

Within-population variation in sexual resource allocation in *Mimulus guttatus*

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Summary

1. The theory of sexual resource allocation in plants assumes (a) that trade-offs occur between the resources allocated to male, female and attractive structures, and (b) that genetic variation occurs, or has occurred, within populations upon which natural selection could act to optimize the relative investment in each structure. These assumptions were tested in a study of 10 clones of *Mimulus guttatus* isolated from a natural population, which varied in the proportion of viable pollen they produced. The experiment was designed to investigate whether plants which invest less in viable pollen can reallocate the resources released to other functions within the same or subsequent flowers or fruits.

2. This study has failed to find any significant difference in other floral characters associated with the variation in pollen quality. Plants producing less pollen do not produce bigger or more numerous flowers, do not invest more in female function (ovaries or fruits), and do not differ in the rate at which flowers and floral components decline in size as the plants age.

3. These results suggest therefore that trade-offs between male and female function do not occur, or at least are not inevitable. There is evidence for a trade-off between primary sexual characters and attractive structures, and between the size of flowers and the rate at which flowers declined in size with time. This indicates that early investment in large flowers may be costly.

4. Pollen–ovule ratios decline with time and size of flower. Most of the floral characters studied were significantly correlated with flower size, but even when this correlation was removed by analysis of covariance, significant genetic variation could still be detected for most characters. This indicates that selection could act to alter the relative contribution of the different components of the flower if it should be advantageous.

Key-words: Genetic correlations, heritability, pollen–ovule ratios, resource allocation, trade-offs

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Introduction

There is considerable interest in the problem of the optimal resource allocation strategy for a hermaphrodite plant between male, female and attractive structures (Charlesworth & Morgan 1991; Brunet 1992; Morgan 1992). There have been a number of theoretical treatments which have determined the optimal allocation strategy between female, male and attractive structures depending on various ecological factors (Charlesworth & Charlesworth 1987; Lloyd 1987; Morgan 1992). All of these models make, *inter alia*, two assumptions.

1. That resources are limiting, so that extra investment in one function must be at the expense of investment in another. Thus there should be a trade-off between the various functions that should be manifested in a negative genetic correlation between investment in different structures.

2. There is, or has been, genetic variance in allocation patterns upon which natural selection can act to optimize investment pattern.

As Charlesworth & Morgan (1991) note, there have been few studies in which these assumptions have been verified empirically in hermaphrodites. Goldman & Willson (1986) review the empirical evidence for trade-offs and show that there are very few relevant data in hermaphrodites, although there are some studies in monoecious or gynodioecious species that suggest that trade-offs occur (Goldman & Willson

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1986; Kohn 1989; Charlesworth & Morgan 1991). Rameau & Gouyon (1991), studying the hermaphrodite plant *Gladiolus*, were able to detect trade-offs between male investment in pollen and both vegetative characters and female functions.

There are more data comparing the allocation of resources between functions in populations or species of varying ecology (for reviews, see Cruden & Lyon 1985; Goldman & Willson 1986; Brunet 1992). In particular, self-fertilization has been associated with a shift from male and attractive structures to female function (Brunet 1992). In *Mimulus*, Macnair & Cumbes (1990) found some evidence of between-population variation within *M. guttatus*, and Ritland & Ritland (1989) found considerable variation in pollen-ovule ratios and allocation to corolla mass between species within the *M. guttatus* group. However, to interpret these between-population or species comparisons requires knowledge of the pattern of variation within populations.

This paper describes an experiment in which a number of clones of *M. guttatus* Fischer ex DC were compared for their allocations to attractive structures (corollas), male structures (anthers) and female structures (ovaries and capsules).

Materials and methods

PROVENANCE OF THE PLANTS

All clones were originally grown from wild-collected seed from the Copperopolis population of *M. guttatus* (Macnair, Macnair & Martin 1989). This population is extremely variable in life-history strategies (H. R. McCombie, personal communication), selfing rates in the absence of pollinators (A. Diaz, personal communication) and percentage of viable pollen produced by plants (Martin 1988). This latter character is highly heritable (Robertson, Diaz & Macnair 1994) and the primary purpose of the experiment described here was to test the hypothesis that plants that invest less in pollen (because they produce a high proportion of inviable, empty pollen grains) will have more resources available to devote to flower or fruit production. Therefore 10 clones were selected from a sample of about 100 that spanned the range of variation from about 30% viable pollen to 100%. The plants were selected on this character alone: variation in other floristic characters can be assumed to be random, unless in linkage disequilibrium with pollen viability.

EXPERIMENTAL DESIGN

Cuttings were taken of the 10 clones and potted in 8.75-cm plastic pots in coco-fibre compost. They were kept under 8-h days under a blackout until well established: this prevents flower initiation. Six ramets of each clone, matched as far as possible for size and

condition, were then transferred to long days (16 h) in the glasshouse. Natural daylight was supplemented with sodium lighting. Before flowering, three were assigned at random to treatment A and three to B. All clones were fully randomized. Each plant was allowed to form two flowering stems (exceptionally, a few had one or three). The plants were inspected every day and all flowers opening on that day recorded. All corollas were removed from flowers on the day of flowering or the day after, and their length and width measured. As the anthers are attached to the corolla in this species, this also destaminates the flowers. Anthers from a sample of all flowers were weighed to 0.01 mg. Anther mass, anther size and pollen number are all highly correlated (Ritland & Ritland 1989; A. W. Robertson, unpublished data). The corollas from the same flowers were dried and weighed. All flowers of plants in treatment A were pollinated on the day of destamination using pooled pollen from a set of clones from the Copperopolis population (it included ramets of the experimental clones, but others as well). Eighteen days later, when the seeds were ripe but the capsules had not dehisced, the capsules were collected and their dry weight determined. The remainder of the flower (i.e. calyx, ovary and pistil), after the corolla had been removed from plants in treatment B, was collected and fixed in 3:1 ethanol:acetic acid. The ovary length and width were measured on a sample of these preserved flowers, using a dissecting microscope with a graticule eyepiece. Units are graticule units. The statistic length \times width (ovary size) is highly correlated with ovule number (Ritland & Ritland 1989; Robertson *et al.* 1993). The flowers selected to weigh anthers or corollas, and to measure the size of ovaries, were chosen so as to span the whole flowering period (approximately every fourth flower for the former and sixth for the latter). In total the 60 plants produced 3676 flowers over a 71-day period. All stems were allowed to finish flowering naturally.

POLLEN VIABILITY

The pollen from a number of flowers from each plant was collected and pooled, and a random sample was stained with aceto-carmin and counted (see Robertson *et al.* 1994). Viability is here defined as the proportion stained. Inviability pollen grains appear to have little or no cytoplasm, and this character has been shown to be heritable (Robertson *et al.* 1994) and highly repeatable both within and between ramets (unpublished data).

ANALYSIS

All statistical analyses were performed using MINITAB (release 7.1). The experimental unit is the individual plant, so all comparisons of treatments or genotypes

used the between-ramet variance as the error. The between-clone variance component is an estimate of V_g , the overall genetic variance between clones. The within-clone variance component is an estimate of V_e . Broad sense heritabilities were calculated as $V_g/(V_g + V_e)$. Technically, the between-clones variance could also be inflated by any carry-over environmental effects from the parent plants from which the cuttings were taken: the long period of growth following cloning (more than 10 weeks before plants flowered) and the small size of the original cuttings, will minimize this contribution to V_g . The genetic correlations were calculated following the methods of Falconer (1989).

Results

As flowering progresses, flowers in this species get progressively smaller (Macnair & Cumbes 1990) and are produced at a slower rate. The sizes of all the floral parts are correlated, both phenotypically and genetically (Macnair & Cumbes 1989, 1990); as the flowers get smaller, so do corollas, ovaries, anthers and capsules. These relationships were confirmed in all plants in this experiment (data not shown). Macnair & Cumbes (1990) used corolla mass as a measure of flower size. In this experiment it is estimated as the product of the corolla length \times width (measured for every flower): this statistic is highly correlated with corolla mass ($r=0.828$, $P<0.001$).

Trade-off between functions can occur in a number of different ways. Suppose that plants producing less viable pollen have as a result 'spare' resources. These could be directly transferred to other functions within the *same* flower, so that ovaries and/or corollas were larger. They could be transferred to flowers that are ripening seeds, so that they produce larger or more numerous seeds. Alternatively, they could be transferred to flowers that are being formed, so producing more flowers, or flowers that decline in size less quickly.

To investigate each of these, the following statistics were calculated for each plant. The regression of anther mass, corolla size, and capsule mass (A plants) or ovary size (B plants), on day that the flower opened (first flower = day 1) was calculated. Day 1 was separately determined for each plant. The regression coefficient (Anthreg, Correg, Capreg and Ovreg) is a measure of the rate at which flowers or flower components decline in size. The majority of these coefficients were significant at the 5% level (Table 1). From the regression equation, the predicted value of anther mass, corolla size, capsule mass or ovary size was calculated for day 20 (approximately the mean flowering date for the whole data set). This is an estimate of the mean adjusted for the regression on flowering day (as in an analysis of covariance). The mean values for these four statistics, as well as the regres-

sion coefficients, mean number of flowers per stem and proportion of viable pollen are given for each of the 10 clones in Table 1. The number of the individual regression coefficients that are significant at the 5% level is also indicated in this table.

Almost all characters show significant genetic variation, as measured by the between-clone term in an analysis of variance. The broad sense heritability for each character in each treatment is also given in Table 1. The only character for which the genetic variance is non-significant is the regression coefficient of ovary size on time: this may be a reflection of the greater error associated with this character as fewer ovaries were scored than other characters. All characters except ovary size and capsule mass were scored in both treatments, and so a two-way analysis of variance can be performed to assess the effect of the difference in treatment on these characters, and the significance of the interaction between genotype and treatment. This is a measure of genotype \times environment interaction. The treatment has a significant effect on all characters except pollen viability. This is the pattern that would be expected, and follows the results of Macnair & Cumbes (1990): plants that are investing in seeds produce fewer and smaller flowers, and their rate of decrease in size is greater. Pollen viability, being an intrinsic feature of pollen, is not affected by the reduction in the number of pollen grains in an anther. Interestingly, there is little evidence for any genotype \times environment interaction in any character, indicating that the different genotypes, despite considerable variation in basic flower number and size, are affected equally by the difference in treatment.

Some justification for the method of adjusting the means given in Table 1 is required. Alternative methods might have included regressing on numerical sequence of flower, rather than flowering day, and using the mean or median value of x for each plant individually, rather than using the same mean value of x for all plants. These alternative methods will, however, tend to obscure the difference between A and B treatments. A plants produce fewer flowers more slowly than B plants, and the flowers decrease in size more quickly (significant treatment terms for the regression statistics, Table 1). To use individual x means or medians would have introduced a systematic difference between the mean covariates used for individuals in different treatments, and thus rendered the difference between treatments difficult to interpret. Similarly, because A plants produce flowers more slowly, the use of numerical sequence rather than day will obscure differences in rate of decline between clones or treatments. The differences discussed here are, however, so large that they are apparent whatever method of analysis is used (data not shown).

The phenotypic, environmental and genetic correlation coefficients between these statistics are given

Table 1. Mean no. of flowers per stem (Nfl), mean anther (Anth) and capsule (Caps) masses, mean ovary (Ov) and corolla sizes (Cor) (length \times width), mean regressions of Anth, Cor, Ov and Caps on day of anthesis (Anthreg, Correg, Ovreg, Capreg), and mean proportion viable pollen (Viab) for each of the two treatments (A, all flowers pollinated; B, all flowers removed after anthesis). Anth, Cor, Caps and Ov are means corrected to day 20 by regression on day of anthesis. Each mean is the mean of three values. Also given is the broad sense heritability (h^2), the no. of significant regressions out of the 30 possible (N sig reg), and the F -values for the test of the between-treatments MS (mean square) and the treatments \times clones MS from ANOVA

Clone	Nfl		Anth		Anthreg		Cor		Correg		Caps		Capreg		Ov		Ovreg		Viab	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1	28.8	40.5	1.62	2.04	48	36	531	629	-14.7	-7.9	15.3	-0.24	1074	-13	0.32	0.33				
2	43.2	50.0	1.57	1.72	23	23	471	512	-6.8	-5.6	13.2	-0.18	1015	-9	0.69	0.65				
3	17.3	22.5	1.49	1.37	33	24	346	391	-6.9	-3.9	8.5	-0.08	689	-6	0.62	0.62				
4	28.1	27.3	1.73	1.71	43	46	497	475	-9.3	-8.5	15.3	-0.29	914	-17	0.86	0.90				
5	32.0	37.8	1.82	2.07	32	31	497	623	-12.8	-12.7	13.6	-0.47	955	-15	0.96	0.98				
6	22.0	38.5	1.43	1.61	40	27	457	540	-15.4	-10.0	9.5	-0.59	784	-12	0.97	0.97				
7	20.0	32.3	1.16	1.43	36	32	310	474	-17.7	-6.7	5.5	-0.42	598	-7	0.50	0.48				
8	21.0	29.0	0.84	0.84	18	17	336	429	-13.4	-8.0	6.4	-0.30	537	-5	0.97	0.98				
9	29.1	33.0	1.58	1.78	36	26	519	624	-11.9	-10.9	17.9	-0.29	997	-15	0.96	0.92				
10	21.8	26.8	1.40	1.63	59	43	429	473	-20.6	-14.6	9.1	-0.65	746	-13	0.38	0.44				
h^2	0.67***	0.54**	0.61***	0.69***	0.41*	0.49**	0.58***	0.63***	0.50**	0.53**	0.61***	0.63***	0.76***	0.29 ^{NS}	0.97***	0.80***				
N sig reg					27	30			29	30		29		23						
Treatment $F_{1,9}$	22.5***		9.4*		9.5*		21.9***		13.9**											0.2 ^{NS}
Interaction $F_{9,40}$	1.1 ^{NS}		0.8 ^{NS}		0.6 ^{NS}		1.1 ^{NS}		1.6 ^{NS}											0.1 ^{NS}

^{NS} Non-significant, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Units for the characters: Anth, mg; Anthreg, mg day^{-1} ; Cor, mm^2 ; Correg, $\text{mm}^2 \text{day}^{-1}$; Ov, $(\text{graticule units})^2$; Ovreg, $(\text{graticule units})^2 \text{day}^{-1}$.

in Table 2. There are no significant phenotypic correlations between the proportion of viable pollen grains and the other characters. There is a moderately large genetic correlation between this character and the regression coefficient of anther mass on time. The regression is positive, indicating that plants producing more viable pollen have less steep negative regressions with time. This relationship is difficult to interpret in terms of costs. Corolla size is correlated with flower number, anther mass, ovary size and capsule mass. This pattern is to be expected (Stanton & Preston 1988; Macnair & Cumbes 1989; Robertson *et al.* 1993). In general, phenotypic, environmental and genetic correlations are of the same order of magnitude. Corolla size shows a negative genetic correlation with the regression coefficients of anther, corolla and capsule on time. This indicates that plants with bigger flowers tend to have steeper negative regressions between floral components and time, suggesting that early investment in large flowers puts a greater drain on a plant's resources, which results in a faster reduction in floral size thereafter. Flower number, although correlated with the measures of floral size, is not generally correlated with the rate at which flowers decline in size (the small significant correlation between flower number and Correg in Table 2 should be viewed with suspicion given the large number of correlations and thus increased probability of making a type 1 error). Thus more flowers are not compensated for in these plants by smaller flowers, as might be expected if all flowers were being resourced from a fixed pool of resources.

The correlations of most characters with corolla size complicate the interpretation of the correlations between other characters. Is the genetic variance of all characters simply a reflection of genetic variation for flower size? And does this genetic variation cause all the genetic correlations? In an attempt to investigate this, analyses of covariance have been performed for all characters using corolla size as the covariate. If all the genetic variation for, say, anther mass is due to the genetic variation for flower size, then the removal of the regression on corolla size should remove all the between-clone variation in anther mass. The results of this analysis are given in Table 3. All characters retain their genetic variation except the capsule characters, indicating that the size and mass of the capsule are primarily determined by the size of the flower from which it developed.

Both anther mass and ovary size can be seen to have genetic variation that is not simply a reflection of flower size variation. Figure 1 plots, for each plant, the residual of the regression of anther mass on corolla size against the residual of ovary size on corolla size. If there was a trade-off between the two, so that relatively low anther mass was associated with relatively high ovary size, then this plot should detect it, with plants showing a negative relationship. In fact, the relationship is positive, with clone 8 having both

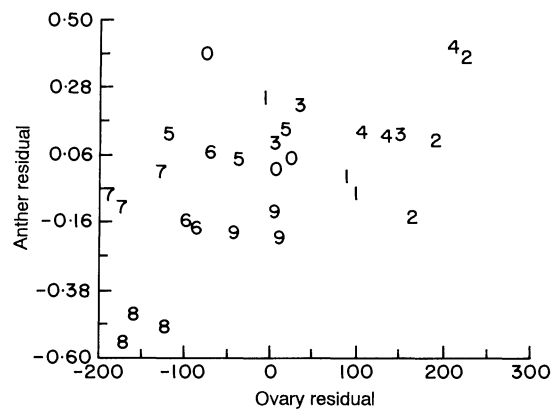


Fig. 1. Plot of the residuals of the regression of anther mass on corolla size (y-axis) against the residuals of the regression of ovary size on corolla size (x-axis). The numbers indicate the clone number (see Table 1, 0 = 10). The correlation between these characters is 0.55 ($P = 0.001$).

relatively small anthers and relatively small ovaries, and clones 2 and 4 having both characters with positive residuals. Robertson *et al.* (1993) also found evidence for a positive relationship between ovary size and anther mass when the effect of flower size was removed. A similar analysis with other character pairs found no similar significant relationships.

The dominating effect of variation in corolla size could also have prevented any negative correlations caused by variation in pollen viability or anther mass from being detected in the correlation matrix (Table 2). An analysis of covariance was therefore performed in which both corolla mass and pollen viability or anther mass were used as covariates. The only character for which a significant effect was found was anther mass when viability was used as a covariate. In this case, there is a significant ($P = 0.05$) positive regression with a slope of 0.46 between viability and anther mass. This relationship is to be expected, as anthers with a low proportion of viable pollen should have a lower mass, but this effect can only be detected when the confounding effect of corolla size has been removed. The slope of the regression leads to the prediction that the difference in anther mass between the plants with most- and least-viable pollen would be 0.304 mg when the effect of corolla size has been removed (cf. the range in mean anther masses 0.843–2.040 mg, see Table 1).

It is possible to look at the effect the variation in anther mass and ovary size has on the derived character, pollen/ovule ratio. The regression of the statistic anther mass/ovary size (which will be correlated with pollen/ovule ratio) on time was calculated for each plant separately, as for the primary characters. In almost every case, the regression was non-significant, but of the 30 plants, 24 had negative regression coefficients. This is very significantly different ($P = 0.001$) from the 15 that would be expected on the null

Table 2. Above the diagonal, phenotypic correlations; below the diagonal, genetic correlations (above) and environmental correlations (below) between characters for the two treatments

	Nfl		Anth		Anthreg		Cor		Correg		Caps		Capreg		Ov		Ovreg		Viab		
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
Nfl	0.42	0.53																			
Anth	0.72	0.55	0.54**	0.53**	0.28	0.24	0.65***	0.60***	0.37*	0.11	0.61***	0.25	0.58***	0.00	0.10	-0.05					
Anthreg	0.19	0.02	-0.69	-0.81	-0.02	-0.29	0.80***	0.79***	0.20	-0.25	0.71***	0.06	0.83***	-0.46***	0.03	-0.13					
Cor	0.30	0.47	0.25	0.52	0.80	0.78	-0.02	0.12	0.47**	0.41	-0.07	0.35	-0.05	0.57***	0.43*	0.22					
Correg	0.57	0.65	0.80	0.78	-0.35	-0.69	-0.60	-0.20	0.08	-0.27	0.86***	-0.02	0.79***	-0.30	0.11	0.04					
Caps	0.64	0.65	0.78	0.82	0.69	0.51	0.16	-0.05	-0.21		0.27	0.74***	-0.10	0.69***	0.26	-0.07					
Capreg	0.02	0.49	-0.45	0.47	0.42	0.52	0.76		0.08			0.28			0.15						
Ov	0.30	0.20	0.04	-0.11	0.40	0.25	-1.10		0.64						-0.04						
Ovreg	0.62	0.20	0.90	0.46	-0.46		0.29		0.76												
Viab	0.55	0.67	0.67	0.83	0.63	0.14	0.97		0.44												
	-0.24	-0.14	-1.14	0.83	0.83	0.56	0.69		0.01												
	0.16	0.06	0.06	0.44	0.44	-0.02	-0.07		0.89												
									0.56												
									0.04												
									0.14												
									0.32												
									-0.23												
									-0.23												

See Table 1 for definitions of abbreviations.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.**Table 3.** Analyses of covariance in which corolla size has been included as the covariate in analyses of each character in each of the two treatments. The MS for each term is given, as well as the significance of this term when tested against the within-clones term. The clones term is a measure of the residual genetic variation when the regression on corolla size has been removed

Term	df	Nfl		Anth		Anthreg		Correg		Caps		Capreg		Ov		Ovreg		Viab		
		A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	
Covariate	1	207***	353***	0.56***	0.59***	8.1**	6.0***	0.8 ^{NS}	3.8**	87.5***	0.1 ^{NS}	14.6***	39 ^{NS}	0.00 ^{NS}	0.01 ^{NS}					
Clones	9	101***	131**	0.09***	0.14***	5.0***	3.1***	59.2**	28.8**	8.8 ^{NS}	9.8 ^{NS}	4.2***	47 ^{NS}	0.19***	0.19***					
Residual	19	14	26	0.01	0.02	0.9	0.3	15.4	7.2	5.0	1.6	0.2	22	0.00	0.01					

See Table 1 for definitions of abbreviations.

^{NS} Non-significant; ** $P < 0.01$; *** $P < 0.001$.

hypothesis that there is no relationship between pollen/ovule ratio and time. The absolute value of the anther mass/ovary ratio is not easily converted to true pollen/ovule ratio, but the average regression coefficient leads to the prediction that pollen/ovule ratios decline by 38% over the 50-day period between the first and last flowers of a typical plant.

Discussion

This experiment was designed to investigate whether plants which invest less in viable pollen can reallocate the resources released to other functions within the same or subsequent flowers or fruits. It is a fundamental assumption of much of current resource allocation theory that trade-offs of this sort occur (Goldman & Willson 1986; Charlesworth & Morgan 1991), but this study has failed to find any significant difference in other floral characters associated with the variation in pollen quality. Plants producing fewer viable pollen grains do not produce bigger or more numerous flowers, invest more in female function (ovaries or fruits), or differ in the rate at which flowers and floral components decline in size as the plants age. These results suggest therefore that trade-offs do not occur, or at least are not inevitable.

It might be suggested that the variation in pollen viability is too trivial to have any effect on other floral components. This argument would suggest that trade-offs do occur, but the changes in other components are so small that they cannot be detected in this experiment. However, it is possible to detect the effect of the pollen viability variation on anther mass, and the magnitude of the effect is equivalent to about 25% of the variation between clone means for this character. Macnair & Cumbes (1990) showed that anther mass made up a significant part (about 8%) of the total cost of producing flowers and fruits in *M. guttatus*, so if the resources released from the anthers had been released to other sexual functions the effect should have been more than marginal if sexual resource allocation theory is generally applicable. The power of this experiment, with over 3000 flowers scored, is relatively high, so that effects too small to be detected really are rather small.

Our negative result with variation in pollen viability contrasts with the results obtained with *Gladiolus* by Rameau & Gouyon (1991). They were able to detect quite large negative correlations between both vegetative characters (stem height) and maternal function (seed weight) and number of full pollen grains produced. It is not clear whether the difference between our results and those of Rameau & Gouyon (1991) could be due to the difference in the provenance of the material used: our population was a natural wild population, while Rameau & Gouyon (1991) studied horticultural clones and crosses, and hybrids with wild species. It is possible that the variation in

such material will be greater than that found within natural populations. Our results are also at variance with the results obtained by Atlan *et al.* (1992) who found a negative phenotypic correlation between the number of full pollen grains per flower and the number of seeds germinating per fruit in *Thymus vulgaris*.

It might also be argued that the variation in pollen viability is caused by genes that have nothing to do with floral development as such, and may be a manifestation of variation in degree of inbreeding (Robertson *et al.* 1993). Why therefore should covariation in other floral functions be expected? Many genes of differing effects are expected to be able to affect male or female function in plants. Perhaps some genes show the expected covariation and some do not (and we have studied genes that do not). This may be true, but it begs the question of what happens to the resources released by the reduction in investment in male function. The power of natural selection to optimize resource allocation between floral components will be constrained if only some genes that reduce the size of one component release resources for other functions within the flower (or plant).

All the floral characters studied had very significant genetic variation, but they were also highly correlated with each other, as shown by Macnair & Cumbes (1990) and Robertson *et al.* (1994). In particular, all characters were very dependent on the overall size of the flower, as also found by Stanton & Preston (1988). It is interesting that the direction and magnitude of the genetic correlations were similar to the environmental correlations. This indicates that genetic and environmental factors reducing flower size have similar effects on the final phenotype and the interrelation of the floral components. However, even when genetic variation in flower size is taken account of, there is still evidence of genetic variation in anther mass and ovary size, but not capsule mass. There is thus evidence that there is genetic variation present that could alter the relative contribution of the different components to the flower if it should be advantageous. The existence of this variance is another assumption of optimal resource allocation theory, but there have been rather few investigations of these characters within natural populations (Mitchell-Olds & Rutledge 1986; Robertson *et al.* 1994).

There was evidence for a negative genetic correlation between size of flower and the rate at which flowers become smaller. This is evidence for a trade-off between early investment in big flowers and their later size. Other examples of trade-offs are more complicated. The variation in anther mass and ovary size was shown to covary, but was positive (Fig. 1). Thus plants with relatively big ovaries also had bigger than expected anthers. This positive correlation was also found by Robertson *et al.* (1994) but does not support theories of trade-offs between male and female function *sensu strictu*, and may be evidence of a trade-off between male and female function and attractive

structures (corollas). If corolla size predicted all the variation in other components, particularly anther mass and ovary size, then there would have been no residual genetic variation in these characters when the regression on floral size had been removed. The residual variation seen in Fig. 1 (a plot of the residuals for anther mass against the residuals for ovary size) shows that some plants have bigger anthers and ovaries than expected on the basis of their corolla size, and other plants smaller. In other words there is variation in the ratio of (anthers and ovaries) : corollas. Recent theory on optimal resource allocation (Charlesworth & Charlesworth 1987; Lloyd 1987; Morgan 1992) has shown that it is necessary to consider allocation to three components of floral function: male, female and attraction. The situation found here illustrates that the question of trade-offs between floral functions is, however, not easy to demonstrate when all three characters are considered.

Flower number was correlated with flower size, possibly indicating that plants which were genetically or environmentally in better condition could produce both more and bigger flowers. However, there was genetic variation in this character that remained when the covariance with flower size was removed. This was not negatively correlated with the rate of decline of flower size, as might have been expected if all flowers were manufactured from a constant pool of resources. Some plants are able to produce more flowers than others. This may be indicative of variation in resource allocation between vegetative and reproductive structures that has been detected between species in the *M. guttatus* complex (Macnair *et al.* 1989) but also within this population (H.R. McCombie & M.R. Macnair, unpublished data).

The pollen-ovule ratio in these plants declines with time. Thus smaller flowers have a lower pollen-ovule ratio. There is considerable evidence that inbreeding plants have reduced pollen-ovule ratios, which has been interpreted as being adaptive (Cruden 1977; Schoen 1982; Brunet 1992). For instance, Ritland & Ritland (1989) found that inbreeding taxa in the *M. guttatus* complex had lower pollen-ovule ratios than *M. guttatus* itself. However, inbreeding plants also have considerably reduced flower size (Ornduff 1969; Wyatt 1988; Ritland & Ritland 1989), and so it is possible, if the within-population variation detected here also applies between species, that the reduction in pollen-ovule ratio is an epiphenomenon of the reduction in flower size, rather than an adaptation *per se*. M. R. Macnair, S. E. Smith, M. Baldwin & S. Huish (in preparation) have evidence that this is indeed so, except in the case of one species in which this developmental relationship is broken.

The overall patterns shown by the results of this study are broadly in agreement with those found by Robertson *et al.* (1993) who looked at the inheritance of many of these characters using a diallel cross. By using cloned plants and scoring every flower pro-

duced, we have been able to study the decline in flower size and test more rigorously for trade-offs than they could; however, because of our design we are unable to investigate the genetic architecture of the characters as they were able to do. The fact that two different experimental designs produce essentially the same answer gives us more confidence in the validity of our conclusions.

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