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Summary

Flowering phenology, that is first flowering date, and duration, synchrony and intensity of the flowering period, have traditionally been thought to have a major impact on plant reproductive success. Many plants are constrained by pollen limitation, and changing flowering phenology can increase pollination success. Also resource availability and herbivorous insects may affect seed production. In two populations of *Cardamine amara*, I investigated how selection acted on phenology-related traits and during which ontogenetic phases of seed production it was expressed. I also examined how the attack of flea beetles from the *Phyllotreta* family influenced fitness and selection. There were great differences in selection between the two populations. In Larslund the flea beetles had great impact on plant fitness, and preferred plants growing in more sunny areas. In Väster-malma on the other hand, there was selection for increased number of flowers, longer duration of the flowering period and to become more synchronous. The observed selection may be the result of both pollen and resource limitation.

Sammanfattning

Blomningsfenologi, det vill säga antal blommor, datumet när första blomman slår ut, längden på blomningsperioden och synkroniseringen inom populationen, har länge ansetts ha stor inverkan på en växts reproduktiva framgång. Många växter är pollenbegränsade och genom att ändra blomningsfenologin kan växten öka sin fröproduktion. Men det är inte bara pollentillgången som styr hur många frön en växt kan producera utan även tillgången på resurser samt interaktioner med herbivorer påverkar utfallet. Jag har i två populationer *Cardmanie amara* undersökt hur selektionen verkar på fenologikaraktärer samt när den uttrycks. Jag har även undersökt hur jordloppor från familjen *Phyllotreta* påverkar fitnessen och selektion. Det visade sig att selektionen skiljer sig kraftigt åt mellan de olika populationerna. I Larslund fanns det en tydlig effekt av jordlopporna vilket resulterade i sänkt fitness hos de plantor som attackerades. Jordlopporna föredrog plantor som växte soligt. I Väster-malma selekterades det för ett ökat antal blommor, längre blomningsperiod samt ökad synkronisering inom populationen. Selektionen kan vara både pollen- och resursdriven.

Introduction

Flowering phenology have traditionally been thought to have a major impact on plants reproductive success (Rathcke & Lacey 1985). Many plants are constrained by pollen limitation (Schemske et. al 1978; Bierzychudek 1981; 1982; Santandreu & Lloret 1999), and changing the flowering phenology may increase the plants' pollination success (Rathcke & Lacey 1985). When studying flowering phenology the most common aspects to investigate is; start of flowering, duration of the flowering period, synchrony of the flowering period with other individuals in the population and the intensity of the flowering period, i.e. number of open flowers per unit time (Ollerton & Lack 1998).

The duration of the flowering period and the number of open flowers at any given time, are correlated to the total number of flowers. The more flowers the plant produce the longer the duration and the more flowers can be open simultaneously. By increasing these character values the plant may become more attractive to pollinators and the chance to become pollinated increases (Thomson 1980; Johnson & Nilsson 1999). However, the plant cannot produce an infinite number of flowers due to resource limitation. In the end the plant has to compromise between attractiveness to pollinators and the alternative use of resources put into flowering (Primack 1987). The cost of producing excess flowers may be relatively low compared to the fitness gained from the increased number of produced seeds (Wise & Cumming 2002). However, increasing pollinator attraction may also lead to increased resource investments in seed production. By aborting fruits in an early stage the plant can calibrate the number of fruits to the available resources (Janzen 1977, Lloyd 1980; Wise & Cumming 2002). This combination of surplus flowers and abortion of initiated pods allows the plant to adjust the number of pods to what is optimal for the year (Stephenson 1981).

Synchrony, the period of time when the plant flowers simultaneously with other members in the population, can be selected both for and against. To flower simultaneously with other members of the population may increase the probability to get pollinated, but may also increase the probability of attracting herbivores and seed predators (Rathcke & Lacey 1985).

Insect herbivores can affect seed production, either directly by feeding on the flowers, fruits and seeds (Pilson 2000; McCall & Irwin 2006), or indirectly by feeding on the plant and thereby reduce the available resources to seed production (Marquis 1984). The effect of herbivory varies from species to species, and effects on seed production may occur the same

season as the attack take place (Hersch 2006; Marquise 1988) or in the year after the attack (Díaz et. al 2003).

By changing flowering time to periods when the seed predators are not active or low in abundance, or by growing in places less preferred by the herbivore, the plant may decrease the effect of herbivores (Pilson 2000). The plant is also limited by its pollinators and therefore restricted to periods when they are active (Ollerton & Díaz 1999). Depending on the local conditions selection can act for increased synchrony or asynchrony (Rathcke & Lacey 1985). The optimal flowering phenology depends on a number of factors and it is important to look at several aspects to understand how selection is acting in a population.

In this study I will:

1) Asses phenotypic selection on first flowering date, duration of the flowering period, synchrony of the flowering period with other individuals in the population and number of flowers in two populations of *Cardamine amara*.

2) Examine if the attack of leaf eating flea beetles influences fitness and alters phenotypic selection on flowering phenology.

3) Investigate during what ontogenetic phases of seed development selection are expressed.

Methods

Study system

Cardamine amara (Brassicaseae) is a perennial rhizomatous herb which can be found in damp and shady environments like forest swamps and stream margins (Mossberg *et al*. 1992). It flowers during May-June with several white flowers assembled in the top of the shoot. The flower has almost 1 cm long petals and purple to amaranthine colored stamens. *Cardamine amara* is the only species of the *Cardamine* family which do not have yellow stamens (Mossberg *et al*. 1992). The flowers opens sequentially and in the studied populations, plants usually produce on average 5.9 (SD = 3.7, range 1-79) flowers (pers. obs.). On average 4.6 (SD = 3.7) pods are initiated and of these 3.3 (SD = 3.2) develops into large seed containing

pods. Each large pod contains 6.7 (SD = 6.0) seeds (pers. obs.). Pollinators in the studied area are not known, but flower visitors include different kind of flies (Lindsey 2005; pers. obs.)

Flea beetles from the *Phyllotreta* family (Coleoptera: Chrysomelidae) are generalists in the *Brassicaceae* family (Dosdall & Stevenson 2005) and attack *C. amara*. Flea beetles damage plants by eating small circular holes in the leaf (Sandhall 2003), often leading to reduced growth and seed production (Dosdall & Stevenson 2005). In some extreme cases the flea beetles can consume whole plant (pers. obs.).

Data collection

I used two populations of *C. amara* at two different locations for my study; Väster-malma and Larslund in the Ludgo Parish, 100 km southwest from Stockholm. A population was defined as a group of individuals of *C. amara* separated from other *C. amara* individuals by at least 50 m. I randomly marked 150 individuals within each population. I visited the populations each 3-4 day during May-June 2008. Both study populations were attacked by flea beetles, *Phyllotreta* sp. The degree of damage was recorded in three categories; no-, light- or heavy damage. Plants with few holes and no damage on the flowers were categorized as light damage while plants with several holes and damaged flowers were categorized as heavy damage. I noted the first date at which the plant had at least one open flower and at each occasion I recorded the number of open flowers and pods until all plants in the population had ceased to flower. The pods were divided into two groups, small pods and large seed containing pods. At the last visit, I measured the height and diameter of the flowering shoot. Just before seed dispersal I collected all pods, and counted seeds at lab.

Digital photos for canopy cover measurements were taken by placing a camera 50 cm above ground next to each individual. The canopy cover was calculated using the free software J-image 1.40 (http://rsb.info.nih.gov/ij/). Plant size was defined as flower shoot height × (stalk diameter)^2. The first flowering date, later referred to as the start date, was given as number of days from first of May. To calculate the start date for the first flower, I used the formula

$$d_n+((d_f-d_n)/n+1)$$

where d_n is the last visit when the plant did not flower, d_f is the first visit when the plant flowered and n is the number of flowers that flowered at the first visit when the plant

flowered. The formula assumes that flowers open linearly, i.e. sequentially and at a constant rate between each visit.

To calculate flowering synchrony (X) for plant i I used the formula of Augspuger (1983, modified from Primack 1980):

$$X_i = (1/(n-1))(1/f_i)\sum e_{j\neq i}$$

where n is number of individuals in the sample, f_i is individual i's total number of flowering days and $e_{j\neq i}$ the number of days individual i and individual j overlap in their flowering.

Selection analyses

The number of seeds was regarded as a measure of plant fitness and relative fitness was calculated by dividing seed production with the population means. The number of flowers, plant size, start date, duration and synchrony were standardized by subtracting the mean of its population from the plants value and divide the difference with the standard deviation of its population. I also included the canopy cover in the analysis to partly adjust for effects of environmental variation.

To investigate if the selection differed between Larslund and Väster-malma I first incorporated the interaction between site and each trait into the analysis. Because the effects of interaction were significant in several cases, I ran selection analyses separately for each population. To test for non-linear selection, I examined the effect of quadratic terms of each trait in models including also linear terms'. I also investigated the effect of all trait combinations, by examining the effects of interaction terms in a model also including the linear terms.

To investigate how attacks by flea beetles influenced average fitness and selection, I ran all models with and without a term describing attack rate. By incorporating the attack into the model, I statistically correct for the effect of the flea beetle attack and estimate how selection should have acted if flea beetles were not present. I also estimated correlation between the traits: number of flowers, start date, duration, plant size and synchrony. Lastly, I performed a logistic regression to asses how canopy cover affected the flea beetle attack.

Mechanism behind the selection

To understand the mechanisms underlying observed selection, I examined how mortality during different ontogenetic stages of fruit and seed development contributed to the total variation in mortality (key factor analysis) (Podoler and Rogers 1975). In my study these ontogenetic stage mortalities refer to the steps: pod initiated, pods retention and seed set. The number of flowers, initiated pods and large seed setting pods were log-transformed. The log-number of initiated pods and large pods were subtracted from the log-number of flowers and pods before the mortality step. Seed mortality was calculated by log-transforming the number of produced seeds and the theoretic maximum number of seeds the plant could have produced and subtracting the log-number actual seed set from the theoretic log-value. The theoretic values were achieved by taking the pod with the highest seed production and multiply the number of seeds with the number of pods on the plant. The total generation mortality, K, was calculated by summarizing the three achieved values from each subtraction, the k-values. To examine the effects of variation in mortality in each stage on variation in total mortality, I used a regression analysis with the submortalities as the response and the total generation mortality as the explanatory variable.

To examine during what ontogenetic phases of seed production effects of different traits were expressed, I performed multiple regressions with number of initiated pods per flower (pod initiation rate), large pods per initiated pod (pod retention rate) and seeds per pod (seed set) as response variables, and number of flowers, plant size, start date, duration and synchrony as explanatory variables.

All analyzes were carried out using the free software R 2.6.2 (R development core team 2008; package car Fox 2007).

Results

Number of flowers, start date and duration were strongly correlated to each other (abs (r) > 0.7 in all cases, table 1). More flowers were correlated to longer duration and earlier start date, and earlier start date were correlated to longer duration of flowering. Plant size was positively correlated to the number of flowers (r = 0.56). Synchrony and duration were negatively correlated (r = -0.67). Synchrony was negatively correlated to the number of flowers (r = -0.52).

Table 1. The correlation between the different plant traits, for each trait the correlation coefficient r is presented.

	Number of flowers	Start	Duration	Plant size	Synchrony
Number of flowers	-				
Start	-0.71	-			
Duration	0.75	-0.76	-		
Plant size	0.56	-0.25	0.36	-	
Synchrony	-0.52	0.41	-0.67	-0.33	-

Flea beetles preferred plants growing in sunnier spots to plants growing more shady (p<<0.001, Fig. 1).
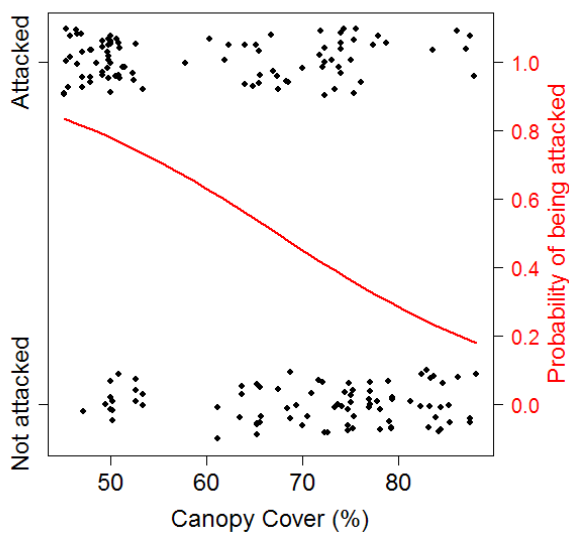
Fig. 1. Probability of being attacked by flea beetles and canopy cover.

Selection analyses

There were interactions between several traits and site (site:synchrony $F_{(1,158)}$=4.0027 p=0.047, site:canopy cover $F_{(1,158)}$=20.3505 p<<0.001), indicating differences in selection between the Larslund and Väster-malma, population.

In Larslund, plants growing shady produced more seeds than plants growing sunnier (Table 2, Fig. 2a). Selection acted for increasing the number of flowers (Table 2). There was also a effect of the quadratic term for number of flowers (Fig. 2b). Of the interaction terms plant size and the number of flowers showed up significant.

Table 2. The results from the selection analysis for linear terms, quadratic terms and the interaction between the plant traits in Larslund, presenting the estimate of the selection (selection), the F-value and p-value for each variable. The column With flea beetle represents how selection is acting when flea beetles are present in the population and the column Without flea beetle shows how selection is acting when the model is corrected for the flea beetle attack.

|	With flea beetle			Without flea beetle		
Character	Selection	F-value	p-value	Selection	F-value	p-value
Linear terms						
Number of flowers	**0.60**	**11.09**	**0.0013**	**0.65**	**14.80**	**<0.001**
Start	-0.20	0.98	0.32	-0.12	0.41	0.53
Duration	-0.065	0.06	0.81	-0.042	0.03	0.87
Plant size	0.11	0.59	0.45	0.047	0.11	0.74
Synchrony	-0.0090	0.0033	0.95	-0.038	0.066	0.80
Canopy cover	**0.053**	**20.44**	**<<0.001**	**0.038**	**10.69**	**0.0016**
Attack					6.35	0.0028
Quadratic terms						
Number of flowers^2	**0.24**	**4.30**	**0.042**	0.20	3.20	0.078
Start^2	0.13	0.92	0.34	0.13	0.98	0.32
Duration^2	0.030	0.033	0.86	0.0093	0.0033	0.95
Plant size^2	-0.17	2.97	0.089	-0.18	3.84	0.054
Synchrony^2	-0.21	2.048	0.16	-0.68	1.53	0.22
Canopy cover^2	-0.0020	2.93	0.16	-0.0014	1.11	0.29
Interaction terms						
Number of flowers : Start	-0.28	0.65	0.42	-0.30	0.82	0.37
Number of flowers : Duration	0.48	0.57	0.45	0.58	1.02	0.31
Number of flowers : Plant size	**-0.41**	**4.65**	**0.034**	**-0.40**	**5.58**	**0.021**
Number of flowers : Synchrony	0.24	0.18	0.67	0.50	0.96	0.33
Start : Duration	0.21	0.40	0.53	0.22	0.57	0.45
Start : Plant size	-0.34	1.44	0.23	-0.32	1.60	0.21
Start : Synchrony	0.21	0.30	0.58	0.18	0.30	0.58
Duration : Plant size	0.057	0.015	0.90	-0.089	0.045	0.83
Duration : Synchrony	0.34	1.69	0.20	0.24	1.082	0.30
Plant size : Synchrony	-0.085	0.049	0.83	-0.27	0.63	0.43
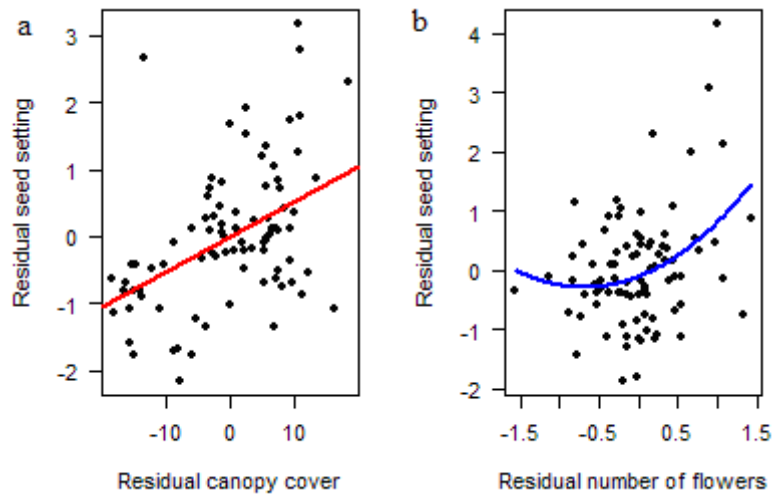
Fig. 2. Partial leverage plot for seed set and a) canopy cover, and b) number of flowers in Larslund.

In Väster-malma there was a linear selection on number of flowers, duration and synchrony (Table 3). Individuals that had more flowers, longer duration of their flowering period and were more synchronous set more seeds (Fig. 3a-c). Canopy cover had no effect on seed set (Table 3). There was also an effect of the quadratic term for start date (Fig. 3d). There was no significant effect of trait interaction terms (Table 3).

Table 2. The results from the selection analysis for linear terms, quadratic terms and the interaction between the plant traits in Väster-malma, presenting the estimate of the selection (selection), the F-value and p-value for each variable. The column With flea beetle represents how selection is acting when flea beetles are present in the population and the column Without flea beetle shows how selection is acting when the model is corrected for the flea beetle attack.

Character	With flea beetle			Without flea beetle		
	Selection	F-value	p-value	Selection	F-value	p-value
Linear terms						
Number of flowers	**0.53**	**5.73**	**0.019**	**0.53**	**5.56**	**0.021**
Start	0.32	2.86	0.094	0.29	2.16	0.15
Duration	**0.50**	**6.00**	**0.017**	**0.48**	**5.03**	**0.028**
Plant size	0.21	2.05	0.15	0.20	1.79	0.18
Synchrony	**0.41**	**8.49**	**0.0047**	**0.41**	**7.77**	**0.0067**
Canopy cover	-0.0070	1.16	0.28	-0.010	1.78	0.19
Attack					0.39	0.68
Quadratic terms						
Number of flowers^2	0.070	0.75	0.39	0.74	0.55	0.46
Start^2	**-0.49**	**6.84**	**0.011**	**-0.51**	**7.11**	**0.0095**
Duration^2	0.028	0.05	0.82	0.050	0.15	0.70
Plant size^2	-0.072	0.43	0.52	-0.081	0.51	0.48
Canopy cover^2	-0.00056	0.39	0.53	-0.00084	0.58	0.50
Synchrony^2	-0.090	1.32	0.25	-0.95	1.39	0.24
Interaction terms						
Number of flowers : Start	0.030	0.0053	0.94	0.061	0.020	0.88
Number of flowers : Duration	0.28	0.31	0.58	0.37	0.48	0.49
Number of flowers : Plant size	-0.052	0.032	0.85	-0.091	0.093	0.76
Number of flowers : Synchrony	0.28	0.23	0.63	0.29	0.24	0.62
Start : Duration	0.35	0.71	0.40	0.38	0.77	0.38
Start : Plant size	-0.19	0.36	0.55	-0.20	0.40	0.53
Start : Synchrony	0.066	0.048	0.83	0.071	0.054	0.82
Duration : Plant size	-0.018	0.0018	0.97	-0.041	0.0088	0.93
Duration : Synchrony	0.014	0.0014	0.97	0.028	0.0049	0.94
Plant size : Synchrony	0.055	0.025	0.87	0.045	0.016	0.90
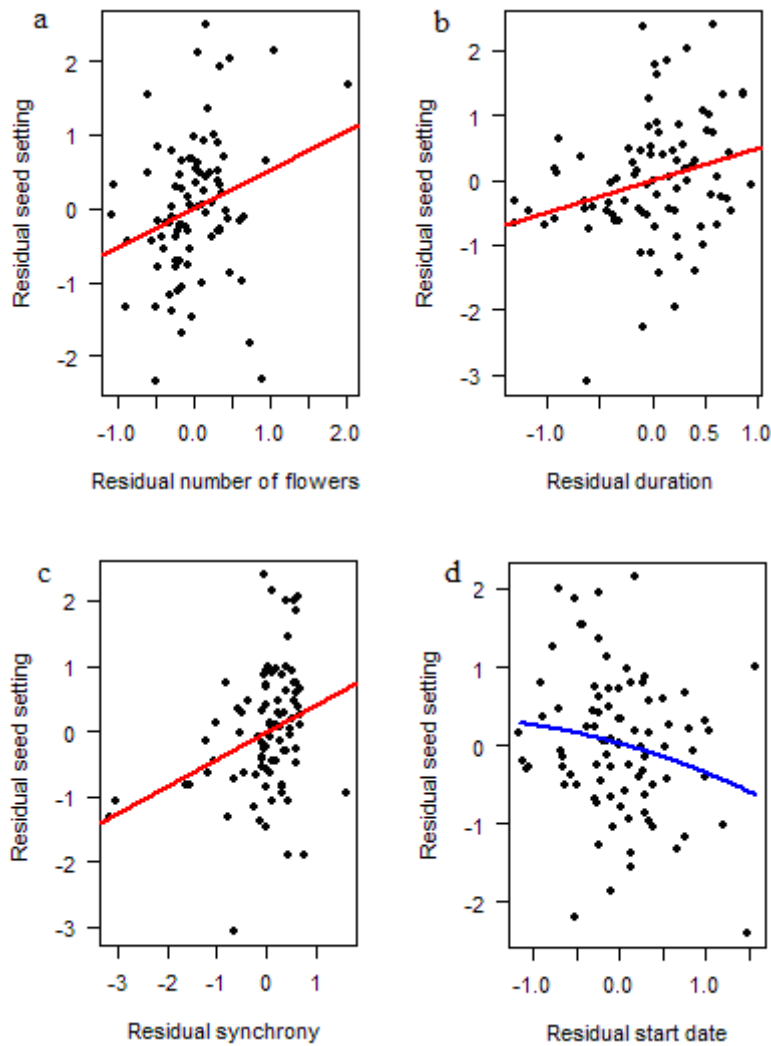
Fig. 3. Partial leverage plot for seed set and a) number of flowers, b) duration, c) synchrony and d) start date in Väster-malma.

The flea beetles affected the fitness of individuals in Larslund population; the more severe the damage from the flea beetles were the less seeds the plant produced (Fig. 4). Overall, damage had very little effect on selection on flowering phenology related traits. However in Larslund, the effect of the quadratic term for number of flowers was not significant when adjusting for damage, but the changes in both estimate and p-value were relatively small (Table 2, Fig. 2b) In the Väster-malma population, flea beetle attack had no significant effect on fitness or selection (Table 3).
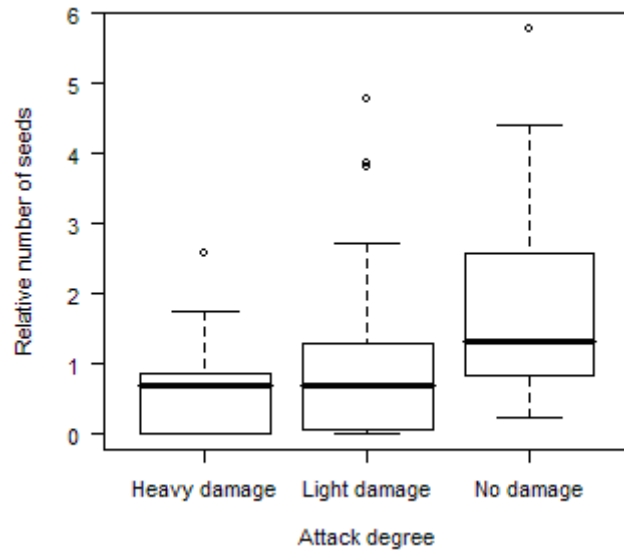
Fig. 4. The number of produced seed in Larslund for the three categories of flea beetle attack.

Mechanisms behind the selection

The key factor analyses showed that mortality during development from small to large pod explained most of the variation in total seed mortality. It had a slope of 0.71 compared to 0.39 for flower to pod mortality and -0.10 for the actual seed set.

In Larslund, shading had positive effect on all three stages; pod initiation, pod retention and seed set (Table 4, Fig 5a-c). As in the selection analysis, non of the plant traits had significant effect on any of the three steps.
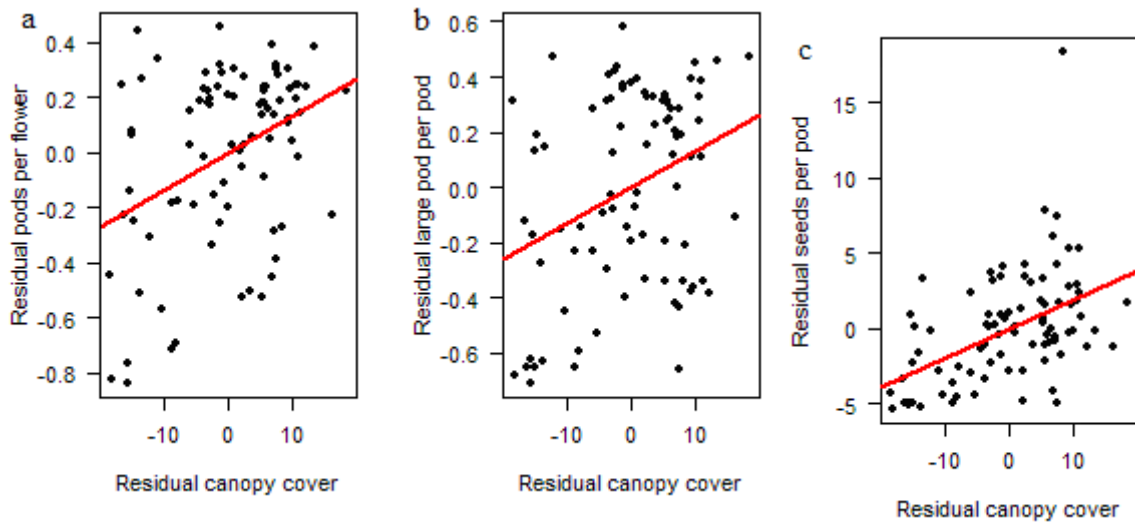
Fig. 5. Partial leverage for the effect of canopy cover on a) pod initiation b) pod retention and c) seed set in Larslund.

In Väster-malma no trait affected the pod initiation (Table 4). Pod retention and seed set were positively affected by duration (Fig. 6a, Fig. 7b). Increased canopy cover had negative effect on both large pod production (Fig. 6b) and seed production (Fig. 7c). This is opposite to the observed pattern in Larslund were increased canopy cover in all three steps increased the survival to the next step (table 4). Later flowering individuals set more seeds per pod compared to earlier flowering individuals (Fig 7a). Increased synchrony led to an increase in seed set (Fig. 7d).
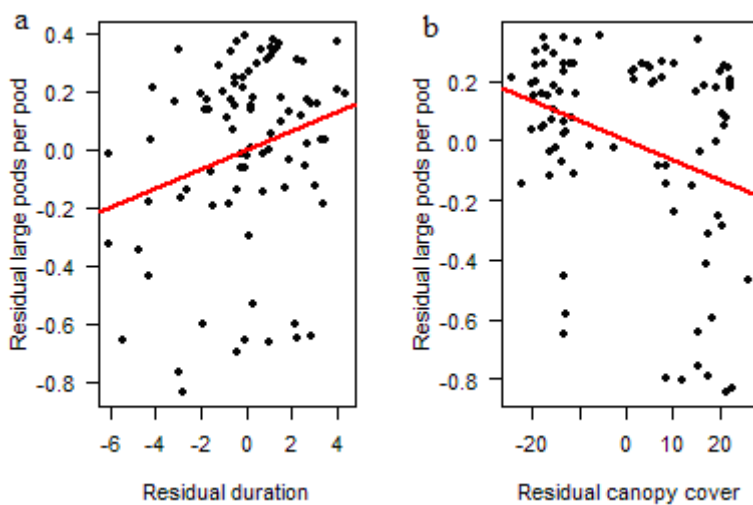
Fig. 6. Partial leverage plot for pod retention and a) duration b) canopy cover in Väster-malma
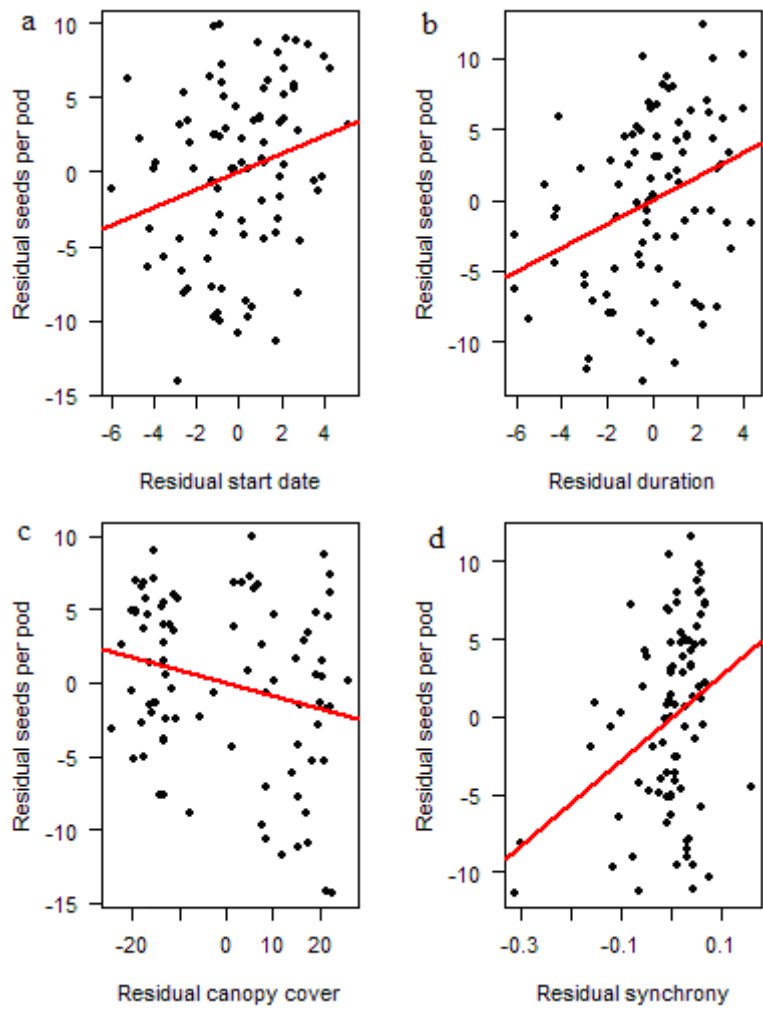
Fig. 7. Partial leverage plot for seed set and a) start date b) duration c) canopy cover d) synchrony in Väster-malma.

Table 4. The effect of plant traits and canopy cover on pod initiation, pod retention and seed set in Larslund and Väster-malma. For each variable the estimated effect on the response variable (Effect), F-value and p-value is presented.

		Larslund			Väster-malma		
Response	Character	Effect	F-value	p-value	Effect	F-value	p-value
Pods initiation	Number of flowers	0.0051	0.078	0.78	-0.010	0.99	0.32
	Start	-0.028	2.41	0.12	-0.0087	1.00	0.32
	Duration	-0.0078	0.19	0.66	-0.0066	0.57	0.45
	Plant size	-1.6E-05	0.0006	0.98	0.00036	0.54	0.46
	Synchrony	0.022	0.24	0.62	0.083	0.085	0.77
	Canopy cover	**0.014**	**13.95**	**<0.001**	0.00034	0.069	0.79
Pod retention	Number of flowers	-0.0025	0.014	0.90	-0.0054	0.092	0.76
	Start	-0.016	0.60	0.44	0.015	1.13	0.29
	Duration	-0.0015	0.0054	0.94	**0.034**	**5.23**	**0.025**
	Plant size	0.00044	0.37	0.54	0.00022	0.070	0.79
	Synchrony	0.53	1.20	0.28	0.66	1.86	0.17
	Canopy cover	**0.013**	**10.09**	**0.0020**	**-0.0067**	**9.30**	**0.0031**
Seed set	Number of flowers	-0.024	0.024	0.90	0.025	0.0059	0.93
	Start	0.011	0.0030	0.96	**0.61**	**5.05**	**0.027**
	Duration	0.17	0.73	0.39	**0.83**	**9.65**	**0.0026**
	Plant size	0.0060	0.73	0.39	0.026	3.02	0.086
	Synchrony	5.87	1.57	0.21	**27.49**	**9.57**	**0.0027**
	Canopy cover	**0.19**	**22.77**	**<<0.001**	**-0.087**	**4.67**	**0.034**

Earlier start date was associated with a higher pod production (Fig. 8a) and a lower seed set (Fig. 8b)
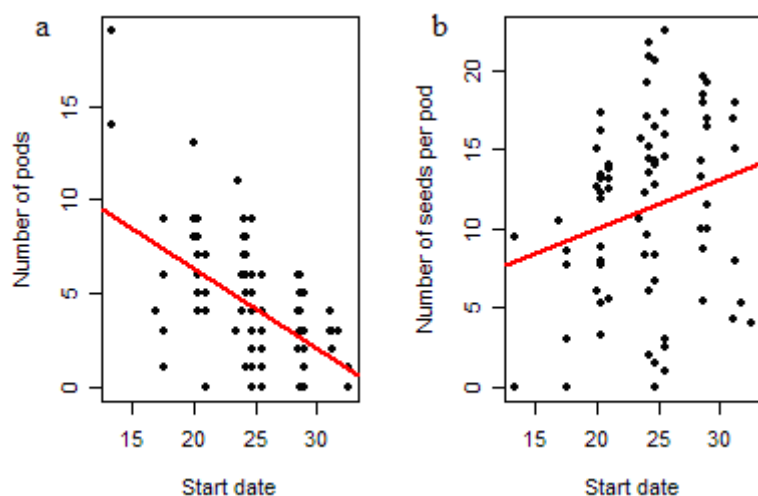
Fig. 8. The relationship between start date and a) pod set and b) number of seeds per pod.

Discussion

My results show that there was selection for increased duration and synchrony in Väster-malma. There was also an effect of the quadratic term for start date. In Larslund, I observed selection on flower number and plants growing shadier had a higher fitness. Although the attack by flea beetles significantly influenced fitness in one of the populations, attack had only small effects on selection. In Väster-malma the observed selection acted on the pod retention and seed set. In Larslund canopy cover affected all ontogenetic steps from pod initiation to seed set.

Number of flowers, duration and start date were correlated. This together with an strong correlation between plant size and number of flowers indicates that these traits are related to plant resource state. Plants with abundant resources can grow taller than plants with lower amounts of resources (Ollerton & Lack 1998). Due to abundance of resources the plant will also be able to produce more flowers and is able to start flowering earlier in the season. More flowers will result in a longer duration of the flowering period as *C. amara*'s flower buds open sequentially. Synchrony was negatively correlated to duration and number of flowers. This is most likely due to the fact that plants with few flowers flower in the middle of the flowering period and therefore achieves a higher synchrony value. Plants with many flowers have a longer duration and are therefore less synchronized because fewer plants flower simultaneously in the population at the start or in the end of their flowering period.

In the Väster-malma population, I found evidence for selection on number of flowers, duration of the flowering period and synchrony within the population. Selection for increasing the number of flowers could be due to that the more flowers the plant has the more pods and seeds can the plant produce (Wise & Cumming 2002). But it could also be that increased number of flowers may increase the attractiveness for pollinator (Johnson & Nilsson 1999) and thereby the probability to become pollinated. Duration and number of flowers is often linked if the flower buds open sequentially, and more buds will automatically increase the flowering period. However, in my analyses I have corrected for number of flowers by. Therefore increased duration has to have an effect on fitness that is not due to more flowers. Increasing the duration of the flowering period does not only increase the chance to be found by pollinators but also increases the chance that the pollinator learns that this is a reliable and stable source of food and thereby begins to visits the plant regularly (Thomson 1980). It could also be that the number of active pollinators varies during the season and by flowering during

a longer period of time the plant is more likely to flower at times when the pollinators are more abundant.

Although both number of flowers and duration were under selection, plant size was not (Table 2). Ollerton and Lack (1998) claims that, due to the often observed correlation between these three traits and the fact that larger individuals often produces more seeds compared to smaller individuals, it is often hard o entangle if the observed selection on number of flowers and duration is due to the trait itself or an effect of plant size. However, in the multiple regression takes into account the effect of plant size and the variable is not significant (F= 0.59 p= 0.45), making it unlikely that the observed selection on number of flowers and duration is due to an indirect effect of plant size.

In this study, selection acted for increased synchrony. Increased synchrony could further decrease the pollen limitation by increasing the number of pollen sources in the area. By becoming more synchronous with the population, the plant will also increase the probability for pollinators to find the population as there are more flowers for the pollinators to find (Rathcke & Lacey 1985).

The analyses showed a negative effect of the quadratic term of start date on seed production, although there was no significant effect of the linear term (Table 3).

In Larslund I found a linear as well as an non-linear selection for an increased the number of flowers. The non-linear selection disappeared when accounting for the flea beetle attack, but the linear selection for increasing the number of flowers remained. I also found an effect of the interaction between plant size and number of flowers. The most likely explanation of this pattern is that the smaller the plant is, the more fitness increases with increasing number of flowers. This could be due to that smaller individuals often are harder to find for the pollinators compared to larger individuals (Vanhoenacker et. al 2006). Increasing pollinator attractiveness will be more important for smaller plants. There was no selection on any other plant traits. Canopy cover had a positive effect on plant fitness.

The shadier the plant grew, the smaller the probability of being attacked were (Fig. 1), and flea beetle attack decreased plant fitness (Fig. 4). This is most likely the reason to why canopy cover had a positive effect on fitness in Larslund: by growing shadier the plants is less likely of being attacked.

The flea beetles were also the reason for the nonlinear selection on flower number. When the analysis was accounted for attacks the effect disappeared. Interesting to note is that the estimate went from 0.24 to 0.20 and the p-value from 0.042 to 0.078 when I corrected for the flea beetles. So the actual effect of the flea beetles seems to be small. Flea beetles had no effects on other traits. Overall, flea beetle attack thus had no or very small effects on selection (Table 2 and 3).

The key factor analysis showed that mortality during pod retention was most important for variation in seed production. Interesting to note is that there is a negative correlation between seed mortality and total generation mortality. This indicates that the plant can calibrate the number of seeds a pod will produce to the available resources. If few pods survive to maturity, they will instead produce a higher amount of seeds compared to when many pods survive.

Canopy cover but no plant traits were correlated with plant fitness in Larslund. An increase in canopy cover positively affected pod initiation, pod retention and seed production. This is still most likely due to the fact that flea beetles prefer plants growing sunnier.

In Väster-malma no trait affected the pod initiation rate. This was unexpected as the selection analysis showed that several aspects of flowering phenology influenced seed production, and one may assume that these relationships are mediated by pollinators and that pollen availability should primarily affect pod initiation. Instead duration and canopy cover influenced pod retention, a step that appears to be more associated with available resources. To be able to keep the initiated pods and develop it into a large seed containing pod, the plant needs resources to pod and seed production. For seed set, duration, synchrony, start date and canopy cover had significant effects. Seed set is most likely a combination of pollination success and resource availability. Although the flower became pollinated, the pollen load does not need to be enough to pollinate all the ovules. The pollen load will therefore limit the maximum number of seeds produced. But after pollination the plant need to have enough resources to be able to develop all pollinated seeds. If it has not enough resources, then plant will be forced to abort seeds until there is a balance between number of seeds and resources.

The duration of the flowering period had a positive effect on both pod retention and seed production. The longer the plant flowered the more large pods survived and the more seeds per pod did the plant produce. It could be, as stated in the selection analysis, that plant that

flowers for a longer period of time have more time to attract pollinators and thereby get a larger pollen load.

Start date had a significant positive effect on seed set, although it did not have any significant effect in the linear selection analysis. It did however have a negative nonlinear effect on seed production. A correlation analysis between start date and number of produced pods and seeds per pod, showed that start date had a negative effect on pod production (Fig. 8a) and a positive effect on seed set (Fig. 8b). This is in line with the key-factor analysis which suggested that *C. amara* compensate for a lower pod production by increasing the number of seeds per pod. This indicates that later flowering plants produces less pods then earlier flowering ones, but instead is able to put more resources into each pod. More available resources results in that the plant is able to produce a higher number of seeds per pod. This pattern has been observed in other study systems (Ehrlén 1992; Guitían et. al 1996).

Increased synchrony has positive effect on the seed set, just as it had in the selection analysis. This is most likely due to pollen limitation: increased synchrony increasing available pollen sources and pollinator attractiveness in the population.

Canopy cover has a negative effect on both large pod survival and seed per pod production. This is the opposite to the effect of increasing canopy cover in Larslund. It could be that pollinators prefer sunnier habitats and therefore visit plants in the shade less often. Another alternative explanation is that the plant has less available resources in the shaded spots due reduced photosynthesis.

Conclusions

There seems to be selection on flowering phenology traits in *C. amara.* Selection acts for increasing the number of flowers, and duration and synchrony of flowering. The observed selection is most likely due to an interaction between pollen and resource limitation. When flea beetles are present in the population they relax the natural selection on flowering phenology traits and change habitat preferences by decreasing reproductive success of plants growing sunnier. For future studies it would be interesting to further investigate the mechanisms behind the observed selection as well as investigating if selection on flowering phenology traits fluctuates between years and populations.

Acknowledgment

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