

# SEXUAL DIMORPHISM IN THE QUANTITATIVE-GENETIC ARCHITECTURE OF FLORAL, LEAF, AND ALLOCATION TRAITS IN *SILENE LATIFOLIA*

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Many species exhibit sexual dimorphism in a variety of characters, and the underlying genetic architecture of dimorphism potentially involves sex-specific differences in the additive-genetic variance–covariance matrix ( $G$ ) of dimorphic traits. We investigated the quantitative-genetic structure of dimorphic traits in the dioecious plant *Silene latifolia* by estimating  $G$  (including within-sex matrices,  $G_m$ ,  $G_f$ , and the between-sex variance–covariance matrix,  $B$ ), and the phenotypic variance–covariance matrix ( $P$ ) for seven traits. Flower number was the most sexually dimorphic trait, and was significantly genetically correlated with all traits within each sex. Negative genetic correlations between flower size and number suggested a genetic trade-off in investment, but positive environmental correlations between the same traits resulted in no physical evidence for a trade-off in the phenotype. Between-sex genetic covariances for homologous traits were always greater than 0 but smaller than 1, showing that some, but not all, of the variation in traits is caused by genes or alleles with sex-limited expression. Using common principal-components analysis (CPCA), a maximum-likelihood (ML) estimation approach, and element-by-element comparison to compare matrices, we found that  $G_m$  and  $G_f$  differed significantly in eigenstructure because of dissimilarity in covariances involving leaf traits, suggesting the presence of variation in sex-limited genes with pleiotropic effects and/or linkage between sex-limited loci. The sex-specific structure of  $G$  is expected to cause differences in the correlated responses to selection within each sex, promoting the further evolution and maintenance of dimorphism.

**KEY WORDS:** CPCA, dioecy,  $G$  matrix, sex-limited gene expression, sexual dimorphism, *Silene latifolia*, size/number trade-off.

Countless plant and animal species exhibit sexual dimorphism in a boggling array of phenotypic traits (see reviews in Owens and Short 1995; Geber et al. 1999; Badyaev 2002). Regardless

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of the ecological and evolutionary forces that ultimately drive such gender differentiation, the presence of sexual dimorphism must involve at least some degree of sex-specific gene expression (Fisher 1958; Lande 1980b; Geber 1999). The genetic architecture that underlies sexually dimorphic traits can influence a variety of evolutionary dynamics that ultimately determine the degree to which phenotypic change in each sex is intertwined.

One approach to understanding genetic architecture is to examine patterns of genetic variances and covariances ( $\mathbf{G}$ ) among traits.  $\mathbf{G}$  is instrumental in understanding the genetic basis of phenotypic variation and in determining the short-term evolutionary response to selection. Because of genetic covariances, selection on one trait may cascade through the phenotype, generating correlated responses in covarying traits. The existence of sexual dimorphism complicates the usual role of  $\mathbf{G}$  in two main ways. First, each sex may have a unique pattern of variances and covariances among traits so that correlated responses to the same selection in males may differ from those in females. Second, patterns of covariance may exist between the sexes that tie together their evolutionary trajectories and determine the degree to which males and females can evolve independently.

The multidimensional  $\mathbf{G}$  matrix for a population can be partitioned to consider these two impacts of sexual dimorphism by distinguishing the covariances among traits within males ( $\mathbf{G}_m$ ) separately from females ( $\mathbf{G}_f$ ), and the covariances of traits between the sexes ( $\mathbf{B}$ ) separately as well, resulting in  $\mathbf{G} = \begin{pmatrix} \mathbf{G}_m & \mathbf{B} \\ \mathbf{B}^T & \mathbf{G}_f \end{pmatrix}$  (Lande 1980b). Differences in the sex-specific matrices ( $\mathbf{G}_m$ ,  $\mathbf{G}_f$ ) indicate the presence of sex-specific quantitative-genetic architecture, which determines how correlated responses to selection are transmitted differently through the phenotype of each sex.  $\mathbf{B}$  reflects the degree to which traits expressed in each sex are coupled, and determines how selection on one sex will effect change in the other sex (Lande 1980b; Mead and Arnold 2004).

The sex-specific selection and gene expression that generate sexual dimorphism are expected to create differences in the underlying structures of male and female  $\mathbf{G}$  matrices through a variety of mechanisms, and the few studies that report  $\mathbf{G}_m$  and  $\mathbf{G}_f$  for sexually dimorphic homologous traits have found evidence for such differences (Hollaway et al. 1993; Guntrip et al. 1997; Arnold and Phillips 1999; Ashman 2003; Jensen et al. 2003; Rolff et al. 2005). Linkage disequilibrium with the sex-determining region on a sex chromosome promotes dimorphism, but autosomal or pseudo-autosomal genes that are expressed differently in the two sexes or act epistatically with respect to sex-determining regions can also contribute significantly to dimorphism (Rice 1984; Lande 1987). Sexual dimorphism is expected to arise from selection that acts on one sex only or unequally in the two sexes (Fisher 1958; Lande 1980b; Geber 1999). Selection can cause  $\mathbf{G}$  to change over time, both by changing allele frequencies (Shaw et al. 1995; Agrawal et al. 2001; Blows et al. 2004) and by generating linkage disequilibrium (Bulmer 1980). Therefore, sex-specific selection acting on sex-limited genes or loci interacting with sex-determining regions can result in differences in  $\mathbf{G}_m$  and  $\mathbf{G}_f$ . Pleiotropic mutations and drift can also have significant effects on the structure of  $\mathbf{G}$  (Lande 1980a; Roff 2000; Steppan et al. 2002; Jones et al. 2003; Mezey and Houle 2003), and when these forces are sex-specific, differences in  $\mathbf{G}_m$  and  $\mathbf{G}_f$  may increase.

The between-sex  $\mathbf{G}$  matrix,  $\mathbf{B}$ , includes diagonal elements that reflect between-sex covariance in homologous traits, and off-diagonal elements that reflect covariances between different traits (Lande 1980b; Meagher 1999). The same forces that generate differences between  $\mathbf{G}_m$  and  $\mathbf{G}_f$  may also cause asymmetry about the diagonal in  $\mathbf{B}$ . Asymmetry in  $\mathbf{B}$  indicates that a covariance between two traits depends upon which sex expresses which trait, and implies that the underlying genetic variance of the traits differs between the sexes. The genetic covariances between males and females for homologous traits (the diagonal elements of  $\mathbf{B}$ ) provide insight into the relative contribution of sex-limited genes to overall trait variation. Homologous traits whose variation is determined exclusively by sex-limited genes will have a between-sex correlation of 0. Conversely, if sex-limited gene expression contributes no variation to a trait, the between-sex correlation for a homologous trait will be 1, even if the trait is highly dimorphic (Lande 1987).

Once sex differences in the quantitative-genetic architecture of  $\mathbf{G}$  are established, they will impact the further evolution of dimorphism. Sex-specific selection can generate dimorphism in genetically correlated traits not under direct selection (Lande 1980b). If additive genetic variance for the trait under selection differs between the sexes and the between-sex genetic correlation is high, sexual dimorphism can still evolve even if selection pressures are equal in the two sexes (Cheverud et al. 1985). Thus, the multivariate sexual dimorphism we observe in most species likely represents the combined effects of direct sex-specific selection and correlated responses to selection determined by sex-specific quantitative-genetic architecture.

We investigated the sex-specific structure of genetic covariances in a population of the dioecious plant *Silene latifolia* (Caryophyllaceae). *Silene latifolia* displays sexual dimorphism in a number of quantitative characters, including morphological, physiological, and allocation traits (Delph and Meagher 1995; Carroll and Delph 1996; Laporte and Delph 1996; Delph et al. 2002). To investigate both the patterns of genetic covariance among traits within each sex and the extent to which genetic architecture differs between the sexes, we estimated  $\mathbf{G}$  (including  $\mathbf{G}_m$ ,  $\mathbf{G}_f$ , and  $\mathbf{B}$ ), the analogously partitioned phenotypic variance-covariance matrix ( $\mathbf{P}$ ), and associated correlations for both  $\mathbf{G}$  and  $\mathbf{P}$  for seven reproductive, morphological, and allocation traits in males and females. To examine patterns of similarity and difference between the sexes, we compared the within-sex matrices for  $\mathbf{P}$  and  $\mathbf{G}$ . We employed common principal-components analysis (CPCA) to detect differences in eigenstructure between the sexes, maximum-likelihood (ML)-based comparison to detect overall differences and the differences related to particular sets of traits, and element-by-element comparison of genetic correlation matrices to pinpoint the pairwise correlations that differed between the sexes.

## Materials and Methods

### STUDY SPECIES

*Silene latifolia* (Caryophyllaceae) is a dioecious, short-lived perennial native to Europe and introduced to much of North America (Gleason and Cronquist 1991). It produces fragrant flowers with white petals in a cymose inflorescence that are pollinated primarily by nocturnal moths (Young 2002). A nine-population study revealed that the sexes are dimorphic for floral and allocation traits (Delph et al. 2002). Flower number exhibited the most extreme degree of dimorphism out of the eight traits investigated, with males making many more flowers than females. In addition, the extent to which other traits were dimorphic varied with the phenotypic correlation of each trait with flower number. Furthermore, artificial selection on flower size resulted in a correlated response in flower number, indicating that a flower size/number trade-off was caused by an underlying negative genetic correlation between the two traits (Delph et al. 2004). In *S. latifolia*, sex is determined by sex chromosomes, and males are the heterogametic sex (Warmke 1946). An examination of gene expression in the sex chromosomes using a quantitative trait locus approach found that portions of the X and Y chromosomes recombine with each other, thus acting as pseudo-autosomal regions. Gene expression for traits that are sexually dimorphic is sex-specific within these regions (Scotti and Delph 2006).

### DATA COLLECTION

Seeds were collected from a wild population in Giles County, Virginia, during the summer of 2002. This population was located in an abandoned field and its adjacent roadside edges, and was composed of several hundred individuals. One seed capsule was collected from each of 103 plants, and seeds were germinated in an Indiana University greenhouse in the fall of 2002. Either a male or a female was chosen from each family to act as a parent in a crossing design that required a total of 50 mothers and 50 fathers. An exception was made for eight families, in which a parent of each sex contributed to the crosses to obtain an adequate number of parents; plants from the same family were not crossed to each other. These parents were mated using a modification of a cross-classified design (Lynch and Walsh 1998, p. 597) in which each mother was mated to a unique set of three fathers (and each father was therefore mated to three mothers) to give 150 full-sibling families nested within both maternal and paternal half-sibling relationships (Fig. 1). We used this cross-classified breeding design because it yielded individuals with a large number of half-sibling relationships across multiple parents. All of these relationships can be simultaneously included in the estimation of variance components using ML methods and a model based on the individual (rather than the family), thus yielding more precise variance component estimates than common alternatives (Shaw 1987; Lynch and Walsh 1998, p. 598; Kruuk 2004).

		Mother					
		1	2	3	4	5	6
Father	51						
	52						
	53						
	54						
	55						
	56						

**Figure 1.** Diagram of the crossing design used in this study. Shaded boxes represent crosses. Extending the diagram out to 50 mothers and 50 fathers would represent all of the crosses we conducted.

In the spring of 2003, seeds from the 150 families were planted in Metromix in celled trays at the Indiana University Botany Experimental Field greenhouse. When seedlings produced two sets of true leaves, we transplanted 10–14 per family into 5-in. clay pots filled with a 50:50 mix of Metromix (Scotts Horticultural Products, Marysville, OH) and potting soil and placed them randomly on benches in a large walk-in outdoor chamber covered by mesh. Both this insect-excluding mesh and adequate spacing among male and female plants prevented fruit set on females. We fertilized the plants approximately every two weeks with half-strength 20:20:20 Peter's solution (Scotts Horticultural Products). Plants were treated with pesticides for thrips and aphids on an as-needed basis.

All traits were measured on three or more female plants per family in all but six families, in which only two female plants flowered. Because the sex ratio was female biased in some families, we obtained data for only two males in 27 families, one male in 22 families, and for no males in 13 families. Three males were measured in all other families. We recorded the date and sex of the first flower opened. On the same day, we selected one leaf at the second node below the first flower and measured the length of this leaf with digital calipers to 0.1 mm. We measured the calyx length to the longest point and diameter at the widest point to 0.1 mm on the third to fifth flower produced. The sixth flower produced was collected the morning after it opened, dried to a constant mass in a 60°C drying oven, and weighed to an accuracy of 1 mg. We attempted to minimize the effect of within-plant variation and/or plant architecture on these measures by always

measuring flowers in the same position on each plant. Thirty days after a plant opened its first flower, we determined the total number of flowers produced by counting open flowers, flowers that would open that evening, and pedicels of flowers that had fallen off. We separated all flowers, buds, and leaves larger than 20-mm long from the stem, dried the stem and leaves to a constant mass in a 60°C drying oven, and weighed them to an accuracy of 0.001 g.

### ESTIMATION OF G AND P

All analyses used data from a total of 965 plants from 150 full-sibling families. To confirm the presence of sexual dimorphism in our study population, we compared traits between males and females using pooled-sample *t*-tests. We also calculated the coefficient of variation for each mean. Before estimating variance components, we ln-transformed all variables to minimize the correlation between the mean and the variance and to normalize distributions.

We estimated **G** (including **G<sub>m</sub>**, **G<sub>f</sub>**, and **B**) using an animal model and restricted maximum likelihood (REML), treating male and female values for characters as separate traits. REML in conjunction with the animal model, so called because the individual and not the family is the experimental unit, is a flexible and powerful approach to estimating variance components, and current computing power has increased its practicality (Kruuk 2004). Note that REML is not sensitive to unbalanced sample sizes within families or missing data (Lynch and Walsh 1998, p. 779), allowing us to include families with biased sex ratios in the analysis. In practice, this analytical approach is also relatively insensitive to departures from normality in the distribution of breeding values (though the breeding values in this study are normally distributed, see below) (Kruuk 2004). In general, the animal model is also capable of estimating variance components more precisely than analysis of variance (ANOVA)-based estimates of half-sibling designs because information from all individuals and relationships is included simultaneously (Kruuk 2004).

We estimated only additive genetic variance and covariance components. Although further partitioning into parental, dominance, and epistatic components is possible with REML (Shaw 1987), we limited the scope of our analysis to focus on possible gender differences in **G**. The REML approach also provides flexibility in partitioning variance caused by known environmental effects. Because we were aware that the position of plants in the mesh chamber potentially influenced some of the traits, we recorded which of the 48 benches the plants occupied and included bench as a fixed effect in the model to remove the variance caused by positional effects and increase our ability to detect and quantify genetic covariance. Thus, our mixed-effects animal model is:

$$\mathbf{y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}_1\boldsymbol{\alpha} + \mathbf{Z}_2\boldsymbol{\gamma} + \mathbf{e}$$

where **y** is the vector of phenotypic values, **X** is a design matrix

that relates the fixed effects to **y**, **β** is a vector of fixed effects (analogous to the true population mean in a univariate model), **Z<sub>1</sub>** is a design matrix that includes information on familial relationships and relates **α** to **y**, **α** is the matrix of additive genetic effects (a random effect in the model), **Z<sub>2</sub>** is the design matrix that relates **γ** to **y**, **γ** is the matrix of fixed effects due to bench, and **e** is the vector of residuals. For this analysis, we used the variance components estimation REML program (v. 5.1.2; Neumaier and Groeneveld 1998, available from <ftp://ftp.zgr.fal.de/pub>) to estimate variance components, additive genetic correlations between traits (**r<sub>G</sub>**), and heritabilities. In addition, we obtained correlations between traits for the variation not assigned to other factors. We refer to these as environmental correlations, although they may also include epistatic and dominance effects.

We used VCE to estimate standard errors (SEs) of heritabilities, genetic correlations, and environmental correlations. These SEs are sensitive to deviations from normality in the sampling distribution (Lynch and Walsh 1998, p. 812) and represent lower limits of the true SE (Wolf et al. 2000). To correct for these problems, we applied Chebyshev's theorem, under which we took  $\pm 4.5$  SEs to obtain a conservative estimate of the 95% confidence interval that is robust to deviations from normality (Lynch and Walsh 1998, p. 813). Estimates within the environmental and genetic correlation matrices (including heritabilities) with 95% confidence intervals not overlapping 0 were determined to be significantly greater than 0 at the  $P < 0.05$  level. In a similar fashion, we tested the between-sex genetic correlations for homologous traits against a null hypothesis of  $r = 1$  by determining whether the 95% confidence intervals overlapped 1. We estimated **P** and the phenotypic correlations, **r<sub>P</sub>**, for all pairs of traits. We conducted a general linear model analysis on each trait with bench as a random effect, saved the residuals from these analyses, and used them to calculate Pearson correlation coefficients between each pair of traits within each sex. To obtain between-sex phenotypic correlations, we generated family means of the residuals for each trait and then calculated Pearson correlation coefficients using the means. We tested whether each phenotypic correlation was significantly different from 0, using a sequential Bonferroni correction to adjust for simultaneous testing in all tests involving phenotypic correlations (Rice 1989).

### MATRIX COMPARISONS

Although several approaches to matrix comparison exist, no single approach provides a problem-free analysis. Therefore, we compared male and female phenotypic and genetic variance-covariance matrices using three approaches. First, we compared the within-sex variance-covariance matrices by using the common principal components software package to implement CPCA (Flury 1988; Phillips 1998). The benefit of CPCA is that it compares matrices within a hierarchical context of eigenstructure

(Phillips and Arnold 1999; Stepan et al. 2002). At the lowest level of the hierarchy, the matrices share no structural elements (unrelated). At the next lowest level, matrices share one principal component only (CPC(1)); the following levels each increase the number of principal components until the matrices share five of the seven principal components (CPC(5)). At the next level, the matrices share all principal components but do not have similar proportionality for these components (full CPC). At the penultimate level, matrices are proportional but differ in the overall amount of variation (proportionality); the final level is one of total similarity (equality). Thus, CPCA tests a spectrum of null hypotheses ranging from equality of matrices, to proportionality of matrices, to various levels of similarity in structure but not magnitude.

We compared the estimates of  $\mathbf{G}_m$  and  $\mathbf{G}_f$  and  $\mathbf{P}_m$  and  $\mathbf{P}_f$  generated by VCE in our CPCA analysis; however, CPCA does not account for the error inherent in these estimates, potentially inflating our chances of obtaining a significant result. We varied the order that the principal components were introduced into the analysis and used the most conservative result. Because CPCA assumes a normal distribution of variables, we used VCE to estimate breeding values (in the form of best linear unbiased predictors, or BLUPs) for each trait in all individuals. These estimates of breeding values were normally distributed in all cases. Although the true distribution of breeding values is not knowable and the estimates may tend toward normality due to the nature of the analysis, the estimates suggest that the assumptions of the CPC package for normality in both  $\mathbf{P}$  and  $\mathbf{G}$  are likely to be satisfied. The  $\mathbf{r}_P$  and  $\mathbf{r}_G$  matrices contained negative eigenvalues and therefore could not be compared with CPCA.

To select the best model of matrix similarity, we used the three approaches described in Phillips and Arnold (1999). First, we used the “jump-up” approach, in which each level of the hierarchy is compared against the null hypothesis of unrelated structure and the lowest level that significantly deviates from an unrelated structure is selected as the model. We also used the “step-up” approach, in which each level of the hierarchy is compared to the next level up and the lowest comparison that is statistically significant indicates the relationship between the two matrices. In the third approach, we used the Akaike information criterion (AIC) to determine the model that demonstrates the best goodness-of-fit given its number of parameters. This “model-building” approach suggested by Flury (1988) takes the model with the smallest AIC as the best fit. During preliminary analyses, we discovered that measurement scale had dramatic effects on the outcome of CPCA. To determine whether the observed differences in eigenstructure were caused only by differences in scale between variables in different matrices, we standardized each trait to a mean of 0 and a variance of 1 and used these values in estimating  $\mathbf{P}$  and  $\mathbf{G}$  as described above. We then compared these estimates of  $\mathbf{P}_m$  and  $\mathbf{P}_f$  and  $\mathbf{G}_m$  and  $\mathbf{G}_f$  using CPCA.

Our second approach to matrix comparison employed an element-by-element comparison of each pair of values in the gender-specific genetic correlation matrices to pinpoint the individual elements that differed between the two sexes. Element-by-element comparisons do not reveal differences or similarities in the multivariate structure of two matrices, but do identify the pairwise correlations that differ and therefore provide insight into the role of individual traits in shaping overall architectural differences. Correlations and heritabilities with nonoverlapping confidence intervals between the sexes were determined to be significantly different from one another.

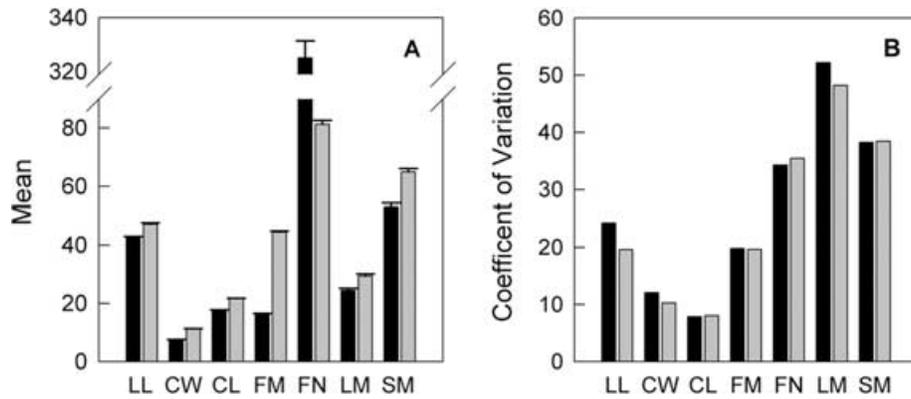
In our third approach to matrix comparison, we used the ML approach developed by Shaw (1991). In this method, the variance parameters of interest are specified in a model and estimated using REML, which maximizes the logarithm of the likelihood of the estimates based on the observed data. Then specific variance parameters are constrained to be identical, and the log-likelihood under this null hypothesis is obtained. Twice the difference in log-likelihood values is expected to be chi-square distributed, with the number of degrees of freedom determined by the number of constrained parameters. The ML method tests the null hypothesis that the two matrices are identical and, unlike the CPCA comparison, accounts for the error inherent in the data. However, power is generally low, decreasing the chance of identifying significant differences in the matrices (Shaw 1991).

We used the software package ASReml v. 2.0 (available from VSN International, Hemel Hempstead, United Kingdom) to conduct an ML-based comparison of  $\mathbf{G}_m$  and  $\mathbf{G}_f$ . We used a model with bench as a fixed effect and male and female additive-genetic (co)variance as separate random effects, and then fit the same model but constrained all the elements of  $\mathbf{G}_m$  and  $\mathbf{G}_f$  to be identical. We also tested two other null hypotheses by constraining subsets of variances and covariances rather than the entire matrix. We constrained all values associated with leaf traits (leaf length and leaf mass) to determine whether these relationships were driving differences in the matrices. We also constrained flower mass and flower number in another comparison to detect whether the most dimorphic traits were associated with differences in the matrices.

## Results

### SEXUAL DIMORPHISM

All of the morphological and allocation traits we measured were sexually dimorphic in this population of *S. latifolia* (Fig. 2A). Females produced longer leaves at the second node, larger flowers (in both dimension and mass), and a greater leaf and stem biomass than males. The only trait that was greater in males was also the most dimorphic, with males producing 4.0 times as many flowers as females. The second most dimorphic trait was flower mass: females produced flowers that were 2.7 times as heavy as male



**Figure 2.** (A) Trait means ( $\pm 1$  standard error [SE]) and (B) coefficients of variation for male and female leaf, floral, and biomass allocation traits in *Silene latifolia*. Black bars represent data from males and gray bars data from females. Within each trait, male and female means are significantly different based on a  $t$ -test and  $P < 0.001$ . LL, leaf length in mm; CW, calyx width in mm; CL, calyx length in mm; FM, flower mass in mg; FN, flower number; LM, leaf mass in cg; SM, stem mass in cg.

flowers. Despite differences in the means, the coefficients of variation for homologous traits were similar between sexes (Fig. 2B). Coefficients of variation were generally greater in flower number and allocation traits than in flower-size traits.

Heritable genetic variation was present for all of the traits we measured (Tables 1 and 2). The flower-size traits in both sexes generally had greater heritabilities than leaf length and flower num-

ber. Heritabilities tended to differ between males and females; leaf mass and stem mass, the two allocation traits, were more heritable in males, but calyx width was more heritable in females.

#### PATTERNS OF COVARIANCE

In males, all but one genetic correlation between traits were significantly different from 0, and 14 of 21 correlations were significant

**Table 1.** The male within-sex genetic variance–covariance matrix ( $G_m$ ) and associated heritabilities (on the diagonal) and genetic correlations (with 95% confidence intervals) for seven traits. Heritabilities and correlations with confidence intervals that do not overlap zero are in bold. The data were ln transformed prior to analysis.

	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass
Variance–covariance							
Leaf length	.01470	.00112	.00135	.00294	–.00600	–.00598	–.00448
Calyx width		.00550	.00156	.00544	–.00299	.00894	.00503
Calyx length			.00439	.00561	–.00617	–.00283	.00224
Flower mass				.01899	–.01880	–.00761	–.00673
Flower number					.03577	.01062	.02158
Leaf mass						.10871	.04564
Stem mass							.07227
Heritabilities and correlations							
Leaf length	<b>.274</b>	.125	<b>.168</b>	<b>.176</b>	<b>–.262</b>	<b>–.150</b>	<b>–.137</b>
	(.200, .349)	(–.006, .255)	(.074, .262)	(.061, .291)	(–.416, –.108)	(–.265, –.034)	(–.255, –.020)
Calyx width		<b>.389</b>	<b>.317</b>	<b>.532</b>	<b>–.213</b>	<b>.366</b>	<b>.252</b>
		(.309, .469)	(.225, .409)	(.432, .632)	(–.372, –.054)	(.254, .477)	(.135, .369)
Calyx length			<b>.759</b>	<b>.615</b>	<b>–.492</b>	<b>–.130</b>	<b>.126</b>
			(.687, .830)	(.516, .714)	(–.632, –.353)	(–.253, –.006)	(.004, .248)
Flower mass				<b>.492</b>	<b>–.721</b>	<b>–.167</b>	<b>–.182</b>
				(.410, .574)	(–.835, –.607)	(–.299, –.036)	(–.315, –.048)
Flower number					<b>.351</b>	<b>–.170</b>	<b>.424</b>
					(.261, .441)	(–.024, –.317)	(.290, .559)
Leaf mass						<b>.531</b>	<b>.515</b>
						(.447, .616)	(.395, .634)
Stem mass							<b>.668</b>
							(.576, .760)

**Table 2.** The female within-sex genetic variance–covariance matrix ( $G_f$ ) and associated heritabilities (on the diagonal) and genetic correlations (with 95% confidence intervals) for seven traits. Heritabilities and correlations with confidence intervals that do not overlap zero are in bold. The data were ln transformed prior to analysis.

	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass
Variance–covariance							
Leaf length	.01307	−.00111	.00299	.00012	.00793	−.00806	.00503
Calyx width		.00831	.00151	.00868	−.00659	.00146	.00273
Calyx length			.00463	.00625	−.00553	.00351	.00087
Flower mass				.01868	−.01733	.00748	−.00435
Flower number					.04573	−.01070	.01744
Leaf mass						.05371	.01004
Stem mass							.04140
Heritabilities and correlations							
Leaf length	<b>.385</b>	−.107	<b>.384</b>	.008	<b>.324</b>	− <b>.304</b>	<b>.216</b>
	(.302, .468)	(−.255, .042)	(.237, .530)	(−.160, .176)	(.142, .507)	(−.502, −.106)	(.010, .423)
Calyx width		<b>.806</b>	<b>.244</b>	<b>.697</b>	− <b>.338</b>	.069	.147
		(.741, .870)	(.117, .370)	(.595, .798)	(−.501, −.175)	(−.112, .250)	(−.041, .334)
Calyx length			<b>.791</b>	<b>.672</b>	− <b>.380</b>	<b>.223</b>	.063
			(.723, .860)	(.563, .781)	(−.543, −.217)	(.044, .402)	(−.126, .251)
Flower mass				<b>.528</b>	− <b>.593</b>	<b>.236</b>	−.157
				(.447, .610)	(−.743, −.443)	(.048, .424)	(−.352, .039)
Flower number					<b>.393</b>	− <b>.216</b>	<b>.401</b>
					(.302, .483)	(−.427, −.005)	(.201, .601)
Leaf mass						<b>.317</b>	.213
						(.235, .399)	(−.008, .434)
Stem mass							<b>.326</b>
							(.237, .415)

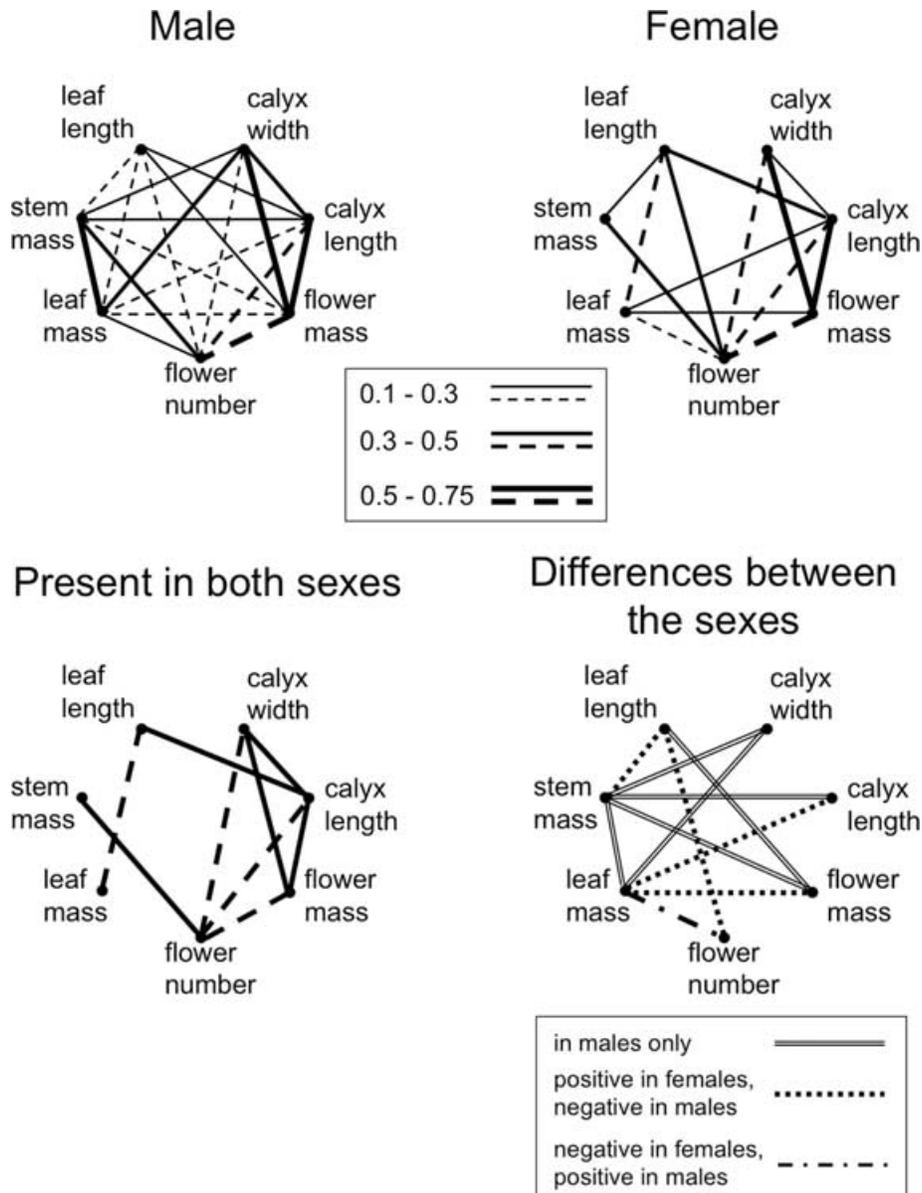
in females (Tables 1 and 2, Fig. 3). Within each sex, correlations among flower-size traits (calyx width, calyx length, and flower mass) and flower number tended to be strong, whereas correlations between leaf length and other traits, as well as between leaf mass and other traits, were somewhat weaker. Between the sexes, correlations among flower-size traits and flower number again tended to be large and were more often significant than correlations between other trait pairings (Table 3). All between-sex genetic correlations for homologous traits in males and females were significantly greater than 0 and significantly less than 1, and were highest for leaf length, calyx width, and calyx length (Table 3).

Phenotypic correlations were sometimes, but not always, similar to genetic correlations (Tables 4 and 5). Phenotypic correlations within flower-size traits were generally somewhat smaller than genetic correlations, but of the same sign. However, phenotypic correlations between flower number and flower-size traits were rarely significant (Tables 4 and 5) whereas genetic correlations between flower number and flower-size traits are significantly negative within and between the sexes (with the exception of male flower number and female calyx width, Tables 1–3). Positive environmental correlations offset the negative genetic correla-

tions between flower number and size, resulting in nonsignificant phenotypic correlations (Fig. 4). In addition, within each sex, the positive phenotypic correlation between stem mass and flower number was greater than the genetic correlation. Strong environmental correlations between flower number and stem mass explain the large phenotypic correlations between these traits (Fig. 4).

**COMPARISONS OF COVARIANCE STRUCTURE**

CPCA suggested that  $P_m$  and  $P_f$  shared some common principal-component structure, but  $G_m$  and  $G_f$  were not similar at all. Using the ln-transformed data, CPCA revealed that  $P_m$  and  $P_f$  shared two principal components in common, regardless of the method of significance testing (Table 6: jump-up;  $\chi^2 = 28.21, P = 0.020$ ). When standardized data (mean = 0, variance = 1 for all traits) were used,  $P_m$  and  $P_f$  were found to share all principal components (full CPC) but were not proportional (Table 7: jump-up;  $\chi^2 = 41.58, P = 0.036$ ). In contrast, CPCA based on the ln-transformed data showed that  $G_m$  and  $G_f$  shared no similar structure; all three significance tests found that the matrices had no principal components in common (Table 6: jump-up;  $\chi^2 = 38.73, P < 0.0001$ ). Analysis of the standardized data gave the same result (Table 7: jump-up;  $\chi^2 = 33.98, P < 0.0001$ ).



**Figure 3.** The statistically significant genetic correlations between traits within males and within females, and the similarities and differences between the sexes. In the top two and bottom left diagrams, solid lines represent positive correlations and dotted lines represent negative correlations. In the male and female diagrams, the thickness of the line indicates the magnitude of the correlation.

The element-by-element comparison of the male and female heritabilities and genetic correlations resulted in eight significant differences between the sexes (Fig. 5). Three heritabilities differed: leaf mass, stem mass, and calyx width. The five correlations that were significantly different involved differences in sign between the sexes; two correlations were associated with leaf length and three were associated with leaf mass (Figs. 3 and 5). Correlations between flower-size traits and flower number were large and similar between the sexes (Fig. 3).

The ML-based comparison of  $\mathbf{G}_m$  and  $\mathbf{G}_f$  also indicated that the matrices were significantly different from one another ( $l =$

46.92,  $df = 28$ ,  $P = 0.014$ ). When variances and covariances associated with leaf traits were constrained to be similar, the unconstrained model was also significantly different from the constrained model ( $l = 22.54$ ,  $df = 13$ ,  $P = 0.048$ ), thus rejecting the null hypothesis that the genetic variance and covariance associated with leaf traits are the same in the two sexes. When flower mass and number are the only constrained traits, the constrained model does not differ from the unconstrained model ( $l = 11.66$ ,  $df = 13$ ,  $P = 0.56$ ), suggesting that the genetic variance and covariance associated with these traits do not differ across the sexes.

**Table 3.** Between-sex genetic covariances (**B**) and correlations (with 95% confidence intervals) for seven homologous traits. Correlations with confidence intervals that do not overlap zero are in bold. The data were ln transformed prior to analysis.

	Male						
	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass
Covariances							
Female							
Leaf length	.01185	−.00188	.00104	−.00084	−.00127	−.01710	−.00275
Calyx width	.00225	.00546	.00190	.00503	−.00180	.00718	.00436
Calyx length	.00367	.00035	.00363	.00479	−.00774	−.00220	.00071
Flower mass	.00634	.00503	.00505	.01214	−.01365	.00627	−.00605
Flower number	.00001	−.00916	−.00741	−.01445	.02378	−.02393	.00569
Leaf mass	−.00167	.00378	.00137	.00956	−.00787	.05552	.02581
Stem mass	.00304	.00185	−.00163	.00222	.00278	−.00181	.03220
Correlations							
Female							
Leaf length	<b>.855</b>	<b>−.222</b>	.137	−.053	−.059	<b>−.454</b>	−.089
	(.784, .926)	(−.399, −.044)	(−.024, .298)	(−.228, .121)	(−.261, .144)	(−.611, −.296)	(−.268, .090)
Calyx width	<b>.204</b>	<b>.808</b>	<b>.315</b>	<b>.400</b>	−.104	<b>.239</b>	<b>.178</b>
	(.115, .293)	(.752, .863)	(.202, .427)	(.282, .519)	(−.263, .055)	(.111, .366)	(.046, .310)
Calyx length	<b>.445</b>	.070	<b>.806</b>	<b>.511</b>	<b>−.602</b>	−.098	.039
	(.360, .530)	(−.041, .181)	(.739, .873)	(.392, .630)	(−.734, −.469)	(−.241, .045)	(−.109, .186)
Flower mass	<b>.383</b>	<b>.496</b>	<b>.558</b>	<b>.645</b>	<b>−.528</b>	.139	<b>−.165</b>
	(.279, .487)	(.390, .602)	(.456, .660)	(.548, .741)	(−.656, −.400)	(−.004, .282)	(−.312, −.017)
Flower number	.001	<b>−.577</b>	<b>−.523</b>	<b>−.490</b>	<b>.588</b>	<b>−.339</b>	.099
	(−.148, .149)	(−.699, −.455)	(−.657, −.389)	(−.632, −.349)	(.434, .742)	(−.507, −.172)	(−.084, .282)
Leaf mass	−.059	<b>.220</b>	.089	<b>.299</b>	<b>−.180</b>	<b>.727</b>	<b>.414</b>
	(−.216, .097)	(.066, .374)	(−.069, .247)	(.148, .451)	(−.341, −.018)	(.596, .857)	(.236, .592)
Stem mass	.123	.123	−.121	.079	.072	−.027	<b>.589</b>
	(−.041, .288)	(−.042, .288)	(−.286, .044)	(−.088, .246)	(−.099, .244)	(−.197, .143)	(.427, .750)

### Discussion

Our investigation into sex-specific expression of floral, leaf, and allocation traits in *S. latifolia* revealed dimorphism in both the trait values and their patterns of genetic integration. All traits were heritable and genetically correlated with many other traits both within and between the sexes, suggesting genetic integration among all measured traits. In addition, sex-limited genetic variation was present for all traits, as demonstrated by between-sex correlations for homologous traits that were less than one.  $\mathbf{P}_m$  and  $\mathbf{P}_f$  matrices were somewhat similar in patterns of covariance, but the  $\mathbf{G}_m$  and  $\mathbf{G}_f$  matrices differed significantly. Although flower mass and number are the traits with the most dimorphic means, genetic correlations among them are similar in the two sexes. The major differences in  $\mathbf{G}_m$  and  $\mathbf{G}_f$  appear to be driven largely by relationships between leaf length and total leaf mass and other characters. The lack of shared structure between  $\mathbf{G}_m$  and  $\mathbf{G}_f$  suggests the presence of sex-limited pleiotropic effects and/or sex-limited linkage disequilibrium among loci influencing traits. The existence of sexually dimorphic genetic integration is

expected to generate sex differences in the response to further selection, and below we show how the observed patterns of within- and between-sex quantitative-genetic architecture could translate simple forces of selection into extensive sexual dimorphism in *S. latifolia*.

### PATTERNS OF COVARIANCE

We detected moderate to high heritability in all measured traits in both males and females. The three flower-size traits (calyx width, calyx length, and flower mass) were the least variable characters, but a relatively large amount of this variation was due to additive genetic rather than environmental factors. Flower number exhibited larger phenotypic variation and somewhat lower heritability than flower-size measures. Flower number may be strongly influenced by environmental as well as genetic factors, and flower number in *S. latifolia* has been shown to change in response to resource availability (Gehring and Linhart 1993; Meagher and Delph 2001). Leaf mass showed the greatest coefficient of variation as well as a relatively low heritability in females; nongenetic

**Table 4.** The within-sex phenotypic variance-covariance matrix ( $P_m$  and  $P_f$ ) and associated phenotypic correlations for seven homologous traits in males and females. Significant correlations, based on Pearson correlation coefficients and a sequential Bonferroni correction, are in bold.

	Male							Female						
	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass
Variance-covariance														
Leaf length	.05358	.00477	.00343	.00885	.00843	-.00300	.01231	.03395	.00123	.00349	.00227	.01405	-.00044	.01894
Calyx width		.01413	.00203	.00934	.00153	.01444	.00584	.01032	.00145	.00944	-.00360	-.00360	.00809	.00495
Calyx length			.00578	.00514	-.00099	-.00002	.00347		.00585	.00507	-.00015	-.00015	.00217	.00598
Flower mass				.03861	-.00697	-.01055	-.00617			.03535	-.01017	-.01017	.01017	.00168
Flower number					.10197	-.00439	.05950				.11650	-.01953	-.01953	.08162
Leaf mass						.20455	.03656					.16944	.16944	.00693
Stem mass							.10818							.12689
Correlations														
Leaf length		.166	<b>.210**</b>	<b>.217*</b>	.116	-.047	.152	.089	<b>.283**</b>	.086	<b>.224**</b>	.000	.000	<b>.297**</b>
Calyx width			<b>.256**</b>	<b>.415**</b>	.019	<b>.271**</b>	.171		<b>.183**</b>	<b>.491**</b>	-.096	<b>.198**</b>	<b>.149*</b>	
Calyx length				<b>.390**</b>	-.093	-.022	.168			<b>.371**</b>	-.031	.083	<b>.214**</b>	
Flower mass					-.120	-.085	-.044				<b>.161*</b>	.135	.041	
Flower number						-.053	<b>.530**</b>					-.152	<b>.666**</b>	
Leaf mass							<b>.235**</b>						.046	

\* $P = .05$ ; \*\* $P = .01$ .

**Table 5.** Between-sex phenotypic covariances and correlations for seven homologous traits. Significant correlations, based on Pearson correlation coefficients between family means and a sequential Bonferroni correction, are in bold.

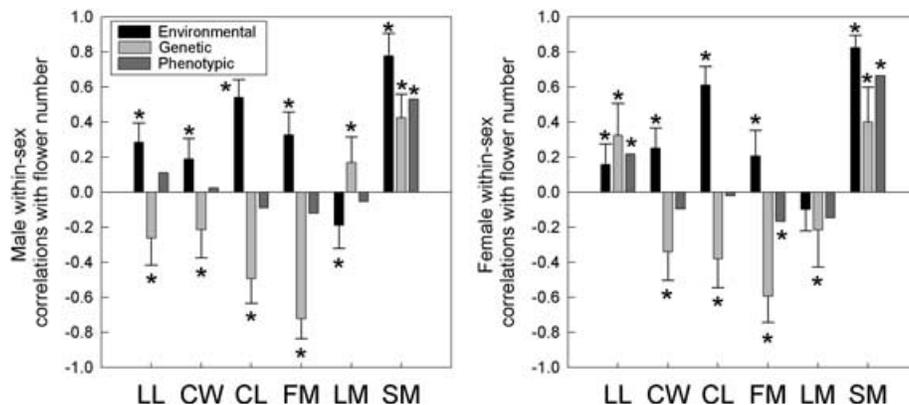
	Male						
	Leaf length	Calyx width	Calyx length	Flower mass	Flower number	Leaf mass	Stem mass
Covariances							
Female							
Leaf length	.01185	-.00188	.00104	-.00084	-.00127	-.01710	-.00275
Calyx width	.00225	.00546	.00190	.00503	-.00180	.00718	.00436
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Leaf mass	-.00167	.00378	.00137	.00956	-.00787	.05552	.02581
Stem mass	.00304	.00185	-.00163	.00222	.00278	-.00181	.03220
Correlations							
Female							
Leaf length	<b>.339*</b>	-.008	.121	-.041	-.038	-.117	-.043
Calyx width	.163	<b>.445**</b>	.250	.239	-.016	.155	.198
Calyx length	.153	.036	<b>.544**</b>	<b>.239*</b>	<b>-.240*</b>	-.012	.123
Flower mass	.243	.264	.353	<b>.333*</b>	-.183	.123	-.002
Flower number	.019	-.171	-.281	-.208	.237	-.132	.040
Leaf mass	-.005	.063	.010	.106	-.110	<b>.319*</b>	.150
Stem mass	.096	.106	-.039	.046	.111	-.015	<b>.344**</b>

\* $P = .05$ ; \*\* $P = .01$ .

effects, including variation in bolting time (Delph and Meagher 1995), may comprise a large proportion of the overall variation in leaf mass.

Flower number was the most dimorphic trait, with males producing four times as many flowers as females. In *S. latifolia*, selection for increased flower number in males might arise from both greater pollinator visitation to plants with more flowers (Shykoff and Bucheli 1995) and the fact that the number of pollen grains per flower does not vary with flower size, requiring an increase

in flower production to increase pollen production (Delph et al. 2004). Negative genetic correlations between flower number and flower size indicate that an increase in flower number must be accompanied by a reduction in flower size (see also Meagher 1992; Delph et al. 2004). This trade-off is most likely a result of limited resources being available for reproduction (Cohen and Dukas 1990; Morgan 1993; Sakai 1993). However, high variation in overall allocation to reproduction, either due to genetic variation in resource acquisition and distribution or environmental variation



**Figure 4.** Environmental, genetic, and phenotypic correlations with flower number within males and females. Error bars represent 4.5 SE from the estimated correlation. Environmental and genetic correlations whose 95% confidence intervals do not overlap zero and phenotypic values significantly different from zero at  $P < 0.05$  are marked with an asterisk. LL, leaf length; CW, calyx width; CL, calyx length; FM, flower mass; LM, leaf mass; SM, stem mass.

**Table 6.** Comparison of the male and female within-sex variance-covariance matrices for phenotypic and genetic estimates from seven traits in *Silene latifolia* (data were ln transformed). With the step-up approach, the higher model is compared against the lower with a  $\chi^2$  test within each row and the lowest order comparison that is significant determines the best-fitting model. The smallest Akaike information criterion (AIC) determines the best-fitting model in the model-building approach. Principal components were reordered in the analysis to give the most conservative results.

Hierarchy		$\chi^2$	df	P	AIC
Higher	Lower				
Phenotypic					
Equality	Proportionality	15.01	1	.0001	92.17
Proportionality	Full CPC	24.86	6	.0004	79.16
Full CPC	CPC(5)	.15	1	.6994	66.31
CPC(5)	CPC(4)	18.23	2	.0001	68.16
CPC(4)	CPC(3)	5.66	3	.1295	53.87
CPC(3)	CPC(2)	19.88	4	.0005	54.21
CPC(2)	CPC(1)	6.00	5	.3066	42.33
CPC(1)	Unrelated	2.33	6	.8870	46.33
Unrelated					56.00
Genetic					
Equality	Proportionality	32.63	1	<.0001	576.75
Proportionality	Full CPC	151.66	6	<.0001	546.12
Full CPC	CPC(5)	.26	1	.6080	406.46
CPC(5)	CPC(4)	225.22	2	<.0001	408.19
CPC(4)	CPC(3)	12.33	3	.0063	186.97
CPC(3)	CPC(2)	63.17	4	<.0001	180.64
CPC(2)	CPC(1)	52.74	5	<.0001	125.47
CPC(1)	Unrelated	38.73	6	<.0001	82.73
Unrelated					56.00

in the availability of resources, can obscure the trade-off between flower size and number in the phenotype (van Noordwijk and de Jong 1986; Houle 1991; Fenster and Carr 1997), and fewer than half of the studies on flower size and number have demonstrated a clear negative relationship between the two (Ashman 1999; Worley and Barrett 2000; Caruso 2004). In this study, we found significant environmental correlations between flower size and number, indicating the presence of nongenetic variation (or nonadditive genetic variation) that caused an increase in both flower size and number in some plants. Therefore, the overall phenotypic correlation between flower size and number was not significant in most cases because negative genetic correlations were counteracted by positive environmental correlations.

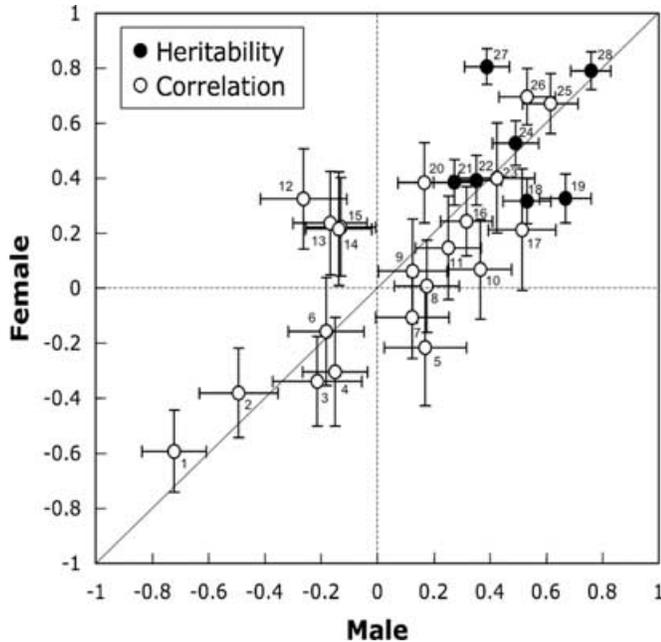
Although we prevented pollination and therefore fruit set on females in this study to minimize environmental variance, other studies in *S. latifolia* suggest that fruit set would not influence genetic correlations among traits. Unpollinated females have been shown to produce more flowers on average than pollinated fe-

**Table 7.** Comparison of the male and female within-sex variance-covariance matrices for phenotypic and genetic estimates from seven traits in *Silene latifolia* (data were standardized to a mean of 0 and a variance of 1). With the step-up approach, the higher model is compared against the lower with a  $\chi^2$  test within each row and the lowest order comparison that is significant determines the best-fitting model. The smallest Akaike information criterion (AIC) determines the best-fitting model in the model-building approach. Principal components were reordered in the analysis to give the most conservative results.

Hierarchy		$\chi^2$	df	P	AIC
Higher	Lower				
Phenotypic					
Equality	Proportionality	.46	1	.4967	42.04
Proportionality	Full CPC	15.15	6	.0191	43.58
Full CPC	CPC(5)	.88	1	.3489	40.44
CPC(5)	CPC(4)	2.95	2	.2293	41.56
CPC(4)	CPC(3)	5.08	3	.1662	42.61
CPC(3)	CPC(2)	9.47	4	.0505	43.54
CPC(2)	CPC(1)	3.57	5	.6133	42.07
CPC(1)	Unrelated	4.50	6	.6091	48.50
Unrelated					56.00
Genetic					
Equality	Proportionality	31.93	1	<.0001	745.16
Proportionality	Full CPC	404.00	6	<.0001	715.23
Full CPC	CPC(5)	1.38	1	.2407	323.23
CPC(5)	CPC(4)	4.41	2	.1104	323.85
CPC(4)	CPC(3)	9.07	3	.0284	323.45
CPC(3)	CPC(2)	136.19	4	<.0001	320.38
CPC(2)	CPC(1)	124.22	5	<.0001	192.19
CPC(1)	Unrelated	33.98	6	<.0001	77.98
Unrelated					56.00

males (Delph and Meagher 1995). However, pollination did not affect relative differences among traits in an artificial selection experiment on flower size (Delph et al. 2004). Therefore, although we expect pollination to affect trait means, we do not expect it to influence the genetic correlations among traits.

Most of the phenotypic correlations not involving the flower number and size trade-off were in the same direction as genetic correlations but were somewhat weaker, which follows the predictions and observations of Cheverud (1988; see also Waitt and Levin 1998). However, phenotypic correlations between stem mass and flower number were greater than genetic correlations in both males and females. Because each new flower is borne on a separate branch, inflorescence structure is most likely driving a high environmental and phenotypic correlation. Plants with more flowers must make more stems, regardless of genetic covariation between the traits. Furthermore, our comparison of **P** resulted in greater similarity between male and female matrices than our comparison of **G** did. Both this finding and the discrepancies in correlations



**Figure 5.** Female genetic correlations and heritabilities for seven traits plotted against the male genetic correlations and heritabilities for the same traits. Error bars are 4.5 SE. Correlations and heritabilities whose 95% confidence intervals do not overlap are marked with an asterisk. Point 1, flower mass/flower number; 2, calyx length/flower number; 3, calyx width/flower number; 4, leaf length/leaf mass; 5, flower number/leaf mass\*; 6, flower mass/stem mass; 7, leaf length/calyx width; 8, leaf length/flower mass; 9, calyx length/stem mass; 10, calyx width/leaf mass; 11, calyx width/stem mass; 12, leaf length/flower number\*; 13, flower mass/leaf mass\*; 14, leaf length/stem mass\*; 15, calyx length/leaf mass\*; 16, calyx width/calyx length; 17, leaf mass/stem mass; 18, leaf mass\*; 19, stem mass\*; 20, leaf length/calyx length; 21, leaf length; 22, flower number; 23, flower number/stem mass; 24, flower mass; 25, calyx length/flower mass; 26, calyx width/flower mass; 27, calyx width\*; 28, calyx length.

involving flower size suggest that  $\mathbf{P}$  would be a poor substitute for  $\mathbf{G}$  in this system; underlying genetic correlations would not always result in similar phenotypic correlations, and phenotypic correlations may not indicate an underlying genetic correlation.

The between-sex genetic correlations for all of the homologous traits were less than 1 but of moderate to high magnitude, indicating that some of the variation in traits is influenced by alleles with common effects in both sexes and some is determined by alleles with sex-limited effects. However, the fixation of advantageous sex-limited alleles will lead to traits that are sexually dimorphic, but lack sex-limited variation. This situation, in which the between-sex correlation of homologous traits approaches one, will constrain the further evolution of dimorphism because selection upon a trait in one sex will result in a highly correlated response in the homologous trait in the other sex (Lande 1987; Meagher 1999). Leaf length, calyx width, and calyx length all

had a between-sex genetic correlation above 0.80, suggesting a limited availability of independent genetic variation in each sex.

#### COMPARISONS OF COVARIANCE STRUCTURE

Both of the approaches that tested a null hypothesis of similarity found significant differences between the gender-specific  $\mathbf{G}$  matrices,  $\mathbf{G}_m$  and  $\mathbf{G}_f$ . CPCA showed significant differences in eigenstructure and the ML-based comparison also showed significant differences overall. The element-by-element comparison of the genetic correlation matrices and the ML-based tests suggested that the multivariate differences are largely attributable to deviations in covariances with the two leaf traits. The trade-off between flower size and number, as well as the genetic correlations among the flower-size measures, appear to be common to both sexes despite involving the most dimorphic characters. In contrast, genetic correlations with the two leaf traits differed between the sexes and appear to be driving gender-based differences in genetic architecture, although the magnitude of dimorphism was less for these traits.

Many studies that compare  $\mathbf{G}$  matrices have found some degree of conservation in structure over long periods of evolutionary time and across population- or species-level phenotypic divergence (Lofsvold 1986; Carr and Fenster 1994; reviewed in Steppan et al. 2002; Baker and Wilkinson 2003; Bégin and Roff 2003, 2004). Conversely, the studies that have explicitly compared  $\mathbf{G}_m$  and  $\mathbf{G}_f$  within species have not found concordance of structure between the sexes. Guntrip et al. (1997) found that dimorphism in body weight and development time in the cowpea weevil was accompanied by differences in within-sex covariance. Another beetle species, the two-spot ladybird, also showed dimorphism in life history and defense traits as well as significant differences in  $\mathbf{G}_m$  and  $\mathbf{G}_f$  (Holloway et al. 1993). Sexual dimorphism in scale counts in garter snakes is accompanied by shared principal-component structure but not proportionality of the  $\mathbf{G}_m$  and  $\mathbf{G}_f$  matrices (Arnold and Phillips 1999). Jensen et al. (2003) used CPCA to compare the  $\mathbf{G}_m$  and  $\mathbf{G}_f$  matrices for morphological traits in the house sparrow and found that the two matrices shared no structural elements in common. In Virginian wild strawberry, a gynodioecious plant species, Ashman (2003) used CPCA to show that female and hermaphrodite  $\mathbf{G}$  matrices for reproductive traits shared eigenvectors but were not proportional in each of three populations studied. Rolff et al. (2005) also used CPCA to show that in the mealworm beetle, male and female  $\mathbf{G}$  matrices of four immune defense and condition traits shared no principal components in common. While these studies vary somewhat in sample size, statistical approach in estimation of genetic parameters, and statistical approach in comparison of matrices, the results are generally consistent with our findings and together suggest that sexual dimorphism is typically accompanied by significant differences in quantitative-genetic architecture.

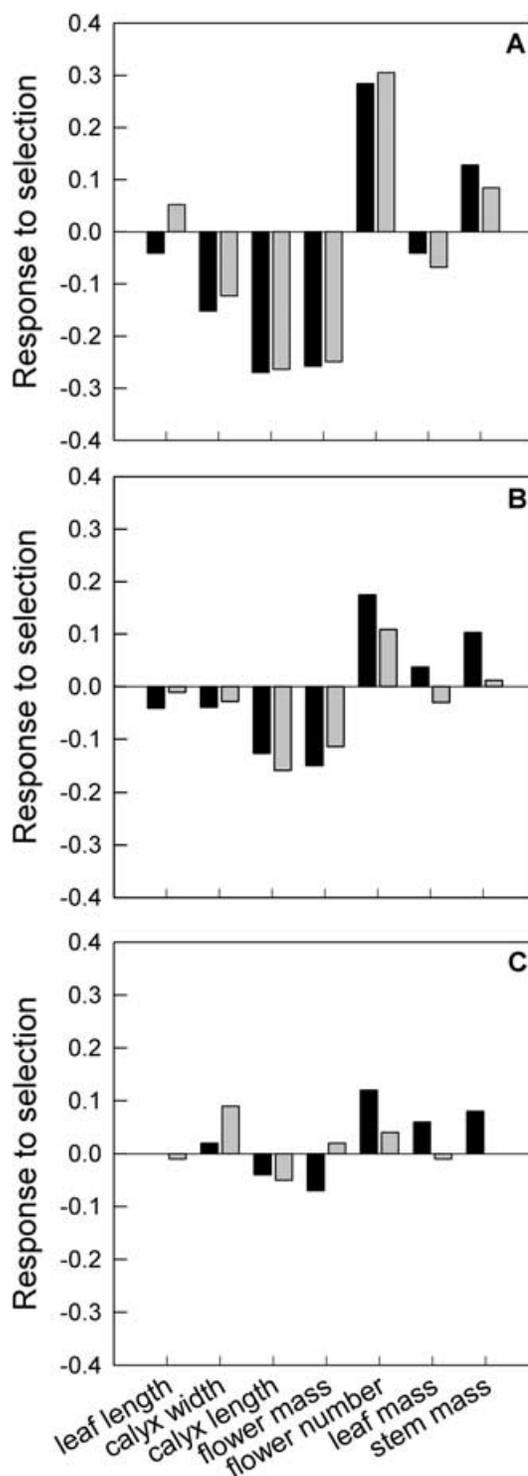
The dramatic differences in  $G_m$  and  $G_f$  indicate the presence of sex-limited genetic covariation and suggest that not only allelic expression but also patterns of pleiotropy differ between sexes. Additionally (or alternatively), linkage between sex-limited loci, possibly due to clustering around the sex-determining region, may contribute to dimorphism in genetic covariance structure. Combined with the observation that strong genetic correlations exist between the sexes for homologous traits, these results suggest that a complex pattern of shared and sex-limited gene expression influences the integration of floral and leaf traits in *S. latifolia*.

### CONSEQUENCES FOR THE EVOLUTION OF SEXUAL DIMORPHISM

The sex-specific partitioning of  $G$  into  $G_m$ ,  $G_f$ , and  $B$  reveals three potentially conflicting ways in which patterns of genetic integration might influence the evolution of sexual dimorphism. First, strong, positive correlations between homologous traits in each sex, as we find on the diagonal of the  $B$ -matrix for *S. latifolia*, will retard further dimorphism that might be favored by opposing selection on individual traits. Second, differences in the structure of the sex-specific matrices,  $G_m$  and  $G_f$ , will cause the transmission of correlated responses to selection to differ in each sex, even in the face of similar vectors of selection. In most cases, such dimorphism of quantitative-genetic architecture should contribute to increased sexual dimorphism and has the potential for sex-specific selection on one or a few traits to result in dimorphism in many aspects of the phenotype. Finally, genetic correlations among different traits across the sexes (the off-diagonal elements of  $B$ ) further link the phenotypic evolution of males and females. In general, asymmetries about the diagonal of  $B$  such as those reported in Table 3 will contribute positively to dimorphism if multivariate selection is similar in each sex.

The consequences of these axes of integration are difficult to predict without a complete picture of multivariate selection. Each of these effects might confound one another depending upon the precise pattern of genetic integration within and between sexes and traits and upon the multivariate pattern of selection. To explore the potential consequences of the observed patterns of genetic covariance on the evolution of sexual dimorphism in *S. latifolia*, we calculated the predicted response to selection under three simple scenarios of sex-specific selection (Fig. 6).

In the first case, selection is assumed not to differ between sexes and to target only an increase in flower number. Despite no difference in overall selection, we predict dimorphic evolution of leaf length in males and females, a trait not experiencing direct selection. This prediction is likely due to the differences in  $G_m$  and  $G_f$  with respect to leaf-length correlations. Other characters under this scenario evolve in the same direction; small differences in males and females may reflect slightly different rates of



**Figure 6.** Predicted response to selection for seven traits in males and females, as calculated with the multivariate breeder's equation using our estimate of  $G$  and three different selection vectors: (A) equal positive selection on flower number in males and females; (B) positive selection on flower number in males only; and (C) positive selection on flower number in males and positive selection of half the strength on flower mass in females. Black bars represent data from males and gray bars data from females. Responses to selection are given in standard deviations.

evolutionary change, but may also be a product of error in our estimations.

In the second case, we assume selection favoring greater flower number only in males (i.e., sexual selection for more elaborate mating displays only in males). Again we predict dimorphic evolution of a leaf trait, in this case leaf mass. Other responses to selection vary in magnitude between the sexes and are generally weaker than in the first scenario. The correlated responses in females reflect the strong genetic correlations between homologous traits as well as correlations among the off-diagonal elements of **B**.

The third pattern of selection is most similar to the pattern predicted to drive the sexual dimorphism observed in *S. latifolia*, where flower number in males experiences strong positive selection (via sexual selection) and flower mass in females experiences slightly weaker positive selection (via fecundity selection) (Delph et al. 2004). In this case, we see dimorphic evolution in several traits, including flower mass, leaf mass, and stem mass, and large differences in magnitude of response in flower number and calyx width.

These calculations show that if selection acts similarly in both sexes, we nevertheless predict a dimorphic change in traits, and when selection acts differently in the two sexes, dimorphism in the responses is even greater. The fundamental sexual dimorphism in genetic covariance structure observed in this study suggests that sex-limited pleiotropic effects and/or linkage will continue to translate selection on floral traits into sexual dimorphism throughout the phenotype.

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