

## Sexual dimorphism and the genetic potential for evolution of sex allocation in the gynodioecious plant, *Schiedea salicaria*

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### Abstract

Sex allocation theory addresses how separate sexes can evolve from hermaphroditism but little is known about the genetic potential for shifts in sex allocation in flowering plants. We tested assumptions of this theory using the common currency of biomass and measurements of narrow-sense heritabilities and genetic correlations in *Schiedea salicaria*, a gynodioecious species under selection for greater differentiation of the sexes. Female (carpel) biomass showed heritable variation in both sexes. Male (stamen) biomass in hermaphrodites also had significant heritability, suggesting the potential for further evolution of dioecy. Significant positive genetic correlations between females and hermaphrodites in carpel mass may slow differentiation between the sexes. Within hermaphrodites, there were no negative genetic correlations between male and female biomass as assumed by models for the evolution of dioecy, suggesting that *S. salicaria* is capable of further changes in biomass allocation to male and female functions and evolution toward dioecy.

### Introduction

Sexual dimorphism is perhaps best known in animals but flowering plants also offer opportunities to test models of sex allocation and processes that may influence the evolution of dimorphism (Delph, 2005). Flowering plants have evolved a great diversity of breeding systems, including gynodioecy (populations with females and hermaphroditic plants), subdioecy (populations with females, males and a few hermaphroditic plants) and dioecy (populations with separate male and female plants). The evolution of dioecy requires selection for separate sexes and changes in allocation of resources in response to selection. Theoretical models suggest two general hypotheses for the evolution of dioecy via gynodioecy. In cases with nuclear inheritance of male sterility, females may be favoured in an otherwise hermaphroditic population if both inbreeding

depression and selfing rates in hermaphrodites are sufficiently high (Charlesworth & Charlesworth, 1978). Dioecy also may be favoured over hermaphroditism if the trade-off in investment in resources to male and female functions results in accelerating fitness returns on the investment of resources in a single sexual function (Charlesworth & Charlesworth, 1981; Charnov, 1982; Lloyd, 1984; reviewed in Campbell, 2000). These mechanisms are not mutually exclusive; both inbreeding depression and reallocation of resources can contribute together to the evolution of dioecy. Moreover, regardless of the selection mechanism, the models assume genetic variation and some form of trade-off in allocation of fixed resources between the two sexual functions. Gynodioecy is a common intermediate in the evolution of dioecy, and once females are present in populations, selection occurs for hermaphrodites that specialize in more male function at the expense of female function (Charlesworth & Charlesworth, 1978; Charnov, 1982; Charlesworth, 1989; reviewed in Charlesworth, 1999; Charlesworth & Guttman, 1999; Ashman, 1999). As the proportion of females in the population increases through time, hermaphrodites that are more specialized for male function are expected to be favoured, until ultimately, the population consists of only males and females.

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The presumed trade-offs in allocation of resources between the two sexual functions assumed in these theoretical models are most directly tested when expressed in a common currency such as biomass or nutrients (Goldman & Willson, 1986; Campbell, 2000). In models for sex allocation, allocation to male function is often measured by the proportion of resources invested in male reproductive structures (i.e. stamens) relative to the total investment in the stamens, in the female reproductive structures of the flower (i.e. pistil) and in seeds (Charlesworth & Charlesworth, 1981; Charnov, 1982; Lloyd, 1984). Assuming similar abilities to acquire resources and heritable variation for allocation of resources to the two sexual functions, these trade-offs should be evident as negative genetic correlations between allocation to male and female functions (Charlesworth & Morgan, 1991), e.g. genotypes allocating more resources to male function should retain less for female function. Genetic variation in the ability to acquire resources tends to generate counter-balancing positive correlations in allocation, however, so that the net genetic correlation can instead be positive (De Jong, 1993). Positive genetic correlations between male and female traits could retard the process of sexual specialization.

For natural populations, we know little about patterns of genetic variation and covariation in underlying traits related to sex allocation in plants. Of the studies that have explored the quantitative genetics of sex allocation traits in hermaphroditic plant species, only a few have focused on common resources such as biomass (reviews in Campbell, 2000; Ashman & Majetic, 2006). Additive genetic variation in biomass invested in stamens has been demonstrated in natural populations of *Ipomopsis aggregata* (Campbell, 1997). Stamen biomass, however, positively rather than negatively genetically correlated with pistil biomass. Other hermaphroditic species (*Lythrum salicaria*, comparing stamen and pistil biomass, O'Neil & Schmitt, 1993; *Begonia semiovata*, comparing mass of male and female flowers, Ågren & Schemske, 1995) also had positive genetic correlations between male and female biomass.

For dimorphic plants, many sex allocation traits have significant broad-sense heritabilities (Ashman, 2003), and negative genetic correlations between male and female traits measured in different currencies have been shown in hermaphrodites of gynodioecious *Thymus* (Atlan *et al.*, 1992; pollen N/flower, N germinating seeds/fruit), gynodioecious *Fragaria virginiana* (Ashman, 1999, 2003; pollen production, fruit set per flower) and subdioecious *Astilbe biternata* (Olson & Antonovics, 2000; percentage fruit set and percentage stamen production). In one of the few tests of sex allocation models in plants using a common currency, Koelewijn & Hunscheid (2000) employed maternal families to estimate broad-sense heritabilities and demonstrated a positive genetic correlation between stamen biomass and pistil biomass in

gynodioecious *Plantago coronopus*. These studies suggest that gynodioecious species may be particularly helpful in revealing how changes in sex allocation occur, but few investigations used breeding designs allowing estimation of additive genetic variation and covariation upon which the response to selection is most dependent (see Ashman & Majetic, 2006). A study using this approach along with a common currency, such as biomass allocation, would test sex allocation theory more directly.

The endemic Hawaiian genus *Schiedea* Chamisso & Schlechtendal (Caryophyllaceae) offers an excellent opportunity to study quantitative genetic changes in the evolution of breeding systems because it includes closely related species with different proportions of females in populations and also variation in male function in hermaphrodites. In *Schiedea*, all 34 species are descended from a common hermaphroditic ancestor, and species have hermaphroditic, gynodioecious, subdioecious or dioecious breeding systems (Wagner *et al.*, 1995, 2005; Weller *et al.*, 1995; Soltis *et al.*, 1996; Sakai *et al.*, 1997). Gynodioecious species with low frequencies of females, such as *Schiedea salicaria* Hillebrand, have hermaphrodites and females with similar female function (Weller & Sakai, 2005). By contrast, females produce over twice as many seeds as hermaphrodites under field conditions in *Schiedea adamantis* St John, a gynodioecious species with 39% females (Sakai *et al.*, 1997). In subdioecious species, the frequency of females is even higher, and females produce nearly all the seeds. These differences in breeding systems are presumably the result of selection on heritable patterns of allocation to male and female functions. Male sterility in the genus *Schiedea* is under the control of a single nuclear gene (Weller & Sakai, 1991); females are homozygous recessive (hh) and hermaphroditic plants are homozygous dominant (HH) or heterozygous (Hh). Quantitative variation in female function of females, and in male and female functions of hermaphrodites, is likely to be controlled by a number of genes. In several species of *Schiedea*, breeding systems may be in transition (e.g. Sakai *et al.*, 1997, 2006; Rankin *et al.*, 2002; Weller & Sakai, 2005), with allocation patterns that are under directional selection.

In this paper, we test basic assumptions of sex allocation theory to predict the potential for evolutionary responses in allocation to male and female functions in *S. salicaria*. *Schiedea salicaria* is a gynodioecious species with a low (12–13%) frequency of females and is especially suitable for this study because it appears close to the initial stages in the transition from hermaphroditism to dioecy. The initial presence of females in this species may be the result of strong inbreeding depression (Sakai *et al.*, 1989) expressed in progeny of largely selfed hermaphrodites (Weller & Sakai, 2005); resource allocation to seed production was very similar for both sexes. In view of conditions that appear to favour the increase in frequency of females (a combination of both high inbreeding depression and high selfing rates), we expected that

the presence of heritable variation for allocation patterns would permit the further divergence of females and hermaphrodites in sexual function. Our goals were to determine: (1) the current extent of sexual dimorphism in biomass allocation between females and hermaphrodites; and (2) the genetic potential for further differentiation of these allocation patterns, based on narrow-sense heritabilities and genetic correlations of allocation measured in a common currency (male biomass vs. female biomass). As assumed by the models, and especially given the range in breeding systems and allocation patterns in closely related species, we predicted that traits associated with male and female functions in *S. salicaria* would show significant narrow-sense heritability. Based on resource allocation theory suggesting that trade-offs between male and female traits are important within hermaphrodites, we predicted that negative genetic correlations should occur between male and female biomass. We also predicted that homologous traits expressed in both females and hermaphrodites (e.g. carpel biomass) are likely to be positively genetically correlated and, if so, may constrain the response to selection for further differentiation of the sexes (Lande, 1980; see also Reeve & Fairbairn, 2001).

## Materials and methods

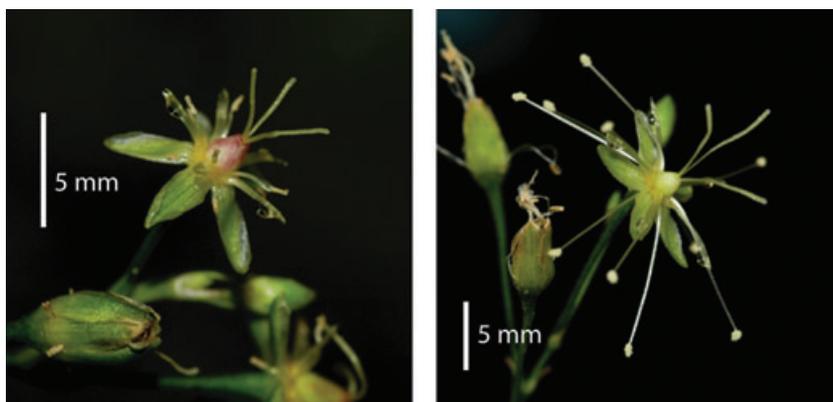
### Study organism

*Schiedea salicaria* is a perennial, woody shrub endemic to steep windswept ridges at approximately 300 m in dry shrublands of the West Maui Mountains of Hawaii (Wagner *et al.*, 2005). Hermaphroditic flowers are capable of self-fertilization. Within a flower, anthers disperse pollen 3–5 days before the stigmas are receptive. In both hermaphrodites and females, the pistils (fused carpels in *Schiedea*) are functional. Female flowers have small, vestigial anthers with no viable pollen (Fig. 1). Flowers of both sexes have functional nectaries but no petals. The flower terminating the main axis of the inflorescence is usually the first to open; terminal flowers (distal flowers

of the secondary axes) are larger and open before lateral flowers (all other flowers on the secondary or tertiary axes). Inflorescences of *S. salicaria* have a mean of nine terminal flowers for both sexes, and 42 and 48 lateral flowers for hermaphrodites and females respectively (Weller *et al.*, 2006). Based on wind tunnel experiments, *S. salicaria* is wind pollinated (Weller *et al.*, 1998). The fruits are capsules, and seeds are released about 3 weeks after pollination.

### Crossing design

Plants used in this experiment were derived from seeds collected from one field population (842) and were grown in the UC-Irvine greenhouses. To have both hermaphroditic and female progeny segregate in each cross, we identified 35 full or half-sib families that had both hermaphrodites heterozygous for the male sterility gene (Hh) and females (hh). Heterozygous hermaphrodites were identified as heterozygous through test crosses or because they had a female rather than a hermaphrodite as a maternal parent. Seventy plants [one heterozygous hermaphrodite (Hh) and one female (hh) from each of the 35 families] were used in pollinations beginning in summer 1999 to produce the plants measured to estimate the additive genetic variance–covariance matrix (*G* matrix) for the focal traits. We employed a partial diallel design similar to that of Meagher (1992), but with larger sample sizes for greater statistical power. Each hermaphrodite used as the pollen donor was crossed with three females randomly chosen from different families, and each selected female was crossed with three hermaphrodites from different families. The full design included 35 paternal half-sibships and 91 (rather than 105) full sibships because a few crosses failed to produce enough progeny. We estimated additive genetic variances by examining the component of variation among paternal half-sib families (see Kearsey, 1965; Shaw, 1987; Meagher, 1992). A nested design was not practical because it would have required many more unrelated females, derived from a population growing on



**Fig. 1** Female (left) and hermaphroditic (right) flowers of *Schiedea salicaria*, showing sepals, nectaries, stamens (on hermaphrodite) and ovary with a three-part stigma. *Schiedea* flowers lack petals. Photos by N. Kawakubo.

steep cliffs where females are rare (12–13%). Full diallels and factorial designs with the large number of parents necessary to minimize inbreeding were also impractical because they would require too many inflorescences to perform all of the crosses. Our plan to use the progeny in a future artificial selection programme also would have made it difficult to combine several separate full factorials.

### Traits

Seeds from the crosses were planted in March 2000 after the required dormancy, transplanted to 5-cm<sup>2</sup> pots in May 2000, and to 8 cm<sup>2</sup> pots in August 2000. Liquid fertilizer (20–20–20) was applied weekly and plants were watered as needed. Sex (hermaphrodite or female) was determined at the time of flowering for 25 plants in every full sibship. We measured the biomass of floral and/or capsule traits for 1082 plants (approximately five plants per sex for each of the 91 full sibships for floral traits; approximately three plants per sex for fruit traits). Biomass was chosen as a measure of common currency because it is relatively convenient compared with other potential measures of common currency such as C or N content. Five female and five hermaphroditic progeny from each sibship were measured in summer–autumn 2000 for floral traits on each of two inflorescences. Because the plants produce both terminal and lateral flowers that differ in size, we measured biomass allocation separately for these two types of flowers, along with estimates of flower numbers to calculate composite measures of biomass allocation. We included measures of both types of flowers because terminal flowers produced more seeds and pollen than lateral flowers, but changes in lateral flower number may be more important in determining allocation patterns (Weller *et al.*, 2006, 2007). Because hermaphroditic flowers mature pollen and stigmas at slightly different times, stamen biomass and carpel biomass of hermaphrodites were measured on different flowers for each plant. As a result, over 4400 flowers of hermaphrodites and over 2100 flowers of females were dissected for floral biomass measures. Three progeny of each sex from each full sibship were used to determine the number of flowers and fruits (capsules) per inflorescence in spring–summer 2001. Two inflorescences per plant were used to determine capsule production, and all flowers on those two inflorescences were hand pollinated three times per week to control background levels of pollination. Two terminal and two lateral fruits (mature but unopen) from each inflorescence were collected (about 4600 fruits) to determine fruit biomass (capsule including seeds) and viable seed biomass. After all fruits had formed, the numbers of terminal and lateral flowers and capsules per inflorescence were counted. For both floral and fruit traits, plant means were calculated by averaging the inflorescence means, and full sibship means were calculated using the plant means. The family mean for each of

the 35 paternal half-sibships was the average of the means of the three full sibships associated with each hermaphrodite.

Flowers of females and hermaphrodites were tagged with extremely fine (30 gauge) electrical wire when first open, and dissected 3 days later when stigmas were receptive to measure the biomass of pistils (carpel biomass) and the combined biomass of sepals and nectaries (referred to as sepal biomass), a measure of floral size. Newly opened flowers were used to weigh the biomass of all stamens in the flower for hermaphrodites (stamen biomass). We were unable to measure the biomass of the vestigial stamens in females because of the very short filament length and small size of these anthers (about half the length of anthers of hermaphrodites). Biomass of all floral and fruit measures were taken after plant material had been thoroughly dried at 67 °C. Biomass was weighed to the nearest 0.001 mg on a Cahn 25 electrobalance (Cahn Instruments, Inc., Cerritos, CA 90701, USA).

Final resource allocation was calculated as the proportion of total male biomass/(total male and female biomass) of an inflorescence, where male biomass was the stamen biomass, and female biomass included both the carpel and capsule biomass. Resource allocation at the time of flowering was calculated in a similar fashion but female biomass included only carpel biomass. Both final resource allocation and resource allocation at the time of flowering were calculated separately for terminal and lateral flowers.

We also combined the data from lateral and terminal flowers to estimate male and female biomass on an average basis. The ‘average’ male biomass of a hermaphroditic flower was calculated as the mean stamen biomass per flower of all lateral and terminal flowers on an inflorescence, weighted by the proportion of terminal and lateral flowers. The ‘average’ female biomass of a female or hermaphroditic flower was calculated as the mean carpel plus capsule biomass (including seeds) per flower for all lateral and terminal flowers on an inflorescence, again weighted by the proportion of terminal and lateral flowers. Throughout the *Results* section, ‘average’ values refer to these weighted values.

### Analysis

Additive genetic variance, narrow-sense heritability and additive genetic covariance were estimated by examining the components of variation among paternal half-sib families (see Kearsley, 1965; Shaw, 1987; Meagher, 1992; Weller *et al.*, 2006). Values were estimated separately for the hermaphroditic and female progeny using Proc Mixed in SAS version 9.1 (SAS Institute, Cary, NC, USA). Restricted maximum-likelihood methods were used because of the unbalanced nature of the design. The model included effects of paternal parent and maternal parent, both specified as random factors because the original parent plants were a random subset

of genotypes in the natural population, and a residual error term. We did not include the interaction term between paternal and maternal parents because the large number of missing cells in a partial diallel design makes interpretation of this interaction term problematic (Searle *et al.*, 1992). In a preliminary analysis including the interaction, only one of 18 interaction terms was significant (not including derived traits; data not shown).

Significance of the paternal effect was tested using a log-likelihood ratio test to compare the full model with a reduced model without the paternal parent effect and only the maternal parent effect and residual error remaining (Littell *et al.*, 1996). The likelihood ratio statistic was compared with the critical value for a chi-squared distribution with 1 d.f. A significant paternal effect implies significant additive genetic variance (assuming no additive-additive epistasis, Lynch & Walsh, 1998). Additive genetic variance ( $V_A$ ) was estimated for each trait in females and in hermaphrodites by multiplying the paternal variance component by four (Falconer & Mackay, 1996). Narrow-sense heritabilities were calculated for each trait in each sex as the additive genetic variance divided by the total variance (paternal variance + maternal variance + error). Coefficients of additive genetic variation ( $CV_A$ ; Houle, 1992) were also calculated for each trait for each sex.

Sexual dimorphism in inflorescence traits was investigated using paired *t*-tests of the means of females and hermaphrodites based on the 35 paternal sibships. Terminal and lateral flowers and fruits were analysed separately. To see which combination of traits (terminal and lateral carpel mass, sepal mass and capsule mass) best differentiated the sexes, we also examined sexual dimorphism in biomass traits by using MANOVA followed by canonical discriminant analysis. Stamen mass was omitted because it was only measured in hermaphrodites; correlated or derived traits (average female mass, average male biomass and viable seed mass) were also excluded.

Genetic correlations between traits were estimated using best linear unbiased predictors (BLUPs) of sire breeding values obtained from PROC MIXED (SAS Institute; Conner *et al.*, 2003). In contrast to simple sample means, BLUPs take into account information on the probability distribution, shrinking the estimates towards the overall mean, which is appropriate for random effects (Littell *et al.*, 1996). Pearson correlation coefficients were calculated between the BLUP values for pairs of traits. Significance levels were adjusted for multiple comparisons using the sequential Bonferroni method, as described by Rice (1989), which does not require independent tests. To examine the genetic variance-covariance matrix (*G* matrix) of females and hermaphrodites, we compared the matrices based on the BLUPs of sire breeding values for the two sexes using common principal component (CPC) analysis (Arnold & Phillips, 1999; Phillips & Arnold, 1999). For this method,

we used the CPC programme of Phillips (1998) to test whether matrices were equivalent, or differed in eigenvalues (reflecting different magnitudes of genetic variances) or eigenvectors (reflecting a different pattern of the genetic covariances). The jump-up approach was used to test each level of hierarchy against the hypothesis that the matrices are unrelated. Although all methods to date for comparing *G* matrices have limitations, the CPC method is perhaps the most widely used (Houle *et al.*, 2002; Mezey & Houle, 2003). A drawback to the method is that because error in estimating the breeding values is not incorporated, the significance of matrix differences might be overestimated. Viable seed mass was not included because it so highly correlated with capsule mass; average female mass and average male mass were not included because they were composites of other traits.

## Results

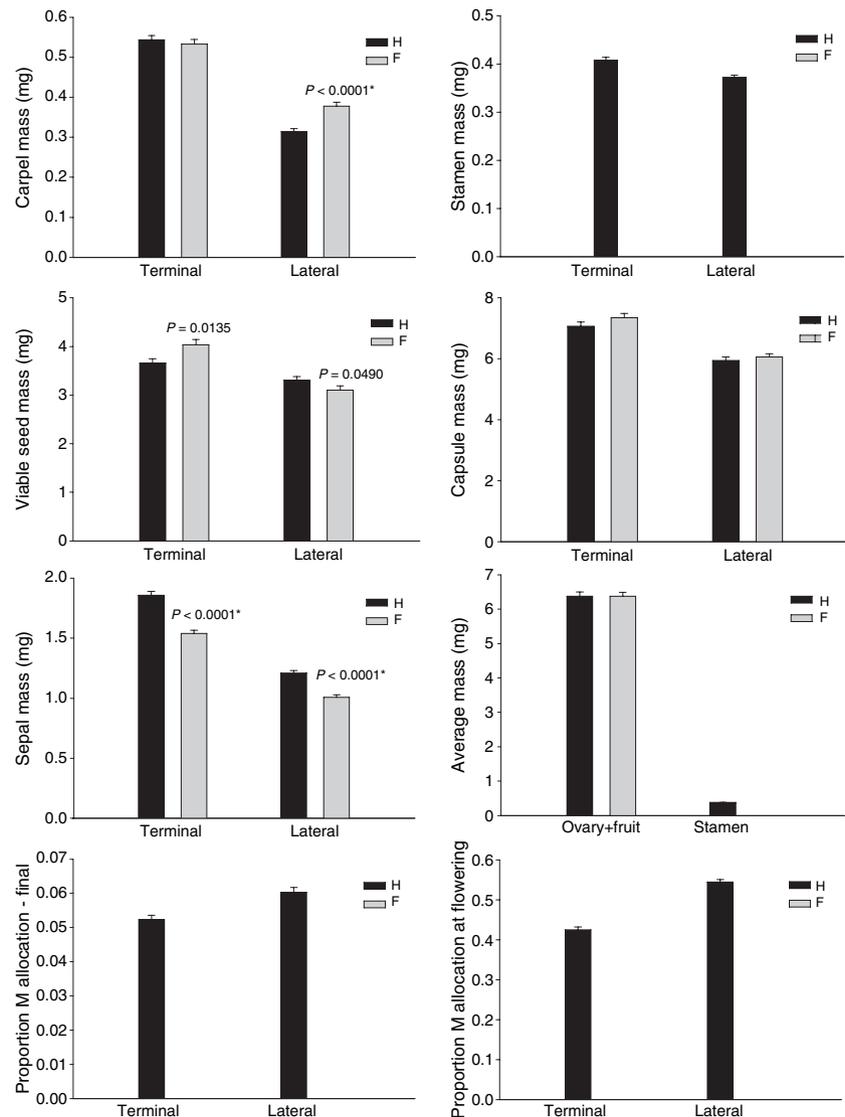
### Sexual dimorphism and allocation patterns

Flowers of hermaphrodites (H) were larger (heavier sepal biomass) than flowers of female (F) plants for both terminal (T) and lateral (L) flowers (Fig. 2). Terminal flowers of females and hermaphrodites were similar in carpel mass, but lateral flowers of females had significantly heavier carpels than those of hermaphrodites (Fig. 2). Females and hermaphrodites did not differ in the later stages of viable seed mass or capsule mass (Fig. 2). Results from a multivariate analysis using carpel, capsule and sepal biomass were consistent with univariate analyses. Using a multivariate approach, there were significant differences between the sexes for biomass (Wilks'  $\lambda = 0.0668$ ;  $F = 67.47$ , d.f. = 6, 29;  $P < 0.0001$ ). The first canonical discriminant function explained 86.5% of the variation between females and hermaphrodites. The standardized canonical coefficients gave the highest weighting to L carpel mass and L sepal biomass, consistent with univariate results.

In hermaphrodites, the relative allocation to male biomass as a proportion of total reproductive biomass differed with the position of the flower (T and L) and the stage of development (flower and fruit). Male biomass was a greater proportion of total reproductive biomass in lateral flowers at both flowering and fruiting [42.5% of T and 54.5% of L flower reproductive biomass (biomass of stamens and carpels); 5.2% of T and 6.0% of L total reproductive biomass (biomass of stamens, carpels, and fruits); Fig. 2]. The difference between terminal and lateral flowers largely reflects the relatively greater reduction in female than male biomass in the lateral flowers of hermaphrodites.

The average female biomass (carpel + capsule mass, weighted by the relative number of terminal and lateral flowers) was similar for female and hermaphroditic plants (Fig. 2).

**Fig. 2** Means of traits (standard errors, SE) for females and hermaphrodites. Terminal and lateral refer to the flower position on the inflorescence and are measures per flower (see text). Biomass measures are in mg. 'Proportion M allocation-final' is the proportion of the carpel + capsule + stamen mass allocated to stamen mass. 'Proportion M allocation at flowering' is calculated in a similar fashion but female biomass includes only carpel biomass. The 'average male mass' and 'average female mass' are the average of the terminal and lateral flowers, weighted by the number of flowers of each type on an inflorescence. The sample size in all cases is  $N = 35$  paternal half-sibships.  $P$  is the significance level for the paired  $t$ -test of females and hermaphrodites for those tests with  $P < 0.05$ . Those levels remaining significant at the 0.05 level after a sequential Bonferroni correction (using the eight measures of carpel, sepal, capsule and viable seed mass) are indicated by an asterisk.



## Heritabilities

Male and female biomass showed significant genetic variation, and patterns varied depending on position within the inflorescence (T and L) and sex (F and H; Table 1). Male (stamen) biomass of hermaphrodites had significant narrow-sense heritabilities in both terminal ( $h^2 = 0.46$ ) and lateral ( $h^2 = 0.30$ ) flowers.

In traits related to female function, patterns of narrow-sense heritability depended upon the sex of the plant and stage of development (flower and fruit). Female traits at flowering and fruit traits at the lateral position of hermaphrodites exhibited the highest heritabilities (or the greatest additive genetic variation). Carpel biomass exhibited significant narrow-sense heritabilities in both sexes ( $h^2$  for FT: 0.37, FL: 0.37, HT: 0.26) except for lateral flowers of hermaphrodites ( $h^2$  for HL: 0.19,

Table 1). At the fruiting stage, lateral fruits of hermaphrodites had high narrow-sense heritabilities in capsule mass ( $h^2 = 0.69$ ) and seed mass ( $h^2 = 0.52$ ). By contrast, the terminal fruits of hermaphrodites did not exhibit significant narrow-sense heritability for capsule and seed mass, and the coefficients of variation were somewhat lower, particularly for viable seed mass. In females, narrow-sense heritabilities for capsule and seed biomass were not significant after Bonferroni correction ( $h^2$  ranging from 0.26 to 0.35). Sepal biomass showed significant narrow-sense heritability for terminal flowers for both sexes ( $h^2 = 0.23$  for F,  $h^2 = 0.28$  for H) but not for lateral flowers.

In hermaphrodites, the proportion of resources allocated to male function at flowering also exhibited high narrow-sense heritability for terminal flowers ( $h^2 = 0.79$ ) but not for lateral flowers ( $h^2 = 0.13$ ). Final resource

**Table 1** Narrow-sense heritabilities ( $h^2$ ),  $\chi^2$  values (the test-statistic associated with the log-likelihood ratio test for an effect of the male parent), coefficients of additive genetic variation ( $CV_a$ ) and significance levels ( $P$ ) of biomass traits in females and hermaphrodites.

Trait	Female (F)				Herm (H)			
	$h^2$	$\chi^2$	$P$	$CV_a$	$h^2$	$\chi^2$	$P$	$CV_a$
Terminal (T)								
T stamen mass	–	–			0.462	20.1	0.0000073	13.83
T carpel mass	0.375	15.5	0.0000825	17.98	0.262	7.7	0.00551	14.12
T sepal mass	0.235	6.9	0.0086196	13.52	0.276	7.8	0.0052246	13.61
T capsule mass	0.261	3.2	0.0736383	12.59	0.234	2.4	0.1213353	12.24
T viable seed mass	0.351	5.3	0.0213254	20.56	0.044	0.1	0.7518296	7.52
T resource alloc.: final	–				0.591	11.3	0.0007751	21.96
T resource alloc.: at flowering					0.785	24.3	0.00000008	15.98
Lateral (L)								
L stamen mass	–				0.296	7.8	0.0052246	9.85
L carpel mass	0.374	13.7	0.0002145	21.30	0.192	4.3	0.0381124	13.55
L sepal mass	0.160	2.8	0.0942643	12.82	0.184	3.8	0.0512526	12.05
L capsule mass	0.326	4.1	0.0428832	13.04	0.692	16.7	0.0000438	20.23
L viable seed mass	0.314	4.4	0.0359389	17.14	0.523	10.7	0.0010714	24.79
L resource alloc.: final	–				0.625	11.7	0.0006250	22.29
L resource alloc.: at flowering					0.133	0.6	0.438578	5.24
Average female mass	0.237	2.3	0.1293740	10.69	0.639	13.2	0.0002799	17.57
Average male mass	–				0.537	8.8	0.0030123	13.23

allocation to male function after fruiting showed significant narrow-sense heritability in both the terminal ( $h^2 = 0.59$ ) and lateral ( $h^2 = 0.63$ ) positions (Table 1). In hermaphrodites, both the average male biomass ( $h^2 = 0.54$ ) and the average female biomass per flower ( $h^2 = 0.64$ ) exhibited high narrow-sense heritability, but in females, the average female biomass showed no significant narrow-sense heritability ( $h^2 = 0.24$ ,  $P = 0.13$ ).

#### Genetic correlations of biomass within each sex

Within hermaphrodites, there were no significant genetic correlations between male and female allocations at the time of flowering (stamen biomass vs. carpel mass) or after fruiting (stamen biomass vs. capsule mass or viable seed mass; Fig. 3) or between average male biomass and average female biomass ( $r = 0.015$ ,  $P = 0.93$ ,  $N = 35$ ). Female biomass at flowering (carpel biomass) did not correlate with the female biomass at fruiting (capsule biomass) in either sex (Fig. 3). Not surprisingly, average female biomass, which includes both capsule mass and the much smaller carpel mass, was highly correlated with capsule mass in females and hermaphrodites ( $r = 0.73$ – $0.96$ ,  $P < 0.0001$  for all) but not with carpel biomass ( $r = 0.09$ – $0.27$ , with  $P = 0.11$ – $0.61$ ).

Sepal mass, a measure of flower size, had significant positive genetic correlations with female biomass at flowering (carpel mass) for both females and hermaphrodites, but not at fruiting (capsule biomass, Fig. 3). The correlation of sepal mass and stamen mass in hermaphrodites was positive but not significant after Bonferroni correction (HT:  $r = 0.46$ ,  $P = 0.005$ ; HL:  $r = 0.44$ ,  $P = 0.008$ ).

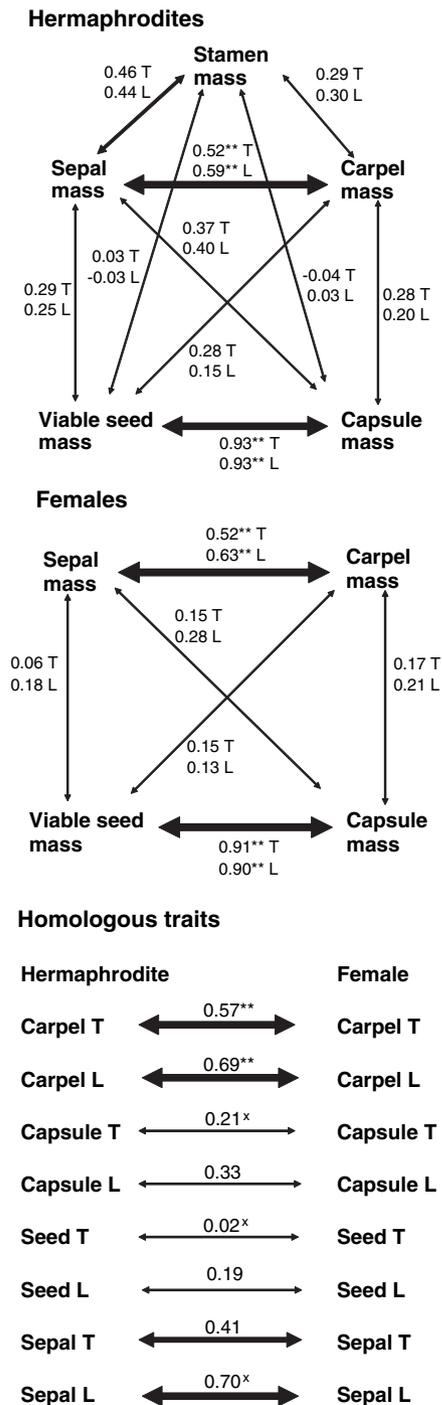
Within a sex, genetic correlations between terminal and lateral flowers for carpel, capsule or stamen biomass were all positive ( $r = 0.42$ – $0.83$ ; none of these correlations were significant after Bonferroni correction except sepal biomass in females).

#### Genetic correlations of homologous traits between sexes

Carpel biomass had significant genetic correlations between females and hermaphrodites for both terminal ( $r = 0.57$ ) and lateral ( $r = 0.69$ ; Fig. 3) flowers. None of the other traits (capsule mass, sepal mass and viable seed mass) with significant narrow-sense heritability had significant correlations between the sexes in either position (T and L). The correlation between the sexes of average female biomass was positive ( $r = 0.41$ ,  $P = 0.015$ ) but not significant after Bonferroni correction.

#### Comparison of matrices of females and hermaphrodites

The genetic variance–covariance matrices for females and hermaphrodites were different in structure. They differed significantly not only from equality ( $P < 0.0001$ ), but also from proportionality ( $P < 0.0001$ ), the latter indicating that the matrices differed in ways beyond simply higher genetic variances in one sex than the other. Furthermore, the matrices did not share any principal components in common (CPC[1],  $P = 0.0022$ ; CPC[2],  $P = 0.0007$ ; CPC[3],  $P = 0.0010$ ; CPC[4],  $P = 0.0005$ ), based on



**Fig. 3** Genetic correlations (using best linear unbiased predictors) of biomass traits within sex (female or hermaphrodite), within position (terminal, T or lateral, L) or between sexes (homologous traits in hermaphrodites and females). Stamen mass was not measured in females.  $\times$  indicates correlations where neither trait had significant narrow-sense heritability.  $**$  indicates correlations significant after adjusting for multiple comparisons using the sequential Bonferroni method (correlations marked by  $\times$  were not included in these adjustments).

the CPC analysis using Flury's jump up approach. This difference between the  $G$  matrices is consistent with the finding that many genetic correlations were substantially less than one.

## Discussion

Models for sex allocation and the evolution of plant breeding systems generally assume that sex allocation patterns exhibit genetic variation and that trade-offs will occur between allocation to male and female functions (Charnov, 1982; reviewed in Campbell, 2000). The use of a common currency such as biomass allows the most direct test of resource allocation theory in the evolution of separate sexes, but there are relatively few studies of natural populations using this approach, in part because of the difficulty in measuring biomass of small floral parts such as stamens. In *S. salicaria*, we found that many biomass traits associated with allocation to female and male functions had significant narrow-sense heritabilities, suggesting the ability of this species to respond to selection for dioecy by altering allocation patterns.

Selective pressures on gynodioecious *S. salicaria* appear to favour the evolution of dioecy. The shift to gynodioecy from hermaphroditism may be relatively recent, given the low frequencies of females (12–13%) and only modest differentiation between females and hermaphrodites in this species (Golonka *et al.*, 2005; Weller & Sakai, 2005). Selection for higher frequencies of females is expected because the high selfing rates found in *S. salicaria* (Weller & Sakai, 2005) should lead to the expression of substantial inbreeding depression (Sakai *et al.*, 1989). Higher frequencies of females are expected to select for hermaphrodites with increased male function, leading to further differentiation of the sexes and potentially the evolution of dioecy (Charlesworth & Charlesworth, 1978; Charlesworth & Guttman, 1999). Although the combination of high selfing rates and substantial inbreeding depression can help to drive the evolution of dioecy, without heritable allocation patterns such as we found, a shift to dioecy would be unlikely.

## Sexual dimorphism between females and hermaphrodites

The morphological differences in floral traits between females and hermaphrodites in *S. salicaria* are small, relative to the differences between the sexes in other dimorphic *Schiedea* species Golonka *et al.* (2005). As anticipated, sexual dimorphism in allocation of biomass was also limited in this species. Females had smaller flowers (sepal mass) than hermaphrodites, for both terminal and lateral flowers. Females had significantly larger lateral carpels than hermaphrodites, but females and hermaphrodites did not differ in viable seed mass or capsule mass. These results are largely consistent with results from field populations, which showed little

evidence for dimorphism in traits related to the number or biomass of seeds (Weller & Sakai, 2005). Based on comparison with other species of *Schiedea*, the difference in sepal size between the sexes in *S. salicaria* is the result of smaller female flowers rather than larger hermaphroditic flowers (Golonka *et al.*, 2005).

Females of gynodioecious taxa often have smaller floral size (reviewed by Delph *et al.*, 1996; Eckhart, 1999). This sexual dimorphism in flower size has been hypothesized to be advantageous because the smaller size of female flowers allows reallocation to greater seed production, or because greater size in hermaphroditic flowers may result in better pollinator attraction and pollen dispersal (Miller & Venable, 2003). In *Schiedea*, smaller flower size did not result in greater seed or capsule mass in females; the average female mass per flower was similar in females and hermaphrodites. *Schiedea salicaria* is wind pollinated, and smaller flower size in females, relative to hermaphrodites, is associated with greater inflorescence condensation (number of flowers per inflorescence length), which may facilitate pollen receipt (Weller *et al.*, 2006). The smaller sepals in females of *Schiedea* may also result from pleiotropic effects of the male sterility mutation (Eckhart, 1999). In hermaphrodites of *S. salicaria*, the genetic correlations of stamen mass and sepal mass are positive (although not significant), suggesting the possibility of developmental correlations. The larger sepal size of hermaphrodites may also reflect selection for protection of larger reproductive structures (Delph *et al.*, 1996; Eckhart, 1999); in *S. salicaria*, hermaphroditic flowers have relatively large ovaries and stamens, whereas female flowers have much smaller vestigial stamens. In *S. salicaria*, sepal biomass has a positive genetic correlation with carpel mass in both sexes, an observation consistent with this protection hypothesis.

### Heritabilities and genetic correlations within females and hermaphrodites

Past selection does not appear to have depleted genetic variation for biomass traits critical in sex allocation in *S. salicaria*. With only one exception, measures of both male and female floral biomass showed significant and relatively high narrow-sense heritabilities, and resource allocation at flowering also exhibited very high heritability for terminal flowers. Final resource allocation to male biomass also had high narrow-sense heritabilities for both terminal and lateral flowers of hermaphrodites, although capsule mass exhibited significant narrow-sense heritability only in the lateral position of hermaphrodites. Using floral traits other than biomass, Mazer *et al.* (1999) also found significant heritabilities in both female (N ovules per flower) and male (N anthers per flower) traits in *Spergularia marina*. In other species, heritabilities of female floral traits (ovule N, pistil mass) have generally been higher than those for male traits (pollen N, stamen

mass; O'Neil & Schmitt, 1993; Fenster & Carr, 1997; Ashman, 2003).

The significant heritability of these biomass and allocation traits in *S. salicaria* suggests the potential for a response to further selection for the evolution of dioecy. The differences in measures by position of the flowers (terminal or lateral) also suggest that changes in inflorescence architecture may impact not only the effectiveness of wind pollination (Weller *et al.*, 2006) but also alter patterns of resource allocation. For example, Ashman & Hitchens (2000) also have found sex-specific intra-inflorescence differences in allocation patterns.

If heritabilities and genetic correlations in the field are consistent with those measured in the greenhouse, further changes in allocation patterns may occur with selection. Our comparisons using CPC indicate that differences between the variance-covariance matrices of females and hermaphrodites have already evolved. As reviewed by Conner *et al.* (2003), however, genetic variances and covariances in the greenhouse may be higher than similar measures in the field. Ideally, field measures are needed to confirm these predictions because of potential genotype by environment interactions and differences in environmental variances. Only a few such studies exist (e.g. Mazer & Schick, 1991; Campbell, 1997; Ashman, 2003) because of the difficulties in carrying out quantitative genetic analysis under field conditions.

### Trade-offs within hermaphrodites

Despite high heritabilities of male allocation, trade-offs between male (stamen) and female (carpel or capsule) biomass in the form of negative genetic correlations within hermaphrodites were not detected in *S. salicaria*. Recent reviews of empirical tests (Ashman, 2003; Ashman & Majetic, 2006) show mixed support for the trade-offs predicted in sex allocation theory (e.g. Charlesworth & Charlesworth, 1978, 1981; Charnov, 1982; Charlesworth & Guttman, 1999). Using a variety of traits, some studies of dimorphic species have shown sex allocation traits with significant broad-sense heritabilities and negative genetic correlations between male and female fertilities in hermaphrodites (Atlan *et al.*, 1992; Olson & Antonovics, 2000; Ashman, 2003). Our results are similar to a number of other studies where there were no apparent trade-offs between allocation to male and female functions within hermaphrodites (O'Neil & Schmitt, 1993; Fenster & Carr, 1997; Campbell, 1997; Koelewijn & Hunscheid, 2000; other studies reviewed in Ashman & Majetic, 2006).

Trade-offs may not be an initial major force promoting the evolution of dioecy in *S. salicaria*. Instead, selfing rates and levels of inbreeding depression are both high in *S. salicaria* (Sakai *et al.*, 1989; Weller & Sakai, 2005), and these are conditions that can favour the evolution of

dioecy (Charlesworth & Charlesworth, 1978), even in the absence of trade-offs and negative correlations between male and female traits. Alternatively, *S. salicaria* is gynodioecious and the frequency of females is low; negative genetic correlations between male and female traits noted in some other gynodioecious species may not have developed yet. Trade-offs may also not be evident because biomass may not be the appropriate currency to detect trade-offs. In addition, trade-offs have been masked by genetic variation in ability to acquire resources (van Noordwijk & de Jong, 1986; Houle, 1991). *Schiedea salicaria* exhibits some genetic variation in photosynthetic rate (Culley *et al.*, 2006), and higher photosynthetic rate could make more carbon available for both stamen mass and carpel and capsule mass. The absence of significant genetic correlations between measures of plant size and inflorescence traits such as terminal or lateral flower number and inflorescence length suggests that genetic variances and correlations for these traits in *S. salicaria* were not due simply to differences in plant size (Weller *et al.*, 2006). Other factors that may influence the correlations between male and female biomass within hermaphrodites include strong selection to maintain positive associations between floral characters (Stanton & Galloway, 1990).

### Genetic correlations between the sexes

Positive genetic correlations between females and hermaphrodites for presumably homologous traits may slow differentiation of the sexes (Lande, 1980; but see also Reeve & Fairbairn, 2001). In *S. salicaria*, only carpel biomass exhibited significant narrow-sense heritability and had a significant positive genetic correlation between females and hermaphrodites after sequential Bonferroni correction. Our use of sequential Bonferroni correction, however, may be overly conservative (Moran, 2003), and the between-sex correlation for sepal mass in terminal flowers ( $r = 0.406$ ) was also significant based on its individual test. The between-sex correlations for capsule mass and for viable seed mass were lower and not significant. Ashman (2003) found a significant between-sex correlation in ovule number per flower and flower number, but no between-sex correlation in females and hermaphrodites for fruit set in wild strawberry.

### Conclusions

Based on analyses of the variance–covariance matrices of females and hermaphrodites in *S. salicaria*, some changes in sex allocation have already occurred in both females and hermaphrodites, more so with flowers than fruit. The narrow-sense heritabilities of reproductive structures, particularly of lateral flowers, suggest the potential for further differentiation of male and female allocations. Despite the positive correlation of

carpel biomass between the sexes that may tend to slow differentiation of the sexes, females and hermaphrodites exhibited sexual dimorphism in lateral carpel biomass, with females having significantly larger carpels and smaller sepals than hermaphrodites. This pattern suggests strong selection for these differences, despite a positive genetic correlation of this trait between the sexes. The lack of negative genetic correlations expected if there is a trade-off between male and female allocations suggests that trade-offs are unlikely to promote the evolution of dioecy. Avoidance of inbreeding depression (Sakai *et al.*, 2006) appears to be a much stronger selective factor than resource allocation in the evolution of sexual dimorphism in *S. salicaria*.

Ongoing work on the phylogeny of this genus (Nepokroeff *et al.*, 2005) may eventually permit phylogenetic analysis of floral morphometrics (e.g. Miller & Venable, 2003) or more generally, of evolution of *G* matrices (Phillips & Arnold, 1999; Steppan *et al.*, 2002). Comparisons of the genetic potential for changes in allocation patterns in closely related congeneric gynodioecious species with greater dimorphism and a higher frequency of females (e.g. *S. adamantis*) may also give insights into the role of resource allocation in the evolution of breeding systems within this group. Artificial selection experiments for allocation traits provide an alternative and potentially powerful approach to answer questions about sex allocation (e.g. Meagher, 1994; Mazer *et al.*, 1999, 2007; Conner, 2003; Delph *et al.*, 2004a, b, 2005). The significant narrow-sense heritabilities of many male and female biomass traits suggest that selection for greater male biomass in hermaphrodites and greater female biomass in females should each result in a strong positive response to selection. Genetic correlations should not greatly affect these responses, with the possible exception of the correlation between the sexes in carpel mass. Comparisons of the predictions based on the variance–covariance matrices presented here and elsewhere (Culley *et al.*, 2006; Weller *et al.*, 2006) and with results of our ongoing artificial selection studies should allow more powerful tests of sex allocation theory and the genetic potential for evolution of sex allocation in this genus.

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