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Genetic potential for changes in breeding systems: Predicted and observed trait changes during artificial selection for male and female allocation in a gynodioecious species

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Abstract

Premise: Evolution of separate sexes from hermaphroditism often proceeds through gynodioecy, but genetic constraints on this process are poorly understood. Genetic (co-)variances and between-sex genetic correlations were used to predict evolutionary responses of multiple reproductive traits in a sexually dimorphic gynodioecious species, and predictions were compared with observed responses to artificial selection.

Methods: *Schiedea* (Caryophyllaceae) is an endemic Hawaiian lineage with hermaphroditic, gynodioecious, subdioecious, and dioecious species. We measured genetic parameters of *Schiedea salicaria* and used them to predict evolutionary responses of 18 traits in hermaphrodites and females in response to artificial selection for increased male (stamen) biomass in hermaphrodites or increased female (carpel, capsule) biomass in females. Observed responses over two generations were compared with predictions in replicate lines of treatments and controls.

Results: In only two generations, both stamen biomass in hermaphrodites and female biomass in females responded markedly to direct selection, supporting a key assumption of models for evolution of dioecy. Other biomass traits, pollen and ovule numbers, and inflorescence characters important in wind pollination evolved indirectly in response to selection on sex allocation. Responses generally followed predictions from multivariate selection models, with some responses unexpectedly large due to increased genetic correlations as selection proceeded.

Conclusions: Results illustrate the power of artificial selection and utility of multivariate selection models incorporating sex differences. They further indicate that pollen and ovule numbers and inflorescence architecture could evolve in response to selection on biomass allocation to male versus female function, producing complex changes in plant phenotype as separate sexes evolve.

KEYWORDS

artificial selection, between-sex correlation, Caryophyllaceae, dioecy, genetic correlation, inflorescence, *Schiedea*, sex allocation

Although hermaphroditism is the most common breeding system in flowering plants, separate sexes (dioecy) have evolved independently in many groups and occur in 5–6% of species (Renner and Ricklefs, 1995; Renner, 2014). This evolutionary change occurs most often through an intermediary of gynodioecy (females and hermaphrodites in a population) rather than androdioecy (Lloyd, 1974). Gynodioecy can evolve through invasion of a gene for male sterility, generating females, which can be followed by the spread of a modifier gene in hermaphrodites that increases pollen production at the expense of ovule production, thereby generating males (Charlesworth and Charlesworth, 1978; Charlesworth, 2013). For dioecy to evolve from gynodioecy by increased sexual specialization, heritable variation for allocation of resources to the two sexual functions must be present. Whereas genetic variance in allocation has been demonstrated in a number of species of plants and animals (O'Neil and Schmitt, 1993; Ågren and Schemske, 1995; Campbell, 1997; Wyman and Rowe, 2014), suggesting the potential for evolution, few studies have examined the actual evolutionary response to artificial or natural selection for increased maleness in hermaphrodites (or males) or increased femaleness in females.

Indeed, despite the long history of artificial selection in crop domestication (Meyers et al., 2012), this method has been used sparingly in investigation of the evolution of reproductive traits related to plant breeding systems (Meagher, 1994; Mazer et al., 1999; Delph et al., 2005). Artificial selection can be a particularly powerful approach to test whether evolution proceeds as predicted under constraints of the genetic variance–covariance matrix (Lande and Arnold, 1983; Falconer and MacKay, 1996; Lynch and Walsh, 1998) and to examine responses of correlated, but unselected, traits (Hill and Caballero, 1992). Among the traits that could correlate genetically with biomass allocation to male versus female function are biomass of other flower parts such as sepals or petals (Campbell, 1997) and number of flowers in an inflorescence (Ågren and Schemske, 1995). In addition, several hypotheses have been proposed for the evolution of dioecy, which would involve evolution of other traits along with primary sex allocation. For example, insect pollination is underrepresented among dioecious plants, and instead, wind pollination and other forms of abiotic pollination are common (Renner and Ricklefs, 1995; Renner, 2014). Wind pollination in turn involves changes in reproductive characters (Friedman and Barrett, 2009) such as more condensed inflorescences and more small pollen grains (Niklas, 1985). So, it is of interest to know whether other traits are genetically correlated with sex allocation traits and are constrained to evolve along with them.

We subjected sex allocation traits in gynodioecious *Schiedea salicaria* to artificial selection and examined both direct and indirect evolutionary responses. This species is a member of the endemic Hawaiian genus *Schiedea* (Caryophyllaceae), which consists of 35 species (Wagner et al., 2005, 2022). Species in this genus have a remarkable range of breeding systems, including not only hermaphroditism, but also different combinations of separate sexes, including gynodioecy, subdioecy, or dioecy in 10 species, leading the genus to be used as a model for understanding the evolution of breeding systems (Weller et al., 1998; Golonka et al., 2005; Sakai et al., 2006). Among hermaphroditic species, biotic pollination promotes outcrossing in several species occurring in mesic forest or shrubland (Weller et al., 2017; Powers et al., 2020), while autogamy is common in wet forests (Weller and Sakai, 2005). Almost all species with separate sexes are wind-pollinated and occur in dry habitats (Weller et al., 1998). Species with wind pollination produce larger quantities of more buoyant pollen than biotically pollinated species and have numerous flowers in condensed inflorescences held above the foliage, occasionally by elongated, subtending internodes. Gynodioecious species in the genus have been used to test models for the evolution of separate sexes. For both *S. salicaria*, with 12–13% females in populations (Sakai et al., 1989; Weller and Sakai, 2005), and *S. adamantis*, with 39% females (Sakai et al., 1997), expression of inbreeding depression in the offspring of hermaphrodites produced following self-pollination appears to favor the outcrossed progeny of females. For full dioecy to evolve from gynodioecy, hermaphrodites are predicted to allocate more resources to male function as females become more common in populations (Charlesworth and Charlesworth, 1978). As argued above, that transition requires genetic variation in sex allocation, which has been demonstrated in a prior study of *S. salicaria* (Sakai et al., 2008; Campbell et al., 2010). That earlier study established significant narrow-sense heritabilities for both per flower male biomass ($h^2 = 0.54$) and female biomass ($h^2 = 0.24$ to 0.64) with no significant genetic correlation between male and female biomass in hermaphrodites (Sakai et al., 2008). It did not, however, examine genetic correlations of sex allocation with inflorescence architecture, which was reported on separately (Weller et al., 2006), or with pollen and ovule numbers.

Here we first made quantitative predictions about evolutionary responses to selection from the genetic variance–covariance matrix and between sex genetic correlations, capitalizing on data obtained in earlier studies of *S. salicaria* (Weller et al., 2006; Sakai et al., 2008; Campbell et al., 2010). We then performed two generations of artificial selection on per flower male biomass in hermaphrodites or on female biomass in females and compared observed responses in a suite of traits to predicted responses. Since the development of methods for including between-sex genetic correlations in predictions of multivariate evolution (Lande, 1980), several studies have examined how multivariate responses to selection in random directions would in theory be influenced by between sex genetic correlations (reviewed by Sztepanacz and Houle, 2019; Cheng and Houle, 2020). It is rare, however, to compare such predicted responses with observed responses in multiple sexually dimorphic traits as was done in birds (Jensen et al., 2008), and we are unaware of previous studies that have done so for a multivariate set of traits in a sexually dimorphic plant.

We addressed the following questions. (1) Which traits are expected to respond to selection on per flower male

biomass in hermaphrodites and female biomass in females? We focused on the potential for response in biomass of individual flower parts, pollen and ovule number, and aspects of inflorescence architecture associated with wind pollination in this genus (inflorescence condensation and pedicel length). Predictions employed estimates of genetic variance and covariance, including between-sex genetic correlations, from re-analysis of earlier experiments in this species (Weller et al., 2006; Sakai et al., 2008). (2) How did per flower male biomass and female biomass respond to two generations of artificial selection for either greater male biomass or greater female biomass? (3) Did other traits show correlated responses to selection, evolving along with the selected traits for sexual specialization, and to what extent are these responses to selection predicted by estimates of genetic variance and covariances from the earlier independent experiments in this species?

MATERIALS AND METHODS

Study species

Schiedea salicaria Hillebrand is found on steep north-facing slopes in remnant, dry shrubland south of Waikapu on West Maui, Hawaii. Plants used in the selection experiment were derived from field-collected cuttings or from seeds collected from plants growing in a single population near the southernmost distribution of the species (Weller and Sakai 842; US). Although *S. salicaria* is now listed as Endangered (Federal Register, 2012), field collections were made before listing. Permission from landowners was obtained before visiting the population. An attempt was made to collect plants from throughout the population to avoid use of closely related plants in the crossing program, as some species of *Schiedea* show inbreeding depression (Sakai et al., 1997; Rankin et al., 2002).

Sex in *Schiedea* is controlled by two alleles at a nuclear locus; females are homozygous recessive (*hh*) and hermaphrodites are either *Hh* or *HH* (Weller and Sakai, 1991). Heterozygous hermaphrodites (*Hh*) were essential for the crossing program because they segregated both females and hermaphrodites in their progeny, which were needed to produce both sexes in subsequent generations of the selection experiment. Seeds from female plants in the field were used to establish plants in the greenhouse because all their hermaphroditic progeny were heterozygous and segregated females and hermaphrodites when crossed to females. Due to the relatively limited number of female (*hh*) plants in the field population (12%), additional females used in crosses were the progeny of seeds collected from some hermaphroditic plants in the field. Hermaphrodites were identified as *Hh* heterozygotes if they produced female offspring when crossed to a female. All plants were grown in the UC Irvine greenhouses in UC mix (University of California mix with 1:1:1 sand: peat: redwood fiber) with added perlite and watered as needed with dilute liquid fertilizer containing 20-20-20 NPK plus micronutrients (Grow More, Gardena, CA, USA).

Baseline generation

The experiment was initiated in summer 1999 by producing a baseline generation (hereafter referred to as "Baseline"), as described by Sakai et al. (2008). We used a partial diallel crossing design for investigation of narrow-sense heritability of traits associated with resource allocation. We aimed to produce 35 paternal half-sib families that contained both heterozygous hermaphrodites and female plants for the Baseline. A potential of 105 full sibships (35 paternal half sibships each with 3 different maternal plants) could have resulted from these crosses, but because some of these crosses failed to produce enough progeny, the Baseline consisted of 91 full sibships distributed across the 35 paternal half sibships.

Trait measurements

We measured several traits in the Baseline (during summer to fall of 2000) and in the later generations (see *Selection program* below). Some (but not all) of these traits were previously analyzed for the Baseline only (Sakai et al., 2008). In each generation, for each full sibship, flowers from each of five hermaphroditic and five female plants were measured for biomass traits on a per flower basis (dried at 60°C to constant mass and weighed to the nearest 0.01 mg). The biomass traits included carpel (technically, the ovary) biomass, stamen biomass (hermaphroditic flowers only), combined sepal and nectary biomass, and capsule biomass (fruit including the seeds). Capsules produced by two terminal and two lateral flowers were collected from each of two inflorescences per plant (Sakai et al., 2008) for three female and three hermaphroditic plants in each full sibship. For inflorescences used to determine capsule and seed biomass, we pollinated all flowers on the inflorescence. Viable seed biomass per capsule was also measured; seeds that were flat and did not roll (relative to normal plump seeds) and less than half the size of normal seeds were categorized as inviable, criteria similar to those used in other *Schiedea* species (Norman et al, 1995; Sakai et al., 1997). Stamen mass was not measured for females, as the vestigial stamens were too small to measure. *Schiedea* flowers have no petals. Traits were measured on two terminal (hereafter T) flowers (first flower to open on a lateral branch of the dichasial cyme) and two lateral (hereafter L) flowers (flowers opening on nodes below the terminal flower of each branch; Figure 1) on each of two inflorescences per plant. We measured both types of flowers because terminal flowers were larger than lateral flowers and differed substantially in ovule and pollen number per flower, and these and other differences may be important in evolutionary transitions from hermaphroditism to dioecy

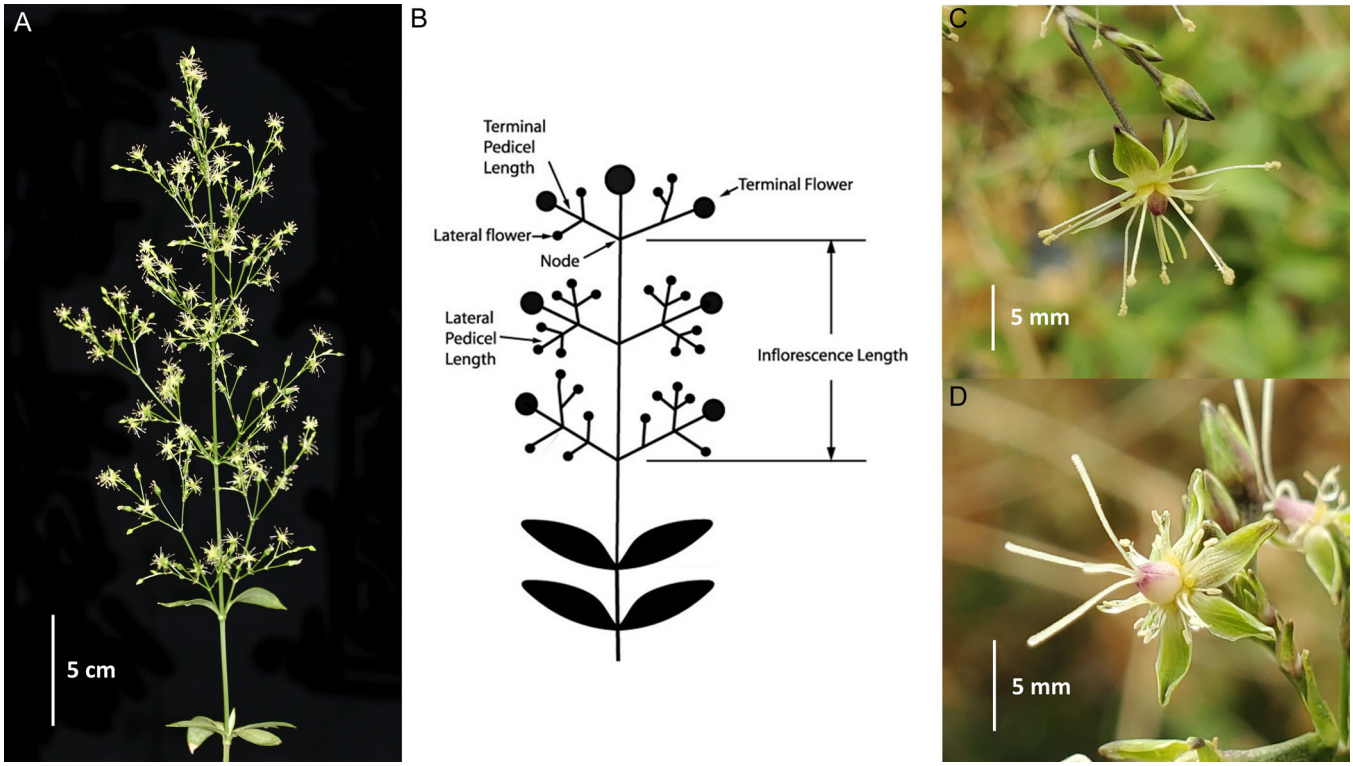

FIGURE 1 *Schiedea salicaria*. (A) Photograph of inflorescence. (B) Diagram of inflorescence illustrating the inflorescence traits included in the selection study. (C) Hermaphroditic flower showing dehisced anthers of a flower in a male stage, with three stigmas that have not yet expanded and become receptive. (D) Female (pistillate) flower showing expanded, receptive stigmas and vestigial stamens.

(Weller et al., 2006, 2007; Sakai et al., 2008). In the Baseline, 21% of the flowers on hermaphrodites and 18% of the flowers on females were terminal flowers. The biomass measures were used in calculating the traits used in selection: average male biomass in hermaphrodites and average female biomass in females. Average male biomass equaled stamen biomass per flower, weighted by the relative number of terminal and lateral flowers per inflorescence. Average female biomass equaled carpel + capsule biomass per flower, also weighted by the relative number of terminal and lateral flowers.

Additional traits that could be associated with correlated responses to selection were also measured including ovule number and pollen number per flower and inflorescence characters (Figure 1). Ovule and pollen numbers were determined on different flowers from the biomass measures, as they required destructive sampling of the carpel and stamens. Pollen number per flower was counted using an Elzone 280 PC Particle Counter for the Baseline and the first generation resulting from selection (hereafter "Gen 1"). In the second generation resulting from selection (Gen 2); see *Selection program* below), pollen number was obtained using hemacytometers instead. For that purpose, anthers were allowed to dehisce in Eppendorf tubes. The pollen was suspended in 400 μL of solution (10:10:12 lactic acid–glycerin–dH2O) and spun on a vortex mixer for 1 min. The sample was then loaded into the hemacytometer chambers, and the number of viable pollen grains in a known volume of the solution was counted using a Zeiss compound microscope at 100× in each of six wells of the hemacytometers. Finally, we measured traits associated with the inflorescence (inflorescence length, number of flowers per inflorescence, and terminal and lateral pedicel length; Figure 1). We calculated inflorescence condensation as number of flowers divided by inflorescence length because it influences wind pollination in *Schiedea* (Weller et al., 1998). The mean of each inflorescence trait on two inflorescences per plant was calculated, and the full sibship means were calculated based on plant means (see Weller et al., 2006; Sakai et al., 2008 for details). For inflorescence traits, all flowers were pollinated (saturating pollinations) on the inflorescences we measured to provide an even background of resource use for the inflorescence.

Selection program

We completed two generations of artificial selection based on either average male biomass in hermaphrodites or average female biomass in females (Figure 2). On the basis of Baseline measures of 1082 plants in 91 full sibships, six "selection" lines were established to produce the next generation (Gen 1), with two control lines (C1, C2), two high male lines (M1, M2) determined by the sibships with the highest mean male biomass in hermaphrodites, and two high female lines (F1, F2) determined by the sibships with

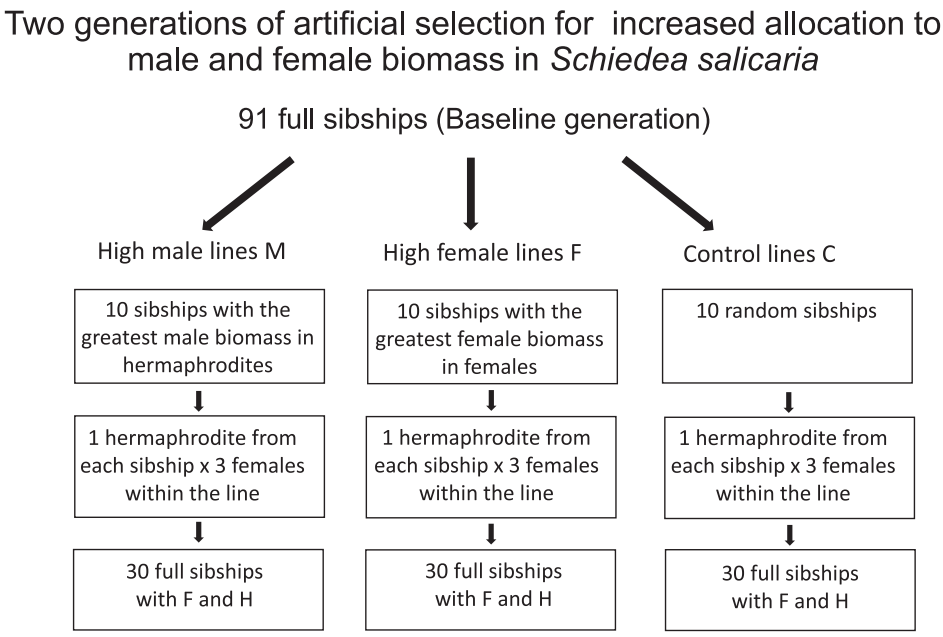

FIGURE 2 Selection program using *Schiedea salicaria* to measure genetic variances and covariances and between sex genetic correlations for biomass and traits associated with reproduction in a sexually dimorphic species. Full-sib families ($n = 91$) from partial diallel crosses were used to create three types of lines: greater male (stamen) biomass in hermaphrodites (High M), greater female (carpel+capsule) biomass in females (High F), or Control (C, no selection for biomass). Each type had two replicate lines. The High M lines were formed by selecting the top 10 full sibships (from the 91 sibships in the baseline generation) with the highest male biomass in hermaphrodites, with the exception that those sibships could not share a parental sibship (to reduce inbreeding). As a result, the 10 sibships selected for the High M lines were drawn from the top 12 sibships for male biomass. Similarly, for the High F lines, the 10 full sibships that had the highest allocation to female biomass were chosen from the top 13 sibships that did not share a parent sibship. Sibships for the Control lines were randomly chosen as long as they did not share a parental sibship. Replicate lines used the same sibships but not the same plants. Each selection line yielded 30 full sibships (180 full sibships for the six lines from Gen 1). At least 10 progeny (5 females and 5 hermaphrodites) were grown from each cross, yielding more than 1800 plants. The entire process was repeated to produce the second generation after selection (Gen 2), although no attempt was made to avoid selecting sibships with shared parents for crossing to form the second generation because of the smaller number of sibships available to choose from relative to the baseline generation.

the highest mean female biomass in female plants. For each of two control lines (C1, C2), 10 full sibships were selected randomly from the 91 full sibships in the Baseline, with the exception that none of the selected Baseline sibships within a line shared a parental sibship. To create the high male lines (M1, M2), we selected the top 10 of the 91 crosses (sibships) with the highest mean male biomass in hermaphrodites from the baseline generation. The high female lines (F1, F2) were based on the sibships with the highest mean female biomass. We made an effort to reduce inbreeding during selection. If one of the parents of a selected sibship overlapped with a parental sibship of a cross already involved in the high male line, then that selected sibship was skipped, and the sibship with the next highest mean male biomass was added until there were 10 sibships. As a result, the 10 selected sibships for high male lines were drawn from the top 12 sibships with the highest mean male biomass from among the 91 full sibships in the Baseline. A similar procedure was used to select the sibships for the high female lines (F1, F2), using females from the sibships with the highest mean female biomass in females. This procedure resulted in 10 selected sibships for the high female lines drawn from the top 13 full sibships for high female biomass from the 91 full sibships in the baseline generation. The two replicate lines in each case were generated from the same full sibships, but using different progeny within those sibships as parents. Within each line, a hermaphrodite from each sibship was crossed to females from three different full sibships to create 30 full sibships for each of the six lines. Pollinations to produce Gen 1 progeny were begun in summer 2002. Traits for Gen 1 were measured during 2003–2004, with plants chosen at random within each sibship for trait measurements. In total, there were 2093 plants in Gen 1, although not every trait was measured on every plant.

The second generation (Gen 2) was produced by identifying the 10 Gen 1 full sibships with the highest mean male biomass in hermaphroditic progeny within each of lines M1 and M2, and the 10 highest mean female biomass in females within each of lines F1 and F2. For the control lines, 10 of the 30 full sibships of Gen 1 in C1 and C2 were chosen at random to create Gen 2. Crosses were made between different full sibships, but no further attempts were made to reduce inbreeding, resulting in the top 10 full sibships being drawn from the 30 sibships in each of M1, M2, F1, and F2, and 10 of 30 full sibships used

for each control line. Seeds for Gen 2 were planted in spring 2006, and trait measurements for Gen 2 were made between 2007 and 2009. Total sample size in Gen 2 was 2168 plants, although not every trait was measured on every plant.

Statistical analyses

Question 1: Predicted responses to selection

To determine expected responses to the artificial selection we imposed, we first reanalyzed the Baseline data sets reported by Sakai et al. (2008) and Weller et al. (2006) along with new measurements of pollen and ovule numbers to estimate heritabilities of the selected traits and genetic correlations of those traits with other measured traits. Although those previous papers provided some of the information needed for predictions of evolutionary responses, they had not focused on the traits we actually selected on and did not analyze genetic correlations between those traits and the nonselected traits of pollen and ovule numbers and inflorescence characters. The narrow-sense heritabilities of average male biomass in hermaphrodites and average female biomass in females were estimated as four times the percentage variance component among paternal parents in analysis of variance with paternal parent and maternal parent as random effects (Falconer and MacKay, 1996). Additive genetic correlations with other traits were estimated by running that same model on all traits and extracting best linear unbiased predictors (BLUPs) for the paternal parents (Conner et al., 2003). Analyses were performed separately for the two sexes where possible. We then calculated Pearson correlations between BLUPs for each pair of traits. Analyses were performed with Proc Mixed in SAS ver. 9.3 (SAS Institute, Cary, NC, USA; see Weller et al. [2006] for further details).

We focused on genetic correlations between four sets of traits. First, we examined the genetic correlation between the two selected traits of average male biomass in hermaphrodites and average female biomass in females, to predict, for example, how average male biomass would respond to selection on high female biomass in high female lines. Second, we examined average biomass of the individual flower parts including carpel mass, capsule mass, sepal mass, and seed mass separately in terminal and lateral flowers. We did not include stamen mass in these genetic correlations because it is essentially the same as average male biomass, which is the weighted average of stamen mass across terminal and lateral flowers. Third, we examined pollen number and ovule number, as those are the currency for some theoretical models of sex allocation (Charlesworth and Charlesworth, 1978). Fourth, we examined genetic correlations with the inflorescence traits related to wind pollination, including inflorescence condensation, total flower number, and terminal and lateral pedicel lengths. Note that because we analyzed genetic correlations separately by sex, some of these correlations are within-sex genetic correlations and some are between-sex genetic correlations.

The response to selection for male mass in males (R_m) was predicted assuming family selection for the male parents and sib selection for the female parents. As described above, in high male lines, male parents were chosen from full-sib families based on the mean value for stamen mass across the family. As stamen mass is not expressed in females, female parents were chosen from families selected based on mean stamen mass in their hermaphroditic full-sib siblings. Following the general expression in Chapter 13 in *Introduction to Quantitative Genetics* (Falconer and MacKay, 1996) and averaging the response to selection through male and female function:

$$R_m = \frac{1}{2}\left(i\sigma_p h^2 \left[\frac{1 + (n-1)r}{\sqrt{n(1 + (n-1)t)}} \right] + i\sigma_p h^2 \left[\frac{nr}{\sqrt{n(1 + (n-1)t)}} \right] \right), \quad (1)$$

where i = intensity of selection, σ_p = phenotypic standard deviation of the trait, h^2 = heritability, $r = 0.5$ (correlation between full sibs), n = number of individuals measured in each full sib family, and t = full sib correlation for the trait. The parts in square brackets modify the expression for response to selection to take into account how the heritability of family means (modification in square brackets to left of plus sign) or heritability of sib means (modification in square brackets to right of plus sign) compares to heritability for individual values. The heritability of family means is r/t times as large as the heritability of individuals, and because selection is based on the observed sample mean for a family rather than the true mean, the variance of observed means is increased by $1/n$ times the within-family variance (see further derivation of the expression in Chapter 13 by Falconer and MacKay [1996]). We used $n = 3$, which approximates the number of plants measured per sex in each full-sib family in each generation. The range was 1–4 plants (mean = 2.8) for average male biomass and 2–5 plants (mean = 3.0) for average female biomass in females per full sib family in the baseline generation. In Gen 1, the intensity of selection, i, = 1.605 for family selection in male parents and 1.564 for sib selection in female parents, corresponding to selecting the top 12 or 13 of 91 full-sib families (linear interpolation from Appendix Table B of Falconer and MacKay [1996]). For the response in Gen 2, we selected 10 families out of 30, making $i = 1.061$. The value for t was determined from the full-sib family variance (i.e., the sum of the male and female parent variances) divided by the total variance (Falconer and MacKay, 1996). The overall response to selection was summed across the two generations. The response to selection on average female biomass in females was predicted in an analogous manner, except using family selection in female parents and sib selection in male parents

because families were selected solely based on average female biomass measured in females.

Indirect responses to selection in genetically correlated traits can be predicted through the concept of co-heritability, which acts similarly to heritability for direct selection. Co-heritability of traits x and y is given by:

$$\text{Co-heritability} = h_x h_y r_A, \tag{2}$$

where h_x is the square root of heritability for trait x and r_A is the additive genetic correlation between the two traits (Falconer and MacKay, 1996; Reeve and Fairbairn, 1996), which can be a within-sex or between-sex correlation. Following the method in Chapter 19 of Falconer and MacKay (1996), we substituted the co-heritability with average male biomass in males (or average female biomass in females) for heritability in Equation 1 to predict the response to selection for non-selected traits. For example, to predict the response for trait x expressed in females in the lines selected for high average male biomass (R_x),

$$R_x = \frac{1}{2}\left(i\sigma_p h_x h_m B_A \left[\frac{1 + (n-1)r}{\sqrt{n(1 + (n-1)t)}} \right] + i\sigma_p h_x h_m B_A \left[\frac{nr}{\sqrt{n(1 + (n-1)t)}} \right] \right), \tag{3}$$

where h_x = square root of heritability for trait x in females, h_m = square root of heritability for average male biomass in males, and B_A = between sex additive genetic correlation of trait x with average male biomass in males. Because the between sex correlation of trait i in males with trait j in females is not necessarily the same as the between sex correlation of trait i in females with trait j in males (Lande, 1980; Wyman et al., 2013), we made separate predictions for traits expressed in the two sexes. Separate calculations by sex allowed us to take full advantage of the knowledge of sex-specific genetic variances and covariances in this species (Sakai et al., 2008; Campbell et al., 2010).

Question 2: Direct responses to selection

To examine the direct response to selection on male biomass, we used ANOVA to compare values for full sib families among the two high male lines, two high female lines and two control lines, with a priori contrast of the average of the two high male lines versus average of the two control lines. Separate analyses were run on average male biomass in hermaphrodites for each generation of selection, using Proc GLM in SAS ver. 9.3. We ran similar analyses on average female biomass in females to examine the direct response to selection on female biomass, with a priori contrast of the average of the two high female lines versus average of the two control lines.

Question 3: Indirect responses to selection and comparisons with predicted responses

Indirect responses for other characters were tested by ANOVA models, with the crossed fixed effects of line and sex of the progeny. For simplicity, we report these results combined by sex of the progeny because it was rare to see differences in the response of the two sexes to selection (almost no interactions between sex and selection line; see Results). Pollen grains were counted only for hermaphrodites, so for terminal and lateral pollen number we used a one-way ANOVA with the fixed effect of line. As for the analyses of direct responses, we further divided the line effect into a priori contrasts. To compare actual with predicted responses, we analyzed responses separately by sex and first standardized them in units of SD of the trait. Then we plotted the actual response in Gen 2 (mean and SE across two selection lines) as a deviation from the control line mean against the predicted response (see Question 1) for the entire set of traits examined in both sexes.

RESULTS

Question 1: Predicted responses to selection

Both of the selected traits (average male biomass per flower and average female biomass per flower) had moderately high narrow-sense heritability in the Baseline ($h^2 = 0.54$ and 0.43, respectively; Table 1). Predicted responses to selection were 0.05 mg in Gen 1 and 0.10 mg summed over both generations for average male biomass (Table 1). For average female biomass, predicted direct responses to selection were 1.09 mg in Gen 1 and 1.62 mg over both generations (Table 1). These total predicted responses are equivalent to 1.48 and 1.14 standard deviations of the trait, for high male and high female selection, respectively. The two selected traits had a nonsignificant additive genetic correlation of –0.09 ($P = 0.60$). Thus, average male biomass in hermaphrodites was not predicted to respond positively to selection on average female biomass in females, nor vice versa.

Average male biomass in hermaphrodites showed evidence of genetic correlation only with sepal biomass and pollen number in hermaphrodites ($P < 0.05$ in Table 2). To balance the chance of type I and type II error in making predictions to selection, we consider values with $P < 0.05$ uncorrected for multiple comparisons as reason to expect a correlated response to selection in a trait with heritable variation. As expected, average female biomass in females showed positive genetic correlations with carpel mass (significant only in hermaphrodites), seed mass measured in females, and capsule and sepal mass in both sexes (Table 2). The genetic correlations of average female biomass with ovule number were weak or zero (r_A = 0.07–0.26, $P > 0.05$ in terminal and lateral flowers in both sexes). That result may reflect relatively low statistical power because, if the two traits (average female biomass and ovule

TABLE 1 Parameter estimates for predicting direct responses to selection on average male biomass in hermaphrodites and average female biomass in females.

	Parameter	Average male biomass (mg)	Average female biomass (mg)
Variance components from mixed model ANOVA	Male parent variance	0.00062*	0.17330
	Female parent variance	0.00067*	0.15480
	Residual variance	0.00334****	1.6675****
Calculated parameters	σ_p	0.068	1.413
	h^2	0.537	0.347
	t	0.279	0.164
	i in Baseline	1.632	1.632
	i in Gen 1	1.061	1.061
Predicted response	Predicted response Gen 1	0.060	1.086
	Predicted response Gen 2	0.0387	0.530
	Total predicted response	0.098	1.616

*$P < 0.05$; **$P < 0.01$; ****$P < 0.0001$.

TABLE 2 Genetic correlations (r_A) between selected traits (average male biomass in hermaphrodites and average female biomass in females) and other measured traits in hermaphrodites and females. Values are obtained from Pearson correlations between best linear unbiased predictors (BLUPs); 95% confidence intervals based on Fisher's z transformation are given in parentheses.

	r_A with average male biomass in hermaphrodites		r_A with average female biomass in females	
Trait	Measured in hermaphrodites	Measured in females	Measured in hermaphrodites	Measured in females
T carpel mass	0.027 (−0.31, 0.36)	−0.141 (−0.45, 0.20)	**0.341* (0.004, 0.60)**	0.165 (−0.18, 0.47)
L carpel mass	0.148 (−0.20, 0.46)	0.037 (−0.30, 0.37)	0.123 (−0.22, 0.44)	0.328 (−0.02, 0.59)
T capsule mass	0.044 (−0.29, 0.37)	−0.182 (−0.48, 0.16)	**0.339* (0.001, 0.60)**	**0.821**** (0.66, 0.90)**
L capsule mass	−0.008 (−0.34, 0.33)	−0.145 (−0.45, 0.20)	**0.340* (0.003, 0.60)**	**0.971**** (0.94, 0.99)**
T sepal mass	0.326 (−0.01, 0.59)	0.086 (−0.26, 0.41)	**0.403* (0.07, 0.65)**	0.282 (−0.06, 0.56)
L sepal mass	**0.373* (0.04, 0.62)**	0.246 (−0.10, 0.53)	0.152 (−0.19, 0.46)	**0.371* (0.04, 0.62)**
T seed mass	0.034 (−0.30, 0.36)	−0.201 (−0.50, 0.14)	0.279 (−0.06, 0.56)	**0.724**** (0.51, 0.85)**
L seed mass	−0.016 (−0.35, 0.32)	−0.055 (−0.38, 0.28)	0.272 (−0.07, 0.55)	**0.882**** (0.77, 0.93)**
T pollen N	**0.513** (0.21, 0.72)**	NA	−0.0004 (−0.33, 0.33)	NA
L pollen N	**0.595*** (0.32, 0.77)**	NA	−0.018 (−0.35, 0.32)	NA
T ovule N	−0.004 (−0.34, 0.33)	0.100 (−0.24, 0.41)	0.257 (−0.09, 0.54)	0.072 (−0.27, 0.39)
L ovule N	0.169 (−0.18, 0.47)	0.139 (−0.21, 0.45)	0.139 (−0.21, 0.45)	0.116 (−0.23, 0.43)
Infl. condensation	−0.180 (−0.48, 0.17)	−0.154 (−0.46, 0.19)	0.005 (−0.33, 0.34)	0.124 (−0.22, 0.44)
Total flower N	−0.265 (−0.55, 0.08)	−0.028 (−0.36, 0.31)	−0.061 (−0.39, 0.29)	0.077 (−0.26, 0.40)
T pedicel length	−0.038 (−0.37, 0.30)	−0.114 (−0.43, 0.23)	−0.049 (−0.31, 0.35)	0.066 (−0.27, 0.39)
L pedicel length	0.011 (−0.32, 0.35)	−0.065 (−0.39, 0.27)	0.023 (−0.31, 0.35)	−0.102 (−0.42, 0.24)

Notes: T = terminal flowers. L = lateral flowers. Bold font indicates values with uncorrected $P < 0.05$.*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

number) are both averaged across the two sexes of progeny, the genetic correlation is positive and statistically significant in terminal flowers ($r_A = 0.36$, $P < 0.05$). Traits contributing to inflorescence architecture showed weak or no genetic correlations with either selected trait (Table 2). Predicted responses to selection in these other, unselected, traits (see Table 3) ranged up to 1.14 SD of the trait over two generations, the value obtained for the predicted response of lateral capsule mass in females to selection on average female biomass in females (Appendix S1).

Question 2: direct responses to selection

In Gen 1, responses to selection were not yet evident for male biomass in the high male lines (Figure 3A); overall ANOVA, $P = 0.55$). The lines had, however, already diverged significantly for female biomass in females (Figure 3B; overall ANOVA, $F_{5,174} = 3.33$, $P = 0.0067$). By Gen 2, the direct responses to selection were highly consistent. The two high male lines had higher average male biomass (means = 0.42 and 0.43 mg) than the two control lines (means = 0.36 and 0.37 mg; a priori contrast, $F_{1,174} = 34.17$, $P < 0.0001$; Figure 3A). Interestingly, average male biomass in hermaphrodites of the two high female lines was intermediate and significantly different from both the two high male lines ($P = 0.0144$) and the control lines ($P = 0.0009$). Similarly, the two high female lines had higher average female biomass in females (means = 8.31 and 8.78 mg) than the two control lines (means = 6.85 and 6.85 mg; a priori contrast, $F_{1,174} = 65.61$, $P < 0.0001$; Figure 3B), with the two high male lines intermediate and significantly different from both the high female lines ($P < 0.0001$) and the control lines ($P < 0.0190$).

Question 3: Correlated trait responses and comparisons with predictions

The measured traits evolved along with the traits under direct selection (Figures 4–6). Significant responses by Gen 2 (Table 4) were seen not only in traits that exhibited strong genetic correlations with the selected traits (Table 2), but also the traits for which no genetic correlation had been detected ($P < 0.05$ for at least one comparison with the control lines for all traits in Table 4). The two control lines were generally similar to each other, supporting the absence of changes due to genetic drift, with the notable exception of terminal and lateral pedicel lengths (Figure 5). Both carpels and capsules became heavier in the lines selected for high female biomass (a priori contrast of female vs. control lines in two-way ANOVA with sex, all $P < 0.0001$; Figure 4A–D). Sepal mass was greater in lines selected for high male biomass and in lines selected for high female biomass (Figure 4E,F), as expected since that trait correlated genetically with both of the selected traits (significant values for $r_A = 0.37$ for lateral sepal mass measured in females with female biomass in females, 0.37 for lateral sepal mass measured in hermaphrodites with male biomass in hermaphrodites, and 0.40 for terminal sepal mass with female biomass in females, Table 2). Seed mass increased in the high female lines ($P < 0.0001$, Figure 5A,B), but surprisingly also in the high male lines ($P = 0.0313$) given the zero or negative genetic correlation with average male biomass (r_A ranged from –0.20 to 0.03 across terminal and lateral flowers measured in hermaphrodites and females). The proportion of flowers that were terminal rather than lateral changed little with two generations of selection. Plants in the high female lines had just a slightly higher proportion of terminal flowers than did plants in the high male lines (19% vs. 18% averaged over sexes; $P = 0.034$).

Pollen number in both terminal and lateral flowers was much higher in the high male selected lines compared to the control lines ($P < 0.0001$, Figure 6A,B). Surprisingly, terminal (but not lateral) pollen number also increased in the hermaphrodites in the high female selected lines (Table 4), but the response of this indirectly selected trait was rather different between the two lines, suggesting a possible effect of genetic drift (Figure 6A). Ovule number in both terminal and lateral flowers was much higher in the female selected lines compared to the control lines ($P < 0.0001$, Figure 6C,D), and interestingly in the male selected lines as well ($P < 0.0001$).

Inflorescence condensation evolved to lower values in the female selected lines ($P < 0.0001$, Figure 5C). That lower inflorescence condensation in the female selected lines reflected evolution of lower total flower number in those lines ($P < 0.0001$) but not in the male selected lines ($P = 0.7448$). Thus, there was a trade-off between evolution of fewer flowers but greater female biomass per flower in the female selected lines, but no change in flower number in the male selected lines. The pedicel lengths (in both terminal and lateral flowers) showed a contrasting pattern in which they became shorter in male selected lines (a priori contrast of two male lines with two control lines, $P = 0.0005$ and 0.0014), while not changing detectably in the two, quite divergent, female selected lines (a priori contrast, $P = 0.1797$ and 0.5716). Most indirectly selected traits exhibited no interaction between selection line and the sex of the progeny ($P > 0.05$ for all traits but one). The exception was terminal capsule biomass (interaction $P = 0.0203$), due to higher values for females in the high female selected lines, but higher values for hermaphrodites in the high male selected lines.

The lines selected for high male biomass and the lines selected for high female biomass diverged from one another in most traits except for carpel biomass and sepal biomass (Table 4). Pollen to ovule ratios diverged, with both pollen and ovule numbers changing to different extents between the high male and high female lines ($P < 0.05$ for all but lateral ovule number in Table 4). There were no detectable average differences in ovule number between hermaphrodites and females ($P = 0.1736$ and 0.0724 for terminal and lateral flowers), nor interactions between progeny sex and

TABLE 3 Estimates of parameters used in predicting indirect evolutionary responses of nonselected traits over two generations of selection. Phenotypic standard deviations (σ_P), heritabilities (h^2), genetic correlations (r_A) and co-heritabilities with the two selected traits are provided along with the predicted responses. Predicted responses are provided in units of the trait. Predicted response in units of SD are provided in Appendix S1. The selected traits are average male biomass in hermaphrodites and average female biomass in females.

Sex of progeny	Trait	σ_P	h^2	r_A with ave. male biomass	Co-herit. with average male biomass in males	t	Predicted response in high male lines	r_A with ave. female biomass in females	Co-herit. with average female biomass	Predicted response in high female lines
Hermaph.	Average male biomass (mg)	0.067	**0.537**	**1.000**	0.537	0.279	0.099	−0.091	−0.067	−0.011
	Average female biomass (mg)	1.402	**0.624**	0.033	0.019	0.229	0.077	0.389	0.202	0.716
	T carpel mass (mg)	0.150	**0.262**	0.027	0.010	0.159	0.005	**0.341**	0.115	0.051
	Lcarpel mass (mg)	0.097	0.192	0.148	0.048	0.160	0.014	0.123	0.035	0.010
	T capsule mass (mg)	1.787	0.280	0.047	0.018	0.124	0.101	**0.339**	0.118	0.647
	L capsule mass (mg)	1.435	**0.617**	−0.009	−0.005	0.182	−0.021	**0.340**	0.176	0.740
	T sepal mass (mg)	0.481	**0.276**	0.326	0.125	0.187	0.177	**0.403**	0.139	0.196
	L sepal mass (mg)	0.340	0.184	**0.373**	0.117	0.181	0.117	0.152	0.043	0.043
	T seed mass (mg)	1.295	0.056	0.034	0.006	0.075	0.024	0.279	0.043	0.180
	L seed mass (mg)	1.037	**0.512**	−0.016	−0.008	0.139	−0.026	0.272	0.128	0.403
	T pollen number (grains per flr)	3665.2	**0.363**	**0.513**	0.226	0.148	2512.07	0.000	0.000	−1.751
	L pollen number (grains per flr)	3381.55	**0.253**	**0.595**	0.219	0.155	2233.19	−0.018	−0.006	−60.377
	T ovule number	7.399	**0.551**	−0.004	−0.002	0.194	−0.051	0.257	0.125	2.702
	L ovule number	6.173	**0.460**	0.169	0.084	0.271	1.437	0.139	0.062	1.057
	Inflor. cond.	8.121	**0.535**	−0.180	−0.096	0.134	−2.391	0.005	0.002	0.060
	Total flower number	23.034	**0.654**	−0.265	−0.157	0.223	−10.369	−0.061	−0.032	−2.130
	T pedicel length (cm)	0.259	**0.789**	−0.038	−0.025	0.353	−0.017	−0.049	−0.029	−0.019
	L pedicel length (cm)	0.204	**0.472**	0.011	0.005	0.251	0.003	0.023	0.010	0.006
Female	Average male biomass (mg)	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Average female biomass (mg)	1.413	0.347	−0.091	−0.067	0.164	−0.282	**1.000**	0.433	1.616
	T carpel mass (mg)	0.157	**0.375**	−0.141	−0.063	0.175	−0.029	0.165	0.066	0.031

(Continues)

TABLE 3 (Continued)

Sex of progeny	Trait	σ_P	h^2	r_A with ave. male biomass	Co-herit. with average male biomass in males	t	Predicted response in high male lines	r_A with ave. female biomass in females	Co-herit. with average female biomass	Predicted response in high female lines
	L carpel mass (mg)	0.132	**0.374**	0.037	0.016	0.205	0.006	0.328	0.132	0.050
	T capsule mass (mg)	1.773	0.167	−0.183	−0.055	0.101	−0.305	**0.821**	0.221	1.226
	L capsule mass (mg)	1.395	0.354	−0.145	−0.063	0.152	−0.266	**0.971**	0.380	1.593
	T sepal mass (mg)	0.429	**0.235**	0.086	0.031	0.152	0.040	0.282	0.090	0.116
	L sepal mass (mg)	0.323	0.160	0.246	0.072	0.147	0.071	**0.371**	0.098	0.095
	T seed mass (mg)	1.358	0.268	−0.201	−0.076	0.070	−0.335	**0.724**	0.247	1.075
	L seed mass (mg)	1.006	0.286	−0.055	−0.022	0.072	−0.070	**0.882**	0.311	1.002
	T pollen number (grains per flr)	NA	NA	NA	NA	NA	NA	NA	NA	NA
	L pollen number (grains per flr)	NA	NA	NA	NA	NA	NA	NA	NA	NA
	T ovule number	7.516	**0.427**	0.100	0.048	0.236	1.026	0.072	0.031	0.657
	L ovule number	6.511	**0.549**	0.139	0.076	0.214	1.418	0.116	0.057	1.056
	Inflor. cond.	6.319	0.254	−0.154	−0.057	0.146	−1.088	0.124	0.041	0.784
	Total flower number	22.827	**0.553**	−0.028	−0.015	0.292	−0.943	0.077	0.038	2.342
	T pedicel length (cm)	0.236	0.109	−0.114	−0.028	0.143	−0.020	0.066	0.014	0.010
	L pedicel length (cm)	0.172	0.325	−0.065	−0.027	0.138	−0.014	−0.102	−0.038	−0.020

Notes: T = terminal flowers. L = lateral flowers. Inflor. cond. = inflorescence condensation. Bold font for heritabilities indicates $P < 0.05$ for paternal variance component. Bold font for genetic correlations indicates $P < 0.05$ for Pearson correlation between best linear unbiased predictors (BLUPs).

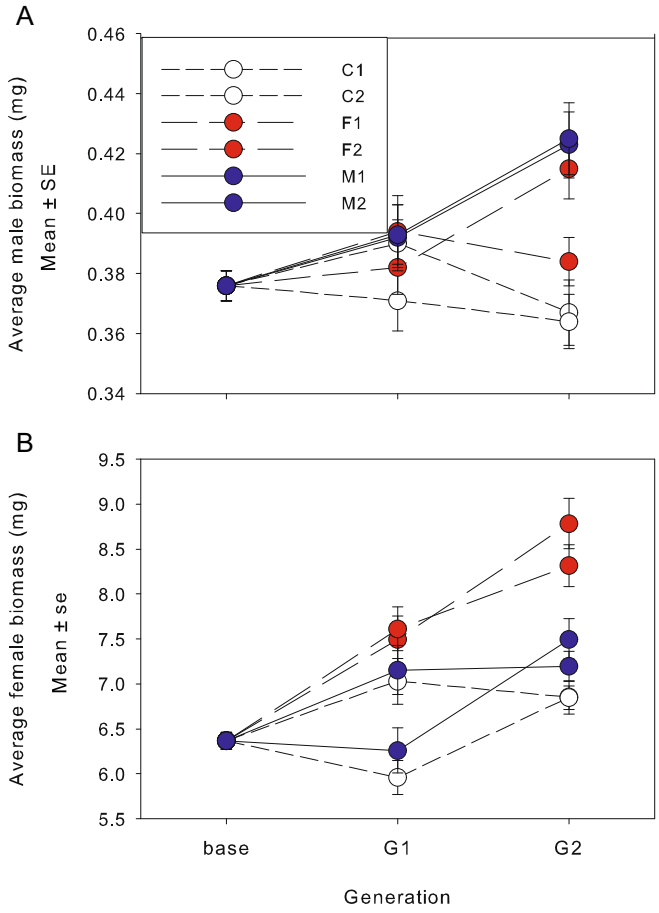

FIGURE 3 Responses to selection over two generations for (A) average male biomass in hermaphrodites and (B) average female biomass in females. Means and standard errors across full-sib family averages are shown for two control lines, two high female lines, and two high male lines.

selection line ($P = 0.7967$ and 0.6040), so differences in ovule number among the six selection lines were similar whether based on measurements in female progeny or hermaphrodites. The ratio of mean pollen number to mean ovule number in hermaphrodites equaled 334 to 344 in the high male lines compared to 291 to 298 in the high female lines, for lateral and terminal flowers, respectively. The high female lines had heavier capsules and seeds, fewer flowers giving rise to less-condensed inflorescences, and longer pedicels than the high male lines (Figures 4 and 5). Thus, we observed divergence in secondary traits along with sex allocation.

Actual responses to selection were predicted moderately well by heritabilities and genetic correlations in the Baseline (Figure 7; Appendix S1). Deviations from predictions were mostly in the direction of larger responses than expected (i.e., a preponderance of points above the 1:1 line in Figure 7), reflecting general increases in size of the flowers. Predictions appeared better in hermaphrodites in the lines selected for high male biomass and in females in the lines selected for high female biomass (Figure 7A, D) than in cases that involved assessing responses in one sex to selection on the other sex, which depended upon the values of between-sex genetic correlations (Figure 7B,C). The direct response to selection on average female biomass was 1.20 SD (SE = 0.166, red circle in Figure 7B), compared to the predicted value of 1.14 SD. In contrast, the direct response to selection on average male biomass was 0.88 SD (SE = 0.016, blue circle in Figure 7A), compared to the predicted value of 1.48 SD. The two selected traits had a nonsignificant additive genetic correlation of −0.09 ($P = 0.60$; Table 2). Thus, they were not predicted to respond positively to selection on the other trait, as nevertheless occurred, making them deviate from the 1:1 line (Figure 7). Carpel biomass, capsule biomass, and sepal biomass all increased in lines selected for high male biomass, even though predicted responses had been small or negative (upward triangles in Figure 7; Appendix S1), also leading to points above the 1:1 line in Figure 7A, C. Pollen number responded nearly as expected in the high male lines, but as noted above unexpectedly increased in the high female lines as well, despite the tendency (nonsignificant) toward a negative genetic correlation with average female biomass (Table 2). Ovule number increased more than expected in both kinds of selection lines, but especially so in the high male lines (Figure 7A). In lines selected for high female biomass, the traits with the responses deviating most from the predicted values included inflorescence condensation and flower number, both of which showed strongly negative responses (points below the 1:1 line in Figure 7B) despite being genetically uncorrelated with the selected trait in the Baseline ($r_A = 0.005$ and −0.06 in hermaphrodites and 0.12 and 0.08 in females, all $P > 0.05$).

DISCUSSION

Test of a fundamental assumption of models for the evolution of dioecy

A fundamental assumption of models for the evolution of dioecy from gynodioecy is that genetic variation in sex allocation allows hermaphrodites to evolve to become more male-like and females to evolve to become more female-like. In a rare test involving artificial selection on either male biomass per flower or female biomass per flower in gynodioecious *Schiedea salicaria*, this was the pattern observed. First, genetic variation in these traits was documented (Question 1), confirming earlier studies (Sakai et al., 2008). Then, in only two generations, per-flower stamen biomass in hermaphrodites responded to artificial selection with an increase of 0.88 SD and per-flower female biomass in females with an increase of 1.20 SD, showing direct responses to selection (Question 2). Pollen and ovule numbers evolved indirectly, with no change in pollen size (unpublished data). Although pollen and ovule numbers both increased in response to both types of selection, pollen number increased more in lines selected for high male

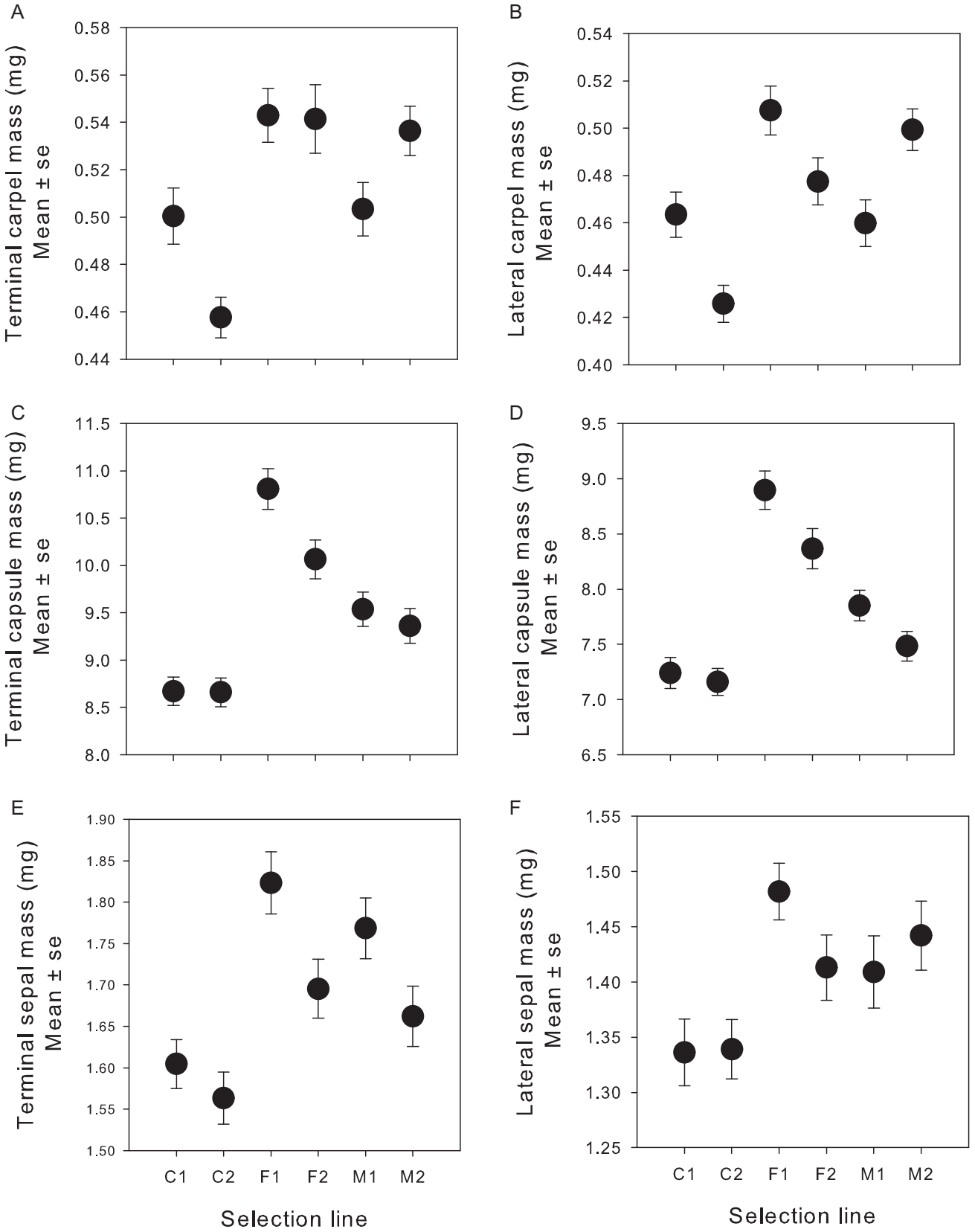

FIGURE 4 Mean trait values for carpel mass, capsule mass, and sepal mass after two generations of selection. Selection lines included two control lines, two lines selected for high average female biomass in females (F1, F2), and two lines selected for high average male biomass in hermaphrodites (M1, M2). Values plotted are means and standard errors across 60 full sib by progeny sex combinations.

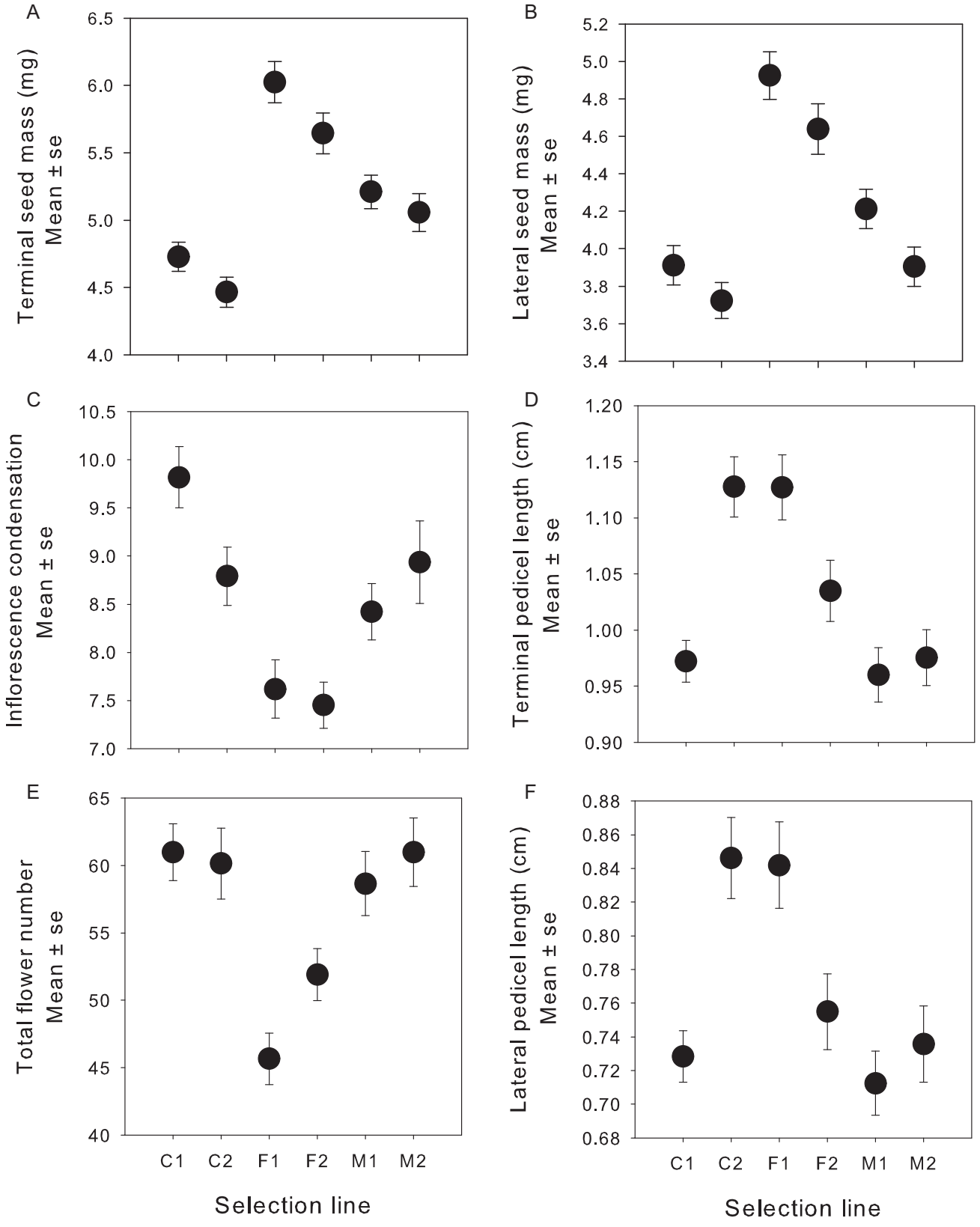

FIGURE 5 Mean trait values for seed mass and inflorescence traits after two generations of selection. Selection lines included two control lines, two lines selected for high average female biomass in females (F1, F2), and two lines selected for high average male biomass in hermaphrodites (M1, M2). Values plotted are means and standard errors across 60 full sib by progeny sex combinations.

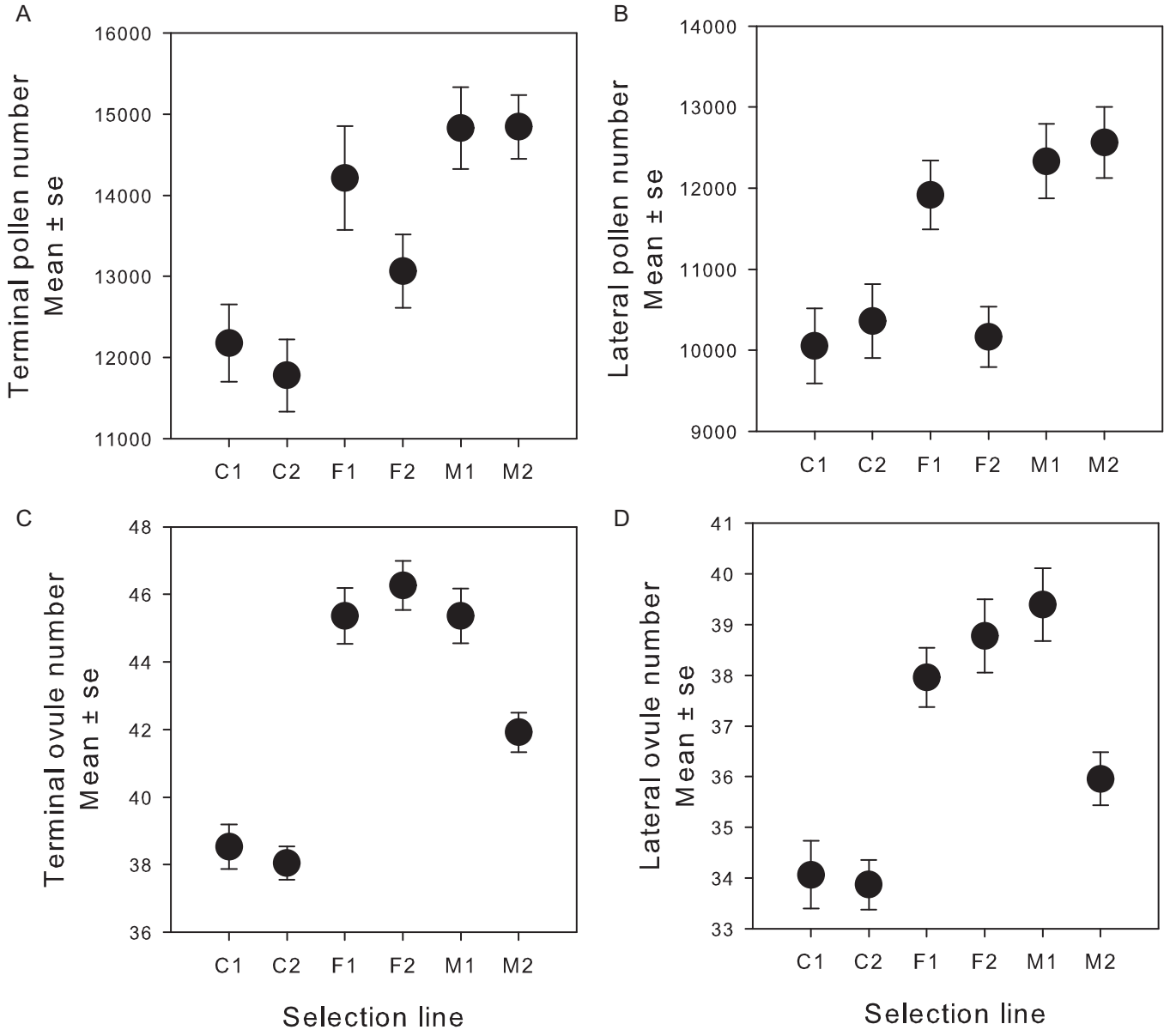

FIGURE 6 Mean trait values for pollen number per flower and ovule number per flower after two generations of selection. Selection lines included two control lines, two lines selected for high average female biomass in females (F1, F2), and two lines selected for high average male biomass in hermaphrodites (M1, M2). (A, B) Values plotted are means and standard errors across 30 full-sib combinations. (C, D) Mean and standard errors across 60 full sib by progeny sex combinations. High female lines (F1, F2) differed significantly from the control lines (C1, C2), except for lateral pollen number (panel B). High male lines (M1, M2) differed significantly from the control lines in all cases. High male lines differed significantly from the high female lines, except for lateral ovule number (panel D). Statistical details are in Table 4.

biomass and ovule number increased more in lines selected for high female biomass, generating a 15% higher pollen to ovule ratio in hermaphrodites in the high male lines compared to in the high female lines (15.47% in terminal flowers and 14.62% in lateral flowers). Similar changes in anther production and ovule number were seen in a study that selected directly on those aspects of gamete production in *Spergulina marina* (Mazer et al., 1999). Whereas that earlier study was important to establishing the assumption of genetic variation in male and female gamete production, our study demonstrates evolution of allocation of resources to the two sexual functions in terms of the common currency of biomass that is used in theoretical models for sex allocation (Charnov, 1982; Lloyd, 1984). That common currency is critical because it allows comparisons of genetic variance in male and female function, and the genetic correlation between them, using a common measurement scale (Sakai et al., 2013). Based on comparisons with observed measurements of evolution in units of standard deviation, we observed very rapid evolution of male and female biomass (Gingerich, 2009). This evolution had been predicted by prior measurements of genetic variances,

TABLE 4 Statistical tests of responses to selection over two generations. Entries are *P* values from a priori contrasts. For directly selected traits (average male biomass and average female biomass), the model included the fixed factor of selection line. For the other traits, the model also included sex of the progeny as a crossed fixed factor.

Trait	High female vs. control lines	High male vs. control lines	High female vs. high male lines
Average male biomass in males	**0.0009**	**<0.0001**	0.0144
Average female biomass in females	**<0.0001**	0.0190	**<0.0001**
T carpel mass	**<0.0001**	**0.0002**	0.0378
L carpel mass	**<0.0001**	**0.0001**	0.1519
T capsule mass	**<0.0001**	**<0.0001**	**<0.0001**
L capsule mass	**<0.0001**	**0.0021**	**<0.0001**
T sepal mass	**<0.0001**	**<0.0001**	0.1289
L sepal mass	**<0.0001**	**0.0011**	0.4099
T seed mass	**<0.0001**	**<0.0001**	**<0.0001**
L seed mass	**<0.0001**	0.0313	**<0.0001**
T pollen N	**<0.0001**	**<0.0001**	**0.0009**
L pollen N	0.0511	**<0.0001**	**0.0002**
T ovule N	**<0.0001**	**<0.0001**	**0.0001**
L ovule N	**<0.0001**	**<0.0001**	0.1376
Inflorescence condensation	**<0.0001**	0.0491	**0.0004**
Total flower number	**<0.0001**	0.7448	**<0.0001**
T pedicel length	0.1797	**0.0005**	**<0.0001**
L pedicel length	0.5716	**0.0014**	**0.0002**

Notes: T = terminal flowers. L = lateral flowers. Bold font indicates cases that are statistically significant ($P < 0.05$) after sequential Bonferroni correction for testing 18 traits.

illustrating the strong power of quantitative genetic theory to predict evolution.

Predicted versus observed responses to selection for sex allocation

We also used within-sex and between-sex genetic correlations to investigate the potential for other traits to be dragged along during evolution of sex allocation. Our study thus provides highly unusual information comparing predicted and observed responses for a multivariate set of traits showing sexual dimorphism (Question 3), with most previous studies predicting observed responses in dimorphic traits limited to one or a small number of traits, as seen in insects (Reeve and Fairbairn, 1996; Fedorka et al., 2007). Although in general larger evolutionary responses were associated with larger predicted responses according to the model, not every trait changed as predicted from point estimates of genetic correlations in the Baseline prior to selection. All biomass traits increased in both lines selected for high male function and lines selected for female function, even though only stamen biomass and sepal biomass had been predicted to respond in the former type of selection. Of particular note is that some aspects of female biomass responded positively to selection for high male function, and stamen biomass responded positively to selection for high female function. That result was not expected, as positive genetic correlations between such traits had not been detected in the Baseline. That result from artificial selection is contrary to the assumption of a genetically based trade-off between investment in male versus female function that is assumed by sex allocation models (Charlesworth and Charlesworth, 1981; Charnov, 1982). Tests with other plant species, most of which used breeding experiments rather than artificial selection, have yielded a variety of results including negative genetic correlation, positive genetic correlation, and no detectable correlation (reviews by Campbell, 2000; Ashman, 2003; Mazer et al., 2007), with some evidence that correlations are more positive in highly selfing species (Mazer et al., 2007). The concordant changes in male and female biomass we observed could have resulted from genetic variation in resource acquisition ability that correlates with overall flower size, including masses of both male and female reproductive parts (De Jong and Van Noordwijk, 1992). Although it is difficult to assess resource acquisition, an earlier study did examine genetic variation in some physiological traits of *S. salicaria* and found genetic correlations of inflorescence number with both stem number and low specific leaf area (thick leaves) (Culley et al., 2006), suggesting the possibility that several resource-related traits vary in tandem.

Potential causes of differences between predicted and observed responses

Discrepancies between the predicted and observed responses could in principle be due to changes in genetic parameters during selection, uncertainty in estimates of these parameters in the baseline generation, or genetic drift. Although changes in the **G** matrix are considered unlikely to appear in only a few generations (Pélabon et al., 2021), we examined that possibility by using a mixed model with fixed effect of the treatment (control vs. high male vs. high female selection) and random effects of paternal parent and maternal parent, separately in each generation. The point estimate of heritability for average male biomass in hermaphrodites changed from 0.54 in the Baseline to 1.23 in Gen 1 and 0.64 in Gen 2. With the higher heritability in Gen 1, responses to selection on that trait could be higher by Gen 2, which could explain some points above the 1:1 observed to predicted line in Figure 5. Heritability for average female biomass in

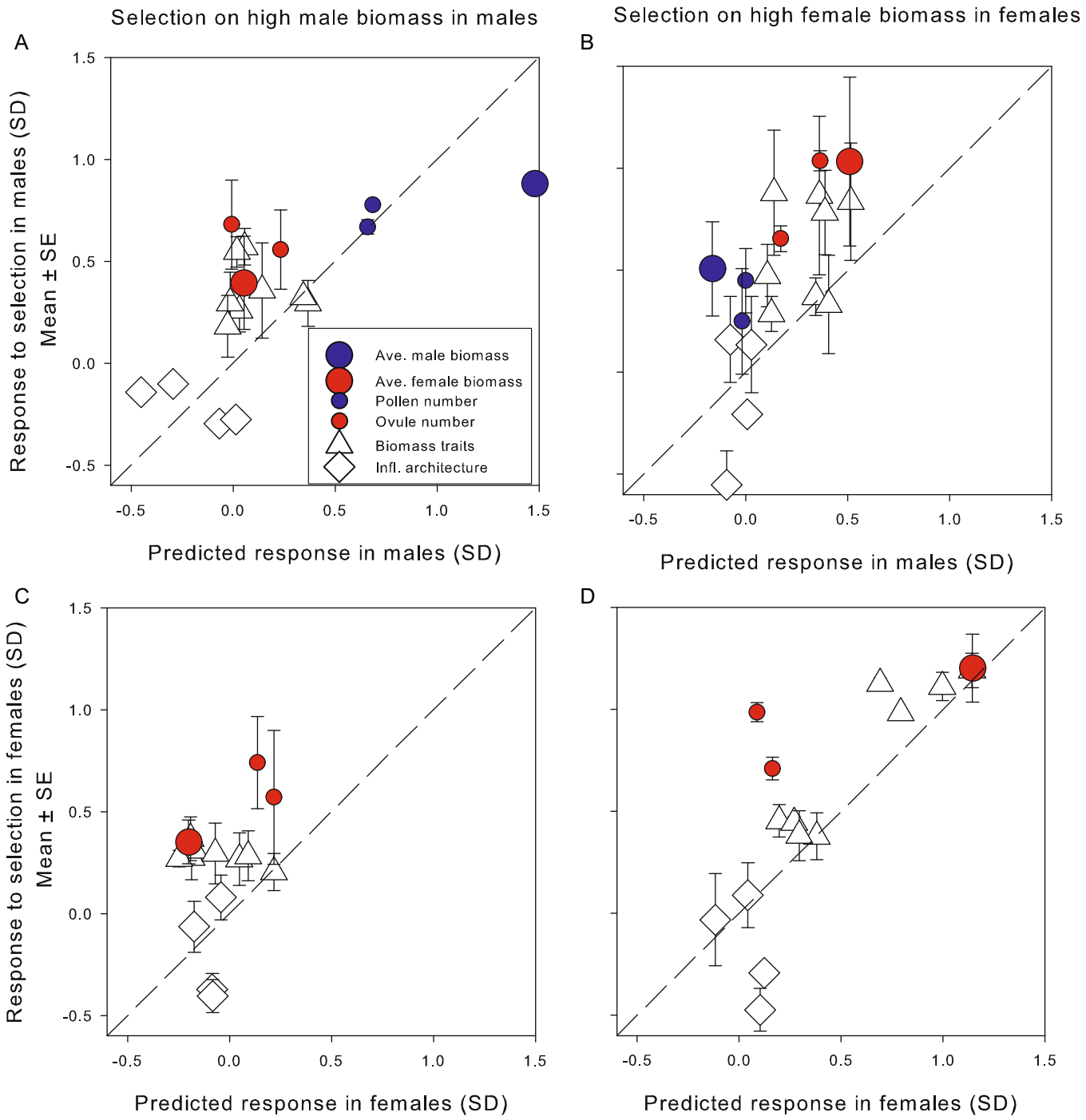

FIGURE 7 Comparison of responses to selection over two generations to predicted values from baseline estimates of genetic parameters. (A) Responses in hermaphrodites to selection on average male biomass in hermaphrodites. (B) Responses to selection in hermaphrodites on average female biomass in females. (C) Responses to selection in females on average male biomass in hermaphrodites. (D) Responses to selection in females on average male biomass in hermaphrodites. Means and standard errors across two lines are shown. Responses are relative to the mean for the two control lines. Both responses and predicted values are expressed in units of SD of the trait in the Baseline generation. Values used in plotting are available by trait in Appendix S1. Large blue circle: average male biomass. Large red circle: average female biomass. Small blue circle: pollen number. Small red circle: ovule number. Upward triangle: biomass traits (carpel mass, capsule mass, sepal mass, and seed mass for terminal and lateral flowers). Diamond: inflorescence architecture (total flower number, inflorescence condensation, pedicel length for terminal and lateral flowers).

females changed less (Appendix S2). In Gen 1, the co-heritability between the two directly selected traits was 0.137 rather than –0.067, potentially explaining part, but not all, of the increase in average female biomass when only male biomass was selected, as illustrated in Figure 7C. The increase in heritability for average male biomass in Gen 1 was generated both by an increase in genetic variation due to the male parent and a decrease in residual variance due to the

environment (Appendix S2). The former might be due to changes in allele frequency, and the latter might have been due to changes in greenhouse conditions over the 10 years of this study. Gen 1 plants grew more slowly than plants in the Baseline because white paint applied to the UC Irvine greenhouse provided too much shade during 2002–2003. Slower growth overall could have potentially changed the impact of variation in resource acquisition.

Since heritability for average male biomass showed such a large change, we made new predictions for evolutionary responses in response to selection on that trait, for traits measured in females that used estimates from Gen 1, rather than the baseline, to predict the response in Gen 2. We chose to examine whether this process would better predict the traits measured in females, as our predictions using just the baseline had done a worse job predicting those responses (Figure 7C) than for traits measured in males (Figure 7A). That refined procedure led to an expected increase of 0.09 SD units for average female biomass in females compared to an actual increase of 0.35 SD units (SE = 0.11) rather than an expected change of −0.20 SD over two generations using only baseline estimates (Appendix S3). For nearly all traits (13 of 14; see Appendix S3), genetic correlations with average male biomass became more positive in Gen 1 than they had been in the Baseline, explaining the increases in indirectly selected traits such as carpel and capsule biomass. As a result, predictions shifted higher for those traits and performed better when genetic parameters were re-estimated in Gen 1 rather than using only baseline estimates. The new predicted values explained 37% of the variance across traits in the actual response of females to selection for high male biomass (Appendix S3B) compared to only 11% (Appendix S3B) using only estimates from the Baseline. This increase in accuracy of predictions suggests that such refined predictions may be useful generally in artificial selection, even though studies typically take the simpler approach of comparing realized heritabilities over many generations with heritability measured in the baseline (Hill and Caballero, 1992).

High uncertainty in estimates of genetic correlations could also play a large role in generating these discrepancies from predicted responses. The estimates of the genetic correlations in the Baseline have wide confidence intervals (Table 2). So even in cases when the best point estimate was slightly negative (e.g., average male biomass with lateral capsule mass estimated at −0.01; Table 2), the true genetic correlation could have been positive (in this case, the upper confidence limit was 0.33). Indeed, for all genetic correlations with the selected traits, the upper confidence limit was positive (Table 2), which could also help to explain the preponderance of observed responses above the 1:1 observed to predicted line (Figure 7).

Genetic drift is another possible explanation for discrepancies between predicted and observed responses. Our use of replicate selection lines allows us to rule out drift as an explanation when both selection lines responded very similarly, as was the case for responses of both male biomass and female biomass to direct selection on those traits. Drift is the most likely explanation for the few cases of differences between replicate lines that received the same selection treatment, such as the two control lines for terminal and lateral pedicel lengths, and also the two high female lines for terminal and lateral pedicel lengths (Figure 5D,F). Evolutionary responses were slight during Gen 1, as expected from the low genetic correlations with the selected traits, and lines receiving the same selection treatment had similar values during Gen 1 (Appendix S4). The two control lines, however, markedly diverged during Gen 2, as did the two high female lines (Appendix S4). We did not attempt to combine effects of uncertainty in estimates of genetic parameters with genetic drift, to find prediction intervals around our predicted responses, as methodology has so far focused on individual selection rather than family or sib selection (Pélabon et al., 2021). It is clear, however, from the wide confidence intervals for genetic correlations, that even without accounting for genetic drift these prediction intervals would also be very wide, as was observed for selection on gland size in *Dalechampia scandens* blossoms (Pélabon et al., 2021).

Evolution of inflorescence architecture as a correlated response

The low genetic correlations between inflorescence architecture traits and the selected traits in the Baseline (r ranged from −0.26 to 0.12; Table 2) would have led to the expectation that inflorescence architecture could evolve independently of sex allocation. But some aspects of inflorescence architecture appeared to show evolutionary responses to artificial selection. High male lines evolved shorter pedicels than in high female lines, whereas high female lines evolved less-condensed inflorescences. We saw no interaction for pedicel lengths between sex of the progeny and selection line, suggesting no increase or decrease in the level of sexual dimorphism for those traits in response to selection on sex allocation. Without knowing how inflorescence architecture would change in a single population with both kinds of selection (hermaphrodites selected to be more male-like and females selected for higher female biomass), it is unclear to what extent such responses would constrain the evolution of inflorescence architecture in natural populations. The fact that evolutionary responses were seen, however, indicates some level of genetic variation (see also Weller et al., 2006), allowing evolution in response to the selection conditions likely in the windy environments where *Schiedea* species with separate sexes have evolved (Sakai et al., 2006).

Our artificial selection generated changes in male and female biomass expected to occur during evolution of dioecy from gynodioecy. But the selection program with separate high male and high female lines differed from selection in nature in two important respects. First, selection in nature would likely favor increased stamen mass of

hermaphrodites and increased female mass of females in the same interbreeding population. A potential area of research for the future would thus be to extend the work here to predict responses of sexual dimorphism in biomass allocation and then perform artificial selection on both hermaphrodites and females in opposing ways in the same selection lines (see Tigreros and Lewis, 2011, for an example of such selection in insects) to test predictions. Second, selection in nature likely involves not only selection on sex allocation, but also simultaneous selection directly on traits involved in wind pollination, as those traits are also important in dioecious breeding systems. The production of numerous hermaphroditic flowers in condensed inflorescences is likely to favor pollen dispersal to female plants (Niklas, 1985), but these traits were not directly selected in our experiment. Many other selection programs can be envisioned, but no single experimental approach is likely to result in the expression of phenotypes characteristic of those found among species of *Schiedea* with progressively greater expression of dimorphism and increased adaptation for wind pollination. That may explain why artificial selection in *S. salicaria* did not lead to phenotypes resembling *S. adamantis*, a related species with a higher frequency of females and traits closer to those of a fully dioecious species, including reduced seed production in hermaphrodites compared to females. Additional traits in *S. adamantis* that resemble those of dioecious species include greater flower number and greater inflorescence condensation, especially in female plants, along with longer pedicels in hermaphrodites than females and more sexual dimorphism in pedicel lengths than in *S. salicaria* (Weller et al., 2007). In contrast to patterns of variation found in *S. adamantis*, artificial selection for higher female biomass in S. *salicaria* resulted in inflorescences with fewer flowers than unselected lines, and reduced inflorescence condensation.

CONCLUSIONS

This study joins a small set of studies that have used artificial selection to investigate the potential for evolutionary responses in traits related to sex allocation in plants (Meagher, 1994; Mazer et al., 1999; Delph et al., 2005). As in those other studies, unselected traits responded along with the directly selected traits, supporting the importance of indirect selection on genetically correlated traits. Using separate estimates of genetic variation in a baseline generation, we were able to demonstrate that evolutionary responses of these traits largely followed predictions from a multivariate selection model that took into account sexual dimorphism and between-sex genetic correlations. These evolutionary changes indicate that biomass of individual flower parts, pollen and ovule numbers, and some aspects of inflorescence architecture could all evolve in response to selection on sex allocation in this endemic Hawaiian plant. Thus, we might expect to see complex changes in plant phenotype as populations evolve completely separate sexes (dioecy) from gynodioecy, as has occurred in this genus (Sakai et al., 2006; Willyard et al., 2011).

AUTHOR CONTRIBUTIONS

D.R.C., A.K.S., and S.G.W. designed the study and wrote the first draft of the manuscript. A.K.S. and S.G.W. directed the lab and greenhouse research and compiled the data for preliminary analyses. D.R.C. performed data curation and the statistical analyses. A.K.S., S.G.W., T.M.C., A.K.D.-W., A.M.A., T.G.W., and T.D. helped standardize lab procedures, maintain plants in the greenhouse, and measure thousands of plants. A.M.A., P.J.N., Q.P.T., T.G.W., A.R.M., A.A.N., B.A., M.K., M.H.T., and T.D. all received UC Irvine UROP awards, and contributed to data collection, analysis, and discussion of parts of this research project.

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DATA AVAILABILITY STATEMENT

The data are deposited in the Dryad Digital Repository at https://doi.org/10.7280/D1W113 (Campbell et al., 2022).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Predicted and observed selection responses after two generations in units of SD.

Appendix S2. Changes in estimates of heritability and genetic correlation for directly selected traits between the baseline generation and generation 2.

Appendix S3. Comparison of fit to predicted evolutionary responses using (A) genetic parameters estimated from the baseline generation only versus (B) genetic parameters estimated separately in the baseline and Gen 1 (G1) generations.

Appendix S4. Responses to selection over two generations (G1 and G2) for (A) terminal pedicel length and (B) lateral pedicel length.

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