

SUPPLEMENTARY DATA 3

Joint effects of flower size and time of deployment on pollen and ovule production and flower femaleness

Regression Model S3.1

In many plant species, *Brassica rapa* included, flower size tends to decrease from first to last flowers on plants. If receptacle width (Fig. S3.1) is a good estimate of flower size, it should decline with day of bud collection (measured as of the plant's flowering onset). We tested the relationship between receptacle width and days since bolting (x) in a simple hierarchical model using receptacle width as the response variable (y). In this model, receptacle width varies as a function of days since bolting:

$$y_i = \alpha + \beta x_i + \varepsilon_i^y \quad \text{for } i = 1 \text{ to } i = n \text{ buds in plant } j,$$

and the partial regression coefficients of this relationship are allowed to vary among plants:

$$\begin{aligned} \alpha_j &= \gamma_0^\alpha + \varepsilon_j^\alpha \\ \beta_j &= \gamma_0^\beta + \varepsilon_j^\beta, \text{ for } j = 1 \text{ to } j = 70 \text{ plants.} \end{aligned}$$

(Model S3.1)

The among plant coefficients γ_0^α and γ_0^β report the overall intercept, and the overall relationship between days since bolting and receptacle width, respectively. ε_j^α and ε_j^β are modeled as being normally distributed with mean 0 and variances σ_α^2 and σ_β^2 , respectively.

Model S3.2

Because receptacle width and days since bolting are negatively correlated (Table S3.1), we developed Model S3.2 to isolate the direct effects of time at the within-plant level from the correlated effects of flower size. This model included predictors days since bolting (x_1), and mean-centered receptacle width (μm) (x_2). The model for adjusted pollen content per bud and total reproductive investment per bud was as follows

Within plants:

$$y_{ji} = \alpha_j + \beta_j^1 x_{1ji} + \beta_j^2 x_{2ji} + \varepsilon_i^y, \quad \text{for } i = 1 \text{ to } i = n \text{ buds in plant } j$$

The partial regression coefficients of the within-plant relationship were allowed to vary among plants:

$$\begin{aligned} \alpha_j &= \gamma_0^\alpha + \varepsilon_j^\alpha \\ \beta_j^1 &= \gamma_0^{\beta^1} + \varepsilon_j^{\beta^1}, \text{ for } j = 1 \text{ to } j = 70 \text{ plants.} \\ \beta_j^2 &= \gamma_0^{\beta^2} + \varepsilon_j^{\beta^2} \end{aligned}$$

(Regression model S3.2A)

Coefficient $\gamma_0^{\beta^1}$ reports the average within-plant relationship between days since bolting and the response variable while controlling for variation in flower size, and coefficient $\gamma_0^{\beta^2}$ similarly reports the average relationship between receptacle width and the response variable while controlling for time. Variances for all among-plant coefficients are modeled as coming from a normal distribution of mean 0 and standard deviation σ_ϕ^2 , where phi is the particular coefficient. Covariances are estimated simultaneously, but not shown for simplicity of model presentation.

We modified the within-plant component of the model regression model S3.2A to model ovules per bud as follows (regression model S3.2B):

$$y_{ji} \sim \text{Poisson}(\lambda_{ji})$$

$$\lambda_{ji} = \exp(\alpha_j + \beta_j^1 x_{1ji} + \beta_j^2 x_{2ji}) \quad \text{for } i = 1 \text{ to } i = n_j \text{ buds in plant } j.$$

where y_{ji} is ovule number in bud i on plant j , and λ_{ji} is the single parameter of the Poisson distribution. λ_{ji} is in turn modeled as a function of α_j , β_j^1 , and β_j^2 . We modeled variation in flower phenotypic femaleness using a logistic-binomial regression at the within-plant level (regression model S3.2C):

$$y_{ji} \sim \text{Binomial}(p_{ji}, \eta_{ji})$$

$$p_{ji} = \text{logit}^{-1}(\alpha_j + \beta_j^1 x_{1ji} + \beta_j^2 x_{2ji}) \quad \text{for } i = 1 \text{ to } i = n_j \text{ buds in plant } j.$$

Phenotypic femaleness is thus modeled indirectly as the number of ovules in bud i of plant j (y_{ji}) given the total reproductive investment of bud i in units of ovule equivalents (η_{ji}). p_{ji} is the probability of a given unit of total reproductive resources being invested in ovules, not pollen, and is modeled as a function of α_j , β_j^1 , and β_j^2 . Regression models S3.2B and S3.2C were identical to S3.2A in their modeling of α_j , β_j^1 , and β_j^2 at the among-plant level.

Table S3.2.1: Estimates (and standard errors) of hierarchical regression model S3.1

RECEPTACLE WIDTH	
WITHIN-PLANT	
intercept	830.0*
(γ_0^α)	(10.4)
days since bolting	-5.72*
(γ_0^β)	(0.47)
VARIANCES	
plant	4173
(σ_α^2)	
days since bolting	7.48
(σ_β^2)	
residual	5989
(σ_y^2)	

* p < 0.05

Table S3.2: Estimates (and standard errors) of within-plant effects for hierarchical regression analyses.

	OVULES	POLLEN	GAMETES	FEMALENESS
REGRESSION MODEL	S3.2B	S3.2A	S3.2A	S3.2C
Family	Poisson	Gaussian	Gaussian	Logistic-binomial
Flower buds	722	722	722	722
Plants	70	70	70	70
WITHIN-PLANT				
intercept	3.22*	30.07*	55.39*	0.42*
(γ_0^α)	(2.11 e-02)	(0.54)	(0.89)	(0.009)
days since bolting	-4.35 e-03*	-0.46*	-0.59*	0.015*
($\gamma_0^{\beta^1}$)	(0.94 e-03)	(0.02)	(0.04)	(0.001)
receptacle width	2.93 e-04*	0.01*	0.02*	-3.0 e-04*
($\gamma_0^{\beta^2}$)	(0.97 e-04)	(0.002)	(0.003)	(1.0 e-04)
VARIANCES				
plant	1.6 e-02	15.9	44.5	1.7 e-02
(σ_α^2)				
days since bolting	2.5 e-07	0.02	6.8 e-02	7.9 e-07
($\sigma_{\beta^1}^2$)				
receptacle width	3.8 e-09	1.2 e-04	1.4 e-04	4.8 e-09
($\sigma_{\beta^2}^2$)				
residual	NA	10.9	25.8	2.7 e-03
(σ_y^2)				

¹Regression Model refers to numbering of statistical models in Supplementary Information 3text

* $p < 0.03$

Figure S3.1: Estimating flower size by the width of the floral receptacle. An image of the ovary, still attached to the receptacle, was captured for every bud dissected. From this image, we measured the width (in micrometers) of the receptacle at its widest point, as indicated by the white line. Measurements were made using ImageJ (Rasband 2007-2011).

