

Plasticity of floral sex allocation within inflorescences of hermaphrodite *Aconitum gymnandrum*

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Abstract

Aims

Sex allocation in plants is often plastic, enabling individuals to adjust to variable environments. However, the predicted male-biased sex allocation in response to low resource conditions has rarely been experimentally tested in hermaphroditic plants. In particular, it is unknown whether distal flowers in linear inflorescences show a larger shift to male allocation relative to basal flowers when resources are reduced. In this study, we measure position-dependent plasticity of floral sex allocation within racemes of *Aconitum gymnandrum* in response to reduced resource availability.

Methods

Using a defoliation treatment in the field applied to potted plants from a nested half-sibling design, we examined the effects of the treatment, flower position, family and their interactions.

Important Findings

Allocation to male function increased with more distal flower position, while female allocation either did not change with position

or declined at the most distal flowers. Defoliation significantly reduced the mass of both the androecium and gynoecium, but not anther number or carpel number. Gynoecial mass declined more strongly with defoliation than did androecial mass, resulting in a significant increase in the androecium/gynoecium ratio as predicted by sex allocation theory. Plastic responses of androecium mass and gynoecium mass were affected by flower position, with less mass lost in basal flowers, but similar plastic magnitude in both sexual traits across flower position lead to consistent variation in the androecium/gynoecium ratio along the inflorescence. A significant treatment*paternal family interaction for the androecium/gynoecium ratio is evidence for additive genetic variation for plastic floral sex allocation, which means that further evolution of allocation can occur.

Key words: *Aconitum gymnandrum*, sex allocation, plasticity, inflorescence architecture

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INTRODUCTION

Sex allocation theory predicts that individuals with fewer resources will be more male-biased while individuals with more resources will be more female-biased, based on the differential fitness gain curves of allocation to male and female functions (Charnov 1982, 1996). The effect of natural variation in plant resource status (with size often used as a proxy measure

of resource status) on sex allocation has been well studied in hermaphrodites (Ashman and Hitchens 2000; Guo *et al.* 2010; Klinkhamer *et al.* 1997; Mazer and Dawson 2001; Méndez and Traveset 2003; Wright and Barrett 1999; Zhao *et al.* 2008a). By contrast, few studies have examined floral sex allocation in response to experimental manipulation of resources in hermaphrodites (West 2009), which would provide a more robust test of conditional sex allocation theory. More studies have

documented the labile sex expression of dioecious and monoecious species under manipulated environmental conditions; these studies generally show that stressed environments cause increases in allocation to male function relative to female function (reviewed in Freeman *et al.* 1980; Lloyd and Bawa 1984). Studies of gynodioecious plants have found that hermaphrodite individuals have plastic sex allocation, which may facilitate the establishment of female individuals in hermaphroditic populations (Delph 1990, 2003; Dorken and Mitchard 2008). Plastic sex allocation in response to variable resources represents a flexible allocation strategy for coping with environmental heterogeneity, and can evolve by natural selection given genetic variation for plasticity (Lloyd and Bawa 1984; Zhang 2006).

Individual plants of most species produce many flowers which vary within individuals for most traits (Cao *et al.* 2015; Diggle 2003; Herrera 2009), and flowers at different positions along the inflorescences can respond differently to resource availability (Herrera 2009; Ashman *et al.* 2001). To date, however, we lack data on how floral sex allocation within an individual plant respond to varying environments, especially whether plastic responses in sex allocation to resource environments are affected by flower position within inflorescences. Intra-inflorescence variation in floral allocation has variously been attributed to competition for resource between basal and distal flowers (Ashman and Baker 1992; Guitián and Navarro 1996; Herrera 1991), architectural effects governed by inherent mechanical limitation of vasculature (Diggle 1995, 1997; Wolfe 1992) or mating environments (Brunet and Charlesworth 1995; Guitián *et al.* 2004; Huang *et al.* 2004). If intra-inflorescence variation in floral allocation is caused by resource competition, when available resources are reduced, basal or early opening flowers are expected to be less affected due to their priority in access to resources moving up the inflorescence, while distal flowers may be more resource restricted and will have relatively more male-biased allocation. Evidence for this would be a treatment by position interaction in a resource manipulation experiment. Alternatively, intra-inflorescence patterns of sex allocation can be adaptations for increased pollination success. In protandrous species, male-biased allocation in distal flowers can increase outcrossing (Brunet and Charlesworth 1995; Gao *et al.* 2015). In this case, altered resources should have consistent effects on different flowers within inflorescences, i.e. there should be similar or no plastic responses of sex allocation to changed resources across the inflorescence. In a resource manipulation experiment, this hypothesis predicts no treatment by flower position interaction.

In this study, we examine the plastic response of floral sex allocation within racemes to experimentally reduced resource availability in *Aconitum gymnandrum*. Previous work on this plant showed significant intra-inflorescence variation of floral sex allocation, with greater anther number and male-biased allocation in more distal flowers within racemes (Zhao *et al.* 2008b). Specifically, we test the theoretical prediction that flowers within inflorescences should become more male in response to reduced resource availability, and whether plasticity is position-dependent.

MATERIALS AND METHODS

Study species

Aconitum gymnandrum Maxim. (Ranunculaceae) is an annual herb, widely distributed in alpine meadows (1600–3800 m) in the Qinghai-Tibet Plateau, China. Individual plants generally produce one erect raceme consisting of 2–30 blue-purple zygomorphic flowers, which open sequentially from bottom to top. Each flower has 6–14 separate carpels (each with 8–14 ovules) surrounded by 30–90 stamens. The galea (or hood), formed from one of five petaloid sepals, contains two stalked petals with nectaries. The species is self-compatible, strongly protandrous like other related species in the same genus, and bumblebee-pollinated. The anthers dehisce over 4–5 days and stigmas become receptive 1–2 days later. Plants commonly bloom from June through August and single flowers last 6–10 days. Fruit maturation requires 20–30 days.

Experimental manipulations and data collection

Seeds were collected in 2007 from a natural population of *A. gymnandrum* at the Research Station of Alpine Meadows and Wetland Ecosystems of Lanzhou University (Hezuo County, E102°53′, N34°55′). We grew 40 plants from these seeds and randomly selected eight to be sires. Each sire plant was used to pollinate four unique randomly selected maternal plants in a nested paternal half-sibling design for a total of 32 full-sib families. On 4 May 2008, seeds from each dam were germinated in Petri dishes with distilled water. Thirty-two 10-day-old seedlings of uniform size from each maternal plant were transplanted in pairs into the center of 26 cm plastic pots filled with mixed soil collected from a local natural site where *A. gymnandrum* grows naturally. The soil had previously been mixed and covered with film for 4 months to eliminate preexisting seeds. We set up two blocks in the field at the research station 5 m apart; each block consisted of eight pots from each maternal family arranged randomly. The pots were watered when needed but were otherwise exposed to the natural environment.

In June 2009, we randomly selected four of the eight pots per family in each block and removed all stem leaves on plant at the time of bud initiation. As carbon gain in *A. gymnandrum* depends mainly on photosynthesis of stem leaves rather than rosette leaves, and rosette leaves were mostly withered at flowering (Zhao *et al.* 2008a), the defoliation treatment should have significantly reduced the photosynthate available to each individual. The plants of the remaining four pots per family were left intact as the control. We randomly chose one of the two plants in each pot and collected two flowers (or one, when flower number per raceme was less than six) for measurements from each of the basal, middle and distal positions of each raceme when the flowers had just opened (the petals extended and the anthers not yet dehisced). During the sampling in this experiment, no fruit set occurred within inflorescences. We recorded the dry mass of the androecium (mass of all stamens of each flower) and gynoecium (mass

of all pistils of each flower) after drying at 80°C for 24 h, and counted the number of anthers and carpels per flower. Due to natural mortality, the final sample was 232 plants in the both treatments.

Statistical analysis

We performed repeated measures mixed-model analysis of variance (JMP 7.0, SAS Institute 2007) on anther and carpel number, androecium and gynoecium mass and the anther/carpel number and androecium/gynoecium mass ratios. We treated the defoliation treatment, flower position (basal, middle, distal) and block as fixed effects, paternal family, maternal family nested within paternal family and plant ID nested within maternal family as the repeated effect, and flower number as a continuous covariate. To evaluate the significance of random effects, the difference of the $-2\log$ likelihood between the full model and the model with that one random term removed was calculated for each of the random effects in the full model. This difference has a χ^2 distribution with df equal to the difference in the number of covariance parameters between the full and reduced models (Littell *et al.* 1996). The full model was run including all interactions, but the dam and Plant ID effects are not presented for simplicity and because they do not test a question of interest. A significant main effect of treatment indicates that the trait responded plastically to resource reduction averaged over the three positions. A significant interaction of treatment and position indicates that flowers in different positions within the inflorescence differed in their plastic response of the trait to defoliation. A significant treatment*paternal family interaction shows that paternal families differed in their responses to defoliation, and is evidence for genotype by environment interaction and additive genetic variance for plasticity (Via 1984). A significant position*paternal family interaction is evidence for additive genetic variation in how that trait changes within the inflorescence.

RESULTS

All measured traits except for gynoecium mass demonstrated significant position effects (Fig. 1; Table 1). The defoliation treatment strongly affected the mass of the male and female parts of the flower (Fig. 1; Table 1). Androecium mass was greatly reduced by resource depletion, and this effect depended on position, becoming stronger in more distal flowers (treatment*position interaction; Table 1; Fig. 1). Gynoecium mass was also greatly reduced by resource depletion (treatment main effect), and the reduction increased across flower position within the inflorescence (marginally significant treatment*position effect). Together, this led to increases in relative allocation to male function with resource depletion and in more distal flowers, as measured by the androecium/gynoecium mass ratio, but no significant treatment*position interaction. This plasticity in relative allocation in response to resources would respond to further

selection, as the significant treatment*paternal family interaction is evidence for genotype*environment interaction, which in turn is evidence for additive variance for plasticity. The significant position*paternal family interaction for androecium mass is evidence for additive genetic variation for the slope of the increase in androecium mass from proximal to distal.

In contrast to the mass traits, resource depletion had no effect on anther or carpel number or their ratio; these traits only varied significantly with position (Table 1). Anther number was much higher in distal flowers and carpel number lower, resulting in a large increase in anther/carpel number ratio in more distal flowers (Fig. 1). Significant treatment*paternal family interaction in anther/carpel ratio also showed additive genetic variation (Table 1). Thus, distal flowers are more male by both the ratio of parts and the ratio of masses.

DISCUSSION

Defoliation significantly reduced the mass of both the androecium and gynoecium, but not anther number or carpel number in *A. gymnanthum*. Gynoecial mass declined more strongly with defoliation than did androecial mass, resulting in a significant increase in the androecium/gynoecium ratio as predicted by sex allocation theory. Plants exposed to environmental stress should allocate more to the relatively cheaper sex (Charnov 1982, 1996). In plants, resource-dependent sex allocation has been regarded as the rule (Klinkhamer *et al.* 1997; Lloyd and Bawa 1984), and this has often been reported in animals and other taxa (see Vizoso and Schärer 2007; West 2009). However, fewer studies have examined response of floral sex allocation in hermaphrodites through experimental manipulation of resources (West 2009). Experimentally resource reduction has been shown to increase allocation to male function in several species (Korpelainen 1998; Lloyd and Bawa 1984; Vaughton and Ramsey 2012). In contrast, a few studies manipulating resource availability have not supported the hypothesis: there were no significant effects on floral sex allocation in the hermaphrodites *Platanthera bifolia* (Vallius and Salonen 2006) and *Hosta ventricosa* (Cao *et al.* 2011), and decreased allocation to male function in response to stress has been reported in hermaphroditic *Spergularia marina* (Delesalle and Mazer 1996) and monoecious *Croton suberosus* (Narbona and Dirzo 2010).

Although the reductions in androecium mass and gynoecium mass were affected by flower position within *A. gymnanthum* inflorescences, with less mass lost in basal flowers, the magnitude of these reductions were similar in both sexual traits across flower position, resulting in a nonsignificant treatment*position interaction for the androecium/gynoecium ratio. This lack of plasticity in relative allocation across floral positions, combined with the fact that, averaged across treatments, allocation to male function increased with more distal flower position while female allocation did not change with position, supports the mating environment hypothesis but not the resource competition hypothesis, in agreement with Zhao *et al.* (2008a) (also see

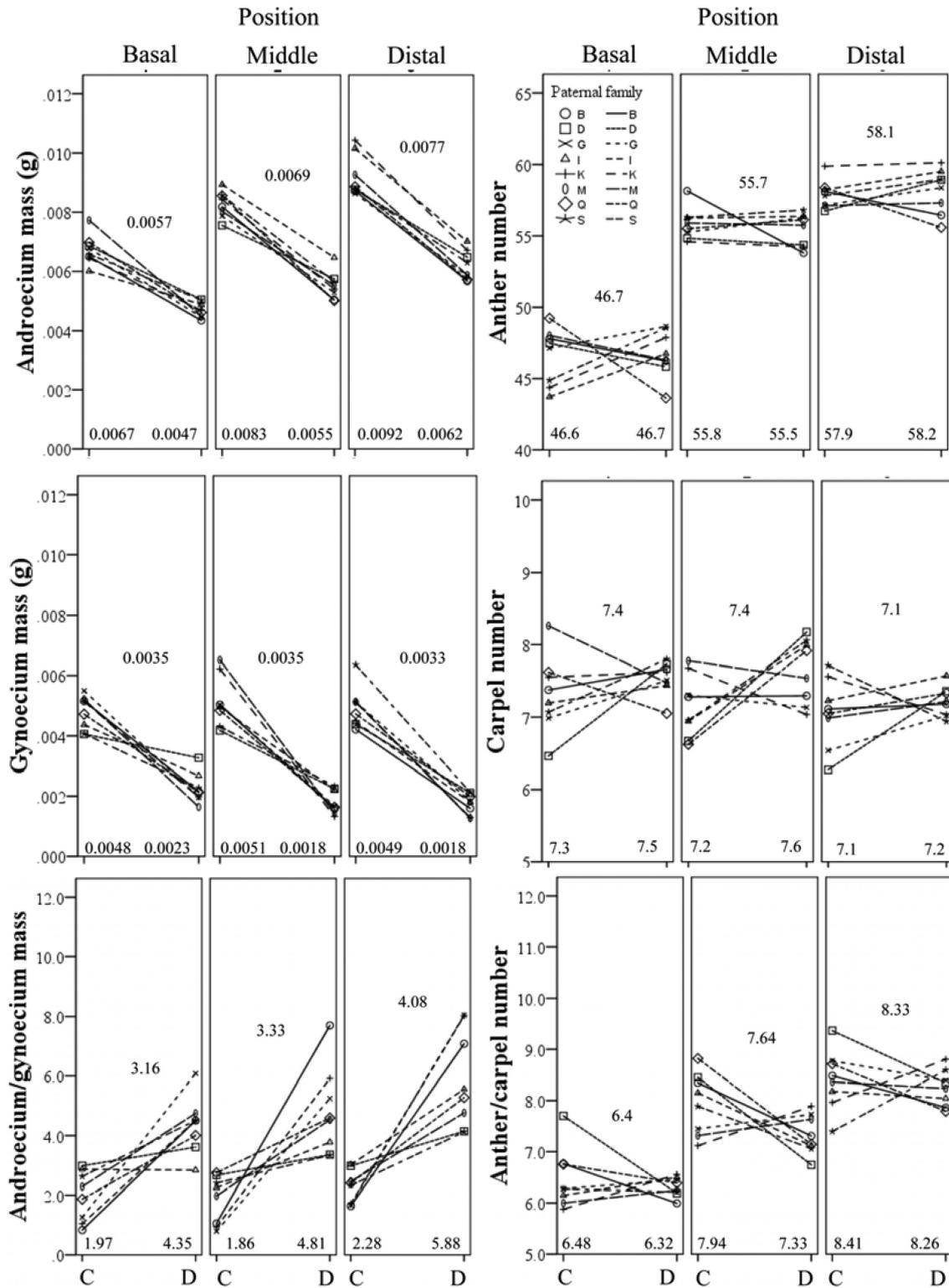


Figure 1 : least square means of sexual traits at different flower positions of *Aconitum gymnantrum* across treatments (control = open circles, defoliation = filled circles; flower positions 1, 2 and 3 are basal, middle and distal within inflorescences, respectively).

Herrera 2009). This consistent male-biased allocation in distal flowers is an ESS in plants with protandry and upward movement of pollinators (Brunet and Charlesworth 1995), both of

which occur in *A. gymnantrum*. Similar to our results for anther and carpel number, Cao et al. (2011) found that defoliation did not change the pattern of intra-inflorescence variation in pollen

Table 1: *F*-statistics (fixed effects) and χ^2 values (random effects) of repeated measures mixed-model on floral sex allocation in *A. gymnanthrum* (androecium/gynoecium mass was ln transformed to improve normality)

Source	DF	Androecium mass (g)	Gynoecium mass (g)	Androecium/ gynoecium mass	Anther number	Carpel number	Anther/carpel number
Position	2	52.2***	0.51	14.33***	256.7***	6.39*	160.1***
Treatment	1	146.2***	80.93***	46.17***	0.001	1.91	1.24
Block	1	3.53	0.8	0.01	0.44	0.22	0.01
Paternal family		0.13	0.5	0.95	0.59	0.03	0.09
Flower number	1	23.3***	47.3***	42.89***	2.64	19.3***	5.06*
Treatment*Position	2	29.49***	3.16†	0.16	0.18	0.65	2.51
Treatment*Paternal family		0.17	4.47*	13.58*	0.33	1.65	6.66*
Position*Paternal family		2.56	0.03	0.79	0.02	3.42†	0.54
R ² of whole model		0.89***	0.82***	0.81***	0.74***	0.58***	0.67***

The significance of random effects (paternal family and the two interactions with paternal family) was assessed using likelihood ratio tests (χ^2 , degrees of freedom = 1). Fixed effects were assessed via *F*-tests. Significant *P* values are in bold. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; †0.05 < *P* < 0.08; *N* = 232.

and ovule number of *Hosta ventricosa*, and Kliber and Eckert (2004) reported that defoliation did not affect the severity of sequential decline in the size of floral organs within inflorescences of *Aquilegia canadensis*.

A significant treatment*paternal family interaction for the androecium/gynoecium and anther/carpel ratio is evidence for additive genetic variation for plastic floral sex allocation in *A. gymnanthrum*. Thus, the plasticity that already exists for these traits (i.e. the significant treatment main effect) can further evolve in response to natural selection. Variation in plasticity among genotypes in response to resource availability was also found in pollen production per anther in hermaphrodites of *Fragaria virginiana* (Bishop *et al.* 2010) and in ovule number per flower of *Campanula rapunculoides* (Vogler *et al.* 1999); however, these studies did not determine proportional sex allocation (the relative allocation of male to female function). Plastic sex allocation in hermaphrodites is favorable in heterogeneous environments by maximizing fitness gain, and plays an important role in sexual system evolution of plants (Delph 2003; Delph and Wolf 2005). Future work should empirically test whether resource-based sex allocation is adaptive.

In conclusion, sexual traits and relative allocation to male and female functions in *A. gymnanthrum* showed plastic responses to flower position and resource treatment. *A. gymnanthrum* increased relative allocation to male function over all flowers in response to reduced resources, and the plastic increase in floral sex allocation did not change with flower position within inflorescences. Both results are predicted by sex allocation theory. Additive genetic variation for plasticity in relative allocation means that further evolution of plastic allocation can occur.

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REFERENCES

- Ashman TL, Baker I (1992) Variation in floral sex allocation with time of season and currency. *Ecology* **73**:1237–43.
- Ashman TL, Hitchens MS (2000) Dissecting the causes of variation in intra-inflorescence allocation in a sexually polymorphic species, *Fragaria virginiana* (Rosaceae). *Am J Bot* **87**:197–204.
- Ashman TL, Pacyna J, Diefenderfer C, *et al.* (2001) Size dependent sex allocation in a gynodioecious wild strawberry: the effects of sex morph and inflorescence architecture. *Int J Plant Sci* **162**: 327–34.
- Bishop EJ, Spigler RB, Ashman TL (2010) Sex-allocation plasticity in hermaphrodites of sexual dimorphic *Fragaria virginiana* (Rosaceae). *Botany* **88**:231–40.
- Brunet J, Charlesworth D (1995) Floral sex allocation in sequentially blooming plants. *Evolution* **49**:70–9.
- Cao GX, Xie T, Wu BX, Yang CP (2015) Floral sex allocation and reproductive success within inflorescences of *Hosta ventricosa*, a pseudogamous apomict. *J Plant Ecol* **8**:142–53.
- Cao GX, Xue L, Li Y, *et al.* (2011) 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. *Ann Bot* **107**:1413–19.
- Charnov EL (1982) *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.
- Charnov EL (1996) Sperm competition and sex allocation in simultaneous hermaphrodites. *Evol Ecol* **10**:457–462.
- Delesalle VA, Mazer SJ (1996) Nutrient levels and salinity affect gender and floral traits in the autogamous *Spergularia marina*. *Int J Plant Sci* **157**:621–31.
- Delph LF (1990) Sex-differential resource allocation patterns in the subdioecious shrub *Hebe subalpina*. *Ecology* **71**:1342–51.

- Delph LF (2003) Sexual dimorphism in gender plasticity and its consequences for breeding system evolution. *Evol Dev* **5**:34–9.
- Delph LF, Wolf DE (2005) Evolutionary consequences of gender plasticity in genetically dimorphic breeding systems. *New Phytol* **166**:119–28.
- Diggle PK (1995) Architectural effects and the interpretation of patterns of fruit and seed development. *Annu Rev Ecol S* **26**:531–52.
- Diggle PK (1997) Ontogenetic contingency and floral morphology: the effects of architecture and resource limitation. *Int J Plant Sci* **158**:S99–S107.
- 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*. Germany: Koeltz, 63–80.
- Dorken ME, Mitchard ET (2008) Phenotypic plasticity of hermaphroditic sex allocation promotes the evolution of separate sexes: an experimental test of the sex-differential plasticity hypothesis using *Sagittaria latifolia* (Alismataceae). *Evolution* **62**:971–8.
- Freeman DC, Harper KT, Charnov EL (1980) Sex change in plants: old and new observations and new hypotheses. *Oecologia* **47**:222–32.
- Gao J, Xiong YZ, Huang SQ (2015) Effects of floral sexual investment and dichogamy on floral longevity. *J Plant Ecol* **8**:116–21.
- Gutián J, Medrano M, Oti JE (2004) Variation in floral sex allocation in *Polygonatum odoratum* (Liliaceae). *Ann Bot* **94**:433–40.
- Gutián J, Navarro L (1996) Allocation of reproductive resources within the inflorescences of *Petrocoptis grandiflora* (Caryophyllaceae). *Can J Bot* **74**:1482–6.
- Guo H, Mazer SJ, Du GZ (2010) Geographic variation in primary sex allocation per flow within and among 12 species of *Pedicularis*: proportional male investment increases with elevation. *Am J Bot* **97**:1334–41.
- Herrera CM (2009) *Multiplicity in Unity: Plant Subindividual Variation & Interactions with Animals*. Chicago, IL: The University of Chicago Press.
- Herrera J (1991) Allocation of reproductive resources within and among inflorescences of *Lavandula stoechas* (Lamiaceae). *Am J Bot* **78**:789–94.
- 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**:1131–4.
- Kliber A, Eckert CG (2004) Sequential decline in allocation among flowers within inflorescences: proximate mechanisms and adaptive significance. *Ecology* **85**:1675–87.
- Klinkhamer PG, de Jong TJ, Metz H (1997) Sex and size in cosexual plants. *Trends Ecol Evol* **12**:260–5.
- Korpelainen H (1998) Labile sex expression in plants. *Biol Rev* **73**:157–80.
- Littell RC, Milliken GA, Stroup WW, et al. (1996) *SAS System for Mixed Models*. Cary, NC: SAS Institute, Inc.
- Lloyd DG, Bawa KS (1984) Modification of the gender of seed plants in varying conditions. *Evol Biol* **17**:255–388.
- Mazer SJ, Dawson KA (2001) Size-dependent sex allocation within flowers of the annual herb *Clarkia unguiculata* (Onagraceae): ontogenetic and among-plant variation. *Am J Bot* **88**:819–31.
- Méndez M, Traveset A (2003) Sexual allocation in single-flowered hermaphroditic individuals in relation to plant and flower size. *Oecologia* **137**:69–75.
- Narbona E, Dirzo R (2010) Experimental defoliation affects male but not female reproductive performance of the tropical monoecious plant *Croton suberosus* (Euphorbiaceae). *Ann Bot* **106**:359–69.
- SAS Institute (2007) *JMP Statistics and Graphics Guide, Version 7.0*. Cary, NC: SAS Institute Inc.
- Vallius E, Salonen V (2006) Allocation to reproduction following experimental defoliation in *Platanthera bifolia* (Orchidaceae). *Plant Ecol* **183**:291–304.
- Vaughton G, Ramsey M (2012) Gender plasticity and sexual system stability in *Wurmbea*. *Ann Bot* **109**:521–30.
- Vizioso DB, Schärer L (2007) Resource-dependent sex-allocation in a simultaneous hermaphrodite. *J Evol Biol* **20**:1046–55.
- Via S (1984) The quantitative genetics of polyphagy in an insect herbivore. I. Genotype-environment interaction in larval performance on different host plant species. *Evolution* **38**:881–95.
- 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). *Am J Bot* **86**:482–94.
- West SA (2009) *Sex Allocation*. Princeton, NJ: Princeton University Press.
- Wolfe LM (1992) Why does the size of reproductive structures decline through time in *Hydrophyllum appendiculatum* (Hydrophyllaceae): developmental constraints vs. resource limitation? *Am J Bot* **79**:1286–90.
- Wright SI, Barrett SCH (1999) Size-dependent gender modification in a hermaphroditic perennial herb. *Proc Roy Soc Lond Biol* **266**:225–32.
- Zhang DY (2006) Evolutionarily stable reproductive investment and sex allocation in plants. In Harder LD, Barrett SCH (eds). *Ecology and Evolution of Flowers*. Oxford: Oxford University Press, 41–60.
- Zhao ZG, Meng JL, Fan BL, et al. (2008a) Size-dependent sex allocation in *Aconitum gymnanthum* (Ranunculaceae): physiological basis and effects of maternal family and environment. *Plant Biol (Stuttg)* **10**:694–703.
- Zhao ZG, Meng JL, Fan BL, et al. (2008b) Reproductive patterns within racemes in protandrous *Aconitum gymnanthum* (Ranunculaceae): potential mechanism and among-family variation. *Plant Syst Evol* **273**:247–56.