Phenotypic but not genotypic selection for earlier flowering in a perennial herb. E. Fogelström, J. Ehrlén. 2018

Appendix S1: Estimating first flowering day

We divided all flowering plants (n=672) into four groups: 1) intact plants (n = 342), 2) grazed plants for which buds had been observed prior to grazing (n = 81), 3) plants grazed before displaying buds during weeks when buds were visible in the plant population (n = 164), and 4) plants that had been grazed before any bud size observations had been made in the plant population (n = 85). Due to the variation in information available, different methods had to be used to estimate first flowering day for the different groups. All analyses were carried out in R version 3.4.2 (R Core Team 2017).

S1:1 Non-grazed plants

First flowering day for non-grazed plants that started flowering between recordings was estimated by adding 1-6 days, based on bud size, to the day of year of the last recording before flowering (Table S1:1). That is, if the largest bud of a plant was recorded as large day 118 of the year, and observed to be flowering the next week, its first flowering day was estimated to day 118 + 2 = 120 of the year. These plants were considered to have "observed" first flowering day and were marked as such in the dataset.

Table S1:1. Bud size categories with description. First flowering day was estimated by adding a specific number of days to the day of year of the observation of a largest bud in a given category.

Bud size, abbreviation, (numeric)	Description	Days from bud size observation until first flowering day
Extra Small, XS (1)	Very small, green bud	6
Small, S (2)	Petals becoming visible	5
Medium, M (3)	Visible part of petals longer than sepal tube	3.5
Large, L (4)	Bud elongated, about to open	2
Extra Large, XL (5)	Bud opening, flowering the next day	1

S1:2 Grazed plants with observed bud size

First flowering day of grazed plants for which bud size had been observed on at least one occasion before grazing (n=81) was estimated using coefficients from a linear model of first flowering day on bud size, aboveground volume and week of observation for plants with observed first flowering day (i.e. the plants referred to in Appendix S1:2, n=342) (Equation S1:1). Bud size per week was the smallest unit in the dataset constructed for this analysis, why measurements from the same intact plant individuals were included repeatedly, week by week ($n_{observations}$=926). Bud size categories were transformed into a numeric scale before analysis (Table S1:1). Week was included as the number of the week when an observation was made, counted from the first week of the year. Variance inflation factors for the covariates were lower than 2. Validation of model assumptions was done by visual inspection.

The correlation coefficient for observed FFD and model estimates of FFD, for plants with observed FFD, was 0.92 (Pearson's product-moment correlation, t_{340}=44.62 $p < 0.001$). The resulting FFDs are presented in figure S1:1.

$FFD\,\tilde{}\,N(\mu_i, \sigma^2)$

$var(FFD) = \sigma^2$

$E(FFD) = \mu_i$

$\mu_i = Bud\ size + Aboveground\ volume + Week\ number$ (Eqn. S1:1)

Table S1:2. Estimates from a linear model of bud size ($n_{observations}$=926), aboveground volume and week of observation on first flowering day of intact flowering L. vernus *individuals (n=81).*

	Estimate	SE	t-value	P-value	Adj.R^2
Intercept	41.522	1.742	23.837	< 0.001	0.75
Bud size	-2.418	0.076	-31.891	< 0.001	
Aboveground volume	0.000	0.000	-4.215	< 0.001	
Week number	5.177	0.103	50.413	< 0.001	

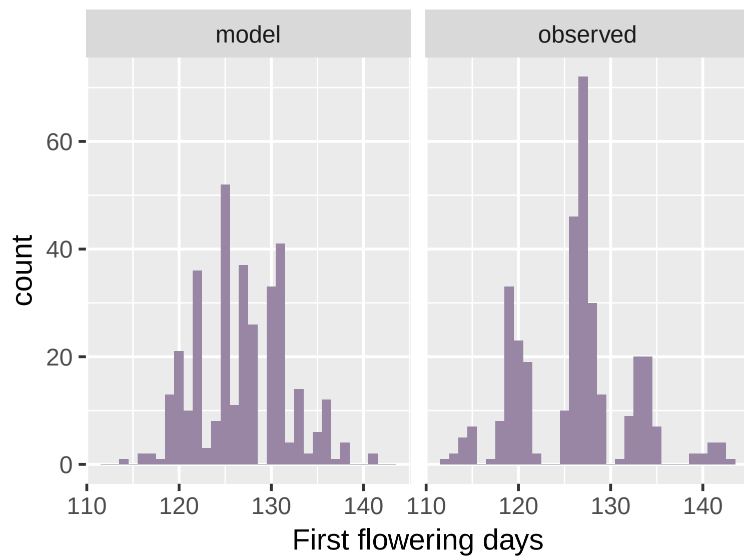

Figure S1:1: Model estimates and observed values, respectively, of first flowering day (day of year) for L. vernus *plants with observed FFD (n=342).*

S1:3 Grazed plants without bud size

Ungulate grazers most often target *L. vernus* plants during flowering (Ehrlén 2002) thus plants that were grazed before buds could be observed were considered flowering if they reached a previously observed threshold aboveground volume of >=230mm^3 (Ehrlén 1995) during the flowering period.

Our estimates of FFD for plants without observed buds were founded upon two assumptions: 1) that flowering plants with no observed bud size were in a smallest possible bud stage (XXS) the week before they were grazed (and that their largest bud would be extra-small (XS) the week after, had they not been grazed), and 2) that the difference in average FFD between plants with XS and small (S) buds corresponded to the difference in average FFD between plants with XXS and XS buds in the same week. To estimate of the difference in FFD of plants that were in bud stage XXS and XS, respectively, in a given week (*w*), we calculated the difference in average FFD ($F\bar{F}D$) of plants in the two smallest observed bud sizes, XS and S, for each week ("interval", Table S1:3). FFD for grazed plants without observed bud size was then estimated by adding the difference in average FFD between intact plants displaying XS and S buds to the average FFD of intact plants who had size XS buds the week before grazing of the focal plant (Equation S1:2, Table S1:2b).

Eqn. S1:2 $FFD_{XXS,w} = FFD_{grazed\ w+1} = F\bar{F}D_{XS\ w} + (F\bar{F}D_{S\ w} - F\bar{F}D_{XS\ w})$

The first buds were visible in the study population during the second week of field measurements (6-10 April 2015). Thus, the first flowering days of plants grazed that week could not be estimated using observed bud sizes in the previous week of measurements. Instead, we estimated the difference in average FFD of intact plants with XS buds the week of grazing of the focal plant, and the week after grazing of the focal plant. This difference, or interval, was then added to the average FFD of intact plants with XS buds (Equation S1:3, Table S1:2a). The distribution of the resulting estimates of first flowering days in the study population are provided in Figure S1:2.

Eqn. S1:3 $FFD_{XXS\ w} = F\bar{F}D_{XS\ w} + (F\bar{F}D_{XS\ w+1} - F\bar{F}D_{XS\ w})$

Table S1:3. Average first flowering day (FFD) of intact L. vernus *plants (n=342) with largest observed bud of size XS and/or S during the weeks of the field study (dates of measurement provided) and the difference (interval) in average FFD between a) intact plants with XS buds the week of grazing of the focal plant and plants with size XS buds the week after grazing of the focal plant, and b) intact plants with size S and XS buds, respectively, per week. FFD for plants grazed before buds were observed was estimated by adding the interval to the average FFD of intact plants with XS buds a) the week before grazing of the focal plant or b) the week before grazing of the focal plant. Week number refers to the week before grazing was observed, when grazed plants were assumed to have buds in a smallest possible stage of development (XXS)*

week	dates	FFD (intact XS)	FFD (intact S)	interval	FFD (XXS)
a					
14	15-03-30 - 15-04-03			6.747	114.306
b					
15	15-04-06 - 15-04-10	117.063	119.058	1.995	121.053
16	15-04-13 - 15-04-17	119.464	123.632	4.168	127.8
17	15-04-20 - 15-04-24	123.236	127.243	4.006	131.249
18	15-04-27 - 15-05-01	128.767	132.818	4.051	136.869
19	15-05-04 - 15-05-08	134.452	137.299	2.847	140.147
20	15-05-11 - 15-05-15	141.18	NaN	NaN	NaN
21	15-05-18 - 15-05-22	NaN	NaN	NaN	NaN

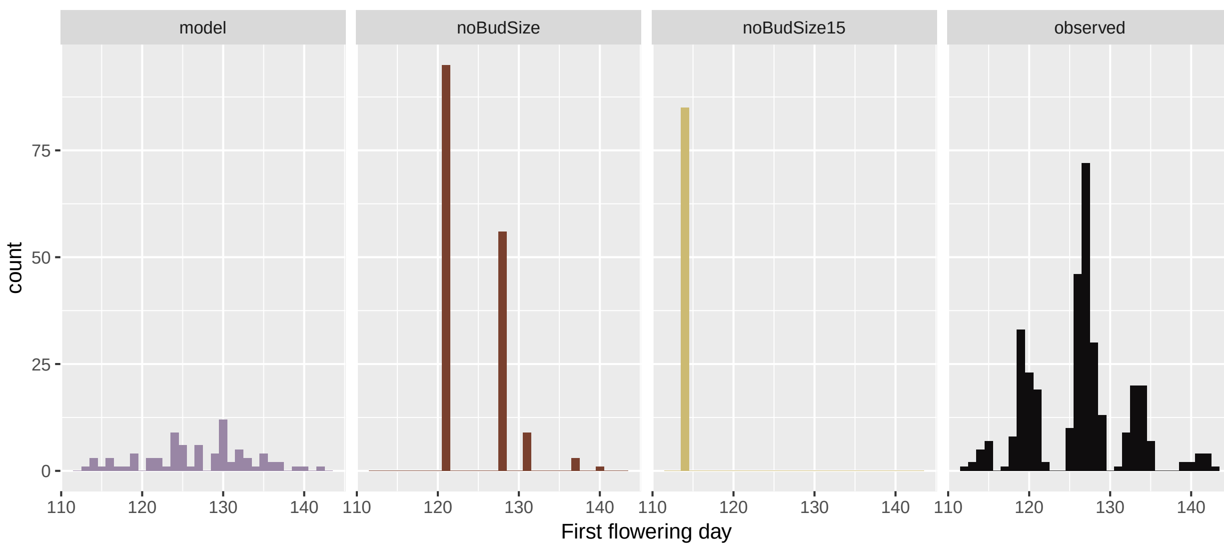

Figure S1:2 Distribution of first flowering days estimated with a linear model ("model", n=81), average first flowering days of intact plants in the smallest observed bud stages the week before grazing ("noBudSize", n=164) and average first flowering day of intact plants the week of grazing of the focal plant ("noBudSize", n=85) and observed first flowering days (day of year) of intact plants (n=342)

References

Ehrlén, J. 1995. Demography of the Perennial Herb *Lathyrus Vernus*. I. Herbivory and Individual Performance. The Journal of Ecology 83:287.

Ehrlén, J. 2002. Assessing the lifetime consequences of plant-animal interactions for the perennial herb *Lathyrus vernus* (Fabaceae). Perspectives in Plant Ecology, Evolution and Systematics 5:145-163.

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Appendix S2: Estimating final shoot height of damaged plants

To estimate height for plant individuals for which no measures of final height were available (n = 599 of 1139 plants, due to grazing or damage post flowering), we estimated the coefficients from a linear model of final shoot height on shoot diameter of non-grazed plants (n = 540). The analysis was carried out in R version 3.4.2 (R Core Team 2017). An initial inspection of the data showed increased variance in final height with increasing shoot diameter, therefore both final height and final diameter were transformed with natural logarithms before analysis. Final height for plants without observed height was estimated by inserting the natural logarithm of the maximum observed shoot diameter of each damaged plant individual into the model formula (Eqn. S2:1, Table S2:1, Figure S2:1). The resulting estimates were then back-transformed into height measures in mm. The correlation coefficient for observed and estimated measures of final height (mm) for intact plants was 0.67 (Pearson's product-moment correlation, t_{538}=21.00, p <0.001). The resulting final height measures are presented in Figure S2:2.

$ln(Height)\tilde{}N(\mu_i, \sigma^2)$

$var(ln(Height)) = \sigma^2$

$E(ln(Height)) = \mu_i$

$\mu_i = \beta_1 + \beta_2 \times ln(diameter)$ (Eqn. S2:1)

Table S2:1 Coefficients from a linear model of final height on final diameter of intact plants (n = 540). Final shoot height and diameter were transformed to natural logarithms before analysis

	Estimate	SE	t-value	P-value	R^2
Intercept	4.728	0.023	210.034	< 0.001	0.53
ln(Final diameter)	0.701	0.029	24.540	< 0.001	

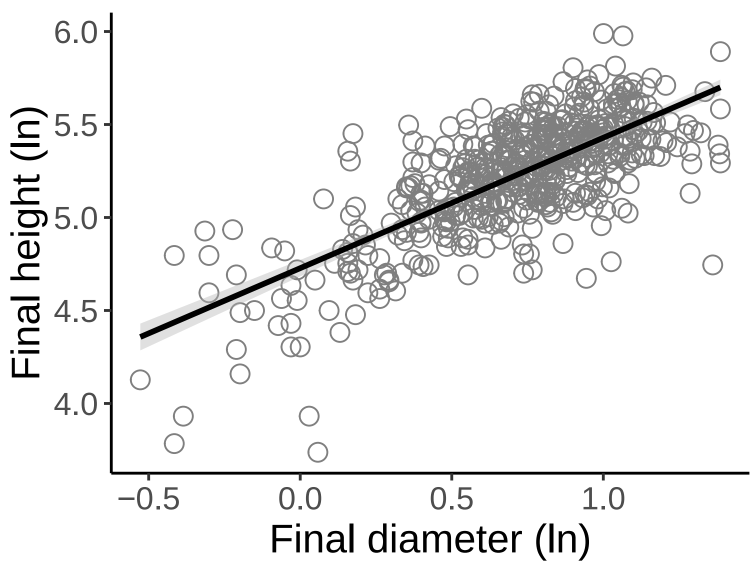

Figure S2:1. Height-diameter relationship from a linear model of final shoot height on diameter of intact L. vernus *plants (n = 540). Final shoot height and diameter were transformed to natural logarithms before analysis*

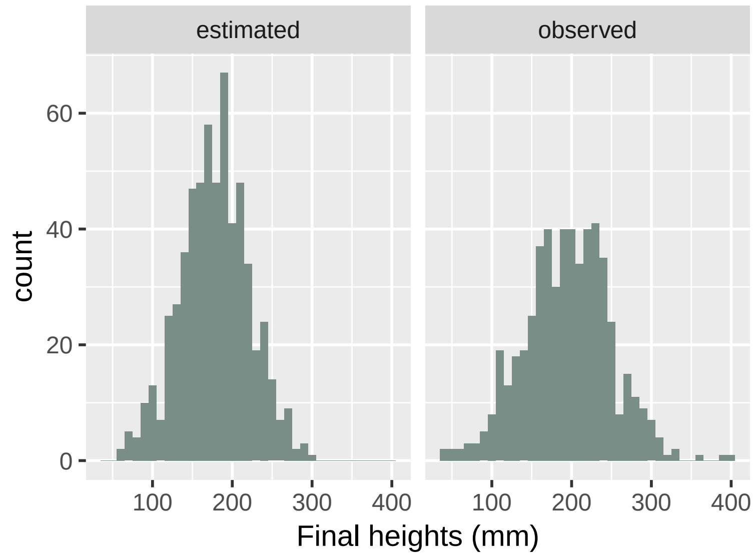

Figure S2:2. Histogram of model estimates and observed values of final heights for the L. vernus *study population (n = 1139)*

References

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Appendix S3: Estimating predation for dispersed seeds

For fruits that had opened prior to examination (27.49% of all collected fruits), the number of seeds and holes on the fruit wall could be counted, as ovules stay attached to the fruit when it opens and seeds leave indentations on the fruit wall. However, seed predation for these fruits had to be estimated from fruits that had not yet opened when checked for seed number and evidence of seed predation (n~~intact fruits~~=1516). We transformed the number of holes made by the seed predator on pod wall and seeds, respectively, into proportions by dividing each measure by the total number of seeds.

This was done for each intact fruit. Linear models could not satisfactory explain the relationship, why we fitted an exponential asymptotic regression model using non-linear least squares (nls) in R version 3.4.2 (R Core Team 2017).

The starting asymptotic value of y was set to 1. The starting value of the rate constant was obtained as the slope from a linear model of the number of holes in pod wall per total number of seeds, on the proportion of seeds preyed upon per total number of seeds (linear model, estimate±SE =0.271±0.008, t= 35.113, p < 0.001) was used as a starting value for the intercept).

The formula that best described the observed data was $y = a(1 - e^{-bx})$ where *a* represents the asymptotic value of y, and *b* is the rate constant (Table S3:1, Figure S3:1).The correlation coefficient for observed and estimated proportions of seeds preyed upon was 0.858 (Pearson's product-moment correlation, t_{1514}=65.083, p <0.001).
We estimated the proportion of seeds preyed upon for open fruits using the formula above, inserting the rate constant of 1.22 from the non-linear least squares model (Table S3:1) and an asymptotic y of 1. The resulting proportions were back-transformed to counts of seeds preyed upon.

Table S3:1. Results from a non-linear least squares model of the proportion of seeds preyed upon on number of holes on pod walls per total number of seeds in fruits that had not open prior to examination (n = 1516).

	Estimate	SE	t-value	P-value
a, Asymptotic value of y	0.998	0.018	54.320	< 0.001
b, Rate constant	1.219	0.052	23.641	< 0.001

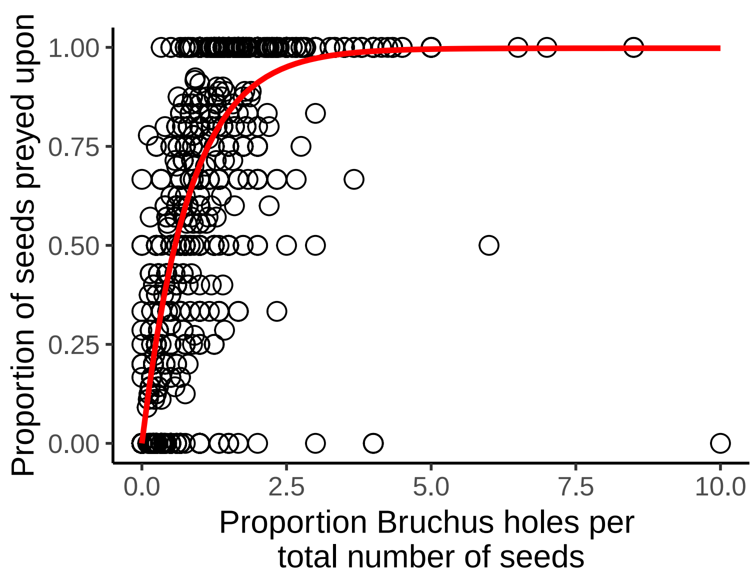

Figure S3:1. The relationship between proportion of seeds preyed upon and number of Bruchus atomarius *holes in the pod wall of* Lathyrus vernus *fruits that had not open prior to examination (n = 1516).*

References

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Appendix S4: Summary statistics

Table S4:1: Summary statistics for the variables used for analyses of L. vernus *plants (n=672).*

Variable	mean	sd	median	min	max
Final shoot height (mm)	208.6	41.3	205.8	109	399
Final shoot diameter (mm)	2.3	0.5	2.3	1	4
Aboveground volume (mm^3)	3668.7	4420.5	2400.5	211.4	46797.2
Number of flowers*	22.6	30.6	14	1	226
First flowering day (day of year)	124.4	6.6	126	112	143.5
Number of fruits	8.2	9	5	0	52
Number of intact seeds	27.7	36	14.9	0	296

*Summary statistics for a subset of 276 plants that had not been grazed (used for analyses containing flower number)

R Core Team. 2017. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.