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RESEARCH PAPER

The dynamics of resource allocation and costs of reproduction in a sexually dimorphic, wind-pollinated dioecious plant

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ABSTRACT

Sexual dimorphism in resource allocation is expected to change during the life cycle of dioecious plants because of temporal differences between the sexes in reproductive investment. Given the potential for sex-specific differences in reproductive costs, resource availability may contribute to variation in reproductive allocation in females and males. Here, we used *Rumex hastatulus*, a dioecious, wind-pollinated annual plant, to investigate whether sexual dimorphism varies with life-history stage and nutrient availability, and determine whether allocation patterns differ depending on reproductive commitment. To examine if the costs of reproduction varied between the sexes, reproduction was either allowed or prevented through bud removal, and biomass allocation was measured at maturity. In a second experiment to assess variation in sexual dimorphism across the life cycle, and whether this varied with resource availability, plants were grown in high and low nutrients and allocation to roots, aboveground vegetative growth and reproduction were measured at three developmental stages. Males prevented from reproducing compensated with increased above- and belowground allocation to a much larger degree than females, suggesting that male reproductive costs reduce vegetative growth. The proportional allocation to roots, reproductive structures and aboveground vegetative growth varied between the sexes and among life-cycle stages, but not with nutrient treatment. Females allocated proportionally more resources to roots than males at peak flowering, but this pattern was reversed at reproductive maturity under low-nutrient conditions. Our study illustrates the importance of temporal dynamics in sex-specific resource allocation and provides support for high male reproductive costs in wind-pollinated plants.

INTRODUCTION

Many dioecious plants display sexual dimorphism in resource allocation across the life cycle. Females and males may differ in both the timing of allocation to various functions and in the absolute amount of resources allocated to above- and belowground vegetative growth and sexual reproduction (Putwain & Harper 1972; Delph 1999). For example, among long-lived trees and woody shrubs, females tend to flower later and be smaller than males at reproductive maturity (Obeso 1997; Nicotra 1999). Sexual size dimorphism (SSD) is often attributed to differences in the costs of reproduction between the sexes (Obeso 2002; Barrett & Hough 2013). Female investment, including the production of fruit and seeds, is thought to be more costly compared to males, which only produce flowers and pollen (Lloyd & Webb 1977; Cipollini & Whigham 1994). Hence, higher female reproductive costs may result in fewer resources available for vegetative growth (Delph & Meagher 1995; Vaughton & Ramsey 2011; Wang *et al.* 2013). Sex-specific differences in the cost of reproduction can also be driven by different resource currencies for female and male function (Ashman 1994). Whereas females need additional carbon for fruit and seed production, males require larger amounts of nitrogen for pollen production (Harris & Pannell 2008; Lankinen *et al.* 2013). Given the potential differences in the resource currencies for female and male reproduction, there is considerable scope for variation in the degree and direction of sexual dimorphism among dioecious flowering plants.

Different resource currencies for female and male reproduction may result in altered patterns of SSD. Whereas male SSD (males larger than females) is common in shrubs (Antos & Allen 1990), female SSD (females larger than males) is reported in wind-pollinated herbs (Conn 1981; Hesse & Pannell 2011; Pickup & Barrett 2012). Male inflorescences (containing pollen) are more nitrogen-rich than seeds and fruit, especially in herbaceous plants (Ishida *et al.* 2005; Kerkhoff *et al.* 2006). It has been suggested that the copious amounts of nitrogen-rich pollen required in wind-pollinated plants may result in higher costs for male reproduction (Harris & Pannell 2008). Therefore, if plants are nitrogen-limited, allocation to male reproduction may compromise resources available for vegetative growth.

Most studies examining the costs of reproduction have used measurements of the amount of aboveground biomass for individuals allowed to reproduce *versus* those prevented from reproducing (*e.g.* Tuomi *et al.* 1983; Fox & Stevens 1991; Thoren *et al.* 1996; Alvarez-Cansino *et al.* 2010). However, relatively few studies have partitioned allocation trade-offs between above- and belowground biomass across the life cycle and how these vary under conditions of nitrogen limitation. Investigations of the allocation trade-offs that incorporate the sources and sinks for carbon and nitrogen are therefore important for understanding how sexual dimorphism is driven by different currencies for female and male reproduction.

Differences between the sexes in their reproductive roles can result in temporal shifts in sexual dimorphism of resource allocation (Wilson 1988; Burd & Head 1992). At flowering, tall male plants may be better able to disperse pollen to more stigmas compared to shorter plants (Niklas & Buchmann 1985). Indeed, in the annual, wind-pollinated *Rumex hastatulus* males are taller than females at flowering, but not at reproductive maturity when females are taller (Pickup & Barrett 2012). Males in this species tend to flower earlier than females, probably because they do not need to accumulate energy for later seed provisioning (Conn & Blum 1981a). This difference in *R. hastatulus* could result in earlier allocation to belowground biomass (roots) to supply nitrogen for pollen production. In comparison, females are more likely to invest in aboveground vegetative growth before flowering in order to acquire sufficient carbon for future seed and fruit production, as proposed for another wind-pollinated annual plant – *Mercurialis annua* (Sanchez-Vilas & Pannell 2011). Few studies have examined sex-specific allocation with respect to both the timing and degree of investment in functionally different plant organs (*i.e.* inflorescences, leaves, shoots and roots). If the temporal reproductive and vegetative requirements differ between the sexes, we may expect to see dynamic changes in the strength and direction of sexual size dimorphism.

Here, we examined the temporal dynamics of sexual dimorphism in *R. hastatulus* by investigating the costs of reproduction and role of resource allocation trade-offs in driving temporal changes in SSD. Annual plants such as *R. hastatulus* are useful systems for studying changes in dimorphism over time because they do not allocate resources to future reproduction, but rather resources are used for a plant's current functional requirements. As in most wind-pollinated species, males of *R. hastatulus* produce large quantities of pollen, which has the potential to limit the vegetative growth of males. We examined sex-specific differences in resource allocation in *R. hastatulus* and how this changes across the life cycle and with different nutrient conditions. Specifically, we asked: (i) are the costs of reproduction higher in males than females due to the high resource demands of pollen production; (ii) does sexual dimorphism in resource allocation increase under stressful environmental conditions (such as nutrient limitation); and (iii) does sexual dimorphism in resource allocation vary across the life span, such that males invest more in reproduction at peak flowering and less during the period when females are maturing seeds? Our experiments provide insights into the dynamics of allocation trade-offs and how sex-specific differences in costs of reproduction influence sexual dimorphism in a dioecious plant.

MATERIAL AND METHODS

Study species and sampled population

Rumex hastatulus (Polygonaceae) is a primarily annual (rarely a short-lived perennial) dioecious weed occurring mainly in sandy well-drained soils in the southeast and central United States (Conn & Blum 1981b). In the field, seeds germinate during winter and early spring and plants flower from March to June, depending on region, with males senescing somewhat earlier than females (see Conn 1981). The species includes two main geographically widespread chromosome races (Smith 1963), the North Carolina karyotype (females = XX, 2n = 8; males = XY_1Y_2, 2n = 9) and the Texas karyotype (females XX, males XY, 2n = 10). In this study, we used seeds collected from 29 maternal plants from a population of the Texas race occurring at Oakwood (Texas; latitude 31°33′39″, longitude 95°54′6″) in May 2009 (see Pickup & Barrett 2013 for further details on the population). In the glasshouse, the life cycle of *R. hastatulus* is approximately 12 weeks from seed germination to reproductive maturity (seed maturation). Sex can be determined ~3–4 days before flowering, based on the morphology of buds and the developing inflorescence. In common with most populations of *R. hastatulus*, the Texas Oakwood population has a female-biased sex ratio (sex ratio = 0.59; Pickup & Barrett 2013).

Seedling germination for experiments

To generate seedlings for both experiments (see below), we germinated seed from 29 seed families. For each maternal plant, we randomly selected 36 seeds, which were soaked in distilled water for 24 h at 5 °C. Seeds were then germinated in Petri dishes on moist filter paper in a growth cabinet maintained at 20 °C for 12 h and 10 °C for 12 h with continuous light. We transplanted seedlings individually after ~14 days to 3-inch pots containing a 1:1 mixture of topsoil and Profile Porous Ceramic Greens Grade (Profile® Greens Grade™, PROFILE Products, LLC, Buffalo Grove, IL, USA) to allow for easier root harvesting, and plants were grown in a glasshouse maintained at 20–24 °C. Approximately 1 cm of Pro-Mix BX (peat moss, vermiculate and perlite) was also added to the pot surface prior to transplantation.

Experiment 1: Costs of reproduction

To examine if there were sex-specific differences in the effect of reproduction on allocation to root and aboveground vegetative growth, we used 80 randomly chosen plants from 21 maternal families. An average of 3.8 ± 0.04 (±SE) seedlings were used from each maternal family (range = 1–7). After 4 weeks of growth, half the plants (n = 40) were randomly allocated to the 'no reproduction' treatment and half were left unmanipulated (allowed to reproduce) in the control treatment. In the 'no reproduction' treatment we prevented reproduction by removing all buds and inflorescences from plants, using fine forceps and scissors, as soon as they became visible. This procedure was carried out on a daily basis for the duration of the experiment. At reproductive maturity (62 days after planting), we harvested plants and separated them into three components: (i) belowground root biomass, (ii) aboveground vegetative biomass (including rosette leaves, stem leaves and stems) and (iii) reproductive biomass (including inflorescences, flowers, and seeds and fruit for females). Only belowground root and aboveground vegetative biomass was available for the 'no reproduction' treatment. We obtained dry weights for each biomass component by drying samples at 55 °C for 3 days before weighing them on a four decimal place gram balance. For belowground root biomass, the root mass was washed prior to drying.

Experiment 2: Temporal dynamics of resource allocation

To examine sexual dimorphism in biomass allocation across the life cycle in *R. hastatulus* and how this varied under high and low nutrients, we randomly chose 360 plants from the 29 maternal families (see germination details above). An average of 12.4 ± 0.95 seedlings were used from each maternal plant. Seedlings were then randomly allocated to either high- or low-nutrient treatments (n = 180 plants in each treatment). The high-nutrient treatment involved a weekly addition of 100 ml high nitrogen (100–200 ppm N) urea-based liquid fertiliser. The low-nitrogen treatment received no fertiliser. As mentioned above, sex can only be accurately determined in *R. hastatulus* a few days prior to anthesis. Therefore, in this experiment, because we used randomly chosen plants at the seedling stage, sex ratios in each treatment were not equal but showed a slight female bias (average sex ratio = 0.55), reflective of the female bias that characterises the population of this species. Plants were positioned in the glasshouse using a randomised complete block design with two blocks.

To assess the temporal dynamics of resource allocation, plants in each nutrient treatment were randomly allocated to one of three harvests (first flowering, peak flowering and reproductive maturity), with 60 plants harvested from each nutrient treatment at each period. For the first sample (first flowering), plants were harvested as soon as they could be sexed, with an average harvest date of 39.0 ± 0.04 days from transplantation (range = 30–52 days). The second and third harvests were undertaken at 51 and 70 days after seedling transplantation, respectively. Plants rapidly senesced following the third harvest, with few males producing new flowers. For plants in the final harvest, we collected senescing flowers and seeds by enclosing inflorescences in mesh bags. For each harvest, we separated plants into belowground root biomass, aboveground vegetative biomass and reproductive biomass, as outlined in Experiment 1.

Statistical analysis

For the first experiment examining sex-specific differences in the effect of reproduction on allocation to root and aboveground vegetative growth, we used a Restricted Maximum Likelihood (REML) linear mixed model. For this model, treatment ('reproduction' and 'no reproduction') and sex were fixed factors and maternal plant was a random factor in the analysis. To investigate if sexual dimorphism in resource allocation varied across the life cycle and under high and low nutrients, we also used a REML linear mixed model. For this model, sex, fertiliser treatment and harvest time were fixed factors in the analysis and maternal plant and block were random factors. However, we removed the random effects of block and maternal plant in our final analysis, as they were non-significant terms in the model. *Post-hoc* comparisons among groups were tested using the least significant difference (LSD) at $\alpha = 0.05$. All analyses were undertaken in Genstat for Windows, version 13 (VSN International, Oxford UK).

RESULTS

Costs of reproduction

Overall, we found that females of *R. hastatulus* had slightly higher biomass than males at reproductive maturity (sex: $F_{1,74} = 3.69$, $P = 0.056$), and that plants prevented from reproducing had significantly more total biomass (aboveground vegetative + root biomass) than those that invested in reproduction (reproductive + aboveground vegetative + root biomass; sex: $F_{1,74} = 6.97$, $P = 0.01$). However, for the treatment that prevented reproduction, there was sexual dimorphism in the response of females and males (sex × treatment: $F_{1,74} = 4.98$, $P = 0.029$). For females there was no significant difference in total biomass between plants that reproduced compared to those that were prevented from reproducing (Fig. 1). In contrast, males that reproduced had 40.4% less total biomass compared to those that did not invest in reproduction (Fig. 1).

For the individual components of biomass, females had higher overall root biomass (sex: $F_{1,74} = 8.53$, $P = 0.005$), and investment in root biomass was higher in plants that were prevented from reproducing (treatment: $F_{1,74} = 14.47$, $P < 0.001$) for both females and males (sex × treatment: $F_{1,74} = 0.00$, $P = 0.952$). Overall, females and males invested similarly in aboveground vegetative biomass (sex: $F_{1,74} = 0.02$, $P = 0.875$), but like root biomass, this was higher in plants that were prevented from reproducing (treatment: $F_{1,74} = 33.56$, $P < 0.001$), with aboveground vegetative investment similar in both sexes across treatments (sex × treatment: $F_{1,74} = 1.27$, $P = 0.263$).

Patterns of resource allocation

We found that the degree and direction of sexual dimorphism in root allocation varied at different life-cycle stages

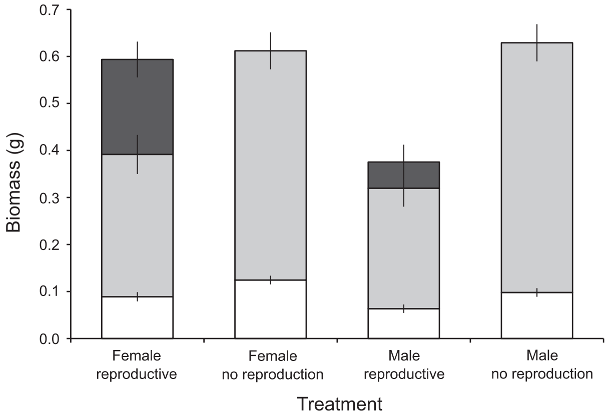

Fig. 1. The effect of reproduction on allocation to root and aboveground vegetative biomass in females and males of *R. hastatulus*. Plants prevented from reproducing are indicated as 'no reproduction', whereas in the other treatment plants were allowed to reproduce. Mean biomass allocated to each plant component: root biomass (white bars), aboveground vegetative biomass (grey bars) and reproductive biomass (dark grey bars). Vertical bars: ±1 SE.

(sex × harvest, $P < 0.001$; Table 1a), but not with nutrient treatment (sex × treatment, $P = 0.677$; Table 1a). There was no difference in root allocation between females and males at first flowering (Fig. 2a). However, at peak flowering, females grown under both low- and high-nitrogen conditions invested proportionally more resources in roots than males (Fig. 2a). In contrast, at reproductive maturity, males under low-nitrogen conditions invested proportionally more resources in root allocation than females (Fig. 2a).

For aboveground vegetative allocation (Table 1b, Fig. 2b), we observed a similar pattern of variation in sexual dimorphism across the three life-cycle stages (sex × harvest, $P < 0.001$; Table 1b), and under high- and low-nutrient conditions (sex × treatment, $P = 0.224$; Table 1b). Females and males had similar allocation to aboveground vegetative biomass at first flowering, while at peak flowering females invested proportionally more resources than males, but this difference was only significant for plants grown under high-nutrient conditions (Fig. 2b). Sexual dimorphism in allocation to aboveground vegetative biomass was reversed at reproductive maturity; males allocated proportionally more resources to aboveground vegetative biomass in both nutrient treatments (Fig. 2b).

Sexual dimorphism in allocation to reproductive biomass varied across life-cycle stages (sex × harvest, $P < 0.001$; Table 1c), but not with nutrient treatment (sex × treatment, $P > 0.05$; Table 1c). There was no difference in reproductive allocation between females and males at first flowering (Fig. 2c), but at peak flowering males invested proportionally more resources than females (Fig. 2c). Sexual dimorphism was then reversed at reproductive maturity, with females investing proportionally more resources than males in reproductive allocation (Fig. 2c). At all three life-cycle stages, high- and low-nitrogen conditions had similar effects on sexual dimorphism in reproductive allocation.

Table 1. Results of the Restricted Maximum Likelihood (REML) linear mixed model analyses examining the effect of sex, nutrient treatment and harvest time on (a) root biomass, (b) aboveground vegetative biomass and (c) reproductive biomass allocation in *R. hastatulus*. Bold values are significant at P<0.05.

	df	*F*	*P*
(a) root allocation			
sex	1	1.82	0.179
treatment	1	**47.75**	**<0.001**
harvest time	2	**111.79**	**<0.001**
sex × treatment	1	0.17	0.677
sex × harvest	2	**9.90**	**<0.001**
treatment × harvest	2	**8.73**	**<0.001**
sex × treatment × harvest	2	0.76	0.470
(b) aboveground vegetative allocation			
sex	1	**32.18**	**<0.001**
treatment	1	**12.02**	**<0.001**
harvest time	2	**674.31**	**<0.001**
sex × treatment	1	1.48	0.224
sex × harvest	2	**119.14**	**<0.001**
treatment × harvest	2	**33.33**	**<0.001**
sex × treatment × harvest	2	1.26	0.285
(c) reproductive allocation			
sex	1	**41.11**	**<0.001**
treatment	1	**65.06**	**<0.001**
harvest time	2	**1031.24**	**<0.001**
sex × treatment	1	2.15	0.143
sex × harvest	2	**151.98**	**<0.001**
treatment × harvest	2	**54.67**	**<0.001**
sex × treatment × harvest	2	1.72	0.181

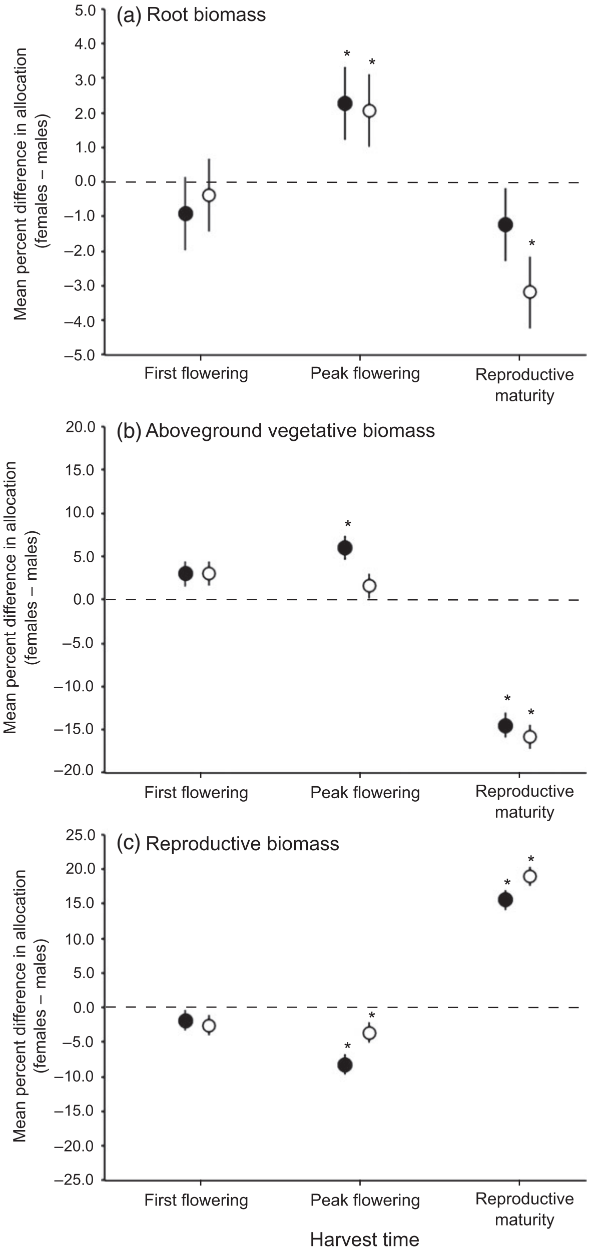

Fig. 2. The mean percentage difference in (a) root biomass, (b) aboveground vegetative biomass and (c) reproductive biomass allocation between females and males of *R. hastatulus* in high- (closed circles) and low- (open circles) nitrogen treatments for three harvest times (first flowering, peak flowering and reproductive maturity). Positive values represent female sexual size dimorphism (SSD, females > males), while negative values represent male SSD (males > females). Vertical bars: ±1 SE. *statistically significant differences between sexes ($P < 0.05$). Note the different *y*-axis scales for each figure.

DISCUSSION

Our study of the dynamics of sexual dimorphism in resource allocation found that compensatory growth was larger in males than females of *R. hastatulus*, suggesting higher male reproductive costs in terms of reduced aboveground vegetative and root growth. At peak flowering females allocated proportionally more resources to roots than males, but this pattern was reversed at reproductive maturity under more stressful (low nutrient) conditions. These results reflect temporal variation between the sexes in their allocation to vegetative tissues and reproduction, and highlight the importance of sex-specific differences in resource currencies for SSD. Although we cannot directly attribute sexual dimorphism in resource allocation to nitrogen limitation, our results provide support for the hypothesis of high male reproductive costs in wind-pollinated plants, and illustrate the importance of temporal dynamics in sex-specific resource allocation strategies. Below we discuss the significance of our findings and particularly the interaction between nutrient currencies and environmental conditions for patterns of SSD in herbaceous plants.

Sex-specific differences in reproductive costs are important drivers of sexual dimorphism in plants. Female reproductive costs have often been considered higher than for males, leading to male SSD; however, this situation may be reversed for wind-pollinated herbaceous plants due to the high nutrient demands of pollen production (Delph 1999; Harris & Pannell 2008). Regardless of pollination system, male reproductive function can be costly in terms of nitrogen requirements (Wright & Dorken 2014), and these requirements can draw on other plant functions to limit vegetative growth. For example, in *Plantago lanceolata* nitrogen allocation to hermaphroditic reproductive parts significantly reduced vegetative growth compared with male-sterile plants (Poot 1997). In dioecious *Sagittaria latifolia,* investment of female biomass in sexual reproduction exhibited a 1:1 trade-off with clonal biomass, whereas male investment in reproduction only produced clonal ramets and corms with low-nitrogen content (Van Drunen & Dorken 2012). In our study of *R. hastatulus,* males appear to have reallocated resources back into aboveground vegetative growth following bud removal, with the amount of growth proportionately higher than the removed biomass. Because female reproduction may not require as much nitrogen, this may account for why they had comparatively high aboveground vegetative growth. When females were prevented from reproducing, they reallocated resources to aboveground vegetative growth equal in proportion to the amount of biomass removed. Because this experiment did not measure the nitrogen content of male and female plant organs, we cannot confidently conclude that differences in biomass allocation are due to contrasting sex-specific nitrogen requirements. Nonetheless, our results suggest that the widespread assumption of higher resource costs for reproduction in females compared with males may not always be warranted, at least in wind-pollinated annual plants.

Unlike females, reproductive effort in males is thought to be more nitrogen- than carbon-limited (Antos & Allen 1990; Ashman & Baker 1992). In our study of *R. hastatulus*, we therefore expected males to invest more in roots for nitrogen acquisition than females. We observed this result at reproductive maturity and to a greater degree in low- as opposed to high-nitrogen conditions, findings consistent with those of Harris & Pannell (2008) on *M. annua*. At reproductive maturity, males likely needed a less extensive root system to acquire the more abundant nitrogen. This could partially explain the reduced sexual dimorphism in root allocation between peak flowering and reproductive maturity under high nitrogen, and the larger allocation to roots at reproductive maturity in males under low-nitrogen conditions. However, we cannot infer that male reproductive effort was more nitrogen- than carbon-limited, because we failed to observe sex-specific differences in reproductive allocation between our nitrogen treatments. We may have not detected a sex × treatment difference because of earlier male than female senescence, despite the synchronised harvest date for both sexes at the end of the experiment. Females may not have fully completed their total reproductive investment at this time.

Similar differential allocation strategies in response to stress (low nutrients) have also been observed in gynodioecious *Phacelia linearis* (Eckhart & Chapin 1997). In our study females invested more in roots than males at peak flowering, when males likely require more nitrogen, which is similar to findings for the sub-dioecious *Honckenya peploides* (Sanchez-Vilas *et al.* 2012). In contrast, for *M. annua,* males have larger proportional root biomass than females during their flowering period (Sanchez-Vilas & Pannell 2011). The difference in our study could be because root production is also a sink for nitrogen, and so during peak flowering males may not be able to afford to divert resources away from reproduction. Females, on the other hand, might invest more in root production at this time, possibly as a strategy to ensure the acquisition of sufficient nitrogen stores for subsequent fruit and seed provisioning (Burd & Head 1992). Patterns of allocation may also respond to stress and competition (see Hesse & Pannell 2011), so it is possible that we could have observed increased female allocation to reproduction at peak flowering if the conditions under which we grew our plants were more stressful.

Assessing the temporal dynamics of SSD can provide insights into trade-offs in resource allocation and how these interact with the contrasting reproductive roles of females and males. In our experiments, we observed the expected pattern of higher male reproductive costs during peak flowering, which likely resulted in reduced root and aboveground vegetative allocation. This reproductive trade-off has also been observed in monoecious *Arum italicum* (Mendez & Obeso 1993) and dioecious *Salix integra* (Tozawa *et al.* 2009). However, we observed the opposite trend during the seed maturation phase of the life cycle, regardless of nutrient treatment (and see Sanchez-Vilas & Retuerto 2012), when females are expected to have the highest reproductive cost (Leigh & Nicotra 2003; Sanchez-Vilas *et al.* 2012). Previous studies of *R. hastatulus* proposed that temporal changes in height dimorphism may reflect the contrasting reproductive functions of the sexes in relation to pollen and seed dispersal (Pickup & Barrett 2012). Males are taller than females at peak flowering, probably to faciliate effective pollen dispersal, but this pattern is reversed at reproductive maturity when seeds of this species are dispersed by the wind. In our study, because reproductive biomass included flowering stems, the increase in male invesment in reproductive biomass at peak flowering may reflect this pattern of height dimorphism.

Similarly, the larger investment of females at reproductive maturity could reflect both the resource investment in seeds and the increase in female stem height, promoting seed dispersal by wind.

In our experiments, sexual dimorphism was not observed prior to flowering but developed rapidly once anthesis began and persisted for the remainder of the life cycle. These changes in growth and development provide support for the hypothesis that sex-specific variations in allocation patterns are driven by the contrasting reproductive roles of females and males (Lloyd & Webb 1977). Furthermore, only when reproduction was prevented was there no sexual dimorphism apparent in root and aboveground vegetative allocation (Fig. 1), providing further evidence that differential reproductive costs are a major cause of sexual dimorphism.

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