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Research Article

# Sexual dimorphism, temporal niche differentiation, and evidence for the Jack Sprat effect in an annual dioecious plant

Qian Yu[1,2], Spencer C. H. Barrett[3], Xin-Jia Wang[2,4], Li Zhong[2,4], Hong Wang[4], De-Zhu Li[1,4]*, and Wei Zhou[1,4]*

[1]Plant Germplasm and Genomics Center, Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

[2]University of Chinese Academy of Sciences, Beijing 100049, China

[3]Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Ontario M5S 3B2, Canada

[4]CAS Key Laboratory for Plant Diversity and Biogeography of East Asia, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, China

*Authors for correspondence. De-Zhu Li. E-mail: dzl@mail.kib.ac.cn; Wei Zhou. E-mail: zhouwei@mail.kib.ac.cn

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**Abstract** Sexual dimorphism in dioecious plants often occurs as a consequence of the different resource requirements of females and males, especially during reproduction. The contrasting reproductive roles of the sexes can influence the phenology of growth, plant size, and flowering time, with implications for the intensity of competitive interactions within and between the sexes. Here, we investigate the influence of contrasting nutrient regimes and intra-sexual and inter-sexual competition on the expression of sexual dimorphism in life-history traits and biomass allocation throughout the life cycle of the dioecious annual *Rumex hastatulus* Baldw. (Polygonaceae). Development of a sex-specific marker enabled us to quantify the influence of competition on sex-specific differences in mortality and vegetative traits. We were particularly interested in determining whether the overall performance of the sexes might differ between the two forms of intra-specific competition, potentially providing evidence for sexual specialization in resource acquisition and niche differentiation. Our results indicated that although patterns of sexual dimorphism were dynamic, they were largely insensitive to nutrient conditions. We found that intra-sexual competition was more severe than inter-sexual competition, differentially affecting mortality and most traits during the vegetative and particularly the reproductive stage of the life history. Female trait values generally increased more under inter-sexual than intra-sexual competition in comparison to males. Our findings are consistent with temporal niche differentiation resulting from sexual specialization for different resource requirements and provide evidence for the "Jack Sprat effect."

**Key words:** competition experiment, dioecy, Jack Sprat effect, niche differentiation, *Rumex hastatulus*, sex-specific genetic marker, sexual dimorphism.

## 1 Introduction

Sexual dimorphism occurs when females and males in dioecious populations differ in morphological, physiological, and behavioral traits. Understanding the proximate and selective mechanisms causing these differences has a long and venerable history, particularly in animal species where sexual selection plays a prominent role in the evolution of sexual dimorphism (Darwin, 1871; Andersson, 1994). In flowering plants, sexual dimorphism can be manifested at different stages of the life history and is often the consequence of the contrasting reproductive roles of the sexes, with fruiting bearing additional resource costs for females that are not incurred by males (reviewed in Lloyd & Webb, 1977; Delph, 1999; Barrett & Hough, 2012). Due to the sessile habit of plants, considerable attention has focused on the role of abiotic factors in differentially influencing the performance of the sexes owing to their contrasting resource requirements (Freeman et al., 1976; Dawson & Geber, 1999; Sánchez-Vilas et al., 2012; Field et al., 2013a; Puixeu et al., 2019). In contrast, although competition is a pervasive feature of most plant populations (reviewed in Keddy, 2001), how sexual dimorphism might influence intra-specific competition and the extent to which it may limit competition between the sexes remains poorly understood.

Sexual dimorphism in the costs of reproduction along gradients of resource availability and in patchy environments can have consequences for the spatial distribution of the sexes and sex ratio of populations. The spatial segregation of the sexes (SSS) has been observed in at least 30 species from

20 families of dioecious plants (Mercer & Eppley, 2010), but the mechanisms causing these patterns are often unclear (Barrett & Hough, 2012). It has been proposed that SSS may reflect an adaptive response to competition between the sexes and that this process can result in niche differentiation. This has been coined the "Jack Sprat effect" in which males and females possess divergent niches and specialize on different resources (Onyekwelu & Harper, 1979; Cox, 1981; Vitale & Freeman, 1986; Sánchez-Vilas & Pannell, 2010). It has been suggested that this form of niche partitioning may represent an evolved response to reduce competition between the sexes (Cox, 1981). However, experimental evidence on the adaptive basis of niche partitioning is generally lacking. Differential mortality among habitat patches in heterogenous environments owing to differences in the costs of reproduction between the sexes could also result in SSS. Indeed, a survey of 32 dioecious species with SSS indicated that differential mortality was the most probable cause in 21 (Bierzychudek & Eckhart, 1988). The extent to which competition within and between the sexes of dioecious populations plays a role in promoting niche differentiation and the Jack Sprat effect therefore remains an open question.

Most research on niche differentiation in dioecious plants has concerned the SSS along environmental gradients, particularly gradients of stress. However, not all dioecious species exhibit SSS and opportunities for niche differences may still occur through temporal niche partitioning owing to sexual dimorphism in growth dynamics. The resource requirements of flowering versus fruiting can result in striking differences between females and males in the timing of height growth, flowering, and senescence, and also in their use of different resource "currencies" (Onyekwelu & Harper, 1979; Cox, 1981; Delph, 1999; Harris & Pannell, 2008; Teitel et al., 2016). Sexual dimorphism in growth dynamics and the timing of investment in reproduction is likely to be especially evident in annual species due to their short life cycles and generally higher reproductive efforts (proportion of resources allocated to reproduction) than perennials (Harper, 1977). Annual dioecious species, therefore, provide tractable experimental systems for investigating the influence of resource availability and intra-specific competition on sexual dimorphism in life-history traits. This contrasts with the majority of dioecious species that are long-lived perennials that are often woody and/or clonal (Renner & Ricklefs, 1995; Vamosi et al., 2003), thus making detection of differences in growth dynamics and competitive interactions between the sexes more challenging to detect without long-term experimental studies.

Here, we investigate the influence of nutrient regimes and intra-specific competition on sexual dimorphism in dioecious *Rumex hastatulus* Baldw. (Polygonaceae). We chose this species because several of its features make it a suitable experimental system for addressing questions concerned with the relations between sexual dimorphism and niche differences between the sexes. Earlier work on this wind-pollinated annual established that females and males differ in a range of secondary sex characters affecting their growth dynamics, flowering time, and patterns of resource allocation (Conn & Blum, 1981; Pickup & Barrett, 2012; Teitel et al., 2016; Puixeu et al., 2019). Although there is no evidence that *R. hastatulus* exhibits pronounced SSS (but see Korpelainen, 1991 for possible SSS in other *Rumex* species), extensive populations surveys report consistently female-biased sex ratios (Pickup & Barrett, 2013). Populations of this colonizer of open habitats are often large in size, with monospecific stands at high density. Thus, intra-specific competition is likely to be a ubiquitous feature of the population biology of the species.

We began our investigation by establishing the extent to which sexual dimorphism was influenced by nutrient regime by comparing life-history traits and patterns of biomass allocation of the sexes throughout the life cycle in a glasshouse experiment. We were particularly interested in determining whether the direction and degree of sexual dimorphism could be modified by resource supply and whether there was evidence of plasticity in sexual dimorphism (Delph & Bell, 2008; Tonnabel et al., 2017; Li et al., 2019). Having established the nature of sexual dimorphism in *R. hastatulus*, we then conducted a competition experiment using hydroponic culture to determine whether the performance of the sexes varied depending on whether they were subject to intra- versus inter-sexual competition. As it was important to examine patterns of mortality and pre-reproductive vegetative traits in the competition experiment, this necessitated development of a sex-specific marker to enable us to determine the sex of non-reproductive plants. Our competition experiment compared sexual dimorphism in a range of life-history traits during the vegetative and reproductive phases of the life cycle. We predicted that if sexual specialization for different resources and evidence for the Jack Sprat effect occur in *R. hastatulus*, intra-sexual competition for resources would be more intense than under inter-sexual competition because plants of the same sex would require the same resources. Thus, we expected the enhanced performance of the sexes when they were grown together, compared with when they were grown with their own sex.

## 2 Material and Methods

### 2.1 Study system

*Rumex hastatulus* (Polygonaceae) is a colonizer of open disturbed fields usually on sandy well-drained soils. It is widely distributed over most of the southern and central United States (Conn & Blum, 1981; Pickup & Barrett, 2013). The wind-dispersed seeds germinate during the winter and early spring and plants flower from March to June, with males senescing earlier than females (Conn & Blum, 1981). The species includes two largely allopatric chromosome races (Smith, 1963; Puixeu et al., 2019; Beaudry et al., 2020), the North Carolina karyotype (females = XX, $2n = 8$; males = $XY_1Y_2$, $2n = 9$) and the Texas karyotype (females = XX, males = XY, $2n = 10$). Populations of the Texas race are distributed across Texas, Oklahoma, Arkansas, and Louisiana, whereas populations of the North Carolina race occur in North Carolina, South Carolina, Georgia, Alabama, and Florida. In this study, we used open-pollinated seeds collected from a population of the Texas race occurring at Rosebud (Texas; latitude 31°7′3″, longitude 96°51′37″) in May 2009 (see Pickup & Barrett, 2013 for further details of the population). In common with most populations of

*R. hastatulus*, this population when sampled exhibited a female-biased sex ratio (sex ratio = 0.65; Pickup & Barrett, 2013). In the glasshouse, the life cycle of *R. hastatulus* is approximately 12 weeks from seed germination to reproductive maturity and the sex of plants can be accurately determined 2–3 days before flowering, based on the morphology of floral buds.

### 2.2 Experiment 1: Phenological differences in growth and reproductive timing between the sexes

To investigate the influence of contrasting growth conditions on the expression of sexual dimorphism throughout the life cycle, we grew plants under glasshouse conditions under high- and low-nutrient conditions. In April 2015, we germinated seeds from three maternal seed families and from each we randomly chose ~300 seeds that were soaked in distilled water for 24 h at 5 °C. We then germinated seeds 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 then randomly selected seedlings and these were transplanted individually into 9-cm-diameter pots (soil volume 340 $cm^3$) containing Pro-Mix BX (peat moss, vermiculate, and perlite) and granular NPK fertilizer (20:20:20) or 6-cm-diameter pots (soil volume 100 $cm^3$), which received no fertilizer (Fig. 1A). Each treatment involved 380 plants and these were positioned in a randomized complete block design on a single glasshouse bench at the University of Toronto maintained at 20–24 °C.

To assess the temporal dynamics of plant growth, we measured the height of the shoot from the pot surface to the tallest point on each plant every two days throughout the life cycle. We recorded the date and sex of each plant on flowering and calculated days to first flower. After reproductive maturity (9 weeks from transplant), we harvested plants and separated them into three components: (i) vegetative biomass (including rosette leaves, stem leaves, and stems), (ii) reproductive biomass (including inflorescences, flowers, and seeds and fruits for females), and (iii) belowground root biomass. The dry weights for each biomass component were obtained by drying samples at 55 °C for 2 days before weighing them on a four decimal place gram balance (CPA224S; Sortorius, Goettingen, German). For root biomass, we washed roots before drying. We also measured at harvest the number of flowering shoots and the width of the tallest shoot.

To investigate patterns of sexual dimorphism in traits under high- and low-nutrient conditions, and whether they varied across the life cycle, we used a Restricted Maximum Likelihood (REML) linear mixed model (R package "lme4"; Bates et al., 2015). In the model, nutrient treatment and sex were treated as fixed effects. We used goodness-of-fit tests (*G*-test) to examine if the sex ratio of the two contrasting nutrient treatments was significantly different from equal numbers of females and males. All analyses were conducted in R version 3.6.3 (R Core Development Team, 2018, https://www.R-project.org/).

### 2.3 Experiment 2: Development of sex-specific molecular marker

Determining the sex of *R. hastatulus* plants during the vegetative phase is not possible based on morphological criteria, thus necessitating development of sex-specific DNA markers. To accomplish this, we used SSR markers. Using 2 × 250-bp read lengths, we sequenced genomic DNA of a male plant on a MiSeq Benchtop sequencer (Illumina, Inc., San Diego, CA, USA). We analyzed raw reads and these were assembled into contigs using Geneious version 6.0 (Biomatters, Auckland, New Zealand). Using BLASTx, the contigs were BLASTed against the NCBI GenBank to identify and exclude contigs with plastid genome hits. Then microsatellites with at least five repeats were identified using QDD version 2.1 Beta (Meglécz et al., 2010). We chose the top 100 loci of the homologous but discrepant microsatellites from QDD, and these were selected for primer design using the software PRIMER version 5.0 (Clarke & Gorley, 2001). These primers were initially tested and optimized using a Veriti 96-well Thermal Cycler Gradient PCR Machine (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 3 min, followed by 28 to 32 cycles at 95 °C for 30 s, at the annealing temperature for each specific primer. Further amplification tests on 43 polymorphic loci were conducted on five female and five male plants of *R. hastatulus*. We separated and visualized PCR products using QIAxcel capillary gel electrophoresis (Qiagen, Dusseldorf, German) with an internal size standard of 10–300 bp or using polyacrylamide gel electrophoresis (PAGE) with Generuler 100 bp plus DNA ladder (Thermo, Rockford, USA).

### 2.4 Experiment 3: Influence of intra- and inter-sexual competition on vegetative and reproductive traits

To determine whether intra- and inter-sexual competition differentially influenced vegetative and reproductive traits of *R. hastatulus*, we conducted a manipulative hydroponics experiment in a glasshouse at the Kunming Institute of Botany, CAS. In April 2017, we germinated *R. hastatulus* seeds from the same source as in Experiment 1. Two seedlings, of

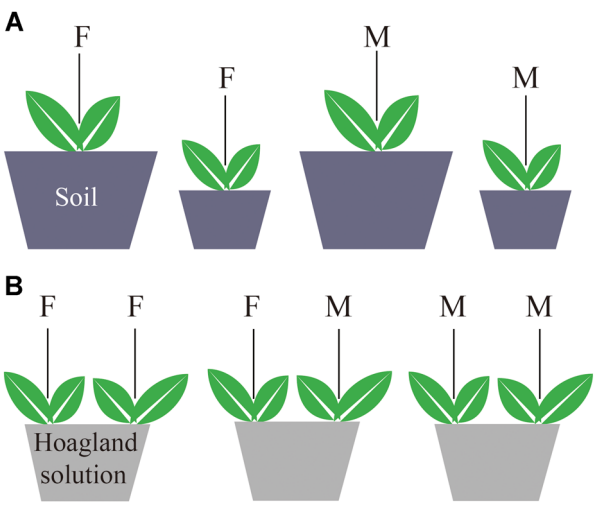


**Fig. 1.** Experimental designs for *Rumex hastatulus* Experiments 1 and 3. **A**, The two sexes were grown singly in pots of two different sizes and nutrient availability. **B**, The two sexes were grown in hydroponic culture using Hoagland solution under either intra-sexual competition (FF, MM) or inter-sexual competition (FM). See Section 2 for details.

unknown sex, were randomly transferred to each pot (diameter: 7.5 cm; 200 ml), which was filled with modified Hoagland nutrient solution. As detailed in Table S1, the composition of the nutrient solution was based on Hoagland standard, with most of the macronutrients diluted 4-fold (see Gibeaut et al., 1997; Tocquin et al., 2003). We added distilled water to the solution in each pot every week to compensate for water loss by transpiration. In total, 600 seedlings of *R. hastatulus* were grown in 300 pots that were positioned on benches in the glasshouse at temperatures ranging from 20 °C to 30 °C using a randomized complete block design.

As shown in Fig. 1B, because the two seedlings are randomly chosen for each pot with unknown sex, there exist three possible sex combinations or competition types: two females (FF), two males (MM), or one female and one male (FM). Both individuals in a pot were used in the subsequent analysis. Therefore, four categories of individuals can be recognized: males from male/male (hereafter M/MM) or female/male pots (M/FM), and females from female/female (F/FF) or female/male pots (F/FM). We determined the sex of individuals in each of the three possible sex combinations, either through use of our sex-specific marker if they did not flower (including mortality and non-flowering plants) or at flowering based on morphology.

To compare the temporal dynamics of mortality of the sexes under intra- and inter-sexual competition, we recorded mortality every three days for the entire duration of the experiment (12 weeks from transfer to pots) and collected leaf material from plants showing clear signs of premature death to determine the sex of dying individuals. During the experiment, we measured traits during the vegetative growth phase (1–4 weeks from transfer date) and the reproductive growth phase (5–12 weeks from transfer date). At 4 weeks, we measured the number of leaves, largest leaf width (the greatest width of the widest leaf), and plant width (the mean of two orthogonal diameters taken at the rosette stage of the plant). In addition, 4 weeks after transfer, 92 of the 300 pots of plants were randomly harvested before flowering and plants were separated into roots and aboveground biomass. Each biomass component was weighted as in Experiment 1. Using the SSR marker, we determined the sex of individuals in the 92 pots of plants. This resulted in a ratio of the following combinations MM = 19: FM = 41: FF = 32 of pots.

At the end of the reproductive stage (12 weeks from transfer date), all remaining pots were harvested. For pots in which both individuals had flowered, the sex combinations were MM = 34, FM = 37, FF = 20 pots. Plants in these pots were separated into the following: vegetative biomass (including roots, leaves, and stems); reproductive biomass (including inflorescence, flowers, seeds, and fruits for females). Total biomass was calculated as the sum of vegetative and reproductive biomass. We obtained dry weights for each biomass component as described for Experiment 1. For pots in which either one ($n = 62$) or both ($n = 47$) of the individuals had not flowered, we harvested leaf material to determine the sex of non-flowering individuals.

To examine if the mortality and sex ratio of non-flowering individuals were significantly different for each sex and sex combination, we used generalized linear models (GLMs) and three-way contingency table (R package "glm2"; Marschner, 2011). We used log transformation of mortality and sex ratio data for non-flowering plants to account for the Poisson distribution, and we considered the sex of individuals and competition type (intra- versus inter-sexual competition) as fixed factors in our analyses. Differences between the sexes and competition types were then tested using analysis of variance (type II analysis of variance [ANOVA]). To examine the influence of intra- and inter-sexual competition on vegetative and reproductive traits, we used linear models (LMs) with sex and competition type as fixed factors in the analyses. For each model, we obtained predicted means and 95% confidence intervals by "PREDICTMEANS" (R package). These analyses were conducted for all pots with two individuals that had successfully reproduced. We tested for differences between the two treatments (intra- and inter-sexual competition) and differences between the four categories (M/MM, F/FM, M/FM, and F/FF) using analysis of variance (type II ANOVA) with least significant differences at $P = 0.05$. All analyses were conducted in R version 3.6.3 (R Core Development Team, 2018, https://www.R-project.org/).

# 3 Results

### 3.1 Differences in flowering time, height, and biomass allocation between the sexes

In both pot-size treatments, the vast majority of *Rumex hastatulus* plants survived to the reproductive stage, with mortality in smaller pots (2.37%, $n = 9$) slightly higher than in larger pots (1.58%, $n = 6$). The sex ratios of the two treatments were very similar and significantly different from 1:1 (large pots = 0.56, $G = 5.95$, $P < 0.05$; small pots = 0.57, $G = 7.79$, $P < 0.01$). Although sex ratios were female biased, the degree of bias was significantly weaker than the source population from which seeds were obtained (sex ratio = 0.65, Pickup & Barrett, 2013).

Despite contrasting pot sizes and nutrient availability, the temporal dynamics of growth of the sexes was remarkably similar between treatments, but with clear differences between the sexes within each treatment (Fig. 2). We detected significant sexual dimorphism in the timing of first flowering in both treatments (Fig. 3A). The onset of flowering was significantly earlier in females than males (sex: $F = 906.50$, $P < 0.001$), and females in small pots flowered about half a day earlier, on average, compared with those in large pots (treatment: $F = 21.89$, $P < 0.001$; Fig. 3A). For males, there was no significant difference in first flowering between treatments (treatment: $F = 1.89$, $P = 0.170$).

Sexual dimorphism in height was evident at first flowering with males significantly taller than females in both treatments (large pots: $F = 126.20$, $P < 0.001$; small pots: $F = 831.50$, $P < 0.001$; Figs. 2, 3B). However, this pattern was reversed after about 45 days, and at harvest, females were significantly taller than males in both treatments (large pots: $F = 88.36$, $P < 0.001$; small pots: $F = 67.40$, $P < 0.001$; Fig. 2). As would be expected given the contrasting nutrient availabilities of treatments, plants of both sexes were significantly taller in large pots than those in the small pots

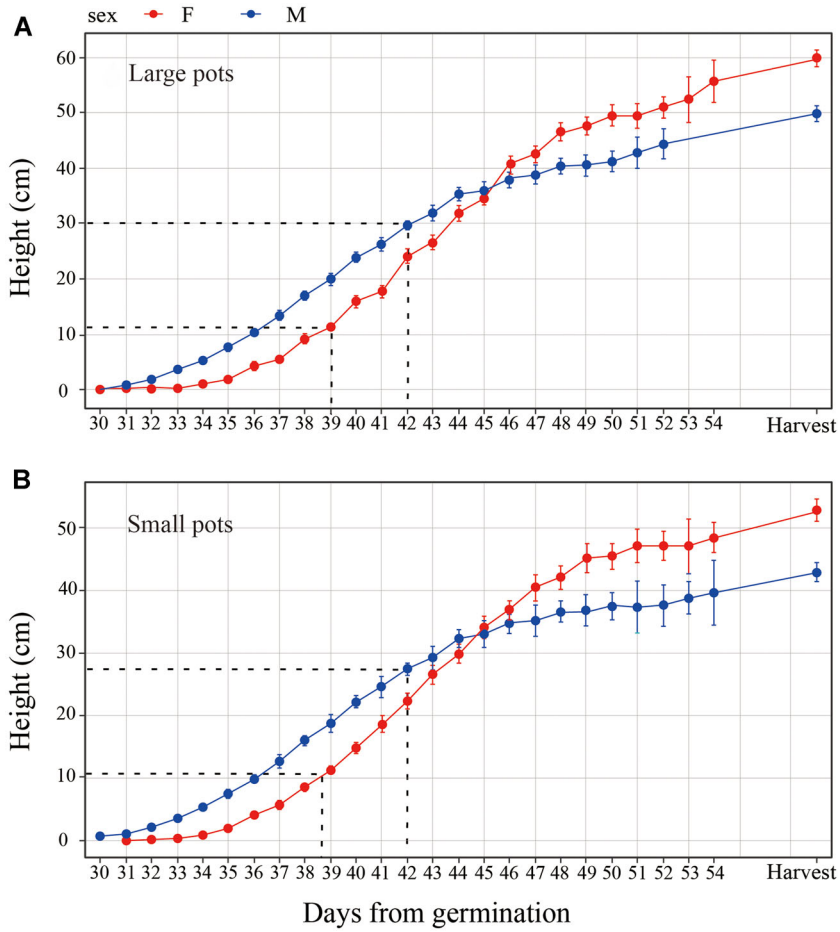


**Fig. 2.** The temporal dynamics of plant height in females (red line) and males (blue line) of *Rumex hastatulus* grown in (**A**) large and (**B**) small pots. The final height was measured at harvest (63 days after germination). Dashed lines indicated the mean date of first flowering and corresponding height of the sexes in each treatment. Vertical bars indicate ± SE.

(treatment: $F = 77.14$, $P < 0.001$, Fig. 2) and this was reflected in the average biomass of plants in large pots compared with small pots (Fig. 4).

Sexual dimorphism in resource allocation was evident in both nutrient treatments. At harvest, females had significantly higher total biomass (root + reproductive + vegetative biomass) than males (sex: $F = 161.45$, $P < 0.001$; Fig. 4). The total biomass of females grown in large pots was 1.91 times higher than males (mean ± SE for female: 0.5086 ± 0.019, male: 0.2655 ± 0.013), and a very similar pattern (ratio of 1.94) was also evident in small pots (female: 0.1869 ± 0.009, male: 0.0962 ± 0.006; Fig. 4). The degree and direction of sexual dimorphism in resource allocation were consistent among three individual components of biomass (Fig. 4). However, there was no difference in inflorescence number between females and males grown in large pots (sex: $F = 5.849$, $P = 0.019$), but females produced significantly fewer inflorescences than males in small pots (sex × treatment: $F = 9.274$, $P < 0.01$; Fig. 3C).

### 3.2 Sex-specific marker

Using the SSR primer RH11, we obtained a locus (GenBank accession MH388808) with a consistent sex-specific pattern present in all females and males. The primer exhibited stable amplification of a 172-bp band in all female plants and a heterozygous band of 162-bp and 172-bp in all males (Fig. 5). To further confirm and validate this sex-specific marker, we tested it using 768 samples (402 female and 366 male plants) from three *R. hastaulus* populations (TX: LA-MAN, LA-BEN, LA-DER; Pickup & Barrett, 2013). This survey resulted in completely accurate identification of all plants of both sexes.

### 3.3 Competitive interactions within and between sexes

#### 3.3.1 Mortality and flowering

At the end of the competition experiment (12 weeks from the beginning of growth under hydroponic conditions), 53 (12.74%) of the 416 individuals suffered mortality before reproducing (Fig. 6A), including 29 females (14.29% of 203 females) and 24 males (11.27% of 213 males); this overall difference in mortality between the sexes was not significant ($Z = 0$, $P = 0.624$). The sex-specific marker revealed that the mortality under intra-sexual competition (14.40%) was significantly greater ($Z = 2.582$, $P < 0.001$) than was recorded in the inter-sexual competition treatment (10.24%). At the end of experiment, the mortality of females experiencing intra-sexual competition (16.67%, 20 of 120 females) was

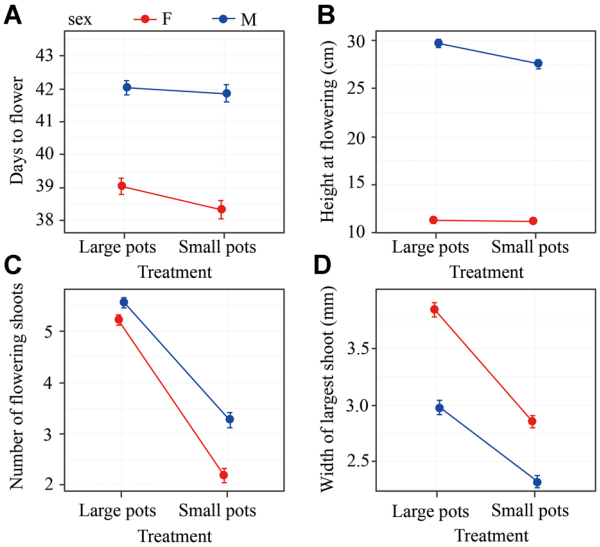


**Fig. 3.** Variation in (**A**) days to flower, (**B**) height at flowering, (**C**) number of flowering shoots, and (**D**) width of largest flowering shoot between females (red line) and males (blue line) of *Rumex hastatulus* grown in large and small pots. Data points are the mean ± SE.

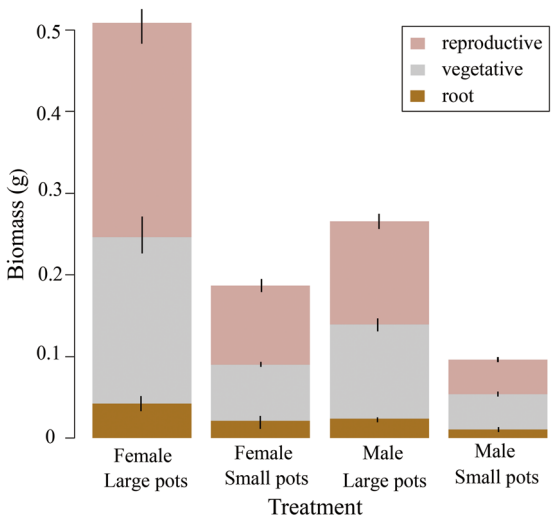


**Fig. 4.** The effect of contrasting nutrient treatments on allocation to root (brown), vegetative (grey), and reproductive (pink) biomass components in female and male plants of *Rumex hastatulus*. Vertical bars indicate ± SE.

significantly higher ($Z = 2.582$, $P = 0.009$) than females in the inter-sexual competition treatment (10.84%, 9 of 83 females; Fig. 6B). A similar pattern was evident in males ($Z = 3.194$, $P = 0.001$), with 12.31% mortality (16 of 130 plants) under intra-sexual competition and 9.64% (8 of 83 plants) under inter-sexual competition (Fig. 6B).

Among the surviving individuals in the experiment, 28.1% (102 of 363 plants) failed to flower before the end of the experiment (Fig. 6C), including 52 females (29.89% of 174 plants) and 50 males (26.46% of 189 plants). The pattern of non-flowering was consistent with the results we obtained for mortality: there were significant differences in non-flowering between intra- and inter-sexual competition treatments ($Z = 1.964$, $P < 0.001$), but overall difference in non-flowering between the sexes was not significant ($Z = 0.082$, $P = 0.431$). At the end of the experiment, the non-flowering percent of females grown under intra-sexual competition (29%, 29 of 100 plants) was significantly lower ($Z = 1.964$, $P = 0.048$) than females grown under inter-sexual competition (31.08%, 23 of 74 plants, Fig. 6C). The percent of non-flowering males grown under intra-sexual competition (29.82%, 34 of 114 plants) was significantly higher ($Z = 2.816$, $P = 0.004$) than males grown under inter-sexual competition (21.33%, 16 of 75 plants).

### 3.3.2 Vegetative traits after 4-week growth

After 4 weeks from the beginning of growth under hydroponic conditions, there was no overall difference in four of the six vegetative traits grown under intra- and inter-sexual competition (dashed lines and color shading, Fig. 7). However, ANOVA results indicated that the number of leaves and plant width in females grown under inter-sexual competition were significantly greater than for females under intra-sexual competition (leaves: $F = 1.75$, $P = 0.042$; Fig. 7A; plant width: $F = 4.67$, $P < 0.001$; Fig. 7C). In males, neither of these traits differed between the two treatments.

The aboveground and total biomass of plants grown under inter-sexual competition were significantly higher than for plants grown under intra-sexual competition (aboveground: $F = 11.68$, $P = 0.01$, Fig. 7D; total biomass $F = 10.31$, $P = 0.002$, Fig. 7F). In contrast, there was no difference in root biomass between the competition treatments (Fig. 7E). ANOVA results for sex differences indicated that females grown under inter-sexual competition had significantly higher aboveground biomass ($F = 2.92$, $P = 0.008$; Fig. 7D) and total biomass ($F = 3.17$, $P = 0.01$; Fig. 7F) than females experiencing intra-sexual competition. In males, there were no significant differences between the two treatments for each of the three biomass traits.

### 3.3.3 Vegetative and reproductive traits after 12-week growth

After 12-week growth, all six vegetative and reproductive traits illustrated in Fig. 8 exhibited significant overall differences between the intra- and inter-sexual competition treatments. ANOVA also revealed conspicuous sex differences, particularly in females in which all six trait values were significantly higher in the inter-sexual competition treatment than in the intra-sexual competition treatment. In contrast, only two trait differences were evident in males, but with similar higher values for plants grown under inter-sexual competition. Several comparisons are worth highlighting. Reproductive biomass in both sexes was significantly higher under inter-sexual than intra-sexual competition (females: $F = 29.12$, $P < 0.001$, males: $F = 29.12$, $P = 0.01$, Fig. 8E). In contrast, for the remaining four biomass measures (Figs. 8B–8D, 8F), only females exhibited higher values under inter-sexual competition, and there were no significant differences evident in males between the two competition treatments for these traits.

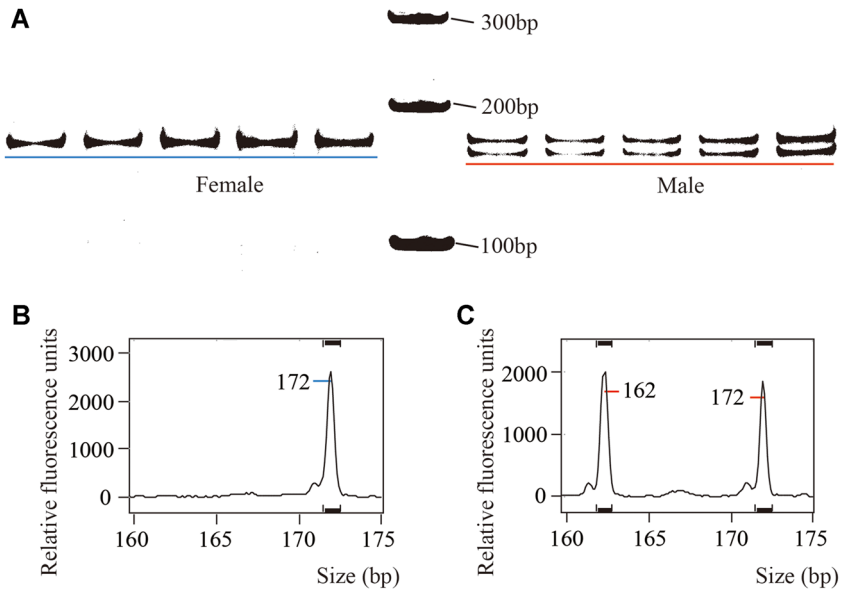


**Fig. 5.** PCR genotyping of sex differences in *Rumex hastatulus* using PAGE and QIAxcel capillary gels. **A,** Sex-specific differences in banding patterns in PAGE gel. After two hours of running the gel, females (blue underline) had only a single 172-bp band, whereas males (red underline) had two bands (162 and 172 bp). The central band is Generuler 100 bp plus DNA ladder. **B,** The electropherogram of females in 172-bp segment in QIAxcel capillary gel. **C,** The electropherogram of males in 162-bp and 172-bp segment in QIAxcel capillary gel.

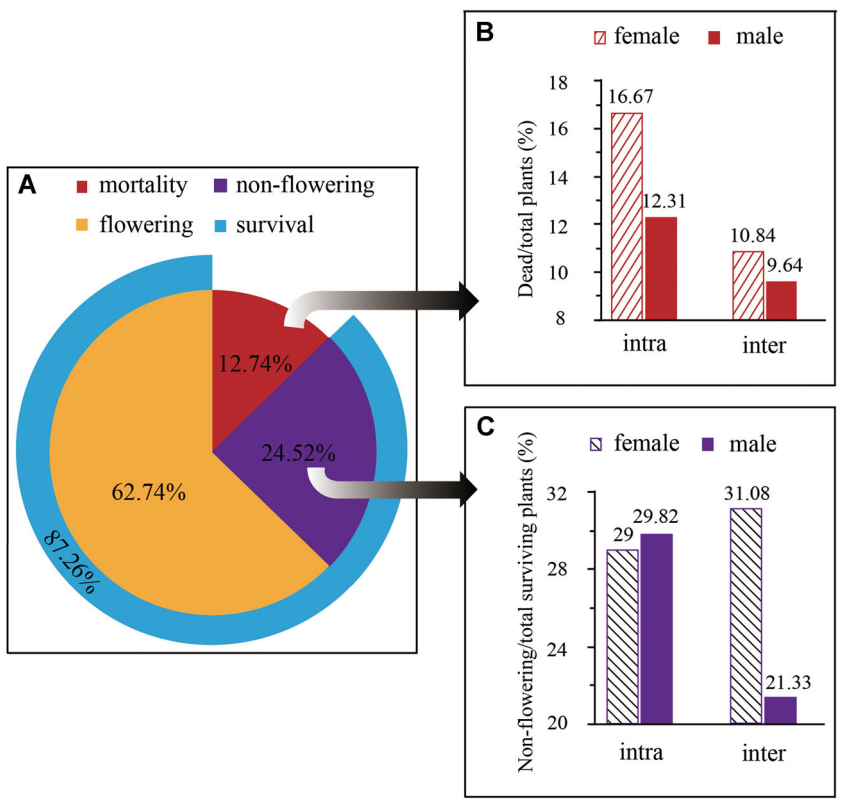


**Fig. 6.** The influence of intra- and inter-sexual competition on survival, mortality, non-flowering, and flowering in female and male plants of *Rumex hastatulus* after 12 weeks growth in hydroponic culture (see Section 2 for details). **A,** State of 416 individuals in the experiment. **B,** Percent mortality of female and male plants grown under intra- and inter-sexual competition. **C,** Percent non-flowering of female and male plants grown under intra- and inter-sexual competition.

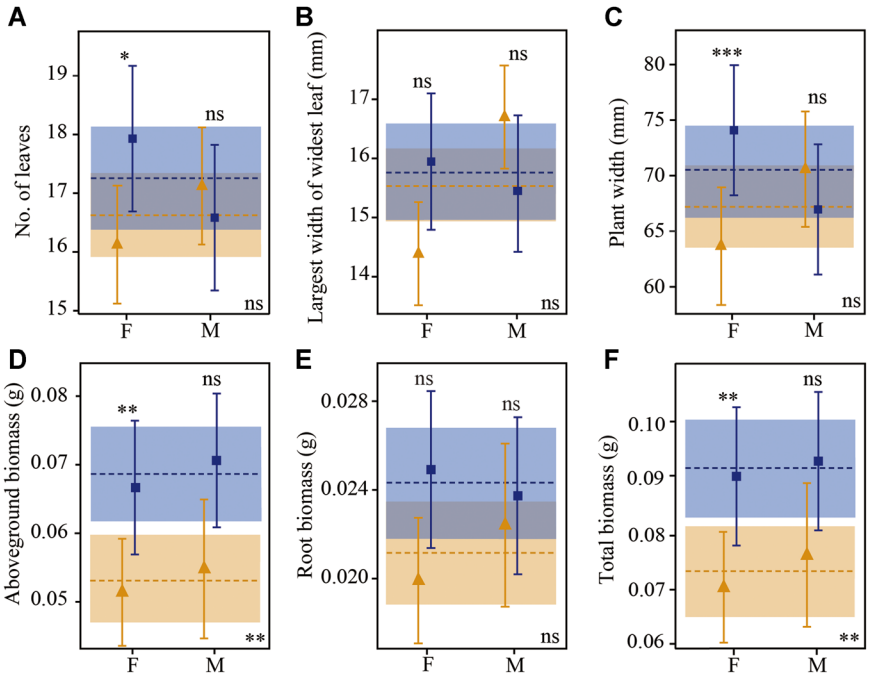


**Fig. 7.** Vegetative trait differences at 4 weeks in *Rumex hastatulus* grown under intra-sexual (orange color) and inter-sexual (blue color) competition in Experiment 3. Individual data points are predicted means and 95% confidence intervals for females (F) and males (M). Dashed lines and color shading are overall values of predicted means and 95% confidence intervals. Traits measured are as follows: **A**, number of leaves. **B**, largest width of widest leaf (mm). **C**, plant width (mm). **D**, aboveground biomass (g). **E**, root biomass (g). **F**, total biomass (g). The significance of treatment differences for each sex and treatment is indicated by stars above the same sex bars and in the lower right corner of each plot, respectively. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$; ns, not statistically significant.

## 4 Discussion

Our experimental investigations of temporal sexual dimorphism in *Rumex hastatulus* revealed several novel findings. There were significant differences between the sexes in growth dynamics, height, and patterns of resource allocation, but these features of life history exhibited striking stability in expression and were relatively insensitive to contrasting nutrient regimes. Our competition experiment revealed that during both vegetative and reproductive phases, the intensity of competition was greater under intra-sexual than inter-sexual competition (Figs. 6–8). Females were also more influenced than males by differences in nutrient supply (Fig. 3) and competitive interactions (Figs. 7, 8). We now compare our results with earlier studies of sexual dimorphism in dioecious plants, discuss their ecological and evolutionary implications, and evaluate evidence for temporal sexual niche differentiation and support for the Jack Sprat effect.

### 4.1 Expression of sexual dimorphism during the life cycle

Previous studies of *R. hastatulus* reported sexual dimorphism in flowering time, plant height, and biomass allocation (Pickup & Barrett, 2012; Teitel et al., 2016; Puixeu et al., 2019). Although the patterns of sexual dimorphism in Experiment 1 were based on samples from a single population of the Texas karyotype, they were in accord with earlier findings from populations sampled throughout the geographical range of both karyotypes of the species (Puixeu et al., 2019). Our fine-scale tracking of height under two nutrient regimes gave a more detailed picture of differences in the growth dynamics of the sexes than previous studies. Throughout the vegetative phase, males were significantly taller than females in both treatments, with females flowering earlier and at a smaller size than males, a pattern consistent with an earlier study (Pickup & Barrett, 2012). Once flowering commenced, female growth increased, and by 45 days, females had overtaken males in height, with males senescing before females. This reversal in height dimorphism did not occur because males stopped growing during flowering; rather it resulted from an acceleration of vegetative growth causing increased female height.

Our finding of later male flowering differs from an earlier study of the North Carolina karyotype of *R. hastatulus*, which reported that males flowered significantly earlier (92.5 days) than females (98.8 days), when grown under contrasting density and nutrient treatments (Conn & Blum, 1981). Males also flower earlier than females in *Spinacia oleracea* L. (Onyekwelu & Harper, 1979) and *Mercurialis annua* L. (Harris & Pannell, 2008), both dioecious wind-pollinated annuals. This pattern of population-level protandry is most often observed in dioecious species and several hypotheses have been proposed to explain earlier male flowering (Lloyd & Webb, 1977; Forrest, 2014). Below we suggest potential proximate and ultimate

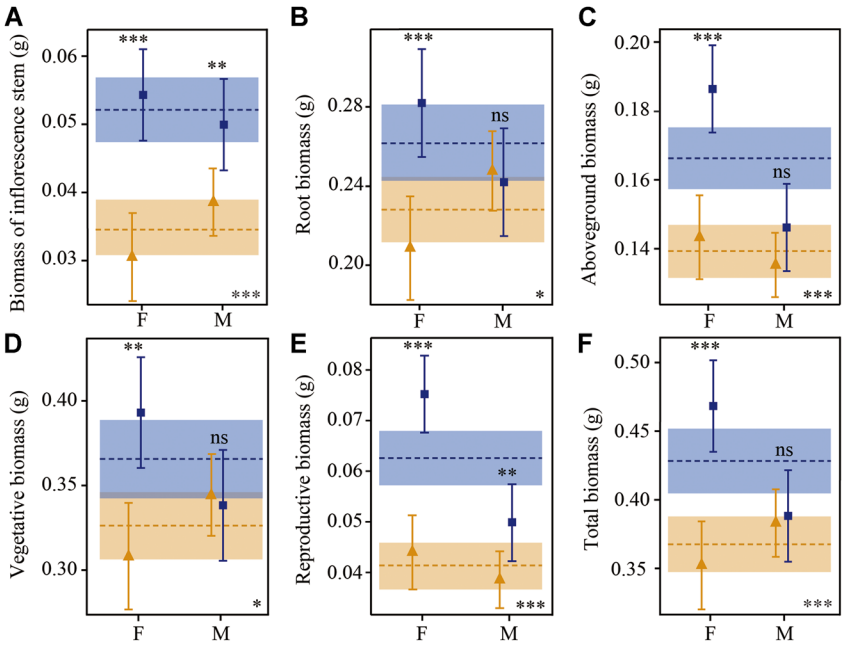


**Fig. 8.** Vegetative and reproductive trait differences at 12 weeks in *Rumex hastatulus* grown under intra-sexual (orange color) and inter-sexual (blue color) competition in Experiment 3. Individual data points are predicted means and 95% confidence intervals for females (F) and males (M). Dashed lines and color shading are overall values of predicted means and 95% confidence intervals. Traits measured are as follows: **A**, biomass of inflorescence stem. **B**, root biomass. **C**, aboveground biomass. **D**, vegetative biomass. **E**, reproductive biomass. **F**, total biomass, all in grams. The significance of treatment differences for each sex and treatment is indicated by stars above the same sex bars and in the lower right corner of each plot, respectively. *$0.01 < P \leq 0.05$; **$0.001 < P \leq 0.01$; ***$P \leq 0.001$; ns, not statistically significant.

factors that might be responsible for delayed male flowering in *R. hastatulus*.

Despite the three-fold difference in soil volume and nutrient availability for plants in the two growth treatments, we observed relatively small differences in trait expression. For example, the respective differences in height of females and males were very similar in the two nutrient regimes (Fig. 2), with the additional biomass of plants in large pots manifested by more flowering shoots of greater width (Figs. 3C, 3D). Sexual dimorphism for some traits increased for plants in small pots without added fertilizer (Figs. 3A, 3C), whereas for other traits, the degree of dimorphism was reduced (Figs. 3B, 3D). However, these differences were relatively small in magnitude, suggesting that sexual dimorphism is functionally constrained in *R. hastatulus*, with relatively little plasticity in direction and degree of phenotypic expression, consistent with the hypothesis that sexual dimorphism plays an important role in the adaptive strategies of the sexes.

The different growth dynamics of the sexes during the life cycle of *R. hastatulus* may be a consequence of their contrasting functional roles during pollination and seed dispersal (Pickup & Barrett, 2012). Males were taller than females at first flowering, owing to more rapid height growth, increased stem elongation, and a delay in onset of flowering. This temporal aspect of sexual dimorphism may result from sex-specific selection associated with the benefits of more effective pollen dispersal by taller plants (Niklas, 1985; Okubo & Levin, 1989; Friedman & Barrett, 2009; Tonnabel et al., 2019). By the same argument, the accelerated growth of females and their taller stature at reproductive maturity could also result from sex-specific selection for improved seed dispersal by wind (Bullock et al., 2003; Tackenberg et al., 2003; Soons et al., 2004; Thomson et al., 2011), resulting in offspring escaping sib competition (Howe & Smallwood, 1982; Levin et al., 2003). Future experimental studies on the reproductive benefits of height variation *R. hastatulus* for successful pollen and seed dispersal would be necessary to test these adaptive hypotheses.

The distinctive temporal reversal of height dimorphism in *R. hastatulus* may also be associated with differences in the timing and nature of resource acquisition by the sexes for reproduction. For example, later male flowering may occur, in part, due to the requirement to acquire nitrogen for the large quantities of pollen typically produced by wind-pollinated species (Ishida et al., 2005). Similarly, post-flowering differences in height growth between the sexes may be associated with the requirement in females for carbon resources for seed production through allocation to increased vegetative structures (Harris & Pannell, 2008). Consistent with this hypothesis, females grown in both nutrient treatments allocated more to vegetative structures than males and overall had nearly double the total biomass (Fig. 4). Additional investigations are required to determine the resource requirements and different nutritional currencies of the sexes in *R. hastatulus*. The observed pattern of larger females than males at reproductive maturity also

occurs in other dioecious herbs; however, this pattern is not evident in woody species (Delph, 1999; Obeso, 2002). Disentangling the proximate physiological and developmental mechanisms associated with costs of reproduction from the potential ecological benefits of sexual dimorphism represents a major future challenge.

### 4.2 Identification of sex-specific marker

In contrast to most sexually dimorphic animals, identification of the sex of dioecious plants before reproduction, or in non-reproductive adults, is usually not possible. This is because secondary sex characters during the vegetative phase of the life history are generally absent or weakly expressed (Lloyd & Webb, 1977; Dawson & Geber, 1999; Delph, 1999; Barrett & Hough, 2012).

The inability to determine the sex of non-reproductives has stymied efforts to address a range of key questions concerned with the mechanisms governing niche differentiation and sex ratio evolution in dioecious plants (Barrett et al., 2010; Field et al., 2013b). However, recent application of molecular approaches to studies of dioecious species has led to the development of genetic markers enabling unambiguous identification of males and females, even at the seed stage (Stehlik & Barrett, 2005). These markers have most often been developed for breeding programs on wild species of potentially important economic importance or in already domesticated species (e.g., Khadke et al., 2012; Cherif et al., 2013; Sun et al., 2014; Wu et al., 2015; Li & Ye, 2020; Wang et al., 2020). Less often they have been applied in ecological and evolutionary studies of dioecious populations (see Eppley et al., 1998; Korpelainen, 2002; Stehlik & Barrett, 2005; Morgan et al., 2020). Table 1 summarizes studies in which sex-specific molecular markers have been identified in diverse wild and domesticated species.

In most studies in Table 1, genetic markers were identified in males not females, including two previous studies of *Rumex* species (e.g., *R. acetosella* L.—Korpelainen, 2002; *R. nivalis* Hegetschw.—Stehlik & Blattner, 2004); however, other patterns have been observed. Our investigation of SSRs in *R. hastatulus* resolved a locus (GenBank accession MH388808) using the primer RH11 that resulted in a consistent sex-specific pattern present in both sexes, but with two bands in males and a single band in females (Fig. 5). By screening a large number of plants (768) from three populations from Texas, we confirmed that this marker could reliably distinguish the sexes of all flowering individuals. Although we have not determined the inheritance of banding patterns, it seems probable that males are heterozygous and female are homozygous for alleles at a single locus with a single fixed difference between the X and Y chromosomes of *R. hastatulus*. A similar pattern has been observed for numerous single-nucleotide polymorphisms associated with the sex chromosomes of this species (Hough et al., 2014; Beaudry et al., 2020; Rifkin et al., 2020). Regardless, this sex-specific marker provided us with a valuable tool for investigating the mortality and growth dynamics of the sexes in our competition experiment.

### 4.3 Sexual dimorphism, competition, and the Jack Sprat effect

In common with most dioecious species, we found that the degree of sexual dimorphism was more accentuated in

**Table 1** Sex-specific molecular makers developed in wild and domesticated dioecious species and observed banding patterns

| Species | Class of marker | Male | Female | References |
|---|---|---|---|---|
| Wild and domesticated species used by humans | | | | |
| *Actinidia arguta* (Sieb. & Zucc.) Planch. ex Miq. | GBS | Two bands | One band | Hale et al. (2018) |
| *Actinidia kolomikta* Maxim. | GBS | One band | Absent | Hale et al. (2018) |
| *Eucommia ulmoides* Oliv. | ddRAD | One band | Absent | Wang et al. (2020) |
| *Gleditsia sinensis* Lam. | SSR | Absent | One band | Li & Ye (2020) |
| *Hippophae rhamnoides* L. | RAPD | Present | Absent | Persson & Nybom (1998) |
| *Humulus lupulus* L. | RAPD | Present | Absent | Polley et al. (1997) |
| *Litsea cubeba* (Lour.) Pers. | SCAR | Absent | One band | Wu et al. (2015) |
| *Phoenix dactylifera* L. | SSR | Two bands | One band | Cherif et al. (2013) |
| *Piper betle* L. | SCAR | Two bands | One band | Khadke et al. (2012) |
| | | One band | Absent | Khadke et al. (2012) |
| | | Absent | A band | Khadke et al. (2012) |
| *Pistacia chinensis* Bunge | SCAR | Absent | One band | Sun et al. (2014) |
| *Pistacia vera* L. | RAPD | Absent | One band | Hormaza et al. (1994) |
| *Poa arachnifera* Torr. | AFLP | One band | Absent | Renganayaki et al. (2005) |
| Wild species used in ecological and evolutionary studies | | | | |
| *Asparagus officinalis* L. | SCAR | One band | Absent | Jiang & Sink (1997) |
| *Distichlis spicata* (L.) Greene | RAPD | Absent | One band | Eppley et al. (1998) |
| *Lodoicea maldivica* (J.F.Gmel.) Pers. | ddRAD | One band | Absent | Morgan et al. (2020) |
| *Rumex acetosa* L. | nuclear | Two bands | Absent | Korpelainen (1991) |
| *R. nivalis* Hegetschw. | SCAR | One band | Absent | Stehlik & Blattner (2004) |
| *Silene latifolia* Poir. | SCAR | One band | Absent | Zhang et al. (1998) |

Note: AFLP, amplified fragment length polymorphism; ddRAD, double-digest restriction site-associated DNA; GBS, genotyping-by-sequencing; Present, number of bands in males unclear; RAPD, random amplified polymorphic DNA; SCAR, sequence-characterized amplified region; SSR, simple sequence repeat.

reproductive than vegetative traits (Lloyd & Webb, 1977; Barrett & Hough, 2012). The variation we observed should result in temporal differences in the competitive ability of the sexes, which likely differ in nutrient requirements for reproduction, but also competition for the same limiting resources. Plant competition occurs both below and above ground and between conspecifics and different species. As *R. hastatulus* is a colonizer of open habitats, it commonly occurs in extensive monospecific stands in which intra-specific competition probably dominates. Our investigation therefore focused on the consequences of intra- and inter-sexual competition on the performance of the sexes.

We predicted that competition intensity in Experiment 3 would be greater between plants of the same sex than when females and males were grown together. This expectation was because plants of the same sex are likely to require the same limiting resources, whereas females and males likely differ in resource use due to their contrasting reproductive investments (Harris & Pannell, 2008). More intense competition in single-sex pots might be expected to suppress overall performance compared with mixed-sex pots in which asymmetric competition between the sexes should be evident. In general, our predictions were supported and our results were consistent with earlier findings in which differences in competitive ability between the sexes have been reported with females more competitive than males (e.g., Conn & Blum, 1981; Bertiller et al., 2002; Eppley, 2006); however, this outcome is often dependent on environmental context.

By using our sex-specific marker, we were able to demonstrate that during the vegetative phase of the life cycle, there was significantly greater mortality (Fig. 6B) and suppressed flowering (Fig. 6C) of plants growing under intra-sexual than inter-sexual competition. However, under inter-sexual competition, males had significantly fewer non-flowering plants than females, a pattern that was not evident under intra-sexual competition in which both sexes exhibited similar proportions of non-flowering plants (Fig. 6C). The greater suppression of flowering in females under inter-sexual competition may be associated with more intense competition they experienced owing to the greater resource acquisition of faster growing and taller males in this treatment.

The aboveground and total biomass of vegetative plants at 4 weeks were significantly lower under intra-sexual than inter-sexual competition, consistent with intense competition for the same resources (Fig. 7). This trend was further accentuated at 12 weeks, where each of the six traits we measured exhibited lower values under intra-sexual than inter-sexual competition (Fig. 8). Generally, where differences in trait values between the competition treatments were evident, females benefitted more than males when growing under inter-sexual competition, with exception of the higher percentage of non-flowering individuals discussed above (Fig. 6C). Higher trait values in females was particularly evident at reproductive maturity for all biomass components (Fig. 8), each of which was significantly greater for females growing with males. In contrast, in males, there were no differences in vegetative traits after 4 weeks between the competition treatments, and at reproductive maturity, only three of the six traits had increased under inter-sexual competition. Our experiment therefore indicates asymmetrical competition, with females more often benefitting from having males rather than females as competitors. Male performance was less affected by the sex of neighbors; however, for a few traits (e.g., inflorescence stems and reproductive biomass Figs. 8A, 8E, respectively), values increased under inter-sexual competition.

How do our results for *R. hastatulus* compare with previous studies on competition within and between the sexes of dioecious species? The answer to this question is not straightforward, as earlier results varied and were sometimes contradictory (reviewed in Varga & Kytöviita, 2012). Different approaches have been used to assess the competitive ability of the sexes. These include the manipulation of plant density (reviewed in Ågren et al., 1999), the growth of sexes in relation to the sex of neighbors (Herrera, 1988; Vasiliauskas & Aarssen, 1992; Zhang et al., 2009), whether males or females differentially affected the subsequent generation growing in the same soil (sex-differential niche modification, Sánchez-Vilas & Pannell, 2010), and competition experiments in which the sexes are forced to compete by growing them together in close proximity, as in our study. These experiments have been conducted under a range of different conditions including glasshouse pot experiments and common garden and field comparisons (e.g., Bertiller et al., 2002; Eppley, 2006; Hawkins et al., 2009; Mercer & Eppley, 2010; Hesse & Pannell, 2011; Sánchez-Vilas et al., 2011; Varga & Kytöviita, 2012; Chen et al., 2014). Not surprisingly, given the range of approaches, environmental contexts, and the taxonomic and life history diversity of study systems (e.g., herbaceous annuals and perennials, shrubs, trees), a variety of outcomes are reported, thus limiting generalizations. In some cases, little evidence for sex differences in competitive ability was found (e.g., Hawkins et al., 2009; Varga & Kytöviita, 2012), whereas in other instances, females were reported to be more competitive (e.g., Herrera, 1988; Bertiller et al., 2002; Eppley, 2006). In studies in which experimental conditions were manipulated (e.g., watering regimes), the outcome of intra- and inter-sexual competition on the performance of the sexes differed and was environment-dependent (e.g., Chen et al., 2014). Probably the most common finding has been that competitive interactions between the sexes are asymmetric, leading authors to infer that niche differentiation was probable in natural populations.

The Jack Sprat effect was first proposed by Onyekwelu & Harper (1979) to characterize niche differentiation between the sexes in *S. oleracea*. These authors also used an earlier study of different seasonal niches in *R. acetosa* L. and *R. acetosella* (see Putwain & Harper, 1972) as evidence for their hypothesis. The concept was later referred to as sexual niche partitioning and further investigated by Cox (1981), with evidence from three dioecious species. Numerous subsequent studies have reported evidence for spatial or temporal niche differentiation between the sexes of dioecious species owing to sexual dimorphism in resource acquisition and allocation (Dawson & Geber, 1999; Hultine et al., 2016). What is currently unclear is the extent to which observed differences between the sexes are simply a consequence of their differences in reproductive biology, related to costs of reproduction and different functional roles of males and females in pollination and seed dispersal (Bierzychudek &

Eckhart, 1988), or alternatively whether they are caused by selection to decrease inter-sexual competition (Cox, 1981). Our studies support the predictions of the Jack Sprat effect by showing both temporal niche differentiation and improved performance of the sexes, especially females, under inter-sexual competition. However, without further work, we cannot reject either non-adaptive or adaptive hypothesis, and, of course, a variety of factors likely contribute to the evolution of sexual dimorphism. Initial differences in resource use associated with reproduction causing temporal niche differentiation may often become further amplified by sex-specific selection and sexual specialization. As is the case with most research on this topic, the extent to which niche differentiation is the cause or consequence of sexual dimorphism in *R. hastatulus* is largely unresolved.

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## Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12753/suppinfo:

**Table S1.** Chemical components of the standard Hoagland and *Rumex hastatulus* nutrient solution system used in Experiment 3.