

Genetics of sex allocation in *Mimulus* (Scrophulariaceae)

C. B. Fenster and D. E. Carr

*Department of Plant Biology, University of Maryland, College Park,
MD 20742-5815, USA, e-mail: cf25@umail.umd.edu*

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Abstract

Theoretical models of the evolution of resource allocation patterns to male and female function make the assumption that there are inherent trade-offs between the two. Here we use a quantitative genetic approach to quantify trade-offs between male and female function and to determine whether plant populations could readily respond to natural selection by quantifying the amount of genetic variation for pollen and ovule production. Both intra- and interspecific crossing designs were applied to two populations of the predominantly outcrossing *Mimulus guttatus* and two populations of the highly selfing congener, *M. micranthus*.

The only significant correlations observed among pollen number, pollen size and ovule number were positive. Positive genetic correlations among the traits were sometimes reduced after removing the effect of flower size but still no significant negative correlations were detected. These results suggest that positive correlations between pollen and ovule production may be due to the joint positive correlation of these characters with the resource pool available for pollen and ovule production, as reflected by flower size. Heritabilities were moderate to high for ovule production but low for pollen number and pollen size and suggest that responses to selection would differ between the two traits. Crosses between the species revealed that there are additional genetic factors contributing to differences between the two species for corolla width, vs. pollen:ovule ratio. This is consistent with the hypothesis that genetic variation for resource acquisition may in part be responsible for the overall lack of a negative correlation between pollen and ovule production and provides a genetic explanation for little evidence of trade-offs between sexual functions in *Mimulus*.

Introduction

Hermaphroditic plants transmit genes to their offspring through male and female function. Therefore differential investment of resources into the production of either pollen or ovules will have important consequences for the plant's total fitness. Among the factors influencing the consequence of varying resource allocation are the correlated responses to selection that may occur in male and female reproductive functions. In response to the frequent observation of the seemingly maladaptive loss of male function and female function in the evolution of gynodioecy and dioecy, respectively, Darwin (1877) proposed a "law of compensation"; reduced investment into one reproductive function may be compensated by the greater resource availability for the other reproductive function.

Darwin's concept of compensation or trade-off between competing functions is fundamental to current models of resource allocation and gender evolution (e.g., Charnov et al., 1976; Charnov, 1982; Charlesworth and Charlesworth, 1987; Morgan, 1992), but empirical data demonstrating the existence of these trade-offs in hermaphroditic plants is equivocal (Goldman and Willson, 1986; Charlesworth and Morgan, 1991; Brunet, 1992). Investigators have examined the empirical question of trade-offs in a number of different ways including the use of quantitative genetic approaches (Charlesworth and Morgan, 1991; Partridge and Sibly, 1991). The quantitative genetic demonstration of trade-offs are negative genetic correlations between traits competing for resources from a limited pool which are ultimately due to pleiotropic gene action (Lande, 1982; Reznik, 1985; Charlesworth, 1990).

Houle (1991) has challenged the expectation of negative genetic correlations between fitness components. His model assumes that phenotypic variation in two fitness components is generated by two independent sets of loci that affect either acquisition of resources for a common pool used by the two components or allocation of resources from that pool to the two traits. While loci affecting allocation will promote negative covariances between the fitness components, loci affecting acquisition will promote positive covariances. Therefore, measurements of the covariance between fitness components should take into account the covariance that can be produced by variation in the size of the available resource pool. In addition, estimates of the number of loci affecting allocation and acquisition are needed for understanding the structure of the covariance between male and female function.

A plant's mating system should affect the relationship between fitness and investment in male reproductive function. Pollen production is expected to decrease as the selfing rate increases resulting in lower pollen-ovule ratios (Charnov, 1979; Charlesworth and Charlesworth, 1981). Most studies that have compared populations or related taxa that differ in their selfing rates have supported these predictions (Morgan and Barrett, 1989; reviewed by Brunet, 1992). The genus *Mimulus* is one such example. Ritland and Ritland (1989) examined pollen-ovule ratios in 8 species and found that pollen-ovule ratios decreased with increasing selfing rates. However, absolute pollen and ovule production by these species appears at odds

with Darwin's "law of compensation". Pollen and ovule numbers are highly positively correlated across *Mimulus* species (Ritland and Ritland, 1989), indicating that as pollen production is reduced in the more selfing taxa there is a concurrent reduction in ovule production.

This study will test Darwin's "law of compensation" by estimating the genetic correlation between male and female traits within flowers of two species of hermaphroditic plants that differ in their mating systems, the highly selfing *Mimulus micranthus* and the more outcrossing *M. guttatus*. Variation and covariation in pollen and ovule production (as measures of male and female reproductive investment, respectively), and corolla width (as a measure of overall flower size and index of the size of the resource pool available for pollen and ovule production) will be assessed. The specific questions asked in this study are: 1) Is there heritable variation for pollen and ovule production and corolla width upon which natural selection can act? 2) How many loci are involved in the differentiation of these two species with respect to pollen and ovule production, pollen-ovule ratio, and corolla size? 3) Is there a trade-off between pollen and ovule production as measured as a negative genetic correlation among these characters? and 4) Is the trade-off or genetic correlation between pollen number and ovule number determined by the size of the resource pool? Because genetic correlations derived from population level analyses may have only short term predictive evolutionary value of trade-offs (Partridge and Sibly, 1991), we also examined the genetic correlations determined from the cross between *M. micranthus* and *M. guttatus*. The pattern of segregation of corolla size, pollen number and ovule number in the cross between the two species should allow exploration of a larger character space (because of the segregation of alleles at many more loci or loci with large effect) compared to the restricted options set of more limited genetic variation at the within population level. Thus we hypothesize that genetic correlations from the between species crosses provide a better opportunity to quantify constraints or trade-offs between pollen and ovule allocation patterns than correlations determined from within population crosses.

Material and methods

Study organisms and sites

Mimulus guttatus DC (Scrophulariaceae) is an annual to perennial herb widely distributed in western North America with moderate to high levels of outcrossing ($t = 0.6-0.9$, Ritland, 1990). *Mimulus micranthus* Heller is strictly annual and is restricted to the coastal range of central California (Munz and Keck, 1968) and is highly selfing ($t = 0.16$, Ritland and Ritland, 1989). Both species are found in moist open habitats such as stream edges and ephemeral pools. Seed families of *M. guttatus* were collected from three populations, two annual: S ($n = 23$ families) and T (approximately 10 k from S, $n = 25$ families) both located in Tuolumne Co., California; and one very large-flowered perennial population: 118 ($n = 12$ families),

Marin Co., California. Population T consisted of approximately 100 flowering individuals, population S numbered in the tens of thousands, and population 118 consisted of several hundred ramets. Seed families of *M. micranthus* were collected from two populations: 301 ($n = 11$ families, Lake Co.), and 305 ($n = 6$ families, Mendicino Co.). Populations S and T of *M. guttatus* and 301 and 305 of *M. micranthus* were used in the population level studies and populations 118 and 301 of *M. guttatus* and *M. micranthus*, respectively, were used in the between species cross. Precise localities are provided in appendix 1 of Fenster and Ritland (1992).

Quantifying the size of the resource pool

We chose corolla width as an index of the resource pool available for pollen and ovule production for several reasons. First, there is an allometric basis for considering corolla width to be an indicator of the amount of resources allocated to both pollen and ovule production. *Mimulus* corolla width demonstrates both positive phenotypic and genetic correlations to other floral characters (Macnair and Cumbes, 1989; Carr and Fenster, 1994; Fenster and Ritland, 1994a). Previous studies documented a positive correlation of corolla size with pollen and ovule production (Ritland and Ritland, 1989; Macnair and Cumbes, 1990; Robertson et al., 1994; Mossop et al., 1994). Hence, corolla width is a good indicator of flower size in *Mimulus*. Also, corolla width is an easily measured character with high repeatability (Fenster and Ritland, 1994b). Third, floral size has been previously used as a measure of the resource pool to determine if sex allocation trade-offs exist (O'Neil and Schmitt, 1993). Fourth, flower size is sensitive to resource availability. The size of later blooming flowers is reduced more on plants producing seed than on plants not producing seed in *M. guttatus* (Macnair and Cumbes, 1990; Mossop et al., 1994), as well as other taxa (e.g., *Solanum hirtum*, Diggle, 1991). Finally, flower size in *M. guttatus* is correlated to water availability such that ramets grown under drought conditions produce smaller flowers with less pollen than genetically identical ramets grown under less stressful conditions (Galloway, unpublished data).

Within population crosses

Crosses and data collection

The following experiments were conducted in the greenhouses at the University of Maryland, College Park to quantify genetic variation and covariation for pollen and ovule production at the population level. Our parental generation for *M. guttatus* was produced from field-collected seed. We sowed approximately 30 field-collected seeds from each of the maternal families from the S and T populations into 68 mm square plastic pots and allowed them to germinate under soil-saturated conditions with ambient daylight for two weeks. Transplants were grown in the same sized pots and watering conditions, but natural photoperiod was

extended to 18 hrs with high intensity sodium-vapor lights. We created 23 and 25 full sib families for the S and T populations, respectively, by reciprocally crossing randomly selected pairs of parents.

We sowed 30 seeds from the 46 and 50 maternal families produced for the S and T populations, respectively, in the same manner as described above. Five seedlings were randomly chosen from each maternal family, providing a total of 10 offspring for each of the 23 and 25 crosses ($n = 230$ and 250 progeny for S and T populations, respectively). Offspring were grown in a randomized block design, with a total of five blocks. One progeny per maternal family was grown in each block, resulting in two members of each full-sib family per block. The S and T offspring generations were grown one month apart, otherwise conditions were identical for both populations.

In both the parental and offspring generations, we measured the width of the corolla to the nearest 0.1 mm using digital calipers as an indication of overall organ size. We measured the first four flowers for the parental and offspring generations for population T and the offspring generations of population S. Only the first flower was measured for the parental generation of population S.

Pistils were collected from the first two flowers produced, preserved in a 3:1 ethanol:glacial acetic acid solution, and stored in microcentrifuge tubes. Ovule number for both the between species and within population crosses was determined by randomly dissecting one of the two symmetrical locules from the ovary from each of the two collected pistils. The ovules from each locule were spread into a microscope slide, stained with lactophenol with 0.1% aniline blue, and counted under a dissecting microscope. To determine pollen production, all four anthers were collected from both the third and fourth flower buds prior to anther dehiscence. The 8 anthers were air-dried for one week in an open microcentrifuge tube before storage. Pollen production and modal pollen size was determined using an Elzone 280PC particle counter. Dehisced pollen from the 8 anthers was suspended in 15 ml of 2% saline and assayed. A mean was calculated from three replicate 0.5 ml subsamples.

To quantify genetic variation for the same characters in *M. micranthus* we examined among family variation for 11 and 6 families from populations 301 and 305, respectively. Families from population 301 and 305 had been maintained in environmental growth chambers for one and three generations, respectively. We sowed approximately 30 seeds from each *M. micranthus* family and grew one randomly selected seedling per family in each of five randomized blocks. Culturing conditions were the same as described above for *M. guttatus*. We took corolla width measurements on the first four flowers. Pistil and anther samples were collected and quantified as described for *M. guttatus*.

Estimation of genetic variance

We estimated narrow-sense heritabilities of corolla width, pollen number, pollen size, and ovule number for the two *M. guttatus* populations by computing both single and mid-parent offspring regressions (Falconer, 1981). For each individual plant, we calculated mean corolla width, mean pollen production, mean pollen size

and mean number of ovules per locule. Single parent offspring regressions were calculated based on the means of the 46 or 50 sire full-sib or maternal full-sib offspring and multiplying the slopes of the regressions by two to estimate the heritabilities. Mid-parent regressions were calculated based on the means of the 23 or 25 full-sib families. The standard error of the heritability based on single parent- and mid-offspring regression is twice the standard error and the standard error of the regression, respectively (Falconer, 1981).

For comparative purposes with *M. micranthus* we also calculated broad-sense heritabilities for *M. guttatus* based on the among family full-sib variance in the offspring generations (23 and 25 full-sib families in S and T populations, respectively). Among family variation in each of the two populations of *M. guttatus* and *M. micranthus* was estimated using 2-way ANOVA with block and family as random effects. The family \times block interaction was tested and found to be not significant and consequently was pooled with the error term. Variance components were estimated using Proc VARCOMP with the restrained maximum likelihood (REML) option (SAS, 1989). Confidence limits lower than 0 or greater than 1 were set arbitrarily at 0 and 1, respectively. For *M. micranthus* the intraclass correlation was not multiplied by two since sibs in a selfer are likely to share many of their genes in common. This is analogous to estimates of broad sense heritabilities based on within vs. between clonal variance (Falconer, 1981). Broad sense heritabilities estimated with and without the among-block variance component in the estimate of total phenotypic variation did not differ appreciably and only the latter are reported.

To determine whether populations and species would respond proportionally to selection on these traits, we transformed additive genetic variation, V_A , and total genetic variation, V_G , to a dimensionless scale by dividing the standard deviation of each by their trait means (Charlesworth, 1984; Houle, 1991). This resulted in coefficients of variation for additive (CV_A) and total genetic (CV_G) variation ($CV_A = 100(V_A^{0.5}/\bar{X})$ and $CV_G = 100(V_G^{0.5}/\bar{X})$).

Phenotypic and genetic correlations

Phenotypic correlations among the characters were determined by calculating the mean measurement for each individual and measuring the covariance among traits across all individuals in a population. Correlations were determined for the offspring generation for the two *M. guttatus* populations.

Genetic correlations among the characters were quantified using several methods. First, for all four *Mimulus* populations, Pearson correlations among the floral characters were calculated using family means. These correlations represent "broad sense" genetic correlations with maternal and non-additive genetic components contributing to the correlation. Secondly, genetic correlations based on the segregation of genes with additive effect were estimated in *M. guttatus* by using covariances derived from the parent-offspring regressions in the S and T populations. We used the mean cross-covariances for each pair of traits (Becker, 1975):

$$r_A = (\text{cov } x_1z_2 + \text{cov } x_2z_1) / (2\sqrt{\text{cov } x_1z_1 \text{ cov } x_2z_2})$$

where x_1 is character x measured on the parent, z_2 is character z measured on the offspring, x_2 is character x measured on the offspring, and z_1 is character z measured on the parent. Henceforth we refer to these as the additive genetic correlations. The 95% confidence intervals of the additive genetic correlations were estimated by calculating the standard errors (Falconer, 1981) and multiplying them by the appropriate t -value. The correlations were considered to differ significantly from zero if the confidence interval did not include zero.

The above correlations included the effect of flower size. To factor out the joint correlation of pollen size, pollen number and ovule number with corolla size we conducted partial correlation analysis on the correlations based on the family means. We used a different approach for the correlations based on the parent-offspring covariance because generation of the standard errors are not as straight forward as those based on the correlation among family means which are Pearson product moment correlations. For these "narrow sense" or additive genetic correlations, we ran separate regressions of pollen size, pollen number and ovule number on corolla width and then calculated the genetic correlations among the residuals. These approaches are similar to strategies others have used to factor out the effect of the resource pool on the correlation between major fitness characters, e.g., multiple regression to factor out the effect of size of female on the relationship between number and size of young in the lizard *Gerrhonotus coeruleus* (Stewart, 1979), PCA to remove the effect of flower size on the correlation between pollen production and ovule number in *Lythrum salicaria* and *M. guttatus*, where a number of floral size characters were quantified (O'Neill and Schmitt, 1993; Robertson, Diaz and Macnair, 1994, respectively), and the same residual analysis that we use to factor out flower size as a determinant in the correlation between pollen and ovule number in *M. guttatus* (Fig. 1 of Mossop, Macnair and Robertson, 1994). The 95% confidence intervals of the additive genetic correlations were estimated by calculating the standard errors (Falconer, 1981) and multiplying them by the appropriate t -value. The correlations were considered to differ significantly from zero if the confidence interval did not include zero.

Between species crosses

Crosses and data collection

To estimate the minimum number of genes responsible for differences in pollen and ovule production between *M. micranthus* and *M. guttatus* reciprocal crosses were conducted between *M. micranthus* (301) and *M. guttatus* (118). The covariance between pollen and ovules in the segregating F_2 was also used to quantify the degree of pleiotropy for loci responsible for differences across the species. Authenticity of F_1 hybrids was verified by heterozygotes at isozyme loci fixed for alternative alleles in the parents. F_2 progeny were produced by intercrossing F_1 individuals. *Mimulus guttatus* parental stocks were maintained through the F_2 by random crossing of individuals and *M. micranthus* parental stocks were maintained by collecting selfed seed.

Parental ($n = 36$ and 51 for *M. micranthus* and *M. guttatus*, respectively), F_1 ($n = 94$), and F_2 ($n = 278$) generations were grown simultaneously in 68 mm plastic pots, at 18 C/14 C day/night with 18 hr days in a growth chamber at the University of Toronto.

Floral measurements to determine corolla width and ovule number followed the methods used in the within population crosses. To determine pollen number per flower on the same flower that the other floral measurements were taken, anthers were removed and stored in 0.5 ml lactophenol solution with 0.1% aniline blue. Later, the anthers were finely chopped, spun on a vortex mixer and the pollen counted on a hemacytometer grid. Two replicates per individual sample were counted and their average value was used in the analyses. Pollen size was estimated by measuring the diameters of six of the counted pollen using an ocular micrometer under $400\times$ magnification and their average values were used in subsequent analyses.

Gene number

From the means and variances of parental and segregating generations the minimum number of factors (gene loci) differentiating the two taxa was estimated with a derivation of the procedure of Wright (1968) and Lande (1981) with its correction (Cockerham, 1986). A model for the estimation of gene number incorporating estimates of dominance ($N_{E(d)}$) based upon the segregating generation (as opposed to the F_1) was used (Fenster and Ritland, 1994a). Characters were left untransformed.

Genetic correlations

Genetic correlations (equivalently, the proportion of genetic factors in common between a pair of traits) were estimated as the segregation covariance in the F_2 , divided by the geometric mean of the segregational variance of the two traits (Humphreys and Nicholls, 1984). We calculated the genetic correlation here as for the within population crosses: first calculating the genetic correlations without excluding the effects of flower size, then calculating the genetic correlations by factoring out corolla size by conducting separate regressions of pollen size, pollen number and ovule number on corolla width and then determining the genetic correlations among the residuals.

Error estimation

Formula for the variance of estimates derived by Taylor series approximations, such as those given for gene number by Lande (1981) and for genetic correlations by Falconer (1981), substantially underestimate the actual variance (Zeng et al., 1990). Thus we used the bootstrap method (Efron and Gong, 1983; Fenster and Ritland, 1994a) to estimate error. In this study, we based 95% confidence intervals for gene number and genetic correlations upon the 2.5 and 97.5 percentiles of the distribution of 1000 bootstrap estimates.

Results

Within population levels of variation

Mimulus guttatus produces three-fold larger flowers, six-fold more pollen per flower, 20% larger pollen grains, and 30% more ovules compared to *M. micranthus* (Tab. 1). There are significant differences between the two *M. guttatus* populations for all traits. Only corolla width and ovule number differed between the two *M. micranthus* populations. Many of the characters also differed between the parental and offspring generations for each of the two *M. guttatus* populations, indicating the extent to which they are influenced by environmental conditions. The variances must be the same for both parent and offspring otherwise estimates of heritabilities may be biased (Falconer, 1981). Only the variances for pollen size differed significantly across generations ($F_{45,219} = 3.6$ and $F_{49,249} = 2.9$, $p < 0.001$, parent vs. offspring for S and T populations, respectively) and may have biased the estimates of their heritabilities downwards.

The narrow-sense heritabilities for all characters in population S were generally equal to or greater than population T (Tab. 2). The regressions between dam and offspring were similar to the regressions based on sires for all traits except for the pollen characters in the S population. Narrow-sense heritabilities for pollen number and pollen size based on sire regression were not significantly different from zero. There was significant among-family variation and hence broad sense heritabilities for all characters for the two *M. guttatus* populations (Tab. 3). In contrast, among family variation was observed only for ovule number in the 301 population of *M. micranthus*. The coefficients of genetic variability were greatest in the S population for all traits except pollen size (Tab. 4). There is little genetic variation for pollen

Table 1. Phenotypic means (and standard deviations) calculated across all individuals from populations S and T of *Mimulus guttatus* (parental and offspring generations) and populations 301 and 305 of *M. micranthus*. Means followed by different letters are significantly different (Tukey's, $p < 0.05$).

Population (generation)	Corolla width (mm)	Pollen per flower	Pollen size (μ)	Ovules per flower
S (parental)	24.2 b (4.7)	15 403.8 b (9 748.4)	26.3 a (3.2)	1 020.3 b (384.3)
S (offspring)	20.6 c (4.4)	20 127.7 b (8 665.3)	27.3 a (1.9)	673.8 c (326.5)
T (parental)	22.9 bc (3.6)	18 541.4 b (9 361.2)	27.0 a (2.1)	1 219.6 ab (368.1)
T (offspring)	27.6 a (3.9)	34 192.1 a (9 500.2)	27.5 a (1.1)	1 348.6 a (348.6)
301	6.4 e (0.9)	2 740.2 c (1 173.6)	22.1 b (1.1)	683.1 c (186.5)
305	8.2 d (1.1)	4 239.8 c (1 954.6)	21.9 b (0.6)	989.6 b (269.2)

Table 2. Narrow-sense heritabilities (and standard errors) of pollen and ovule characters based on dam, sire, and midparent regressions for the S and T populations of *Mimulus guttatus*.

Character	Population S			Population T		
	Dam	Sire	Mid-parent	Dam	Sire	Mid-parent
Corolla width	0.313 (0.184)	0.442 (0.180)	0.376 (0.165)	0.296 (0.132)	0.0 (0.148)	0.157 (0.138)
Pollen #	0.392* (0.154)	0.240 (0.158)	0.322** (0.105)	0.143 (0.175)	0.146 (0.171)	0.259 (0.147)
Pollen size	0.048** (0.010)	0.0 (0.010)	0.0 (0.067)	0.089** (0.018)	0.0 (0.008)	0.032 (0.078)
Ovule #	0.468** (0.170)	0.384* (0.172)	0.483** (0.131)	0.309* (0.124)	0.416** (0.129)	0.402** (0.119)

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

size in either species and no observable genetic variation for pollen number in *M. micranthus*. *Mimulus micranthus* and the T population of *M. guttatus* have equivalent amounts of genetic variation for the remaining characters (Tab. 4).

Genetic basis of between species differences

The same relative differences among floral character between *M. guttatus* and *M. micranthus* were observed in the experiment to quantify the genetic differences among species and are not presented here. Many loci contribute to the differences between the two species for corolla width and pollen number (Tab. 5). Lower

Table 3. Broad-sense heritabilities (and standard errors) for pollen and ovule characters for two populations of *M. guttatus* and two populations of *M. micranthus*. Standard errors could not be calculated for heritabilities of zero.

Character	<i>M. guttatus</i>		<i>M. micranthus</i>	
	S	T	301	305
Corolla width	0.800*** (0.149)	0.280*** (0.075)	0.294 (0.139)	0.196 (0.200)
Pollen #	0.390** (0.112)	0.384** (0.142)	0.0	0.0
Pollen size	0.200** (0.118)	0.396** (0.143)	0.068 (0.134)	0.060 (0.209)
Ovule #	0.574** (0.167)	0.293** (0.125)	0.473*** (0.023)	0.390 (0.241)

** $p < 0.01$, *** $p < 0.001$.

Table 4. Coefficients of variation for additive (CV_A) and total genetic (CV_G) components of variation for two populations of *M. guttatus* and two populations of *M. micranthus* (CV_G only).

Trait	<i>M. guttatus</i>				<i>M. micranthus</i>	
	S	T	S	T	301	305
	CV_A	CV_A	CV_G	CV_G	CV_G	CV_G
Corolla width	12.96	0.0	17.44	8.55	5.34	3.34
Pollen number	21.09	10.62	26.89	17.22	0.0	0.0
Pollen size	0.0	0.0	3.11	2.52	1.30	6.71
Ovule number	30.03	16.67	36.71	13.99	18.78	16.99

estimates of the number of loci are associated with pollen size and pollen:ovule ratio. A major gene may be responsible for differences in ovule number.

Phenotypic and genetic correlations

We did not observe any significant negative phenotypic correlations among the traits. Corolla width is significantly positively phenotypically correlated with ovule number and only weakly correlated with pollen number and size in both *M. guttatus* populations. Pollen number is correlated with pollen size but only weakly correlated with ovule number in both *M. guttatus* populations. Fewer significant correlations were observed in the two *M. micranthus* populations (Tab. 6). Ovule production was strongly correlated with corolla width in population 301 and pollen production was strongly correlated with ovule production in population 305.

We limit presentation of genetic correlations to traits which demonstrated significant genetic variation. Thus we report only genetic correlations among corolla size, pollen number, pollen size and ovule number for the S and T populations based on variation among family means, and among corolla size, pollen number and ovule number for the S population based on the parent-offspring covariance. All traits demonstrated significantly increased variances in the F_2

Table 5. Minimum estimate of number of loci differentiating *Mimulus micranthus* from *M. guttatus* for acquisition of resources (corolla width) and allocation of resources (pollen/ovule number) to male and female function. Lowest-bound for 95% confidence intervals are provided for all traits except ovule number where 95% upper-bound estimate is provided.

Trait	Minimum number of loci
Corolla width	8.7 (6.8)
Pollen number	13.5 (7.1)
Pollen size	4.0 (2.8)
Ovule number	1.0 (1.8)
Pollen:ovule ratio	3.0 (1.7)

Table 6. Phenotypic and genetic correlations among reproductive characters for two populations of *Mimulus guttatus*, two populations of *M. micranthus*, and for the F_2 of the cross between the two species. Full-sib correlations are based on the correlations among full-sib maternal family means of the offspring generation and the additive genetic correlations are based on the parent-offspring covariance. The genetic correlations derived from the F_2 reflect the covariation of traits due to the segregation of genes and factors out the correlation due to environmental effects (95% C.I. are presented below the correlation). The partial or residual genetic correlations factor out the joint correlation of the traits with corolla size. Genetic correlations are only presented for the *M. guttatus* populations because only these populations demonstrated genetic variation for the reproductive traits.

	Corolla width pollen number	Corolla width pollen size	Corolla width ovule number	Pollen number pollen size	Pollen number ovule number	Pollen size ovule number
Phenotypic						
<i>M. guttatus</i> S	0.22	-0.02	0.42**	0.35*	0.23	0.03
<i>M. guttatus</i> T	0.06	0.08	0.34**	0.22*	-0.02	0.13
<i>M. micranthus</i> 301	0.07	-0.13	0.67***	-0.18	-0.06	-0.20
<i>M. micranthus</i> 305	0.40	0.25	0.14	-0.17	0.53**	-0.04
Genetic full-sib						
<i>M. guttatus</i> S	0.72***	-0.01	0.91***	-0.08	0.68***	0.07
<i>M. guttatus</i> T	0.02	0.40*	0.68**	-0.32	0.38	0.23
Partial genetic full-sib						
<i>M. guttatus</i> S	effect removed	effect removed	effect removed	-0.19	0.09	0.19
<i>M. guttatus</i> T	effect removed	effect removed	effect removed	0.36+	0.50**	-0.06
Additive genetic covariance						
<i>M. guttatus</i> S	0.44	-	0.95***	-	0.28	-
Residual additive genetic covariance						
<i>M. guttatus</i> S	effect removed	effect removed	effect removed	-	-0.267	-
Genetic segregation covariance						
<i>M. guttatus</i> (118) × <i>M. micranthus</i> (301)	0.46 (-0.38-1.00)	-1.80 (-5.00-0.50)	-0.02 (-0.89-0.60)	0.38 (-0.04-1.18)	1.71 (0.67-4.33)	1.54 (0.00-3.74)
Residual genetic segregation covariance						
<i>M. guttatus</i> (118) × <i>M. micranthus</i> (301)	effect removed	effect removed	effect removed	0.13 (-0.37-1.20)	0.11 (-1.00-1.28)	0.01 (-0.80-0.87)

+ 0.10 > p > 0.05, * p < 0.05, ** p < 0.01, *** p < 0.001.

generation compared to the F_1 of the cross between *M. guttatus* and *M. micranthus* and thus were considered to have significant heritabilities in this cross.

No significant negative genetic correlations were observed among the traits. Only significant positive or weak positive or weak negative correlations were quantified for the associations among corolla width, pollen number, pollen size and ovule number. Strong positive genetic correlations were observed for corolla width with both pollen number and ovule number in population S based on the correlation among family means (Tab. 6). Population T demonstrated significant positive correlations of corolla width with both pollen size and ovule number based on the correlation among family means. The genetic correlation between ovule and pollen number was higher and only significant in the S population compared to the T population. The correlation between pollen and ovule production increased in the T population when the joint correlation of pollen and ovule number with corolla size was removed by partial correlation analysis (Tab. 6). In contrast, the correlation between pollen number and ovule number was lowered in the S population after removing the effect of corolla width. Pollen size was marginally positively correlated with pollen number in the T population when the joint correlation with corolla size was removed using partial correlations (Tab. 6). Using the covariance between parent and offspring to estimate the genetic correlations for traits varying because of the segregation of genes with additive effect, we observed positive correlations of pollen number and ovule number with corolla size in the S population, 0.44 and 0.95, respectively, but only the latter correlation was significantly greater than 0 (Tab. 6). The covariance between parent and offspring resulted in an estimate of the correlation between pollen and ovule number of 0.283 in the S population but this was not significantly different from 0 (95% CI = $-0.296-0.668$). After removing covariation due to a joint correlation with corolla width by correlating the residuals of pollen number regressed on corolla width with the residuals of ovule number regressed on corolla width, the correlation between pollen and ovules in the S population was lowered to -0.267 . This correlation also did not differ significantly from 0 or from the estimate prior to removing variation due to corolla width (95% CI = $-0.79-0.252$).

Based on the segregation covariance in the F_2 of the cross between *M. micranthus* and *M. guttatus*, corolla width was positively correlated with pollen number (0.46, 95% CI = $-0.38-1.00$), negatively correlated to pollen size (-1.80 , 95% CI = $-5.00-0.5$) and uncorrelated with ovule number (-0.02 , 95% CI = $-0.89-0.60$) (Tab. 6). However, the confidence intervals around the correlations were large and none of these correlations differed significantly from 0. Ovule number and pollen number had a significantly positive correlation (1.71, 95% CI = $0.67-4.33$) and it is likely that the correlations of pollen number and ovule number with pollen size are positive since the confidence intervals just barely overlapped with 0 (0.38, 95% CI = $-0.04-1.18$ and 1.54, 95% CI = $0.00-3.74$, respectively). All three correlations involving pollen number, pollen size and ovule number were lowered when their joint correlation with corolla width was removed. The correlation of 1) pollen number with pollen size was lowered to 0.13 (95% CI = $-0.37-1.20$), 2) pollen size with ovule number was lowered to 0.01 (95% CI = $-0.80-0.87$) and 3) pollen

number with ovule number was lowered to 0.11 ($-1.00-1.28$) (Tab. 6). The correlation of pollen number with ovule number was significantly lowered after removing the effects of their joint correlation with corolla size since the 95% CI of each estimate was nonoverlapping.

Discussion

Levels of heritable variation for pollen and ovule production

Considerable phenotypic variation was observed for all measured traits for the four *Mimulus* populations examined. However, the degree to which this variation was genetically determined differed between male and female traits and among species. In contrast to female traits (ovule number), male traits (pollen number and pollen size) generally had lower heritable variation. Lower heritabilities were observed in selfing *M. micranthus* versus mixed-mating *M. guttatus*. Differences in heritable variation among the traits and between species does not appear to be solely a function of greater environmental sensitivity (Houle, 1991) since the coefficients of genetic variation are similarly low for pollen number and size compared to ovule number and are generally lower in *M. micranthus* compared to *M. guttatus*.

Lower levels of genetic variation for the pollen characters indicate that a response to selection would be slower for these androecial characters compared to ovule number (Falconer, 1981; Lande and Arnold, 1983). There is limited data on natural populations with which to compare our results. Mazer (1992) and Mazer and Schick (1991) observed large environmental effects on the estimations of heritability of pollen number and volume and ovule number in *Raphanus raphanistrum*. Significant paternal effects were found for pooled estimates across environments for pollen number/flower, and ovule number at intermediate density. No variation among sires was observed for pollen volume. Heritabilities were generally higher for pistil mass than stamen mass in two populations of *Lythrum salicaria* (O'Neil and Schmitt, 1993). In an independent study of a different *M. guttatus* population, Robertson et al. (1994) and Mossop et al. (1994) observed additive genetic variation for ovule number and pollen viability but none for pollen number. In contrast, Carr and Fenster (1994) and Robertson et al. (1994) observed considerable genetic variation for other floral characters such as flower size, degree of spatial separation between stigma and stamens, and autofertility in *M. guttatus*. Similar patterns were observed for *Raphanus spp.* (Mazer and Schick, 1991; Mazer 1992; Conner and Via, 1993) and *L. salicaria* (O'Neil and Schmitt, 1993). This overall lack of genetic variation for pollen production in the few hermaphroditic populations sampled is limited evidence that consistent natural selection may be acting to reduce genetic variation for this trait to levels beyond the power of our experiments to detect (Fisher, 1958; Falconer, 1981).

Greatly reduced genetic variation in *M. micranthus* relative to *M. guttatus* has also been observed for molecular markers (Fenster and Ritland, 1992) and other floral traits (Carr and Fenster, 1994). Many factors may be responsible for the

relative amounts of genetic variation maintained in these two species with contrasting mating systems, including differences in the relative role of founding events and bottlenecks (Hillel, Feldman and Simchen, 1973; Schoen and Brown, 1991) and mating system (Charlesworth and Charlesworth, 1995).

Number of loci differentiating species of mimulus for pollen and ovule production

Despite the present lack of significant heritable variation for pollen number in *Mimulus* populations, both pollen and ovule production have apparently undergone evolution. The between species crosses suggest that the reduction of pollen number and size in *M. micranthus* is due to the fixation of alternative alleles at many loci. This is especially so for pollen number. Our estimator of gene number has several assumptions which potentially bias the number downwards (Wright, 1968; Mather and Jinks, 1982; Zeng et al., 1990). Thus, gene number estimates provided here are minimum estimates and only provide information on the number of linkage groups segregating alleles which are responsible for trait differentiation across the taxa. The large number of gene differences for pollen number correspond to the results of Fenster and Ritland (1994a) and Macnair and Cumbes (1989) who observed many genes responsible for differentiation in floral size and levels of herkogamy in the crosses of *M. guttatus* with two small flowered selfers, *M. micranthus* and *M. cupriphilus*, respectively.

Given the heritable variation for ovule number in our *Mimulus* populations, the single gene estimate for differences in ovule was unexpected. Taking the estimate at face-value, the results suggest that differences in ovule production between the selfing and outcrossing taxa reflect genetic differences that are unrelated to variation observed at the population level. However, our estimate may underestimate the number of genes if the loci affecting ovule production are linked. Further crosses, to the F_3 and beyond, would allow the contribution of linkage to be quantified (Mather and Jinks, 1982). In addition, mapping QTL with molecular markers (e.g., maize, Doebly and Steck, 1991) would help to resolve the issue of a single gene versus many linked genes.

Trade-off between pollen and ovule production

Models of sex allocation which describe the conditions for gender evolution, in particular the conditions which favor dioecy or hermaphroditism, assume a trade-off between pollen and ovule production (Charnov et al., 1976; Charlesworth and Charlesworth, 1981; Lloyd, 1988). Plants which invest less in pollen are expected to have more resources available for ovule production and *vice versa*. However, the observed genetic correlation between two traits is the weighted average of the number of genes contributing to positive and negative correlations (Mather and Jinks, 1982; Cheverud, 1984). Thus a net overall positive genetic correlation will exist between pollen and ovule production if there is more genetic variation affecting acquisition than allocation of resources. Similar arguments have been put

forth to explain observed positive correlations among life-history traits when negative ones were expected (van Noordwijk and de Jong, 1986; Charlesworth, 1990; Houle, 1991; de Jong and van Noordwijk, 1992).

We did not observe any evidence for a trade-off between allocation to male and female function within a flower at either the within population or between species level. The only significant correlations observed among pollen number, pollen size and ovule number were positive. Even when we factored out the positive correlation of these traits with corolla width (our measure of the total amount of resources available for sexual reproduction) the correlations between male and female function either remained positive or were weakened but were never significantly negative.

We estimated that the number of loci contributing to differences between *M. micranthus* and *M. guttatus* in the resources available (as measured by corolla width) to pollen and ovule production were roughly three times greater than the number affecting allocation patterns (pollen:ovule ratio). These results provide a partial genetic explanation for the observation of a positive genetic correlation between male and female function since the relative number of loci affecting acquisition vs. allocation of resources can dictate whether compensation through negative genetic correlations will occur (Houle, 1991). We expect that genes affecting pollen and ovule production will have negative pleiotropic effects but that genes affecting flower size and overall amount of resources available to male and female function will have positive pleiotropic effects on pollen and ovule production. However, the relationship between corolla width and pollen and ovule production was not uniform among populations or between correlations measured at the within vs. between species level. For example, corolla width was positively correlated with pollen number in the S population and in the between species cross but not in the T population. Ovule number was positively correlated with corolla width in both S and T populations but not in the between species cross. These results indicate that the relationship of resource availability with pollen and ovule production may be complex or that corolla width may not be a valid indicator for resources available for pollen and ovule production in all populations of *Mimulus*.

The occurrence of trade-offs via pleiotropic gene action are expected to be most pronounced when either physiological or functional constraints are operating (Partridge and Sibly, 1991). Differences among *Mimulus* populations with respect to their genetic correlation structure also provide evidence that trade-offs between pollen and ovule production are not inherent in *Mimulus*. Rather, the correlations among the traits may reflect linkage disequilibrium generated via selection or drift and not pleiotropic gene action. Additional evidence is provided by the contrast between the two species. *Mimulus micranthus* maintains similar levels of genetic variation for ovule number but has much reduced variation for pollen number and size compared to *M. guttatus*. We can only conclude that different sets of loci are responsible for ovule and pollen production.

The absence of significant negative genetic correlations among pollen and ovule traits is concordant with other studies examining genetic control of allocation patterns to male and female function in *M. guttatus* (Mossop et al., 1994; Robertson et al., 1994), *Begonia semiovata* (Ågren and Schemske, 1995), *L. salicaria*

(O'Neil and Schmitt, 1993), and *R. sativus* (Mazer and Schick, 1991; Mazer, 1992). Several other studies have reported that per flower production of both pollen and ovules is reduced in selfers compared to related outcrossing taxa, suggesting a positive correlation between the two traits, e.g. *Eichhornia* (Barrett, 1985) and unreported taxa (Ornduff, 1969). However, both Lloyd (1965) and Wyatt (1984) reported no consistent differences in ovule production between outcrossing and selfing taxa of *Leavenworthia* and *Arenaria*, respectively, although pollen production was lower in the selfers.

It should be emphasized that we examined trade-offs between male and female function at only one level. Our estimate of female function included only ovule number and excluded seed production. Thus a more general result may have been generated if we had examined the trade-off between seed production and pollen production. At the phenotypic level, fruit production has been shown to decrease subsequent pollen production in a number of hermaphroditic species (e.g., Silvertown, 1987; Stanton et al., 1987) including *M. guttatus* (Macnair and Cumbes, 1990). Similarly, trade-offs between pollen and fruit production are suggested by reports that females have higher seed-set than hermaphrodites in gynodioecious species (e.g., Kohn, 1989; Delph, 1990; Eckhart, 1992; Ashman, 1994). The only studies that have documented a genetic trade-off between male and female function have done so by demonstrating a negative genetic correlation between pollen production and seed-set (Rameau and Gouyon, 1991; Atlan et al., 1992; Garnier et al., 1993; but see Meagher, 1992, 1994). However, post anthesis investment into developing seeds likely affects both ovule and pollen production, as is indeed observed in *M. guttatus* (Macnair and Cumbes, 1990). Thus it is not clear whether trade-offs between pollen production and seed-set actually represent allocation trade-offs between male and female function since seed production also influences future female function.

Evolutionary consequences

One might argue that the lack of negative correlation observed between pollen and ovule production within populations might be an artifact of limited within population genetic variation for pollen production. However, the segregating F_2 of the cross between *M. micranthus* and *M. guttatus* contained ample genetic variation for both traits. The genetic correlations measured in the between species cross do not necessarily provide information on how the traits may have evolved in any given population in the evolution of *M. micranthus* from *M. guttatus*. However, they might be expected to demonstrate the dominant correlations that underlay the evolution of the two species. If so, then there appears to be a considerable constraint to gender specialization in *Mimulus*. The loss of one sexual function is unlikely to result in increased compensation in the other. The large reduction of both ovule and pollen production with the evolution of selfing in *M. micranthus* may reflect a reduction in resources available for flower production.

An important question then is why has *M. micranthus* evolved limited resources available for sexual reproduction. Flower size is positively genetically correlated to

date of first flower in some populations of *M. guttatus* (Carr and Fenster, 1994) and flowers of *M. micranthus* develop at both a faster rate and shorter period than flowers of *M. guttatus* (Fenster et al., 1995). Furthermore, in *M. guttatus*, selfing ensures early fertilization of ovules under field conditions compared to outcrossing flowers which may require several days of pollinator visits for full seed-set (Dudash and Ritland, 1991). Thus the evolutionary changes reported here may reflect selection for rapid development in *M. micranthus* and hence limited resources available to both male and female function in this selfing species.

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