

Sexual dimorphism in a dioecious population of the wind-pollinated herb *Mercurialis annua*: the interactive effects of resource availability and competition

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- **Background and Aims** Male-biased sex allocation commonly occurs in wind-pollinated hermaphroditic plants, and is often positively associated with size, notably in terms of height. Currently, it is not well established whether a corresponding pattern holds for dioecious plants: do males of wind-pollinated species exhibit greater reproductive allocation than females? Here, sexual dimorphism is investigated in terms of life history trade-offs in a dioecious population of the wind-pollinated ruderal herb *Mercurialis annua*.
- **Methods** The allocation strategies of males and females grown under different soil nutrient availability and competitive (i.e. no, male or female competitor) regimes were compared.
- **Key Results** Male reproductive allocation increased disproportionately with biomass, and was greater than that of females when grown in rich soils. Sexual morphs differentially adjusted their reproductive allocation in response to local environmental conditions. In particular, males reduced their reproductive allocation in poor soils, whereas females increased theirs, especially when competing with another female rather than growing alone. Finally, males displayed smaller above-ground vegetative sizes than females, but neither nutrient availability nor competition had a strong independent effect on relative size disparities between the sexes.
- **Conclusions** Selection appears to favour plasticity in reproductive allocation in dioecious *M. annua*, thereby maintaining a relatively constant size hierarchy between sexual morphs. In common with other dioecious species, there seems to be little divergence in the niches occupied by males and females of *M. annua*.

Key words: Life history trade-offs, competition, wind pollination, separate sexes, sex allocation, sexual size dimorphism, reproductive allocation, resource availability, *Mercurialis annua*.

INTRODUCTION

How should plants allocate their limited resources to growth and maintenance on the one hand, and male vs. female reproduction on the other? One key prediction is that plants should allocate more resources to the sexual function that has the greater marginal rate of return (Charlesworth and Charlesworth, 1981; Charnov, 1982). If seeds are dispersed relatively widely, approximately linear fitness gain curves are expected for the female function of plants, regardless of the pollination mode (e.g. Charnov, 1982; Lloyd, 1984; De Jong and Klinkhamer, 1994). However, fitness gains through the male function are likely to differ between wind- and animal-pollinated plant species (e.g. Burd and Allen, 1988; Charlesworth and Morgan, 1991; Klinkhamer *et al.*, 1997; Friedman and Barrett, 2009).

In animal-pollinated plants, gains in male fitness are expected to flatten off with increasing investment, because pollinators visit only a small number of plants so that related pollen grains end up competing with one another (e.g. Lloyd, 1984; Harder and Thomson, 1989; Brunet, 1992). In contrast, a more linear increase in male fitness is expected for wind-pollinated plants, because siring success is likely to be determined by lottery-model competition and air currents are not likely to saturate with pollen (e.g. Charlesworth and

Charlesworth, 1981; Charnov, 1982; Lloyd, 1984). Because the capacity to allocate resources to reproduction tends to increase with plant size, and pollen dispersed from greater heights might have greater outcross siring success, particularly in herbs, the male fitness gain curve might even accelerate under wind pollination (e.g. Burd and Allen, 1988; Klinkhamer *et al.*, 1997; Sakai and Sakai, 2003). In this case, we should expect wind-pollinated plants to allocate disproportionately to their male function. This prediction is well corroborated for wind-pollinated hermaphroditic species (e.g. Lloyd and Bawa, 1984; McKone and Tonkyn, 1986; Burd and Allen, 1988; Solomon, 1989; Ackerly and Jasienski, 1990; Aizen and Kenigsten, 1990; Fox, 1993; Lundholm and Aarssen, 1994; Pannell, 1997a; McKone *et al.*, 1998; Friedman and Barrett, 2009). However, should it also apply to dioecious species, where the life history of males and females is able to diverge?

Males and females of many dioecious species differ from one another in terms of their life histories, including their phenology (e.g. Bullock and Bawa, 1981), reproductive allocation (e.g. Lloyd and Webb, 1977), physiology (e.g. Dawson and Geber, 1999) and allocation towards defence (e.g. Agren *et al.*, 1999; Cepeda-Cornejo and Dirzo, 2010). Because reproductive investment habitually results in a reduction of resources available to other demands (Harper,

1977; Stearns, 1992), sex-differential reproductive allocation should result in sexual dimorphism in size (e.g. Lloyd and Webb, 1977; Meagher, 1984; Delph and Meagher, 1995; Delph, 1999; Geber *et al.*, 1999; Obeso, 2002). In this sense, we may expect males of wind-pollinated herbs to be smaller than females, in terms of their vegetative biomass, if male fitness gains flatten off more slowly than female gains, or if they accelerate, as it is expected they might.

Life history trade-offs between, for example, growth (and thus size) and reproduction, are likely to be more marked when resources are scarce (Conn and Blum, 1981; Allen and Antos, 1988; Antos and Allen, 1990; Delph, 1990, 1999; Ashman and Baker, 1992; Barrett and Pannell, 1999). For instance, in the gender-dimorphic plants *Phacelia linearis* (Eckhart and Chapin, 1997) and *Salix arctica* (Dawson and Bliss, 1989), the degree of size dimorphism increased in more stressful environments. If trade-offs between growth and reproduction are indeed steeper under resource limitation, as these studies suggest, any size disparity between males and females of dioecious species might be expected to be accentuated when resources are scarce (e.g. Wallace and Rundel, 1979; Gehring and Linhart, 1993; Dorken and Barrett, 2004; Sakai *et al.*, 2006), particularly if this disparity is a direct result of differences in reproductive effort between males and females.

Of course, allocation decisions and growth–reproduction trade-offs can also depend on competition between neighbouring plants (Harper, 1977; Grace and Tilman, 1990; Tilman, 1994), as these may differentially deplete the local resource environment in a way dependent on their reproductive requirements. First, we may expect the sex investing relatively little in reproduction to be competitively superior in growth (Lloyd and Webb, 1977). Second, as a result of selection for sexual specialization in resource acquisition and utilization, males and females may occupy divergent niches within the environment (i.e. niche partitioning; Freeman *et al.*, 1976; Onyekwelu and Harper, 1979; Cox, 1981; Vitale *et al.*, 1987; Bierzychudek and Eckhart, 1988; Freeman *et al.*, 1997; Dawson and Geber, 1999; Sanchez-Vilas and Pannell, 2010). As a result of niche partitioning, competition between males and females should be alleviated compared with that between plants of the same sex (e.g. Zhang *et al.*, 2009). However, although few experimental studies have manipulated the sexual context of competition (Agren *et al.*, 1999, and references therein; Eppley, 2006), there appears to be no clear directionality in the outcome of intraspecific competition in dioecious plants (Putwain and Harper, 1972; Conn and Blum, 1981; Lovett Doust *et al.*, 1987; Miller and Lovett Doust, 1987; Vasillauskas and Aarssen, 1992; Hawkins *et al.*, 2009).

Here, we test the joint effects of resource availability and competition on sexual dimorphism in life history trade-offs in a dioecious population of the wind-pollinated herb *Mercurialis annua*. In contrast to most dioecious plants (e.g. Lloyd and Webb, 1977; Bullock and Bawa, 1981; Lovett Doust *et al.*, 1987; Antos and Allen, 1990; Zimmerman, 1991; Delph, 1999; Obeso, 2002), females of *M. annua* are larger than males, a fact that has been attributed to the greater reproductive burden borne by males (Harris and Pannell, 2008), and even hermaphrodites are larger than males of the species in androdioecious populations (Hesse and Pannell, 2011). In the current study, we chose to

manipulate: (a) soil nutrient availability, because males of *M. annua* are known to be nitrogen-greedy (Harris and Pannell, 2008), and their growth should thus be compromised in poor soils; and (b) the absence/presence of competition between plants of varying sexual identities, because this may help us to evaluate whether selection has favoured traits that enhance specialization of males and females to a particular niche. Note that the competition treatment resulted in competition not only for soil nutrients but also for light (see below).

The following specific predictions were tested. First, selection in *M. annua* should favour increased allocation to pollen production with increases in above-ground vegetative biomass, as predicted for wind-pollinated species more generally. Second, females should exhibit lower reproductive allocation and larger sizes. Third, sex-differential allocation trade-offs should be enhanced in nutrient-poor soils, thereby enhancing sexual size disparities. Fourth, male growth should be more negatively affected by competition as they incur higher reproductive costs; moreover, if males and females occupy different niches, competition should be more severe between plants of the same sex.

MATERIALS AND METHODS

Study species

Mercurialis annua is a wind-pollinated annual plant that occurs in disturbed habitats across Europe and the Mediterranean Basin, where it is mostly dioecious (Durand, 1963). Males produce green staminate flowers that are held on erect axillary peduncles, whereas females produce green dehiscent capsules in leaf axils that each contains two seeds. Within two weeks of seed germination, plants begin flowering and continue to do so throughout the season. Because males and females produce and disperse their pollen grains or seeds as they grow (e.g. Pannell, 1997a), there is no sharp transition between vegetative and reproductive growth in *M. annua*. For our experiment, seeds were bulk-collected from approx. 50 females in a dioecious *M. annua* population near Sitges in Catalonia, northeastern Spain.

Experimental design

Seeds were thoroughly mixed and sown in seed trays in a glasshouse in early summer. At the onset of flowering, sexes were identified and transplanted into 4 inch pots containing nutrient-poor sandy soil. The experiment was set up outdoors in a blocked, fully crossed two-factorial design. Males and females were randomly assigned to the following treatments in each of 30 blocks, yielding a total of 360 pots: competition (no, male or female competitor) and soil nutrient availability (rich or poor soils). Thus, focal plants were placed alone in pots or in competition with an individual of the same or opposite sex, and half of these plants were grown in rich soils. Nutrient-rich plants received 100 mL of 1 mL L⁻¹ Phostrogen (Cambridge, UK) fertilizer every 2 d (Phostrogen contains equivalent molarities of nitrogen, potassium and phosphorus, plus micronutrients). Nutrient-poor plants received 100 mL of water every 2 d and 100 mL of 0.125 mL L⁻¹ Phostrogen fertilizer once a week.

Pots were spaced 20 cm apart in their blocks to prevent above-ground competition among plants assigned to different treatments; at the end of the experiment plants continued to grow as isolated individuals among non-competitor pots, whereas neighbouring plants in competitor pots had overlapping branches, indicating that plants in this treatments competed not only for below-ground resources, such as soil nutrients, but very probably also for above-ground resources, such as light.

Allocation measures

After 6 weeks, we harvested the above-ground portions of the focal plants, which were assigned *a priori*, recorded their height and dissected them into vegetative and reproductive components. Note that at the time of harvest, the production and dispersal of pollen and seeds had reached a quasi steady state, correlated with plant size (Pannell, 1997a), with approx. 15 % of all seeds already dispersed (data not shown). Thus, while our allocation assays were based on a snapshot in time, this snapshot can be taken as roughly representative of allocation patterns during the most productive period of growth (i.e. after early establishment but prior to senescence). Previous work with *M. annua* has shown that this estimation of pollen and fruit production provides an effective measure of reproductive allocation (Pannell, 1997b; Buggs and Pannell, 2006; Eppley and Pannell, 2007; Dorken and Pannell, 2008; Harris and Pannell, 2008; Pujol and Pannell, 2008).

Data analyses

For both sexes, we first tested whether the relative absolute reproductive biomass R was proportional to the relative absolute vegetative biomass V , using a log-linear model specified as $\log R = \log a + c \log V$ (Klinkhamer *et al.*, 1992). This model allowed us to test whether allocation to reproduction increases ($c > 1$) or decreases ($c < 1$) more than linearly with size. We tested whether the fitted exponent c differed significantly from 1 by comparing the t -value of the fitted model with that of a model where the exponent c was set to 1 (Venables and Ripley, 2002). For subsequent analyses the ratio of reproductive vs. vegetative dry biomass was used as a measure of reproductive allocation, because reproductive and total biomass are likely to be correlated (Samson and Werk, 1986; Klinkhamer *et al.*, 1992).

Linear mixed-effect models were used to test for differences between the sexes in their response to competition (three levels) and soil nutrient availability (two levels), with block treated as a random effect. To meet the assumptions of the statistical model, measures of plant size were transformed using natural logarithms, and reproductive allocation was arcsine-square root-transformed. Initially, shoot height was included in the analyses to account for initial size differences (mean height \pm s.d. for males = 68.11 ± 23.82 and for females = 66.74 ± 21.13 mm), but this term was never significant and was dropped from the models. Tests of significance were carried out using an F -test of likelihood ratios, based on marginal sum of squares. *Post-hoc* comparisons between means were tested using a Tukey procedure for multiple

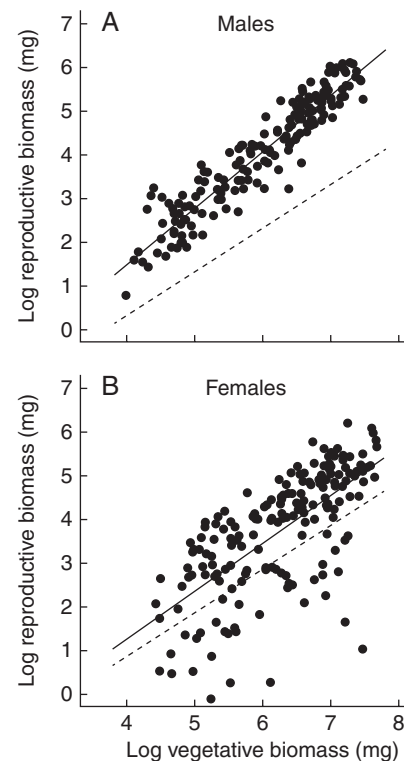


FIG. 1. Log-linear relationship between reproductive and above-ground vegetative dry biomass for (A) male individuals, where $\log(R) = -3.66 + 1.29\log(V)$, $R^2 = 0.88$; and (B) female individuals, where $\log(R) = -3.14 + 1.10\log(V)$, $R^2 = 0.46$. Note that the analysis was carried out on the pooled data set for each sex, regardless of treatment. The dashed line gives a log-linear relationship with $c = 1$ for each of the regressions.

comparisons, with $\alpha < 0.05$. Data analyses were performed using R Version 2.2.2 (<http://www.r-project.org>).

RESULTS

Reproductive allocation

Reproductive allocation increased with increasing biomass in males ($c = 1.29$; $t = 8.41$, $P < 0.0001$ for $c > 1$; Fig. 1A), but not in females ($c = 1.10$, $t = 1.13$, $P = 0.26$; Fig. 1B). Males exhibited greater reproductive allocation than females (mean reproductive allocation \pm s.e. for males across all treatments = 0.17 ± 0.01 and for females = 0.11 ± 0.01 , $n = 180$, $P < 0.001$; Table 1), but only in rich soils (sex \times nutrient treatment interaction: $P < 0.001$; Table 1 and Fig. 2B). While male reproductive allocation decreased under limited soil nutrient availability (*post-hoc* comparison: $P < 0.05$), female reproductive allocation did not differ significantly across resource treatments (*post-hoc* comparison: $P = 0.78$). The extent of sex-differential reproductive allocation depended on the competitive environment (sex \times competition treatment interaction: $P < 0.01$; Table 1 and Fig. 2A). *Post-hoc* comparisons indicated that, although competition did not significantly affect male reproductive allocation, it did affect female reproductive allocation: females allocated a relatively larger proportion of their resources to reproduction when competing with another female than when growing alone in a pot ($P < 0.05$).

TABLE 1. Summary of linear mixed-effects models of reproductive allocation and vegetative biomass for *M. annua* males and females (sex) grown in contrasting resource and competitor environments

	Reproductive allocation	Vegetative biomass
Resource	$F_{1,319} = 38.83^{***}$	$F_{1,319} = 240.34^{***}$
Competitor	$F_{2,319} = 1.34$	$F_{2,319} = 14.11^{***}$
Sex	$F_{1,319} = 69.13^{***}$	$F_{1,319} = 4.77^*$
Resource \times competitor	$F_{2,319} = 0.65$	$F_{1,319} = 4.04^*$
Resource \times sex	$F_{1,319} = 22.97^{***}$	$F_{1,319} = 0.73$
Competitor \times sex	$F_{2,319} = 6.52^{**}$	$F_{2,319} = 0.31$
Resource \times competitor \times sex	$F_{2,319} = 0.03$	$F_{2,319} = 0.62$

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

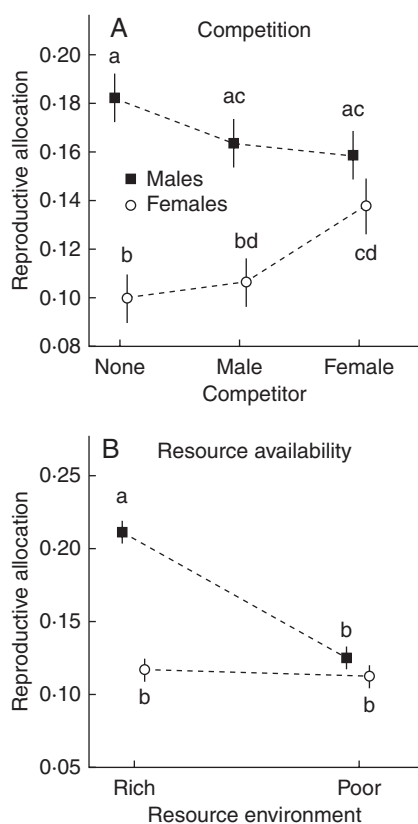


FIG. 2. Reproductive allocation for males and females, as indicated, as a function of (A) the competitive environment (none, male or female competitor) and (B) the availability of resources (resource-rich and resource-poor soils). Results from the different resource levels, and competition treatments were pooled, respectively. For each individual, reproductive allocation was calculated as the ratio between reproductive and vegetative dry biomass. Letters denote significant differences ($P < 0.05$), calculated using Tukey *post-hoc* tests. Error bars show \pm s.e.

Vegetative biomass

Females had a greater above-ground vegetative biomass than males (mean vegetative biomass \pm s.e. for males across treatments = 570 ± 32 mg and for females = 718 ± 40 mg, $n = 180$, $P < 0.05$; Table 1). Neither nutrient availability nor

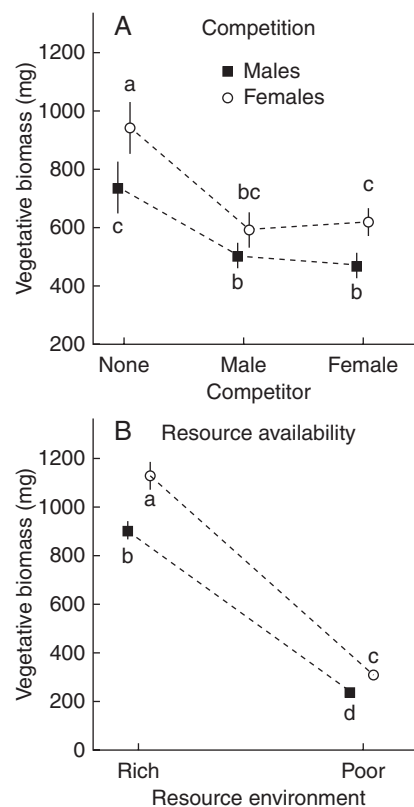


FIG. 3. Above-ground vegetative biomass for males and females, as indicated, as a function of (A) the competitive environment (none, male or female competitor); and (B) the availability of resources (resource-rich and resource-poor soils). Results from the different resource levels, and competition treatments were pooled, respectively. Letters denote significant differences ($P < 0.05$), calculated using Tukey *post-hoc* tests. Error bars show \pm s.e.

competition had a significant effect on the relative sizes of males vs. females (as indicated by non-significant two-way interactions: $P > 0.05$; Fig. 3 and Table 1). Unsurprisingly, plants were smaller when grown in poor soils (mean vegetative biomass \pm s.e. across treatments in rich soils = 1017 ± 30 mg and in poor soils = 271 ± 13 mg, $n = 180$, $P < 0.001$; Table 1), and this depended on the competitive environment (nutrient \times competition treatment interaction: $P < 0.05$; Table 1). *Post-hoc* comparisons indicated that although the sexual identity of the competitor did not significantly affect vegetative biomass in rich soils, it did so in poor soils: only those plants competing with a male were significantly smaller in terms of their vegetative biomass than those growing alone (Fig. 4).

DISCUSSION

Sexual dimorphism under optimal soil conditions

We found that male reproductive allocation increased more than linearly with increases in vegetative biomass, and was greater than that of females. Male-biased reproductive allocation is relatively uncommon in angiosperms, and is often associated with wind pollination (monoecious plants: Schoen and Stewart, 1986; Burd and Allen, 1988; Solomon, 1989; Traveset, 1992;

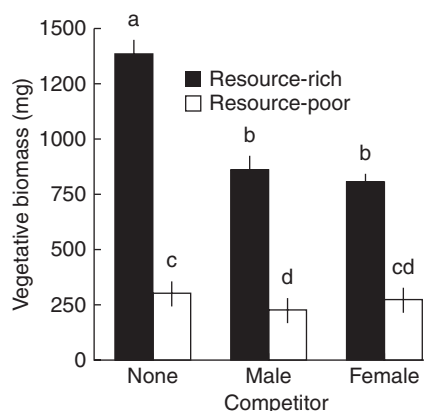


FIG. 4. Combined above-ground vegetative biomass of males and females as a function of competition (none, male and female competitor) in resource-rich and resource-poor conditions, as indicated. Letters denote significant differences ($P < 0.05$), calculated using Tukey *post-hoc* tests. Error bars show \pm s.e.

Fox, 1993; McKone *et al.*, 1998; Paquin and Aarssen, 2004; Friedman and Barrett, 2009; and gender-dimorphic plants: Ramadan *et al.*, 1994; Leigh *et al.*, 2006). This association may be explained in terms of the relative shapes of male vs. female fitness gain curves (e.g. Klinkhamer *et al.*, 1997; Delph, 1999; Culley *et al.*, 2002; Zhang and Jiang, 2002).

As explained in the Introduction, large size, expressed notably in terms of height, may have a direct effect on male fitness in wind-pollinated plants because of the increased siring potential that should result from wider dispersal of pollen (Charlesworth and Charlesworth, 1981; Charnov, 1982; Burd and Allen, 1988; Charlesworth and Morgan, 1991; De Jong and Klinkhamer, 1994; Klinkhamer *et al.*, 1997; McKone *et al.*, 1998). This means that it might pay males to invest little in reproduction while small, and to increase reproductive allocation when the benefits of pollen production are enhanced by greater size, especially in terms of height. In contrast, female fitness is more expected to saturate with size (e.g. De Jong and Klinkhamer, 1994), because seedlings from plants producing many seeds are likely to have to compete among themselves for limited local resources (De Jong and Klinkhamer, 1994; Campbell, 2000). Although these ideas have been largely developed for hermaphroditic species (Golonka *et al.*, 2005; Friedman and Barrett, 2009), our results indicate that they similarly apply to species with separate sexes.

Males were smaller in terms of their above-ground vegetative biomass than females, most probably as a result of a steeper growth–reproduction trade-off in males. It is important to note that the timing of investment in growth vs. reproduction within a growing season may vary between sexes, which could offset trade-offs based on the amount of investment (Bell, 1980; Delph, 1999). That is, male growth may be compromised early in the growing season when plants flower and need to produce nitrogen-rich pollen, whereas female growth may be reduced during the production of carbon-rich fruits. Although earlier male reproductive maturity has been reported for several dioecious species (Putwain and Harper, 1972; Lloyd and Webb, 1977; Delph, 1990; Nicotra, 1998), including *M. annua* (Harris and Pannell, 2008), the sexes in our

experiment did not differ significantly in height as they commenced flowering.

The effects of soil nutrient availability on sexual dimorphism

In contrast to our predictions, size disparities, in terms of biomass, were not greatly affected by soil nutrient availability. There are two possible explanations for this observation. First, because asymmetrical competition can greatly compromise fitness, selection may favour plasticity in reproductive allocation to maintain a rather constant size hierarchy across different environmental conditions. Other studies have also found little effect of resource availability on size dimorphism, possibly as a result of plasticity in reproductive investment (e.g. Popp and Reinartz, 1988; Niesenbaum, 1992). Nevertheless, we found that males, but not females, decreased their reproductive allocation in poor soils. This result might be expected if, as we have argued, male fitness in wind-pollinated species is less negatively affected by reduced allocation at small than at large sizes than is female fitness. Another possible explanation for the small effect of soil nutrients on size dimorphism is that plants may have changed their relative allocation towards roots in response to nutrient availability (e.g. Gehring and Linhart, 1993; Gedroc *et al.*, 1996; Dawson and Geber, 1999). Harris and Pannell (2008) found that the sexes of dioecious *M. annua* adjusted their root–shoot ratios in response to resource availability in hydroponic conditions, but only when resources were very abundant. Further analysis of root–shoot ratios in the context of reproductive investment by plants with different genders would be beneficial, particularly for roots harvested from soil.

The effects of competition on sexual dimorphism

We found that competition affected the reproductive allocation of females, but not that of males. In particular, female reproductive allocation was higher when females competed with one another rather than with a male. The cause of this pattern is puzzling, not least because seed set can be pollen limited when competition for pollen grains is fierce (e.g. Groom, 1998; Ashman *et al.*, 2004; Knight *et al.*, 2006); however, it is unlikely that females experienced pollen limitation under the current experimental conditions, given that plants were spaced relatively close together and that seed set in *M. annua* becomes pollen limited when plants are isolated by several metres (E. Hesse and J. R. Pannell, unpubl. data).

It was predicted that competition should (a) more negatively affect the growth of males, as males incur higher reproductive costs than females, and (b) be alleviated between plants of the opposite sex if sexes occupy divergent niches (e.g. Freeman *et al.*, 1976; Cox, 1981; Bierzychudek and Eckhart, 1988). However, competition in our experiment had no strong effect on the relative sizes of males vs. females, suggesting that niche partitioning may not play an important role in dioecious populations of *M. annua*. Other studies have similarly found little evidence for niche partitioning for gender-dimorphic plants (e.g. Onyekwelu and Harper, 1979; Miller and Lovett Doust, 1987; Vasillauskas and Aarssen, 1992), probably because plants with different genders actually overlap substantially in their resource acquisition strategies (but see

Sanchez-Vilas and Pannell, 2010). Again, it would be interesting to know whether sexes of *M. annua* show different responses to competition in terms of their relative allocation towards roots.

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