia* did decline among sequential flowers but ovules declined more rapidly, resulting in higher pollen–ovule ratios in later blooming flowers. The resource-competition hypothesis, which predicts that variation in temporal floral allocation results from competition for resources, incorporates the possibility of a trade-off (e.g. between the investment in female and male function). A shift from female- to male-biased sex allocation in sequential flowers is, however, predicted by the mating-environment hypothesis in protandrous species (Brunet and Charlesworth, 1995).

Brunet and Charlesworth (1995) proposed that temporal variation in the mating environment might select for a change in the relative investment in female vs. male function among sequential flowers. Consistent with the mating-environment hypothesis, studies have found an increased allocation to male function in late flowers in protandrous plants (Brunet, 1996; Vogler *et al.*, 1999; Mazer and Dawson, 2001; Hiraga and Sakai, 2007; Delesalle *et al.*, 2008; Zhao *et al.*, 2008; Brookes and Jesson, 2010; but see Itagaki and Sakai, 2006) and an increase in allocation to female function in protogynous species (Thomson, 1985; Huang *et al.*, 2004; Guitián, 2006). However, in these studies temporal variation in floral allocation could not be completely confirmed because temporal effects were confounded with positional effects. With such confounding effects removed, our results in *A. jasionifolia* provide stronger support for the prediction of the effects of dichogamy on floral sex allocation.

Previous studies have identified two major factors governing variation in floral longevity: the benefit of successful pollination and the cost of floral maintenance (Primack, 1985; Ashman and Schoen, 1994; Rathcke, 2003; Giblin, 2005; Clark and Husband, 2007). In some species, however, rapid removal or receipt of pollen did not shorten the male or female phase of manipulated flowers, as might be expected if doing so minimizes the cost of maintenance. Instead, a minimum floral longevity appears to exist (Devlin and Stephenson, 1984; Ishii and Sakai, 2000). For example, within the sequentially blooming inflorescences of *Narthecium asiaticum* (Liliaceae), the longevity of lower- and middle-positioned flowers was significantly greater than the length of time required for the completion of male and female functions. An alternative hypothesis for the decline of floral longevity within inflorescences is that longevity of early flowers creates a large display size, increasing pollinator attractiveness (Ishii and Sakai, 2001). However, the pollinator-attractiveness hypothesis cannot be applied to *A. jasionifolia*, because earlier flowers (distal) with longer duration than other flowers within the inflorescences wilted before later flowers opened, such that they did not increase the floral display for subsequent flowers (Fig. 2). Floral longevity may be shortened by unfavourable weather, such as high temperature (Motten, 1983; Schoen and Ashman, 1995). The temporal decline of floral longevity in *A. jasionifolia* is unlikely to be attributable to temperature change given that air temperature in our study site tends to decrease during the flowering season from July to August. To our knowledge, the finding that the duration of the female phase declines concurrently with a decline in floral sex allocation within inflorescences has not previously been reported.

Observations from other species suggested that floral longevity was related to pollen or ovule production per flower and flower size (Stratton, 1989; Ashman and Schoen, 1996), but it remained unclear whether variation in floral sex allocation affected floral longevity. An investigation of petal abscission in 12 hybrid tea rose (*Rosa hybrida*) cultivars suggested that floral longevity was inversely related to their flower size, and fertilization had little effect on the time to abscission in most cultivars (van Doorn and Schröder, 1995). In general, floral longevity declines with flowering time; for example, a seasonal decline in male-phase duration has been reported in the protandrous species *Chamerion angustifolium* (Sargent and Roitberg, 2000), and longevity has been found to be shorter for late compared with early flowers in the sequentially blooming plant *Narthecium asiaticum* (Ishii and Sakai, 2001). Both floral longevity and the total amount of nectar were found to vary with flower position in the inflorescences of *Platanthera chlorantha*, in which lower flowers had more nectar and greater longevity than upper flowers (Stpiczyńska, 2003). Although both floral longevity and floral sex allocation show similar trends to those reported here in the two sequential flowers in *Aquilegia buergeriana*, their relationship remains largely unexplored (Itagaki and Sakai, 2006). We found a positive relationship between flower size and floral longevity, as well as floral sex allocation and duration of the female phase within inflorescences of *A. jasionifolia*. Compared with early flowers, late flowers produced fewer ovules but occurred in a mating environment with higher pollen–ovule ratios. Therefore, in this environment with greater pollen availability, a shorter duration of female phase may have been favoured to reduce the cost of flower maintenance (Brunet and Charlesworth, 1995). In contrast, to maximize the probability of adequate pollen receipt for the fertilization of a large number of ovules, early flowers may benefit from a long female-phase duration. Greater variation in female-phase than male-phase duration was observed between two populations of *Campanula rotundifolia*, and both phases were affected by mating opportunities (Giblin, 2005). The relatively constant duration of the male phase of individual flowers in *A. jasionifolia* may reflect a minimum duration for completing pollen removal (Devlin and Stephenson, 1984; Ishii and Sakai, 2000; Evanhoe and Galloway, 2002; Clark and Husband, 2007; Castro *et al.*, 2008).

The styles of species in the family Campanulaceae are sensitive to touch or pollen removal, which provides a mechanism by which male-phase duration can be adjusted (Nyman, 1993). A significantly extended male phase in bagged flowers compared with open-pollinated flowers in *A. jasionifolia* is consistent with such a mechanism. In this protandrous species, it seems that female phase was triggered by pollen removal from pollen-collecting hairs of the style, which represents the cessation of male function. Indeed, we observed that male phase was one day longer in 2008 than in 2010, and pollinator visits were abundant in 2010 (Liu and Huang, 2013). The variability in floral longevity has been considered a plastic response to pollinator abundance (Giblin, 2005; Clark and Husband, 2007; Castro *et al.*, 2008; Forrest and Thomson, 2010).

In conclusion, we have demonstrated that *A. jasionifolia* adjusts the relative investment into male and female functions in a pattern that follows the flowering sequence, independent of the spatial position of flowers in the inflorescence. By separating the typically confounded factors of flower position and flowering order, our detection of a positive relationship between floral sex allocation and floral longevity supports the mating-environment hypothesis.

ACKNOWLEDGEMENTS

We thank laboratory members Y.-B. Gong, X.-X. Tang, Q. Fang, and Y.-Y. Mao for their help in the field study, Z.-D. Fang and staff from Shangri-La Alpine Botanical Garden for their logistical support, L.D. Harder for comments on data analysis, and W.S. Armbruster, M. Burd, S. Corbet, L. Delph, D. Eaton, B. Montgomery, and J. Pannell for providing valuable comments on the manuscript. This work was supported by grants from the National Science Foundation of China (Nos. 31030016 and 31270281) to S.Q.H.

REFERENCES

Ashman, T.-L. and Hitchens, M.S. 2000. Dissecting the causes of variation in intrainflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *Am. J. Bot.*. **87**: 197–204.

Ashman, T.-L. and Schoen, D.J. 1994. How long should flowers live? *Nature*, **371**: 788–791.

Ashman, T.-L. and Schoen, D.J. 1996. Floral longevity: fitness consequences and resource costs. In *Floral Biology: Studies on Floral Evolution in Animal-Pollinated Plants* (D.G. Lloyd and S.C.H. Barrett, eds.), pp. 112–139. New York: Chapman & Hall.

Ashman, T.-L., Knight, T.M., Steets, J.A., Amarasekare, P., Burd, M., Campbell, D.R. *et al.* 2004. Pollen limitation of plant reproduction: ecological and evolutionary causes and consequences. *Ecology*, **85**: 2408–2421.

Brookes, R.H. and Jesson, L.K. 2010. Do pollen and ovule number match the mating environment? An examination of temporal change in a population of *Stylidium armeria. Int. J. Plant Sci.*, **171**: 818–827.

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

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

Buide, M.L. 2008. Disentangling the causes of intrainflorescence variation in floral traits and fecundity in the hermaphrodite *Silene acutifolia. Am. J. Bot.*, **95**: 490–497.

Castro, S., Silveira, P. and Navarro, L. 2008. Effect of pollination on floral longevity and costs of delaying fertilization in the out-crossing *Polygala vayredae* Costa (Polygalaceae). *Ann. Bot.*, **102**: 1043–1048.

Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.

Clark, M.J. and Husband, B.C. 2007. Plasticity and timing of flower closure in response to pollination in *Chamerion angustifolium* (Onagraceae). *Int. J. Plant Sci.*, **168**: 619–625.

Dafni, A. 1991. Advertisement, flower longevity, reward and nectar protection in Labiatae. *Acta Horticulturae*, **288**: 340–346.

Delesalle, V.A., Mazer, S.J. and Paz, H. 2008. Temporal variation in the pollen–ovule ratios of *Clarkia* (Onagraceae) taxa with contrasting mating systems: field populations. *J. Evol. Biol.*, **21**: 310–323.

Devlin, B. and Stephenson, A.G. 1984. Factors that influence the duration of the staminate and pistillate phases of *Lobelia cardinalis* flowers. *Bot. Gaz.*, **145**: 323–328.

Diggle, P.K. 2003. Architectural effects on floral form and function: a review. In *Deep Morphology: Toward a Renaissance of Morphology in Plant Systematics* (T. Stuessy, E. Hörandl and V. Mayer, eds.), pp. 63–80. Königstein: Lubrecht & Cramer.

Emms, S.K. 1996. Temporal patterns of seed set and decelerating fitness returns on female allocation in *Zigadenus paniculatus* (Liliaceae), an andromonoecious lily. *Am. J. Bot.*, **83**: 304–315.

Evanhoe, L. and Galloway, L.F. 2002. Floral longevity in *Campanula americana* (Campanulaceae): a comparison of morphological and functional gender phases. *Am. J. Bot.*, **89**: 587–591.

Forrest, J. and Thomson, J.D. 2010. Consequences of variation in flowering time within and among individuals of *Mertensia fusiformis* (Boraginaceae), an early spring wildflower. *Am. J. Bot.*, **97**: 38–48.

Giblin, D.E. 2005. Variation in floral longevity between populations of *Campanula rotundifolia* (Campanulaceae) in response to fitness accrual rate manipulation. *Am. J. Bot.*, **92**: 1714–1722.

Guitián, P. 2006. Phenotypic gender variation within inflorescences of the protogynous species. *Helleborus foetidus* L. (Ranunculaceae). *Plant Syst. Evol.*, **258**: 137–146.

Haig, D. and Westoby, M. 1988. On limits to seed production. *Am. Nat.*, **131**: 757–759.

Harder, L.D. and Johnson, S.D. 2005. Adaptive plasticity of floral display size in animal-pollinated plants. *Proc. R. Soc. Lond. B*, **272**: 2651–2657.

Harder, L.D. and Routley, M.B. 2006. Pollen and ovule fates and reproductive performance by flowering plants. In *Ecology and Evolution of Flowers* (L.D. Harder and S.C.H. Barrett, eds.), pp. 61–80. Oxford: Oxford University Press.

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

Hong, D.-Y. 1983. Campanulaceae, Goodeniaceae, Stylidiaceae. *Flora Reipublicae Popularis Sinicae Tomus*, **73**(2). Beijing: Science Press.

Huang, S.-Q., Tang, L.-L., Yu, Q. and Guo, Y.-H. 2004. Temporal floral sex allocation in protogynous *Aquilegia yabeana* contrasts with protandrous species: support for the mating environment hypothesis. *Evolution*, **58**: 1131–1134.

Ishii, H.S. and Sakai, S. 2000. Optimal timing of corolla abscission: experimental study on *Erythronium japonicum* (Liliaceae). *Funct. Ecol.*, **14**: 122–128.

Ishii, H.S. and Sakai, S. 2001. Effects of display size and position on individual floral longevity in racemes of *Narthecium asiaticum* (Liliaceae). *Funct. Ecol.*, **15**: 396–405.

Ishii, H.S. and Sakai, S. 2002. Temporal variation in floral display size and individual floral sex allocation in racemes of *Narthecium asiaticum* (Liliaceae). *Am. J. Bot.*, **89**: 441–446.

Itagaki, T. and Sakai, S. 2006. Relationship between floral longevity and sex allocation among flowers within inflorescences in *Aquilegia buergeriana* var. *oxysepala* (Ranunculaceae). *Am. J. Bot.*, **93**: 1320–1327.

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

Liu, C.-Q. and Huang, S.-Q. 2013. Floral divergence, pollinator partitioning and the spatiotemporal pattern of plant–pollinator interactions in three sympatric *Adenophora* species. *Oecologia*, **173**: 1411–1423.

Lloyd, D.G. 1980. Sexual strategies in plants I. A hypothesis of serial adjustment of maternal investment during one reproductive season. *New Phytol.*, **86**: 69–79.

Lloyd, D.G. and Bawa, K.S. 1984. Modification of the gender of seed plants in varying conditions. *Evol. Biol.*, **17**: 255–338.

Lloyd, D.G. and Yates, J.M.A. 1982. Intrasexual selection and the segregation of pollen and stigmas in hermaphrodite plants, exemplified by *Wahlenbergia albomarginata* (Campanulaceae). *Evolution*, **36**: 903–913.

Mazer, S.J. and Dawson, K.A. 2001. Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): ontogenetic and among-plant variation. *Am. J. Bot.*, **88**: 819–831.

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

Motten, A.F. 1983. Reproduction of *Erythronium umbilicatum* (Liliaceae): pollination success and pollinator effectiveness. *Oecologia*, **59**: 351–359.

Muchhala, N., Brown, Z., Armbruster, W.S. and Potts, M.D. 2010. Competition drives specialization in pollination systems through costs to male fitness. *Am. Nat.*, **176**: 732–743.

Nyman, Y. 1993. The pollen-collecting hairs of *Campanula* (Campanulaceae) II. Function and adaptive significance in relation to pollination. *Am. J. Bot.*, **80**: 1437–1443.

Primack, R.B. 1985. Longevity of individual flowers. *Annu. Rev. Ecol. Syst.*, **16**: 15–37.

Rathcke, B.J. 2003. Floral longevity and reproductive assurance: seasonal patterns and an experimental test with *Kalmia latifolia* (Ericaceae). *Am. J. Bot.*, **90**: 1328–1332.

Sargent, R. and Roitberg, B. 2000. Seasonal decline in male-phase duration in a protandrous plant: a response to increased mating opportunities? *Funct. Ecol.*, **14**: 484–489.

Schoen, D.J. and Ashman, T.-L. 1995. The evolution of floral longevity: resource allocation to maintenance vs. construction of repeated parts in modular organisms. *Evolution*, **49**: 131–139.

Stephenson, A.G. 1981. Flower and fruit abortion: proximate causes and ultimate functions. *Annu. Rev. Ecol. Syst.*, **12**: 253–279.

Stpiczyńska, M. 2003. Floral longevity and nectar secretion of *Platanthera chlorantha* (Custer) Rchb. (Orchidaceae). *Ann. Bot.*, **92**: 191–197.

Stratton, D.A. 1989. Longevity of individual flowers in a Costa Rican cloud forest: ecological correlates and phylogenetic constraints. *Biotropica*, **21**: 308–318.

Thomson, J.D. 1985. Pollination and seed set in *Diervilla lonicera* (Caprifoliaceae): temporal patterns of flower and ovule deployment. *Am. J. Bot.*, **72**: 737–740.

Thomson, J.D. 1989. Deployment of ovules and pollen among flowers within inflorescences. *Evol. Trends Plants*, **3**: 65–68.

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

Van Doorn, W.G. and Schröder, C. 1995. The abscission of rose petals. *Ann. Bot.*, **76**: 539–544.

Vogler, D.W., Peretz, S. and Stephenson, A.G. 1999. Floral plasticity in an iteroparous plant: the interactive effects of genotype, environment, and ontogeny in *Campanula rapunculoides* (Campanulaceae). *Am. J. Bot.*, **86**: 482–494.

Wesselingh, R.A. and Arnold, M.L. 2003. A top-down hierarchy in fruit set on inflorescences in *Iris fulva* (Iridaceae). *Plant Biol.*, **5**: 651–660.

Wyatt, R. 1982. Architecture: how flower number, arrangement, and phenology affect pollination and fruit-set. *Am. J. Bot.*, **69**: 585–594.

Zar, J.H. 1996. *Biostatistical Analysis*, 3rd edn. Englewood Cliffs, NJ: Prentice Hall.

Zhao, Z.-G., Meng, J.-L., Fan, B.-L. and Du, G.-Z. 2008. Reproductive patterns within racemes in protandrous *Aconitum gymnandrum* (Ranunculaceae): potential mechanism and among-family variation. *Plant Syst. Evol.*, **273**: 247–256.