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Article in *Journal of Systematics and Evolution* · June 2023

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Research Article

Functional consequences of temporal reversal of height dimorphism for pollen and seed dispersal in a dioecious plant

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Received 6 December 2022; Accepted 25 April 2023;

Abstract The adaptive significance of phenotypic differences between females and males can provide insights into sex-specific selection and the evolution of sexual dimorphism. Dioecious plants commonly exhibit sexual dimorphism in height, although its ecological and evolutionary significance have rarely been examined experimentally. Here, we investigate the functional consequences of the temporal reversal of height dimorphism for pollen and seed dispersal in dioecious *Rumex hastatulus* Baldw., a species in which males are taller than females at flowering and the reverse pattern occurs at fruiting. Populations of this colonizing weed are wind-pollinated and seeds are wind-dispersed. In a glasshouse experiment we manipulated the height of pollen donors and using sex-specific genetic markers compared the paternal success of males of contrasting height and investigated whether seed families showed evidence of sexual dimorphism in early life-history traits. In a second glasshouse experiment using fruiting plants we also examined how female height influenced the distance that seeds were dispersed. We found that taller males had significantly higher siring success than males of equivalent height to flowering females. Similarly, taller females dispersed fruit to greater distances than shorter females. Female seeds were significantly heavier than male seeds and germinated more rapidly, although early seedling growth was greater in males. Our study suggests that the striking sex reversal of height in *R. hastatulus* likely functions to optimize the contrasting reproductive functions of the sexes by promoting increased pollen and seed dispersal distances. Improved dispersal quality could limit inbreeding and reduce local mate and resource competition within populations.

Key words: dioecy, plant height, pollen dispersal, *Rumex hastatulus*, seed dispersal, sexual dimorphism.

1 Introduction

In flowering plants, males and females of dioecious species often differ in traits other than their primary sex organs. This sexual dimorphism encompasses diverse features of vegetative, floral and inflorescence morphology, as well as several life-history traits (Lloyd & Webb, 1977; Geber et al., 1999; Barrett & Hough, 2013). Although sexual dimorphism in angiosperms is often less conspicuous than in most animals, traits of many species clearly exhibit sex differences, and

these have evolved multiple times convergently among distantly related dioecious lineages (Delph, 1999; Tonnabel et al., 2014; Welsford et al., 2016).

Sexual selection theory proposes that the evolution of sexual dimorphism occurs in response to sex-specific selection associated with intra- and intersexual competition for access to mates (Darwin, 1871; Andersson, 1994; Arnold, 1994), and a growing number of published works on the measurement and mechanisms of sexual selection in plants has accumulated in recent years (Skogsmyr &

Lankinen, 2002; Delph & Ashman, 2006; Moore & Pannell, 2011; Lankinen & Karlsson-Green, 2015; Tonnabel et al., 2021). Despite these advances, few experimental studies have directly investigated the reproductive consequences of sexual dimorphism, particularly for vegetative traits (but see Tonnabel et al., 2019, 2022), and most experimental studies of sexual dimorphism have investigated animal-pollinated species in which floral traits commonly serve to attract and reward pollinators and are often directly associated with reproductive success through male function (e.g., *Silene* L., Delph & Ashman, 2006; Delph & Herlihy, 2012; *Fragaria* L., Ashman, 2000; Ashman et al., 2000).

Wind-pollinated species can also display striking sexual dimorphism (e.g., *Mercurialis* L., Harris & Pannell, 2008; Tonnabel et al., 2019; *Rumex* L., Pickup & Barrett, 2012; Yu et al., 2022; *Leucadendron* R.Br., Welsford et al., 2016) but the functional significance of dimorphic traits is often less well understood. In particular, plant height is a key trait that often differs between the sexes of wind-pollinated species (Friedman & Barrett, 2009). In addition to directly influencing the strength of intra- and interspecific competition for light, plant stature may also affect aspects of pollen dispersal by wind, especially the distance that pollen grains travel from maternal parents. Pollen release from anthers located at greater heights should result in more extensive horizontal dispersion prior to settling from the airstream (Niklas, 1985) thus potentially increasing mating opportunities and mate diversity (Aljiboury & Friedman, 2022). Plant height should not only have direct effects on mating success by influencing pollen dispersal distances, it could also play an important role in the dispersal of fruits and seeds in species with wind-dispersed diaspores (Levin et al., 2003; Thomson et al., 2011). Thus, in species in which wind is the primary vector for both pollen and seeds, taller stature is likely to be beneficial. In dioecious species that are wind-pollinated and wind-dispersed this may require differences in the timing of height growth to optimize the contrasting reproductive functions of the sexes if height improves dispersal quality, although to our knowledge this has not been investigated.

Here, we evaluate the reproductive consequences of sexual dimorphism in plant height on the dispersal of male gametophytes (pollen) and diaspores (fruits and seeds) using the dioecious annual *Rumex hastatulus* Baldw. (heartwing sorrel). This species exhibits striking differences in height, with males significantly taller at the onset of flowering and females taller than males at fruiting (Pickup & Barrett, 2012; Puixeu et al., 2019; Yu et al., 2022). It has been hypothesized that this temporal change in height dimorphism during the annual life history may be adaptive in promoting more effective pollen and seed dispersal as this species is wind-pollinated and has wind-dispersed achenes (Pickup & Barrett, 2012; Puixeu et al., 2019). We addressed this hypothesis in glasshouse experiments by manipulating the height of both males and females. Also, by using sex-specific genetic markers and measuring paternity following pollen dispersal we also investigated whether sexual dimorphism was evident for several often-neglected early life-history stages in which the sexes cannot be distinguished without markers.

2 Material and Methods

2.1 Study system

Rumex hastatulus Baldw. (Polygonaceae) is a dioecious colonizing plant with uniovulate flowers and winged achenes comprised of two main chromosome races (Smith, 1963; Beaudry et al., 2020): the North Carolina karyotype (females = XX, $2n = 8$; males = XY₁Y₂, $2n = 9$) and the Texas karyotype (females = XX, males = XY, $2n = 10$). In the experiments described below, we used open-pollinated offspring of a population of the Texas race occurring at Rosebud (Texas, USA; latitude 31°7'3", longitude 96°51'37") sampled in May 2009 (see Pickup & Barrett, 2013 for further details of the population). In this population males are significantly taller than females at the onset of flowering whereas the reverse pattern occurs at fruiting (Yu et al., 2022). This feature of reverse sexual dimorphism in height is a general feature of populations of both karyotypes of the species (Pickup & Barrett, 2012; Puixeu et al., 2019).

2.2 Experiment 1: Influence of male height on pollen dispersal and siring success

To investigate the influence of differences in male height on pollen dispersal and siring success, we undertook a manipulative experiment in a glasshouse at Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences (Kunming, China). In March 2021, we soaked ~2000 seeds from five seed families in distilled water for 24 h at 4 °C to stimulate germination. The seeds were then placed on moist soil in a tray in a growth cabinet maintained at 25 °C for 12 h and 10 °C for 12 h with continuous light. At 14 days we randomly selected 1000 seedlings of a similar developmental stage (with four leaves), and these were transplanted individually into 22 cm diameter pots (volume 4395 cm³) containing Pro-Mix BX, General Horticulture, Inc., Red Hill, PA, USA (peat moss, vermiculate, and perlite). All pots were randomized by position on a single bench in a glasshouse maintained at temperatures ranging from 20 °C to 30 °C. One week after transplanting, we determined the sex of each plant using a molecular marker (RH11; GenBank accession MH388808) which gives a consistent sex-specific pattern differentiating females and males (Yu et al., 2022). Male and female plants were then separated to prevent pollination from occurring.

Prior to flowering, the plants were set out in the design illustrated in Fig. 1 with two males in the vertex of the triangular sector at ground level on soil and four radii of five females at increasing distances from the two pollen donors (radii = 1, 2, 4, and 6 m, respectively) also at ground level. One male was unmanipulated (hereafter tall or taller male) and the other (hereafter short or shorter male) was adjusted to the mean height of females in the experiment by adjusting the pot height in which it was growing by sinking the pot into the soil surface in the glasshouse. The two males were of similar size and produced equivalent numbers of flowers. The 20 female pollen recipients in the experiment were selected to all have similar flowering times and height. We conducted two consecutive replicates during May–June 2021 of the pollination experiment with identical designs using early blooming plants in the first experiment and later blooming plants in the second replicate. The height of the tall and short

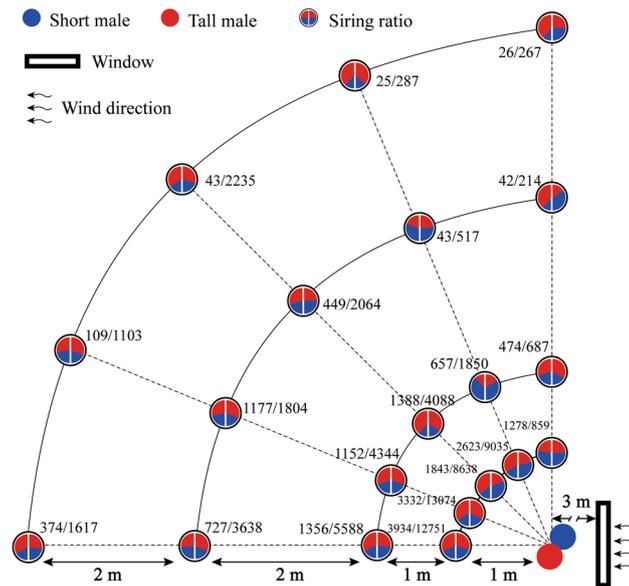


Fig. 1. Diagram of the pollen dispersal experiment in dioecious *Rumex hastatulus*. Blue and red circles represent short and tall male pollen donors, respectively. Bicolored circles represent 20 female pollen recipients. Pies inside circles represent siring ratios of tall male (red) versus short male (blue) in replicate 1 (left pie) and replicate 2 (right pie). Numbers beside circles indicate the total harvested fruit from each family in replicate 1 (left) and replicate 2 (right). Rectangle with arrows indicates the open window of the glasshouse through which wind was allowed to blow.

males in the first replicate were 40.6 and 34.5 cm, respectively, and 47.2 and 43.9 cm, respectively, in the second replicate. The average height of female plants in replicates 1 and 2 were mean $34.5 \text{ cm} \pm \text{SD } 3.4$ and $40.5 \text{ cm} \pm \text{SD } 3.5$. Plants in both replicates experienced unidirectional wind flow as windows on one side of the glasshouse were left open during the entire duration of each experiment (see Fig. 1). After each experiment, each of which lasted for 5 days, we transferred all females to an isolated glasshouse to avoid conspecific pollen contamination and these were maintained until fruits matured.

We randomly chose 45 fruits from each one of the 20 female parents in each replicate (900 seeds total) and the seeds were germinated on Petri dishes (diameter = 8.5 cm) with agarose culture medium (1% agarose) with 10 seeds in each Petri dish. Sixteen days after germination, fresh leaf tissue was collected from each of the seedlings and this was dried in silica gel. We extracted total genomic DNA from dried leaf tissue of seedlings and parents using a modified CTAB protocol (Doyle, 1991). Quantification of DNA was checked by electrophoresis on 0.8% agarose gels, and the concentration was determined using a SmartSpec Plus Spectrophotometer (Bio-Rad, Hercules, CA, USA).

We used three primer pairs (RH06, RH58, and RH95) to carry out PCR amplification using the following protocol: 20 μL total reaction volume containing 10 μL MasterMix (Tiangen Biotech, Beijing, China; including 3 mM MgCl_2 , 100 mM KCl, 0.5 mM of each dNTP, 20 mM Tris-HCl, pH 8.3, and 0.1 U Taq polymerase), 0.3 μL of each primer, 8.4 μL deionized water, and 50 ng genomic DNA. The genotype of tissue was identified under the following conditions: 95 °C for 4 min followed by 30 cycles at 95 °C for 30 s, at the annealing temperature for each primer for 30 s, and 72 °C for 30 s for

extension, and a final extension step at 72 °C for 10 min. We visualized amplicons using QIAxcel capillary gel electrophoresis (Qiagen, Valencia, CA, USA) and these scored with GeneMarker version 2.2 (Soft Genetics, State College, PA, USA).

We undertook paternity analysis on each seedling using CERVUS version 3.0.7 (Marshall et al., 1998), a program based on a maximum likelihood method to assign parentage to offspring (Meagher, 1986). CERVUS uses multilocus genotypes to calculate a likelihood ratio for each candidate parent-offspring pair, then estimates the difference in LOD scores between the most likely and the next most likely parent. We carried out a simulation of 10 000 cycles using allele frequencies observed in the parental and offspring genotypes, with female parent as the “known parent” and the two males in each experiment (tall and short male) as “candidate parents,” 1.0 as the sampled candidate male parent proportion because both males were screened, and 0.012 as the error rate derived from genotyping and/or mutation. Both strict and relaxed confidence intervals specified as 95% were recorded for each offspring.

2.3 Experiment 2: Influence of female height on fruit dispersal

To investigate the influence of female height on fruit dispersal, we traced the settling location of wind-dispersed fruits from tall and short females according to the design illustrated in Fig. 2A, which was replicated three times from June to July 2021. The female plants that were used in the experiment had nearly mature fruits that would have fallen from plants within a few days. We marked all nearly mature fruits on each plant using two contrasting colors of SugarMan natural pigment (Fuzhengdonghai Inc., Guangzhou, China) corresponding to

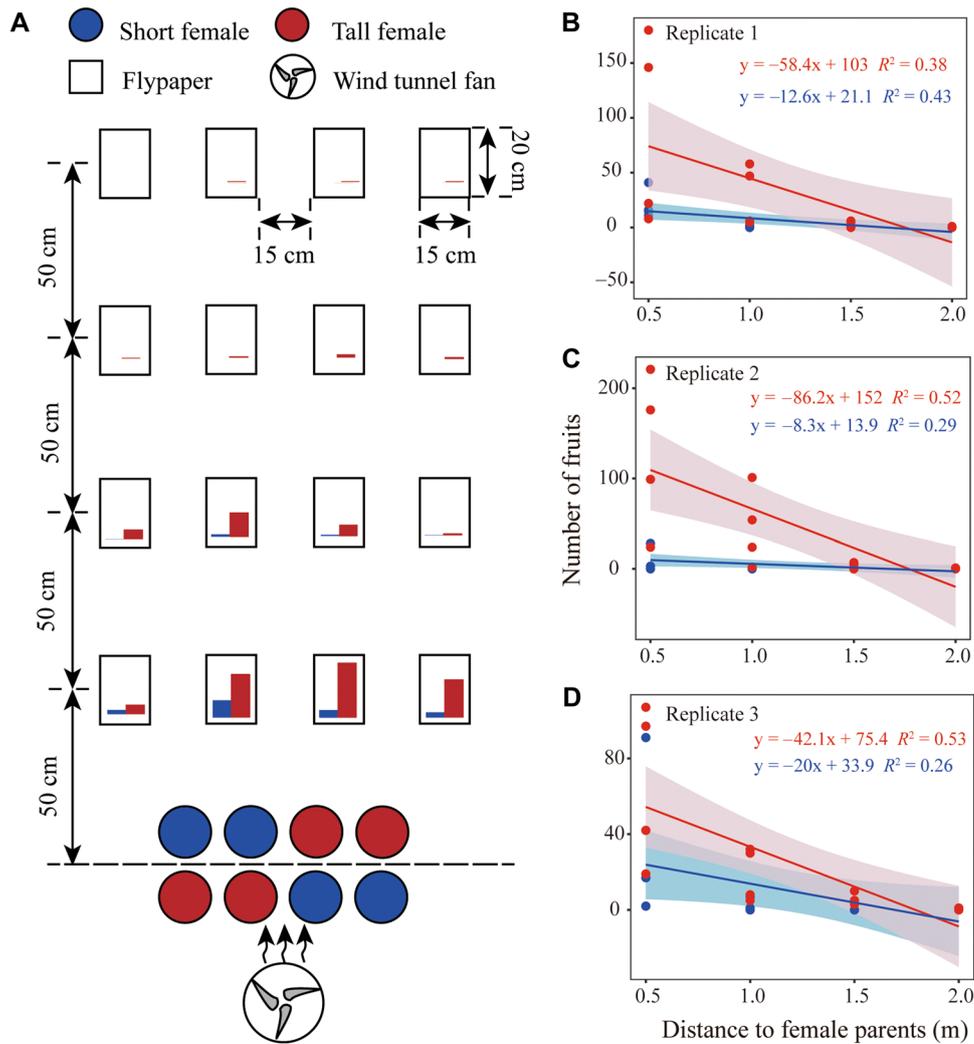


Fig. 2. **A**, Diagram of the fruit dispersal experiment in dioecious *Rumex hastatulus*. Blue and red circles represent short and tall females with fruit, respectively. Rectangles represent strips of flypaper that served to trap dispersing fruit. Red and blue bars within each rectangle indicate the relative frequency of trapped fruits (summed for the three replicates) from tall and short females, respectively. The position of the electrical fan and wind direction is also indicated. **B–D**, Relations between female height and fruit dispersal number in three replicate experiments. Lines and bands represent the linear tendency and 95% confidence intervals. Red and blue indicate tall and short females, respectively.

whether females were tall (red) or short (blue). The pigment was diluted 50 times with water and applied to fruits with a hand-held sprayer. In each replicate, eight individuals were arranged in two rows with two tall females (red fruit) and two short females (blue fruits) within each row (Fig. 2A). The tall females were not manipulated and represented the average height of females producing fruit. In contrast, the shorter females were adjusted, by sinking pots into the soil as in Experiment 1, to the average height of males that had stopped growing in height when females were maturing fruit.

To determine the dispersal distances of fruits from females of contrasting height, we arranged four sheets of sticky flypaper (15 cm x 20 cm) at four distances (0.5, 1.0, 1.5, and 2.0 m) from focal females. We used an electric fan to simulate natural wind, which in wild populations of *R. hastatulus* promotes fruit shedding and dispersal. The

fan remained on for 7 h/day during the 3-day duration of each replicate. At the completion of each experiment the sticky flypaper strips were photographed and the number of blue and red fruits counted using ImageJ version 1.4 (NIH, Bethesda, MD, USA).

2.4 Experiment 3: Sexual dimorphism in early life history stages

In addition to plant height, sexual dimorphism may be expressed at other stages of the life cycle and such differences may often be overlooked, especially in annuals during early phases of growth. To investigate the potential expression of sexual dimorphism in the early life history stages we sexed the seed progeny of females in Experiment 1 using the genetic marker and measured four traits: diaspore weight, seed weight, germination time, and the time of

expansion of the first true leaf. Sample sizes for each trait are provided in Fig. 3 and all offspring were germinated and grown from the 40 female families used in Experiment 1 in a single experiment with seedlings grown under glasshouse conditions.

2.5 Statistical analyses

To determine the effect of tall versus short plants on siring success and seed dispersal distance we used one-way ANOVA implemented in lmerTest package in R version 4.1.1 (R Core Team, 2021). Due to the relatively small number of females used as pollen recipients in Experiment 1 ($n = 20$ total and $n = 5$ at each distance class), we also used a bootstrap-based method (the permutation test) to predict differences in siring success between tall and short males. In these analyses, we applied the symmetry test on the paired data from tall-male sired and short-male sired offspring in each family by using the coin package in R version 4.1.1 (R Core Team, 2021). To investigate the influence of plant height on variation in seed set in Experiment 1 and fruit dispersal distance in Experiment 2, we performed simple linear regression analyses by using the lm function of the stats package in R version 4.1.1 (R Core Team, 2021). We used one-way ANOVA followed by post hoc Tukey tests to determine whether there were significant differences at early stages of the life history of *R. hastatulus*.

3 Results

3.1 Male height and siring success

We harvested 21 052 fruits (=seeds) with mean and SD of seeds per female of 1052.50 ± 1132.11 and 74 660 seeds with mean \pm SD of 3733.00 ± 4055.80 from replicates 1 and 2, respectively. There was a striking variation in the number of seeds produced per female both within and between replicates (Fig. 1). Females used in the second replicate produced on average more than three times more seeds than earlier blooming females. This difference resulted from their larger size and flower production compared to earlier flowering females in the first replicate.

The ANOVA results revealed that seed set in females decreased significantly with increasing distance from the two male pollen donors (replicate 1: $F = 10.011$, $P < 0.001$; replicate 2: $F = 8.189$, $P = 0.002$). The regression analyses indicated a significantly negative relation between the number of seeds and the distance to pollen sources in both replicates (replicate 1: $R = -435.81$, $P < 0.001$; replicate 2: $R = -1344.7$, $P = 0.002$; Figs. 4A, 4B).

CERVUS identified the male parent for 631 of 851 (74.15%) and 797 of 900 (88.56%) offspring in replicates 1 and 2, respectively. In both replicates, taller male parents exhibited significantly higher siring success than the shorter male parents (replicate 1: taller male parent offspring = 377, shorter male parent offspring = 254; $F = 7.937$, $P = 0.007$; replicate 2: taller male parent offspring = 434, shorter male parent offspring = 363; $F = 4.738$, $P = 0.035$; Figs. 4C, 4D). This biased siring success of taller males was also verified by the permutation test of symmetry based on the total number of families (replicate 1: $Z = 2.595$, $P = 0.009$; replicate 2: $Z = 2.079$, $P = 0.037$). However, this siring asymmetry was not consistently evident at each of the four distance classes.

For example, only for families at distance 1 m ($Z = 2.107$, $P = 0.035$) and 6 m ($Z = 2.166$, $P = 0.030$) in replicate 1 and 2 m ($Z = 2.079$, $P = 0.037$) and 6 m ($Z = 2.064$, $P = 0.038$) in replicate 2 did taller males exhibit significantly greater siring success compared with shorter males (Figs. 4E, 4F). Consistent with this heterogeneous distribution of siring superiority of taller males, there was no significant association between the ratio of offspring sired by taller male parents (seedlings of taller male parents/total seedlings) and the distance to pollen donors (replicate 1: $P = 0.387$; replicate 2: $P = 0.212$). Siring success was also not significantly affected by the height of female pollen recipients (replicate 1: $P = 0.371$; replicate 2: $P = 0.465$).

3.2 Female height and fruit dispersal distance

A total of 1567 red fruits and 284 blue fruits of *Rumex hastatulus* were recorded on the flypaper traps across the three replicates. Most fruits were trapped at the 1 m distance class (replicate 1: 97.72%; replicate 2: 97.93%; replicate 3: 95.06%) (Fig. 2A). Of the fruits ($n = 54$) that were dispersed for distances greater than 1 m, 96.29% ($n = 52$) originated from taller females (Fig. 2A). For each of the replicates, the trapped fruit number from taller females was significantly higher than from shorter females (replicate 1: 485 vs. 86, $G = 307.63$, $P < 0.001$; replicate 2: 717 vs. 56, $G = 669.77$, $P < 0.001$; replicate 3: 363 vs. 144, $G = 97.78$, $P < 0.001$) (Figs. 2B–2D). This pattern was evident for each distance class (Fig. 2A).

3.3 Sexual dimorphism in early life-history traits

Sixteen days after the germination of offspring from Experiment 1, we sampled leaf material and determined the sex of plants by using the sex-specific marker. In total, 92.56% (833/900) of the seedlings were successfully identified as either female or male. For all four early life traits that we compared there were significant differences between the sexes (Fig. 3). Diaspores and seeds of females were significantly heavier than those of males (diaspores: female = 0.662 ± 0.007 , male = 0.634 ± 0.007 , $F = 6.654$, $P = 0.010$; seeds: female = 0.320 ± 0.003 , male = 0.306 ± 0.004 , $F = 5.659$, $P = 0.017$). Mean seed weight was positively correlated with diaspore weight ($P < 0.001$). Female seeds germinated significantly earlier than male seeds and the expansion of their first true leaves was also earlier than in males (days to germination: female = 21.44 ± 0.819 , male = 29.87 ± 1.042 , $F = 41.21$, $P < 0.001$; days to expansion of first true leaf: female = 33.64 ± 0.561 , male = 38.58 ± 0.943 , $F = 22.68$, $P < 0.001$).

4 Discussion

We found that in our glasshouse experiments taller plants of *Rumex hastatulus* on average dispersed both pollen and seeds to further distances than shorter plants. This finding is consistent with the hypothesis that the reversal of height dimorphism from flowering to fruiting between the sexes of *R. hastatulus* could have an adaptive basis by improving dispersal quality, particularly in populations with high conspecific plant density. We now discuss the potential fitness consequences that might accrue from the reversal of

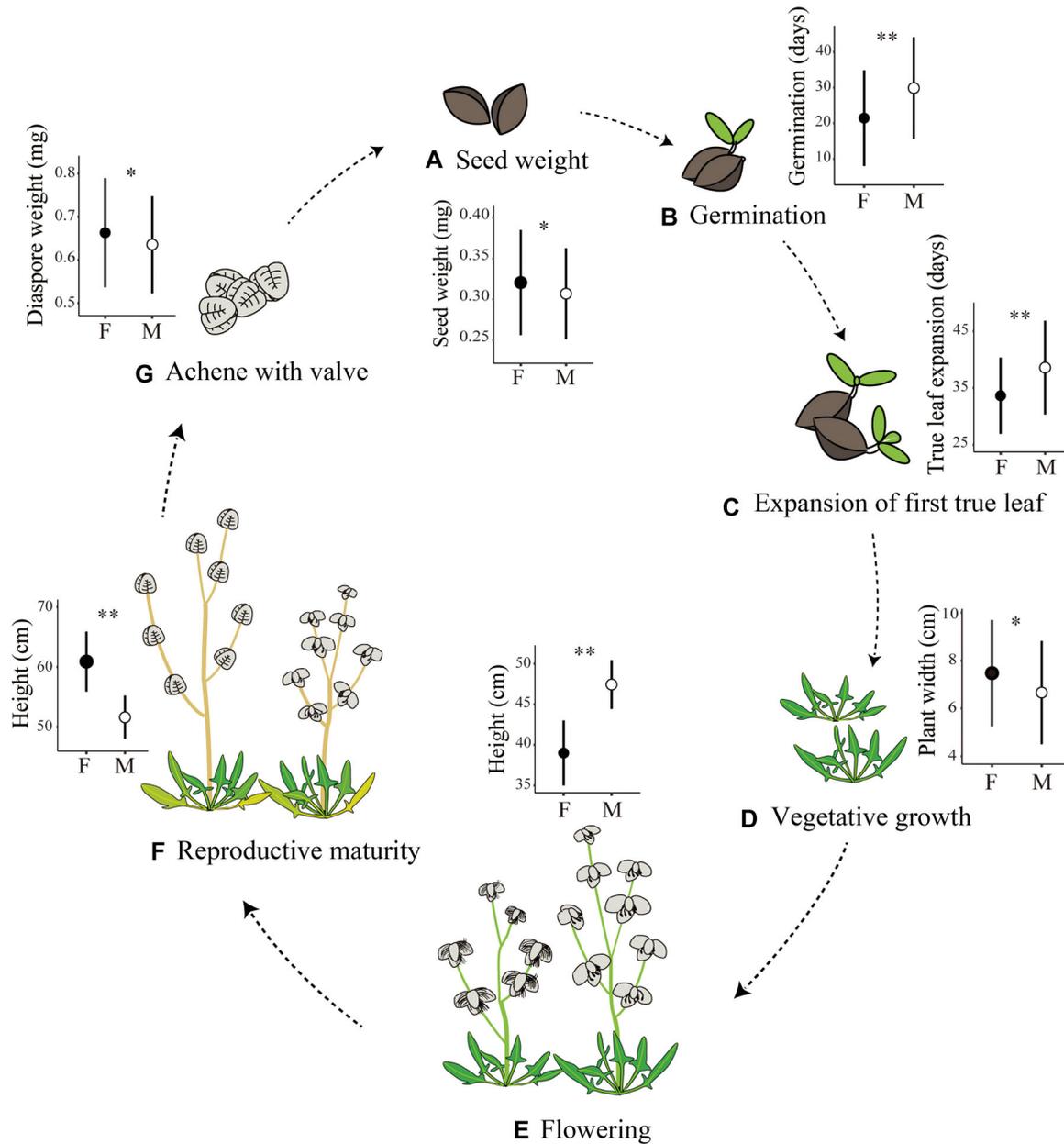


Fig. 3. Summary of sexual dimorphism in *Rumex hastatus* during its annual life history. **A**, Seed weight ($n = 833$). **B**, Germination day ($n = 691$). **C**, Time of expansion of first true leaf ($n = 359$). **D**, Greatest plant width during vegetative growth ($n = 317$). **E**, Height at start of flowering ($n = 126$). **F**, Height at reproductive maturity ($n = 126$). **G**, Weight of achene with valve ($n = 833$). F, female; M, male. Bars with dots indicate the mean and SD. Significant differences are indicated above bars by $*P < 0.05$ or $**P < 0.01$.

height dimorphism, evaluate the extent to which our controlled experiments under glasshouse conditions have relevance to pollen and seed dispersal in natural populations, and consider the challenges in disentangling cause and consequence in the evolution of sexually dimorphic traits.

4.1 Plant height, pollen dispersal, and siring success

Sexual selection that increases male mating success is considered to be a major selective force in the evolution of plant reproductive and vegetative traits (Lloyd, 1984; Delph & Ashman, 2006; Moore & Pannell, 2011). But direct

experimental evidence is surprisingly sparse, especially for wind-pollinated dioecious species (but see Tonnabel et al., 2019). It has frequently been argued that taller plants should have higher fitness gain through male function than shorter plants. Wind-dispersed pollen usually has a leptokurtic distribution from point sources (Bateman, 1947; Gleaves, 1973; Levin & Kerster, 1974), so that pollen dispersal distance likely increases when pollen is released from stamens at elevated heights. Pollen release from a greater height should result in longer dispersal distances as wind velocity and turbulent flow increases with elevation

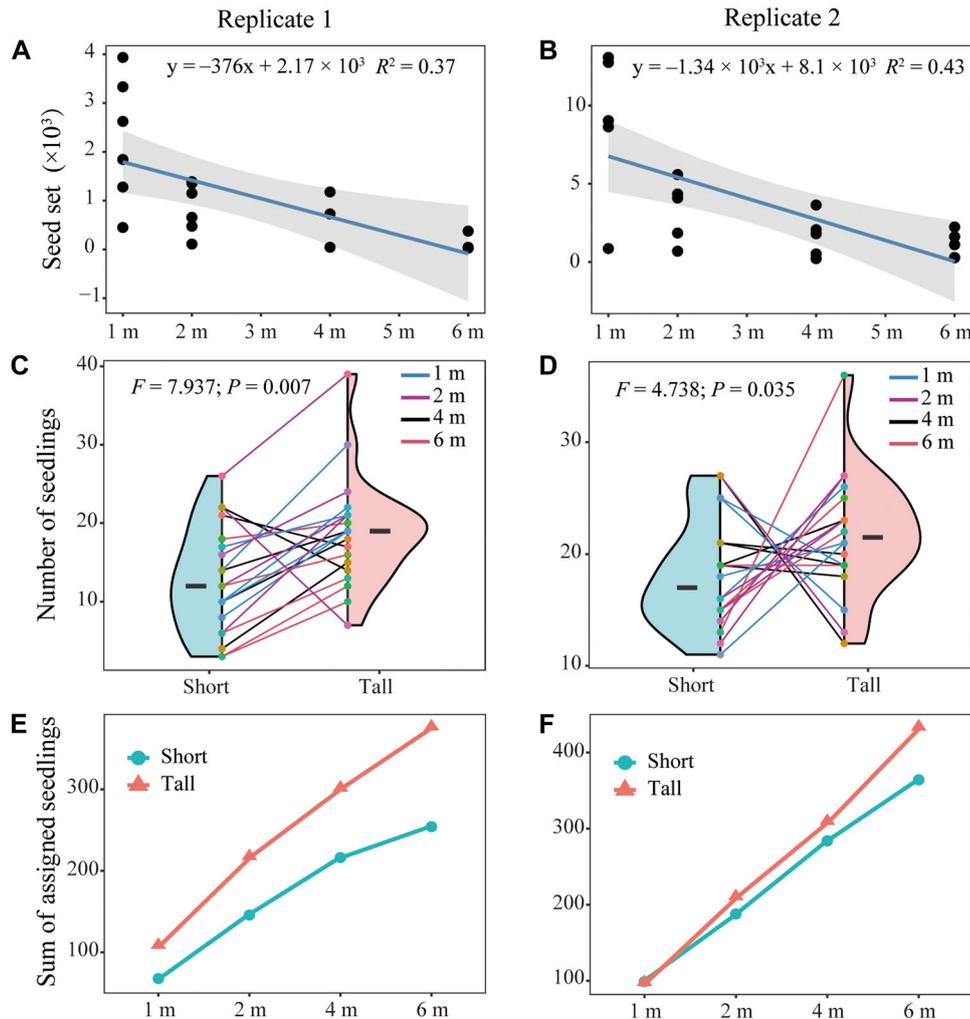


Fig. 4. Seed set and paternity assignment for each family of *Rumex hastatulus* in the two replicates of Experiment 1. **A, B,** Seed number per family and relation between seed number and the distance to pollen donors, respectively. Regression lines and bands represent linear tendencies and 95% confidence intervals. **C, D,** Split-violin plots of the assigned seedlings sired by short and tall males, respectively. Colored lines join siring by short and tall males for each family, with the same color indicating families from the same distance category. **E, F,** Illustration of the cumulative sum of seedlings sired by short and tall males in the two replicates, respectively.

(Niklas, 1985; Burd & Allen, 1988; Okubo & Levin, 1989; Klinkhamer et al., 1997; Sakai & Sakai, 2003; Friedman & Harder, 2005; Harder & Prusinkiewicz, 2013).

Studies of sex allocation in wind-pollinated species indicate that larger plants may disproportionately invest in male function. Such “budget effects” (larger resource pools in larger plants) may therefore also contribute toward greater siring ability (Klinkhamer et al., 1997; Nakahara et al., 2018; Tonnabel et al., 2019). Therefore, to determine whether male–male competition has selected for increased height it is important to distinguish the relative importance of the direct effects of height per se on siring ability versus the influence of higher male allocation to pollen, which could simply be a result of fecundity (natural) selection.

We found an effect of male plant height on pollen dispersal distance and siring success in our glasshouse experiment, although this varied in magnitude depending

on the distance of females from males between the two replicates (Fig. 1). Cases where there was a positive influence of height on siring success are unlikely to be explained by differences in budget effects associated with greater pollen production in taller plants because the pollen donors used in each replicate were chosen to be of similar size and flower production. The contrasting release height of pollen was not associated with differences in absolute size between “taller” and “shorter” plants but instead was achieved by the contrast in positioning of pots in the glasshouse soil bed. Nevertheless, under field conditions it seems probable that both budget and direct effects of height on siring success will occur because height in *R. hastatulus* is correlated with inflorescence size and flower production (Puixeu et al., 2019). In common with most annuals, *R. hastatulus* exhibits a high degree of phenotypic plasticity in height and flower production.

Why might greater pollen dispersal distances in taller rather than shorter plants improve fitness? The most obvious benefit of increased pollen dispersal distance is that it is likely to provide more access to ovules on conspecific plants, thus reducing local mate and resource competition among siblings and limiting local inbreeding (Lloyd, 1982; Klinkhamer et al., 1997). Also, by enlarging the effective mating neighborhood, greater pollen dispersal distances should increase mate diversity and the genetic heterogeneity of paternal offspring, which may aid in bet-hedging in variable environments (Simons, 2011; Barrett & Harder, 2017). Mating with multiple partners also has the benefit of increasing the likelihood of producing superior genotypes (Williams, 1975). Although empirical data linking pollen dispersal to mate diversity is sparse, experimental studies on wind-pollinated dioecious *Mercurialis annua* L. indicated that increased pollen dispersal distances allowed males to gain more mates and provided evidence for sexual selection among male plants (Tonnabel et al., 2019).

4.2 Advantages of height for seed dispersal distance

A variety of morphological, demographic, and atmospheric factors influence seed dispersal by wind; however, seed-release height has been shown both theoretically and empirically to be one of the most important determinants governing dispersal distance (Sheldon & Burrows, 1973; van Dorp et al., 1996; Travis et al., 2010; Skarpaas et al., 2011; Thomson et al., 2011; Zhu et al., 2016; Bullock et al., 2017; DiTommaso et al., 2018; Schupp et al., 2019; Mao et al., 2022). Our experimental results were generally consistent with these findings (Fig. 2), on average taller plants dispersed seeds further than shorter plants, although most seeds were dispersed within 1 m of the maternal parent, regardless of height.

Reports that taller plants disperse seeds further than shorter plants are more extensive than equivalent reports for pollen dispersal. Indeed, a positive relationship between plant height and mean dispersal distance occurs irrespective of dispersal syndrome (Thomson et al., 2011). This contrast between dispersal propagules probably reflects the fact that pollen grains are orders of magnitude lighter than most seeds. Because of their minute size, pollen grains are more likely than seeds to be influenced by updrafts, turbulence, and wind speed, atmospheric conditions that may tend to nullify height differences. These factors are known to influence seed dispersal distance in species with morphological adaptations such as samaras (e.g., *Acer* L.) and pappi (e.g., Asteraceae). The small wings on *R. hastatulus* achenes probably increase seed dispersal distances during strong wind gusts in natural populations but were probably of less importance under our experimental conditions.

There are several reasons why it may be beneficial for maternal parents to disperse seeds widely. These include the avoidance of seed and seedling enemies likely at higher density near other conspecifics (Howe & Smallwood, 1982; Comita et al., 2014), opportunities for long-distance colonization of new sites allowing bet-hedging in uncertain environments (Greenwood-Lee & Taylor, 2001; Nathan, 2006), and the reduction of local resource competition among siblings (Hamilton & May, 1977), which may be especially important in dioecious species due to the nature of their seed shadows compared with hermaphroditic populations

(Lloyd, 1982; Heilbut et al., 2001). *Rumex hastatulus* is a colonizing weed of open fields that often occurs in large dense monospecific populations (e.g., population size estimates: 32 populations > 1000, 24 > 10 000, $n = 46$; Pickup & Barrett, 2013). Under these demographic conditions, intraspecific competition is likely to be intense. Plant height could therefore play an important role in reducing local mate and resource competition at the pollen and seed dispersal stages of the life cycle.

4.3 Prereproductive sexual dimorphism

In flowering plants, sexual dimorphisms are most conspicuous once flowering has commenced and, as a consequence, there has been limited investigation of the extent to which they are apparent during the prereproductive phase of the life cycle (Lloyd & Webb, 1977; Barrett & Hough, 2013). For example, in an experimental study of the dynamics of growth and resource allocation in *R. hastatulus*, sexual dimorphism was not observed prior to flowering (Teitel et al., 2016). Using genetic markers to assay seed families from Experiment 1 we found evidence of sexual dimorphism at very early stages of the life history, including differences in seed weight, seed germination rate, and the timing of first true leaf emergence. Females had significantly heavier seeds that germinated and produced true leaves sooner than males (Fig. 3). Although female seedlings were wider in their growth pattern (Fig. 3), they were of reduced height compared to males for most of their prereproductive phase (data not shown), supporting a previous temporal study of the dynamics of prereproductive height growth in which measurements were taken every 2 days throughout the life cycle of *R. hastatulus* (see fig. 2 in Yu et al., 2022).

To our knowledge the only other reports that we are aware of involving significant differences between the sexes of dioecious species in seed weight involve *Spinacia oleracea* L., a species with environmental sex determination (Freeman & Vitale, 1985; Freeman et al., 1994), and *R. nivalis* Hegetschw (Stehlik & Barrett, 2005), in which markers were also used to identify sex. In both cases, male seeds were significantly heavier than female seeds, the reverse pattern to that observed in *R. hastatulus*. At this stage it is unclear whether the sex differences we report here in early life history stages occur more generally in *R. hastatulus* and are intrinsic potentially adaptive features of sexual dimorphism. This possibility would be worth investigating in a large sample of populations to determine whether sexually dimorphic seed provisioning by maternal parents occurs, as predicted by sex ratio theory for dioecious plants (de Jong & Klinkhamer, 2002).

5 Conclusions

We conclude by discussing several limitations of our glasshouse studies. First, our pollen dispersal experiment involved only two replicates. Although the results of both trials detected an advantage of height to siring success, it clearly would be important to replicate these results with larger sample sizes, especially of male plants. Second, our investigation was carried out under glasshouse conditions with plant height manipulated by pot position relative to the substrate, and simulated wind provided by either an electric

fan in the seed dispersal experiment, or by proximity to an open window in the glasshouse in the pollen dispersal experiment. In both circumstances the absence of strong wind gusts, updrafts, and turbulence, which characterize atmospheric conditions in natural populations, almost certainly meant that the dispersal dynamics of seeds, and especially pollen, were simplified in our experiments. Field-based studies of dispersal distances based on natural variation in height, although technically challenging, are therefore needed to confirm that the qualitative results we obtained also characterize natural populations in which plants of varying height will be growing intermixed with one another.

Finally, our findings are consistent with the hypothesis that the reversal of height dimorphism represents an adaptive strategy associated with the contrasting reproductive roles of females and males, as initially proposed by Pickup & Barrett (2013). But whether temporal changes in height dimorphism originally evolved directly by sex-specific selection to optimize siring success and promote more proficient seed dispersal is not possible to determine at this stage. The reversal of height dimorphism might have developed as a consequence of divergence between the sexes in growth rates, phenology, and resource allocation associated with the timing of their most costly activities. Rapid stem growth in male seedlings preflowering (see fig. 2 in Yu et al., 2022) and in females postflowering, and the concomitant earlier senescence of males, directly influence height dynamics during the annual life cycle of *R. hastatulus* (Conn, 1981; Pickup & Barrett, 2013; Teitel et al., 2016). Therefore, whether reversal of height dimorphism is a secondary consequence of costs of reproduction and/or is maintained in contemporary populations by selection because it is beneficial to fitness by increasing pollen and seed dispersal quality will require measurements of sex-specific selection on components of sexual dimorphism throughout the life history.

Acknowledgements

The authors thank David Timerman for advice on wind pollination. This research was supported by the Strategic Priority Research Program of the Chinese Academy of Sciences (XDB31000000) to De-Zhu Li, the National Natural Science Foundation of China (31971394) to Wei Zhou, the Key Basic Research Program of Yunnan Province (202201AS070057, 202101BC070003, and 202103AC100003) to Wei Zhou, and a Discovery Grant (RGPIN/06442-2017) from the Natural Sciences and Engineering Research Council of Canada to Spencer C.H. Barrett.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Wei Zhou, Spencer C.H. Barrett, and De-Zhu Li planned and designed the research. Qian Yu, Zhi-Hua Zeng, Qing-Hong

Feng, Xin-Jia Wang, Li Zhong, Hua-Ying Sun, and Hong Wang performed experiments. Wei Zhou, Qian Yu, and Zhi-Hua Zeng analyzed the data. Wei Zhou, Spencer C.H. Barrett, and Qian Yu wrote the article.

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