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Predicting evolution of floral traits associated with mating system in a natural plant population

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Abstract

Evolution of floral traits requires that they are heritable, that they affect fitness, and that they are not constrained by genetic correlations. These prerequisites have only rarely been examined in natural populations. For *Mimulus guttatus*, we found by using the Riska-method that corolla width, anther length, ovary length and number of red dots on the corolla were heritable in a natural population. Seed production (maternal fitness) was directly positively affected by corolla width and anther size, and indirectly so by ovary length and number of red dots on the corolla. The siring success (paternal fitness), as estimated from allozyme data, was directly negatively affected by anther–stigma separation, and indirectly so by the corolla length–width ratio. Genetic correlations, estimated with the Lynch-method, were positive between floral size measures. We predict that larger flowers with larger reproductive organs, which generally favour outcrossing, will evolve in this natural population of *M. guttatus*.

Introduction

Many hermaphroditic organisms can reproduce by self-fertilization as well as by cross-fertilization (Barrett & Eckert, 1990). There have been many theoretical models and predictions of the evolutionary dynamics of selfing. Genes for selfing can be selected for because they have a two-fold advantage when they are transmitted via self-pollen in addition to outcross-pollen (Kimura, 1959). However, selfing can be selected against when it results in reduced fitness because of inbreeding depression (Charlesworth & Charlesworth, 1999) or pollen discounting (Holsinger & Thomson, 1994). Stable mixed mating systems may evolve when there is variation in inbreeding depression (Cheptou & Mathias, 2001) or when populations are genetically structured and have biparental inbreeding (Uyenoyama *et al.*, 1993; Ronfort & Couvet, 1995).

In natural plant populations, selfing rates are affected by the availability of pollinators and florar characteristics that either facilitate selfing or outcrossing. A reduced distance between the anthers and the stigma may increase the chance for autogamous selfing (Ritland & Ritland, 1989; Dole, 1992; Robertson *et al.*, 1994; Karron *et al.*, 1997; Motten & Stone, 2000), whereas outcrossing or geitonogamous selfing may be increased by traits involved in pollinator attraction such as the size and shape of flowers (Ashman & Stanton, 1991), and nectar and pollen production (Robertson *et al.*, 1999). Moreover, it has been suggested that developmentally stable symmetric flowers have higher rewards and are therefore more frequently visited by pollinators than developmentally unstable asymmetric ones (Möller & Sorci, 1998; Giurfa *et al.*, 1999, but see Midgley & Johnson, 1998).

One important prerequisite for the evolution of mating systems is heritable genetic variation. Because accurate determination of individual selfing rates requires large sample sizes (Ritland & El-Kassaby, 1985), studies on its heritability are rare (Damgaard & Loeschcke, 1994; Karron *et al.*, 1997). However, the heritability of the mating system can be inferred by determining the heritabilities of floral traits that either facilitate selfing or outcrossing.

Another important prerequisite for the evolution of mating systems is the correlation of floral variation with

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fitness. Most studies on selection measure fitness as the number of seeds produced by a plant, which is a maternal fitness component. For the evolution of floral traits, the paternal fitness component (i.e. the success of a plant as a pollen donor) can also be critical (Stanton *et al.*, 1986) but has rarely been assessed (Campbell, 1989; Conner *et al.*, 1996; O'Connell & Johnston, 1998; Smouse *et al.*, 1999; Morgan & Conner, 2001).

Even if there is heritable variation in and selection on floral traits, their evolution may be constrained if they cannot evolve independently as a consequence of genetic correlations (Lande & Arnold, 1983). Moreover, when traits are not under direct selection, they may still evolve indirectly because of genetic correlations with other traits under selection. Therefore, to predict evolutionary responses, we also need to estimate genetic correlations between traits.

There have been few integral evolutionary studies that assessed all these parameters within natural plant populations (but see Campbell, 1996). Heritabilities and genetic correlations, which require knowledge on the relatedness between individuals, have often been estimated under artificial greenhouse conditions (Farris, 1988). These estimates may differ from those in the natural population of origin because of genotype-by-environment interactions and differences in environmental variation. Some studies have reduced this problem by planting seedlings of known pedigree into the field (Simms & Rausher, 1989; Young *et al.*, 1994; Campbell, 1996). Unfortunately, however, this was not always done into the field of origin (Mitchell-Olds & Bergelson, 1990a,b; Conner *et al.*, 2003). For a good understanding of evolution in natural plant populations, heritabilities and genetic correlations should be estimated *in situ* in these populations. This is especially true for studies on mating system evolution, as rates are affected by interactions with natural pollinators (Barrett & Harder, 1996) and the genetic structure of the population (Levin & Kerster, 1974).

With the advent of increasingly sophisticated molecular genetic techniques, new methods have been developed to study evolution in natural populations. Relatedness among individuals in natural population can be inferred by using molecular markers (Ritland, 1996a; Thomas & Hill, 2000). Heritabilities of and genetic correlations between traits can then be estimated from regression of similarity in trait values between individuals on their pairwise relatedness (Ritland, 1996b). Moreover, other methods which do not require molecular markers have been developed to estimate heritabilities (Riska *et al.*, 1989) and genetic correlations (Lynch, 1999) in natural populations. Molecular genetic analysis of offspring allows estimation of selfing rates (Ritland & El-Kassaby, 1985), and selection through paternal fitness (Smouse *et al.*, 1999; Morgan & Conner, 2001).

Here, we examine individual outcrossing rates, heritabilities and genetic correlations of floral traits, and maternal and paternal selection upon floral traits, in a natural population of the yellow monkeyflower, *Mimulus guttatus* (Fig. 1), using both marker and nonmarker based methods. *Mimulus guttatus* is insect-pollinated and has a mixed mating system (Fenster & Ritland, 1994; Leclerc-Potvin & Ritland, 1994). Differences in the selfing rate are found among closely related taxa within the *M. guttatus* complex (Ritland & Ritland, 1989), and among populations of *M. guttatus* (Ritland & Ganders, 1987), indicating that the mating system can evolve in this species.

Fig. 1 Illustration of flowers of *Mimulus guttatus* (drawing by V. Pasqualetto).

We address the following questions: (1) Do floral traits affect individual outcrossing rates? (2) Is there heritable genetic variation in floral traits? (3) Does selection favour floral traits through maternal fitness? (4) Does selection favour floral traits through paternal fitness? (5) Are floral traits genetically correlated? The results of our study allow us to predict short-term evolutionary changes of mating system in this natural population of *M. guttatus*.

Materials and methods

Study species and study population

The yellow monkey flower *M. guttatus* (Scrophulariaceae) is an annual or perennial herb that is native to western North America and has become naturalized in eastern North America, New Zealand and Europe. The species occurs in moist habitats such as small streams, wet meadows, and on wet bluffs along the sea.

Shoots of *M. guttatus* consist of 0.1–1 m high stems which bear two opposite 1–5 cm long egg- or hearth-shaped leaves at each node. Side branches and single

flowers (Fig. 1) are produced from meristems in the axils of the leaves. Stems may layer and root at the nodes resulting in vegetative reproduction. The funnel-shaped zygomorphic yellow flowers are 1–4 cm in length and have conspicuous red dots at the mouth and inside the funnel. It has been suggested that the red dots might direct pollinators to the two pairs of anthers in the flower (Robertson *et al.*, 1999), and might thus affect outcrossing rates. Flowers produce no or very small amounts of nectar (Robertson *et al.*, 1999), and are mainly pollinated by bumblebees (Kiang, 1972). The stigma consists of two curved lobes and closes after mechanical stimulation by pollinators (Ritland & Ritland, 1989). When flowers abscise their corollas with connected stamen, the anthers are dragged along the stigma, which may result in delayed autogamous selfing (Dole, 1992). Each fruit may produce up to 500 small seeds of ca. 0.02 mg.

Our study population was located on a wet bluff along the sea in Lighthouse Park (49°19′N and 123°13′W) 10 km north-west of Vancouver, British Columbia. The population consisted of ca. 3000 flowering shoots in a 100 m^2 area.

Measurements

Parent generation in the field

From 21 to 24 May 2002, we collected the most recently opened flower from each of 287 randomly chosen plants in the population. We marked the plants with tape. As measures of flower size and shape, we measured the width and length of the corolla, and calculated their ratio. We measured the length of the pistil and the longest pair of stamens, and calculated the anther–stigma separation as their difference. As measures of male reproductive effort, we measured the length of the anther sac on the left longest stamen, which correlates positively with pollen production (Ritland & Ritland, 1989). We determined the proportion of nonviable pollen under a light microscope after staining with lactophenol-analine blue. As measure of female reproductive effort we measured the length of the ovary, which correlates positively with the number of ovules (Ritland & Ritland, 1989). We counted the number of red dots on both the left and right half of the flower. As a measure of developmental instability, we calculated fluctuating asymmetry (FA) in the number of red dots as the absolute difference between the ln-transformed number of red dots on the left and right half of each flower. This measure of FA is independent of the size of the flower (correlation between FA and corolla width is −0.013, $P = 0.827$, $n = 282$). In another study, in which we repeated the counts of the red dots on 14 flowers, the variation of the flower half-by-plant interaction was significantly higher than the variation between the repeated measures ($F_{13,28} = 18.37$, $P < 0.001$) indicating that the measurement error in FA (1.07% of total variation) is negligible (Palmer & Strobeck, 1986).

In the period from mid-June to August, we revisited the population every week and collected from each measured plant one full seed capsule as close as possible to the position of the measured flower. When plants had finished flowering, we counted the final number of full seed capsules. We weighed the seeds of each collected seed capsule and calculated the number of seeds per capsule as 16.8 + 45386.0 × seed weight (g). This relation had been determined with regression analysis for a subsample of seed capsules of which we had both counted and weighed the seeds ($R^2 = 0.805$, $F_{1,26} = 107.26$, $P < 0.001$). As measure of fitness, we counted the total number of seeds per plant by multiplying the number of seeds per capsule with the number of full seed capsules per plant.

Offspring generation in the greenhouse

On 28 August 2002, we sowed the seeds into 67 multi-top trays with each 36 cells of 5 × 6 × 6 cm filled with commercial potting compost in a greenhouse with additional lighting to extend the daily light period to 16 h. For each maternal plant (seed family), we sowed five to 10 seeds in each of 10 randomly chosen cells with the restriction that seeds of the same seed family were not sown into more than one cell of the same tray. During the next month, we thinned seedlings to one per cell. To keep the soil permanently wet and cool, plants were automatically flooded every second hour during the daily light period. Trays were assigned to new random positions in the greenhouse every second week until plants were too large to be moved without damaging them.

From 27 October to 1 November 2002 (9 weeks after sowing seeds), we measured on these offspring the same floral traits as we measured on their parents in the field (see previous section). These measurements were taken on three randomly chosen offspring in each of the 230 seed families that had germinated. Because not all plants were flowering, the total number of measured plants was 492 instead of 690.

Allozymes

In October and November 2002, we collected fresh corollas of each flowering offspring plant (1794 in total) for allozyme analyses. Corollas were put in microcentrifuge tubes on ice, and grinded the same day in an extraction buffer containing 0.011 M germanium oxide, 0.021 M diethyldithiocarbamic acid, 1.3 mM PVP-40T, 0.039 M sodium tetraborate, 0.021 M sodium bisulphite, 0.262 M ascorbic acid, and 1 mL of a 0.16 M phosphate buffer (pH = 7.0), 1 mL dimethyl sulfoxide, one drop of 2-phenoxyethanol and one drop of 2-mercaptoethanol per 10 mL of extraction buffer. The allozyme extract was kept at −80 °C until use.

We used an electrode buffer of 0.04 M citric acid monohydrate adjusted to pH 6.1 with N-(3-aminopro-

pyl)-morpholine, and 11% starch/5% sucrose gels in electrode buffer diluted 1 : 20 with water (Ritland & Ganders, 1987) to assay the following five polymorphic enzyme systems: aconotase, isocitrate dehydrogenase, malic enzyme, phosphoglucomutase and 6-phophogluconic dehydrogenase (two loci). In addition, we used an electrode buffer of 0.19 M boric acid with 0.04 M lithium hydroxide at pH 8.3, and 13% starch/5% sucrose gels in electrode buffer diluted 1 : 10 with a gel buffer containing 0.05 M Tris-HCl and 0.007 M citric acid monohydrate adjusted to pH 8.3 with lithium hydroxide to assay the following two polymorphic enzyme systems: alcohol dehydrogenase and phosphoglucoisomerase.

Analyses

Outcrossing rates and inference of maternal allozyme phenotypes

We assayed the 1794 offspring of 230 maternal plants in the field for the eight allozyme loci. We used the computer program MLTR (Ritland, 2002) to estimate outcrossing rates of maternal plants with progeny arrays larger than four. This program was also used to infer allozyme phenotypes of maternal plants from their progeny arrays.

Estimates of heritability of floral traits

Initially, we used a marker-based method for estimating heritabilities (Ritland, 1996b), implemented in the program MARQ (http://www.genetics.forestry.ubc.ca/ritland/programs.html; accessed 11 July 2004). Because this method needs significant variance of actual relatedness (Ritland, 1996b), which did not occur in our data-set, we do not present these results. Instead, we estimated heritabilities from offspring-single parent regressions (Falconer & Mackay, 1996), i.e. as

$$h^2_{OP} = 2B \qquad (1)$$

where B is the slope of the regression of offspring trait values on the parent trait values. However, h^2_{OP} is dependent not only on the additive genetic variation in the parent generation, but also on the additive genetic variation in the offspring generation grown in the greenhouse (appendix of Lande in Coyne & Beecham, 1987). As a consequence, estimates of h^2_{OP} may not be fully representative for the heritabilities in the natural population. Therefore, we also used a method developed by Riska *et al.* (1989) to calculate the heritability in the natural population from the offspring–parent regression, which corrects for the additive genetic variation of the offspring generation in the greenhouse,

$$h^2_{\text{Riska}} = \gamma^2 h^2 = 4B^2\left(\frac{\sigma^2_{\text{PP}}}{\sigma^2_{\text{GO}}}\right) \qquad (2)$$

Here γ is the additive genetic correlation between a trait in the natural population (parents) and the greenhouse (offspring), σ^2_{PP} is the phenotypic variance in the natural population, and σ^2_{GO} is the additive genetic variation in the greenhouse. We estimated the latter from analysis of variance on the offspring plants under the assumption that offspring of the same seed family are half sibs. This assumption is justified by the fact that the correlation of outcrossed paternity (r_{p}) calculated from the allozyme data, which can be viewed as the proportion of full-sibs among outcrossed progeny (Ritland, 1989), was low for this population ($r_{\text{p}} = 0.116$). Because of this assumption, and because $\gamma \leq 1$, the Riska-estimator is a minimum estimate of the actual heritability in the natural population. To get unbiased estimates and SE of h^2_{OP} and h^2_{Riska}, we calculated pseudovalues of jackknifing by using the statistical software Genstat (Payne *et al.*, 1993). We used the Z-test to determine significance of the estimates. Prior to all analyses, the proportion of nonviable pollen was log10-transformed to improve normality.

Estimates of selection on floral traits through maternal fitness

To determine selection differentials (S) for each floral trait through maternal fitness, we used univariate regression of the relative number of seeds produced by each maternal plant (i.e. total number of seeds produced by a plant divided by the population mean) on each floral trait. In addition, we determined selection gradients (β) to remove the effect of indirect selection on each floral trait because of associations with the other floral traits from multivariate regression of relative seed production on all floral traits (Lande & Arnold, 1983). To allow comparisons between selection coefficients, we standardized the coefficients by expressing them in units of standard deviations. Because the assumption of multivariate normality was violated, we used a jackknife procedure to get unbiased estimates of selection coefficients and standard errors (Mitchell-Olds & Shaw, 1987). We used the Z-test to determine the significance of the selection coefficients.

Estimates of selection on floral traits through paternal fitness

To determine selection on each of the eight floral traits through paternal fitness, we used the Gradient Estimate program described in Morgan & Conner (2001). This program calculates from the allozyme data, the chance X_{om} that an offspring o has been fertilized by a male m. Subsequently it uses a maximum-likelihood procedure for the regression of relative male reproductive success on the floral trait values of the males to estimate the selection gradients (Smouse *et al.*, 1999; Morgan & Conner, 2001). Because the maximum-likelihood procedure did not converge for the multivariate regression, we summed the X_{om} values for each male m to calculate the paternal fertility of each plant, which was subsequently regressed on the floral trait values. We standardized regression coefficients by expressing them in units of standard deviations, and used a jackknife procedure to

get unbiased estimates of selection coefficients and standard errors (Mitchel-Olds & Shaw, 1987). We used the Z-test to determine significance levels of the selection coefficients.

Estimates of genetic correlations between floral traits

Initially, we used a marker-based method for estimating genetic correlations among floral traits implemented in MARQ. Because this method has a large estimation variance when estimates of the additive genetic variances of traits are not very precise, as was the case in our study, we do not present these results. Instead, we calculated genetic correlations between traits x_i and x_j from offspring–parent regression (Falconer & Mackay, 1996), i.e. as

$$r_{OP}(x_i, x_j) = \frac{0.5[Cov(x_{i,O}x_{j,P}) + Cov(x_{j,O}x_{i,P})]}{\sqrt{Cov(x_{i,O}x_{i,P}) \cdot Cov(x_{j,O}x_{j,P})}}. \quad (3)$$

Here Cov stand for covariance, and the suffixes *O* and *P* refer to offspring and parent values, respectively. Because the estimates of genetic variance and covariance components partly depend on trait values measured on the offspring grown in the greenhouse, estimates of r_{OP} may not be fully representative for genetic correlations in the natural population. Therefore, we also calculated genetic correlations between traits x_i and x_j by using a method developed by Lynch (1999) which does not require knowledge about the relatedness between measured individuals in the natural population:

$$r_{\text{Lynch}}(x_i, x_j) = \frac{0.5[Cov(x_{i,k}x_{j,l}) + Cov(x_{j,k}x_{i,l})]}{\sqrt{Cov(x_{i,k}x_{i,l}) \cdot Cov(x_{j,k}x_{j,l})}}. \quad (4)$$

Here the suffixes *k* and *l* refer to individuals of the parent generation in the field. This method assumes that shared environmental effects do not contribute to the phenotypic resemblance between relatives. Because the precision of this estimate increases with the fraction of pairs consisting of related individuals (Lynch, 1999), we only paired individuals that were in close proximity of each other (Lynch, 1999; Réale & Roff, 2001), and each individual was paired only once. For each r_{OP} and r_{Lynch}, we calculated the jacknife estimate and its SE, and used the Z-test to determine significance levels.

Predicted standardized response to selection.

We calculated the predicted response to selection (R) expressed in terms of standard deviations for each floral trait by using the breeders' equation for multivariate selection (Roff, 1997):

$$R_{xi} = h^2_{xi}\beta_{xi} + \sum_{j}^{n-1} r_{xixj}h_{xi}h_{xj}\beta_{xj} \quad (5)$$

Here, h^2, β, and r stand respectively for the heritabilities of, the standardized selection gradients on, and genetic correlations between the n traits. The first component of the equation represents the response to direct selection on trait *xi*, and the second component represents the indirect response because of correlations with the other n-1 traits (*xj*).

For these analyses, we used the heritabilities estimated with the Riska-method (eqn 2), the standardized maternal selection coefficients, and the genetic correlations estimated with the Lynch-method (eqn 4). Because some of the heritability estimates were negative (i.e. biologically impossible), only estimates with $P < 0.1$ were included.

Results

Floral traits and outcrossing rates

Means and standard deviations of floral traits and fitness in the natural population of *M. guttatus* are summarized in Table 1. The population multi-locus outcrossing rate ± SE was 0.710 ± 0.027. This was larger than the averaged single-locus outcrossing rate of 0.630 ± 0.027, which indicates that there was also biparental inbreeding in the population of *M. guttatus*. The individual multilocus outcrossing rate was significantly negatively correlated with the number of open flowers per plant and significantly positively with anther–stigma separation (Table 1).

Table 1 Mean ± SD for untransformed data and ± upper SD/lower SD for log-transformed data (proportion of nonviable pollen) after back-transformation of floral traits and fitness, and phenotypic correlations (r_P) between these traits and the multilocus outcrossing rate in the natural population of *Mimulus guttatus*. *P* is the significance level, and *n* is the number of replicates.

Trait	Mean ± SD	r_P	*P*	*n*
Number of open flowers	2.4 ± 1.8	−0.158	0.023	205
Corolla width (mm)	23.04 ± 5.13	−0.084	0.236	203
Corolla length–width ratio	1.30 ± 0.17	−0.040	0.569	203
Anther–stigma separation (mm)	2.85 ± 1.17	0.190	0.007	200
Anther length (mm)	1.59 ± 0.29	−0.078	0.271	202
Proportion of nonviable pollen	0.087 ± 0.240/0.028	0.028	0.694	198
Ovary length (mm)	6.03 ± 1.09	0.041	0.559	204
Number of red dots	72.6 ± 22.6	0.106	0.132	204
Fluctuating asymmetry in number of red dots	0.100 ± 0.078	−0.029	0.678	205
Number of seeds per plant	4642 ± 7600	−0.080	0.236	199

Heritability of floral traits

Heritabilities calculated from offspring–parent regression (eqn 1) were significant for all floral traits with the exception of the proportion of nonviable pollen (Table 2). After correction of these heritability estimates for additive genetic variation of the offspring generation in the greenhouse by using the Riska-method (eqn 2), the heritability was significant for anther length, ovary length and number of red dots on the corolla, and marginally significant for corolla width (Table 2).

Selection on floral traits

Selection through maternal fitness

In univariate regression of relative fitness measured as the total number of seeds per maternal plant on floral traits, selection differentials were significantly positive for corolla width, anther length, ovary length and number of red dots on the corolla, and marginally significantly positive for the proportion of nonviable pollen (Table 3). In multivariate regression of relative fitness on all eight floral traits, selection gradients were only significant for corolla width and anther length (Table 3).

Selection through paternal fitness

In univariate regression of relative fitness measured as the proportion of offspring fertilized by each plant on floral traits, selection differentials were significantly and marginally significantly negative for anther–stigma separation and corolla length–width ratio, respectively (Table 3). In multivariate regression of relative fitness on all eight floral traits, however, the selection gradient was only significant for anther–stigma separation (Table 3).

Genetic correlations between floral traits

Genetic correlations between floral traits calculated from offspring–parent regression were positive and significant between the size measures corolla width, anther length, ovary length and number of red dots on the corolla (Table 4). Moreover, anther–stigma separation was significantly positively correlated with ovary length and number of red dots on the corolla (Table 4). Genetic correlations between the proportions of nonviable pollen with the other traits could not be calculated from offspring–parent regression because of the estimated negative genetic variance for this trait (see Table 2).

Although genetic correlations calculated by using the Lynch-method were generally higher than the estimates from offspring–parent regression, estimates from both

Trait	Offspring–parent regression	Riska estimator
Corolla width	0.213 ± 0.068***	0.087 ± 0.059†
Corolla length–width ratio	0.108 ± 0.074†	0.020 ± 0.065
Anther–stigma separation	0.280 ± 0.105**	0.055 ± 0.056
Anther length	0.297 ± 0.057***	0.323 ± 0.162*
Proportion of nonviable pollen	−0.108 ± 0.175	−0.035 ± 0.050
Ovary length	0.267 ± 0.073***	0.120 ± 0.061*
Number of red dots	0.396 ± 0.056***	0.388 ± 0.109***
Fluctuating asymmetry in number of red dots	0.167 ± 0.095*	−0.028 ± 0.140

†$P < 0.1$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 2 Heritabilities ±1 SE of floral traits of *Mimulus guttatus* estimated by using offspring–parent regression (eqn 1) and the Riska-method (eqn 2). Presented values are means ±1 SE of pseudovalues of jackknifing, and significance levels were determined with one-sided *Z*-tests.

	Maternal selection coefficients		Paternal selection coefficients	
Trait	S	β	S	β
Corolla width	0.469 ± 0.041***	0.334 ± 0.145*	0.065 ± 0.046	−0.052 ± 0.153
Corolla length–width ratio	0.030 ± 0.063	0.098 ± 0.066	−0.060 ± 0.035†	−0.075 ± 0.085
Anther–stigma separation	0.085 ± 0.076	0.002 ± 0.072	−0.092 ± 0.030**	−0.118 ± 0.036**
Anther length	0.477 ± 0.047***	0.235 ± 0.118*	0.049 ± 0.049	0.011 ± 0.049
Proportion of nonviable pollen	0.126 ± 0.072†	0.019 ± 0.070	0.058 ± 0.058	0.046 ± 0.054
Ovary length	0.484 ± 0.038***	0.074 ± 0.121	0.057 ± 0.061	0.063 ± 0.094
Number of red dots	0.345 ± 0.041***	−0.054 ± 0.065	0.082 ± 0.127	0.086 ± 0.161
Fluctuating asymmetry in number of red dots	−0.031 ± 0.055	−0.017 ± 0.052	0.004 ± 0.031	−0.001 ± 0.032

†$P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Table 3 Maternal and paternal standardized selection coefficients on floral characteristics of *Mimulus guttatus*. Means ± SE of regression coefficients are based on pseudovalues of jackknifing. Standardized selection differentials (S) of univariate regressions indicate total (direct and indirect) selection on each trait, and standardized selection gradients (*beta*) of multivariate regression indicate direct selection on each trait. Significance levels were determined with two-sided *Z*-tests.

Table 4 Genetic correlations between floral traits of *Mimulus guttatus* as estimated from offspring–parent regression (eqn 3; above the diagonal) and with the Lynch-method (eqn 4; below the diagonal). Estimates of the genetic correlations are means (SE) based on pseudovalues of jackknifing, and their significance levels were determined with two-sided *Z*-tests.

Trait	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
(1) Corolla width		0.067 (0.441)	0.293 (0.239)	0.868 (0.139)***	–	0.686 (0.113)***	0.394 (0.129)**	−0.082 (0.300)
(2) Corolla length–width ratio	0.181 (0.262)		0.083 (0.314)	−0.015 (0.231)	–	0.259 (0.302)	0.456 (0.299)	−0.764 (0.653)
(3) Anther–stigma separation	0.307 (0.211)	0.207 (0.354)		0.065 (0.211)	–	0.634 (0.172)***	0.462 (0.179)***	−0.154 (0.292)
(4) Anther length	0.918 (0.063)***	0.446 (0.255)†	0.456 (0.224)*		–	0.556 (0.122)***	0.282 (0.103)*	0.010 (0.243)
(5) Proportion of nonviable pollen	0.663 (0.337)*	−0.174 (0.419)	−0.236 (0.469)	0.721 (0.309)*		–	–	–
(6) Ovary length	0.972 (0.029)***	0.377 (0.216)†	0.362* (0.197)	0.940 (0.038)***	0.804 (0.312)**		0.386 (0.119)**	−0.204 (0.277)
(7) Number of red dots	0.971 (0.067)***	0.404 (0.284)	0.698 (0.241)**	0.899 (0.074)***	0.420 (0.313)	0.934 (0.050)***		−0.124 (0.209)
(8) Fluctuating asymmetry in number of red dots	−0.816 (0.352)*	−0.641 (0.434)	0.013 (0.493)	−0.668 (0.354)†	−0.865 (0.430)*	−0.706 (0.348)*	−0.783 (0.344)*	

†$P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

methods were highly correlated ($r = 0.780$, Mantel $Z = 9.495$, $P < 0.01$; Table 4). In addition to the significant genetic correlations detected by using offspring–parent regeression, the Lynch-method revealed that the proportion of nonviable pollen was significantly positively correlated with the size measures corolla width, anther length and ovary length, and negatively with fluctuating asymmetry in the number of red dots on the corolla (Table 4). The latter was also significantly negatively correlated with the size measures corolla width and ovary length, and number of red dots on the corolla (Table 4).

Predicted responses to selection

Based on heritabilities estimated with the Riska-method, genetic correlations estimated with the Lynch-method and standardized maternal selection gradients, the floral size measures corolla width ($R = 0.0446$ SD), anther length ($R = 0.1009$ SD) and ovary length ($R = 0.0766$ SD), and number of red dots on the corolla ($R = 0.1344$ SD) are predicted to respond to selection. For corolla width 37.7% of the predicted response was indirect because of the genetic correlation with anther length, and for anther length 24.8% of the response was indirect because of the genetic correlation with corolla width. For ovary length and number of red dots on the corolla, 100% of the predicted responses were indirect because of genetic correlations with corolla width and anther length.

Discussion

Floral traits and selfing

We found that the individual outcrossing rate of *M. guttatus* decreases with the number of open flowers on a plant and with increased anther–stigma separation. A positive correlation between anther–stigma separation and outcrossing rate was also found for *M. guttatus* in previous studies (Ritland & Ritland, 1989; Dole, 1992; Robertson *et al.*, 1994), and for *Ipomea purpurea* (Ennos, 1981) and *Datura stramonium* (Motten & Stone, 2000). In *M. guttatus* autogamous selfing because of anther–stigma contact may occur when the lower stigmatic lob curles and touches the anthers, and during corolla abscission when the anthers are dragged along the stigma (Dole, 1992). Other studies, however, did not find evidence that corolla dragging contributes to selfing in *M. guttatus* (Dudash & Ritland, 1991; Leclerc-Potvin & Ritland, 1994).

Because a large number of open flowers on the same plant increases the probability of geitonogamous selfing, whereas a small anther–stigma separation is likely to increase autogamous selfing, the results indicate that both modes of selfing are important in the natural population of *M. guttatus*. Moreover, the larger multi-locus

than single-locus outcrossing rate indicates that also biparental inbreeding occurs in this population (Ritland, 1984). This is in line with results reported by Leclerc-Potvin & Ritland (1994) who also found geitonogamous and autogamous selfing, and biparental inbreeding in a natural population of *M. guttatus*. In this study, we did not further consider the evolution of the number of open flowers because it is directly correlated with fitness.

Although the size, shape and symmetry of flowers (Ashman & Stanton, 1991; Möller & Sorci, 1998), and allocation to male and female reproductive structures (Cruden, 1977) are thought to be important for mating system evolution, correlations of outcrossing rate with corolla width, corolla length–width ratio, anther length, proportion of nonviable pollen, ovary length, number of red dots on the corolla and fluctuating asymmetry in number of red dots were not significant in *M. guttatus*. This, however, could be a consequence of the low power of estimating individual selfing rates from small progeny arrays (Ritland & El-Kassaby, 1985). Another possibility is that the floral traits that increase outcrossing rates are correlated with the ones that decrease outcrossing rates and vice versa (Table 4).

Heritability of floral traits

As a consequence of a lack of variation in the actual degree of relatedness, we could not detect significant heritabilities of floral traits with the Ritland-method (Ritland, 1996b). However, heritabilities estimated from regression of greenhouse-grown offspring on field-grown parents indicated a significant heritable genetic basis for most floral traits (Table 2). Moreover, heritabilities calculated with the Riska-method were significant or marginally significant for the number of red dots on the corolla and the size measures anther length, ovary length and corolla width (Table 2). For the other floral traits, Riska-estimates of heritabilities were close to zero. However, because the correlation between the additive genetic variance in the natural population and the one in the greenhouse (γ in eqn 2) is likely to be <1, the actual heritabilities of floral traits in the natural population are likely to exceed the Riska-estimates. Moreover, our Riska-estimates may underestimate the heritabilities in the natural population because the seed families grown in the greenhouse might in addition to half-sibs consist of full-sibs which would decrease our estimate of the additive genetic variation in the greenhouse (σ^2_{GO} in eqn 2). Because the Riska-estimates are minimum values of the true heritability, our results strongly indicate that floral traits, and especially the floral size measures, are heritable in the natural population of *M. guttatus*.

Other studies on heritabilities of floral traits of *M. guttatus* measured in either natural populations (Ritland & Ritland, 1996) or greenhouse environments (Dole, 1992; Robertson *et al.*, 1994) also found significant heritable variation in floral traits. Although these studies also show that heritability estimates may differ between populations, they all show that there is a genetic basis for variation in floral traits of *M. guttatus*. This was also shown in a recent study by Fishman *et al.* (2002), who found that differences in floral traits between *M. guttatus* and its highly selfing close relative *M. nasutus* have a polygenic basis, although each quantitative trait locus had a relatively small effect. Studies on other plant species, generally also found heritable genetic variation in floral traits (Schoen, 1982; Campbell, 1996), indicating that selection in the past has not depleted all genetic variation in these important plant traits.

Most other estimates of heritabilities in natural populations come from studies on birds and mammals (Weigensberg & Roff, 1996). The magnitude of these estimates is often of the same order as the ones estimated in the laboratory (Weigensberg & Roff, 1996). Estimates of heritabilities in unmanipulated natural plant populations, however, are scarce. In a previous study on *M. guttatus* (Ritland & Ritland, 1996), two of six natural populations had enough variation in the actual degree of relatedness to estimate heritabilities with the Ritland-method. A few other studies have estimated heritabilities in natural populations by planting individuals of known pedigree in their population of origin (Simms & Rausher, 1989; Young *et al.*, 1994; Campbell, 1996). Most studies on heritabilities in natural plant populations have used regression of greenhouse-grown offspring on field-grown parents (e.g. Young *et al.*, 1994; Ritland & Ritland, 1996). To our knowledge, only our study and the one of Ritland & Ritland (1996) corrected the estimates from offspring–parent regression for the additive genetic variation of the offspring generation in the greenhouse. Although most of these studies indicate that quantitative traits may be heritable in natural plant populations, more studies are required to test whether heritabilities in natural plant populations differ from the ones measured in greenhouse environments.

Selection on floral traits

We found significant positive maternal selection differentials for the size measures corolla width, anther length and ovary length, and number of red dots on the corolla (Table 3). Moreover, we found a marginally significantly positive selection differential for the proportion of nonviable pollen (Table 3), which might indicate a trade-off between a paternal fitness component and maternal fitness. A more likely explanation, however, is that this association is a consequence of positive correlations between the proportion of nonviable pollen and the floral size measures (Table 4). In multivariate regressions only the selection gradients for corolla width and anther length were significant, indicating that the proportion of nonviable pollen, ovary length, and number of red dots are not under direct selection. This is surprising for ovary

length because it is positively correlated with the number of ovules (Ritland & Ritland, 1989), and thus with potential seed production. Corolla width is likely to increase seed production by increasing outcrossing because of attraction of more pollinators (Ashman & Stanton, 1991). Anther size, however, is likely to increase seed production by increasing selfing because of higher loads of self-pollen on the stigma (Damgaard & Abbott, 1995).

We used inferred paternity of offspring from allozyme data to estimate selection through paternal fitness (Smouse *et al.*, 1999; Morgan & Conner, 2001; Burczyk *et al.*, 2002). Because we sampled only about 10% of the potential paternal plants in the population, the chance of detecting significant selection coefficients was relatively low (Morgan & Conner, 2001). Nevertheless, we were able to detect a significant negative paternal selection differential for anther–stigma separation, indicating that paternal fitness is increased when the anthers and stigma are close together. In *Ipomopsis aggregata* paternal fitness estimated in terms of the amount of pollen transfer was also promoted by reduced anther–stigma separation (Campbell, 1989). Moreover, we found a marginally significant negative paternal selection differential for the corolla length–width ratio. This indicates that relatively less elongated flowers are promoted through paternal fitness. In multivariate regression, however, the corresponding selection gradient was not significant, indicating that flower shape is not under direct selection through paternal fitness.

Our results show that traits that are under selection through maternal fitness are not necessarily under selection through paternal fitness and vice versa. Although O'Connell & Johnston (1998) did not find differences in selection on floral traits through maternal and paternal fitness components in *Cypripedium acaule*, Campbell (1989) found differences in selection on floral traits through materal and paternal fitness in *I. aggregata*. Therefore, selection through paternal fitness should not be neglected in studies on plant evolution.

In our analyses, we assumed that inbred and outbred offspring do not differ in fitness. Inbreeding depression, however, might reduce the benefit of selfing (Charlesworth & Charlesworth, 1999). Inbreeding depression has been found in some populations of *M. guttatus* (Willis, 1993, 1999), but not in all (Latta & Ritland, 1994; Carr *et al.*, 1997), suggesting that it may have been purged from some populations (Lande & Schemske, 1985). We cannot exclude that there might have been early-acting inbreeding depression in our study population resulting in low levels of seed germination or seedling survival. However, the correlation between the individual multilocus outcrossing rate of parent plants and the average number of flowers produced by their offspring was negative ($r = -0.118$, $P = 0.104$, $n = 190$), indicating that there is no late-acting inbreeding depression in our study population.

Predicted responses to selection on floral traits

Predicted responses to selection have been calculated for natural populations of vertebrates (Grant & Grant, 1995; Kruuk *et al.*, 2002), but only rarely for plant populations. One notable exception is the study of Campbell (1996) who combined estimates of heritability and genetic correlations with selection gradients estimated in a previous study (Campbell, 1989) on the same natural population to predict evolutionary responses of floral traits of *I. aggregata*. However, as far as we know, our study is the first one that estimated all parameters that are necessary to predict evolutionary responses simultaneously in a natural, unmanipulated plant population.

We found that the size measures of corolla width, anther length and ovary length, and number of red dots on the corolla are subject to natural selection via maternal fitness in this natural population of *M. guttatus*. Moreover, there was significant heritable genetic variation for these traits, indicating that they are likely to respond to selection. Estimates of predicted responses to selection indicate that these traits are likely to increase in the next generation with approximately one tenth of their SD, which is of the same magnitude as the ones found for *I. aggregata* (Campbell, 1996). It should be noted, however, that the predicted responses to selection have high uncertainty as a consequence of the combined estimation errors of its compounds (i.e. heritabilities, selection gradients, and genetic correlations). The high genetic correlations between size measures, as estimated with both offspring–parent regression and the Lynch-method (Table 4), indicate that selection may partly act indirectly through selection on correlated traits. Indeed, about 25% of the predicted responses to selection for corolla width and anther length were because of indirect selection, and 100% of the predicted responses of ovary length and number of red dots were because of indirect selection.

Our predicted standardized responses to selection are not corrected for selection through paternal fitness components. The traits that were under selection through paternal fitness, i.e. corolla length–width ratio and anther–stigma separation, did not have significant heritabilities, when estimated with the Riska-method. Offspring–parent regression, on the other hand, indicated that they may be heritable. If they are heritable, anther–stigma separation and corolla length–width ratio are expected to decrease in the next generation through paternal fitness. However, because both traits are positively correlated to anther length, which is under strong positive selection through maternal fitness, they may not respond to selection or might even respond in the opposite direction.

In conclusion, when we assume that selection pressures do not vary greatly between years, we expect that in the natural population of *M. guttatus*, plants will evolve larger flowers with larger reproductive organs, and unchanged

or slightly increased anther–stigma separation. These are traits that are generally associated with outcrossing taxa of *Mimulus* (Ritland & Ritland, 1989; Fenster & Ritland, 1994), indicating that plants in our study population of *M. guttatus* will evolve a more outcrossing mating system as a consequence of selection on floral traits.

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