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Plant-herbivore synchrony and selection on plant flowering phenology

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**Abstract.** Temporal variation in natural selection has profound effects on the evolutionary trajectories of populations. One potential source of variation in selection is that differences in thermal reaction norms and temperature influence the relative phenology of interacting species. We manipulated the phenology of the butterfly herbivore *Anthocharis cardamines* relative to genetically identical populations of its host plant, *Cardamine pratensis*, and examined the effects on butterfly preferences and selection acting on the host plant. We found that butterflies preferred plants at an intermediate flowering stage, regardless of the timing of butterfly flight relative to flowering onset of the population. Consequently, the probability that plant genotypes differing in timing of flowering should experience a butterfly attack depended strongly on relative phenology. These results suggest that differences in spring temperature influence the direction of herbivore-mediated selection on flowering phenology, and that climatic conditions can influence natural selection also when phenotypic preferences remain constant.

**Key words:** *Anthocharis cardamines; Cardamine pratensis; herbivore preference; natural selection; thermal reaction norm; trophic interaction.*

**Introduction**

The timing of life-cycle events is of fundamental importance for most organisms. In seasonal environments, timing within years will have a strong influence on interactions with the local abiotic and biotic environment and thus the performance of individuals (Forrest and Miller-Rushing 2010, Visser et al. 2010, Ehrlén 2015). Selection on phenology can be mediated by abiotic factors, as most organisms must synchronize major life-history events, e.g., migration and reproduction, with favorable environmental conditions (Rathcke and Lacey 1985, Visser et al. 2010). Selection on phenology is also mediated by biotic interactions, in terms of positive fitness effects of synchronizing with mutualists, or negative effects of being synchronized with antagonists (Elzinga et al. 2007, Kolb et al. 2007, Yang and Rudolf 2010). In many systems, selection on timing of reproduction is therefore the combined result of selection from multiple biotic and abiotic agents (Schluter et al. 1991, Elzinga et al. 2007, Kolb et al. 2007, Ehrlén 2015).

Selection on many traits, including seasonal timing of reproduction, has been found to vary over space and time (Siepielski et al. 2009, 2013). In plants, such spatiotemporal variation in selection on timing of reproduction might have several different causes. Variation in the relative abundance of antagonists and mutualists can cause variation in strength and direction of selection among populations or seasons (Kudo 2006, Elzinga et al. 2007, Kolb et al. 2007, Weese et al. 2010). In addition, variation in phenotypic preferences of interacting species among populations or years can result in variation in selection (Wise et al. 2009, Sato et al. 2014). Moreover, also when interaction intensities and phenotype preferences are constant, genotypic selection might vary if genotype–phenotype relationships vary. For example, imagine a herbivore with a preference for host plants in a specific stage of floral development, where the herbivore and the plant differ in thermal reaction norms. In such a system, among-year variation in temperature would result in variation in phenological synchrony between the interacting species. In years when herbivores co-occur with early flowering plant individuals, the herbivore would mediate selection for late flowering. In years when herbivores co-occur with late flowering plants, the direction of selection on flowering time would be reversed (Fig. 1). While evidence of spatiotemporal variation in phenotypic selection mediated by species interactions is fast accumulating, we still know little about the mechanisms underlying this variation (Gienapp et al. 2014, Ehrlén 2015). Studies examining how differences in reaction norms between interacting species influence their relative phenology and trait preferences, and how this influences natural selection are therefore much needed.

In this study, we experimentally investigated whether differences in synchrony between a plant and its main
herbivore affected the herbivore’s preference for plant phenotypes differing in floral development stage, and how differences in synchrony given herbivore preference for a given floral development stage translate into selection acting on plant genotypes differing in flowering phenology. As a model study system, we used a phenological specialist, the orange tip butterfly (*Anthocharis cardamines* L.) and its main host plant, the cuckooflower (*Cardamine pratensis* L). The young larvae of *A. cardamines* feed almost exclusively on early developmental stages of the fruits (Wiklund and Åhrberg 1978, Courtney 1982, Dempster 1997). Butterflies must therefore synchronize their development with plant flowering and fruit initiation and oviposit on plants with floral organs in a stage that maximizes offspring fitness (Wiklund and Åhrberg 1978, Dempster 1997). For plants, butterfly attack has strong negative effects on fitness (Arvanitis et al. 2007, 2008, König et al. 2014). In this system, plant and butterfly development differ in sensitivity to temperature (Phillimore et al. 2012, Posledovich 2015). In accordance, a field study carried out in Southern Sweden indicates that timing of *A. cardamines* oviposition relative to flowering of *C. pratensis* differ among years (König et al. 2015). Based on this, we made two predictions. First, because the growth and development of the butterfly larva needs to be closely timed with the development of the host plant’s fruits, we predicted that the butterfly will show constant phenotypic preference for a given floral development stage over the flowering season, irrespective of mean plant development at the time of butterfly oviposition (Fig. 1a). Second, we predicted that if there is genetic variation for flowering time, then a constant floral development stage preference of the butterfly should result in that selection acting on host plant flowering phenology is contingent on synchrony. More specifically, differences in mean floral development in host plant populations at the time of butterfly oviposition should result in that early or late flowering genotypes are used for oviposition to different degrees (Fig. 1b–d). We tested these two hypotheses by experimentally manipulating relative phenology and introducing newly eclosed and recently mated naïve females to genetically identical plant populations that differed in their mean floral development stage. The results show that butterfly preference for plant floral development stage is unimodal and not influenced by relative timing, and that as a result of this preference, the probability that plant genotypes differing in timing of flowering should experience a butterfly attack strongly depends on relative timing.

**Methods**

**Study system**

*Cardamine pratensis* L. (Brassicaceae) is a perennial rosette herb that is common in most parts of Europe as well as in Central Asia (Hultén and Fries 1986). For this study, we used the tetraploid subspecies *pratensis* that
occurs naturally in southern Sweden where the experiment was carried out. Individuals of this subspecies produce one to several inflorescences that reach 15–50 cm in height, and have up to 30 pink or white flowers (Lökvist 1956). Flowers are self-incompatible and flowering is initiated in May and lasts for 6–7 weeks (Lökvist 1956, Arvanitis et al. 2007). During flowering, *C. pratensis* is frequently attacked by the butterfly herbivore *A. cardamines* L., which is ophiogonaphous on Brassicaceae plants (Wiklund and Åhrberg 1978, Wiklund and Friberg 2009). The butterfly flies for 3–4 weeks in May–June. Female butterflies use the flowers as a cue to find suitable host plant individuals and oviposit on buds or young flowers (Wiklund and Åhrberg 1978). When the larva hatches, after 7–10 d, it is dependent on flowers and young siliques as a food source (Wiklund and Åhrberg 1978, Courtney and Duggan 1983), but can eventually consume also vegetative parts of the plant (Arvanitis et al. 2008), and thus have strong negative effects on plant fitness (König et al. 2014).

### Plants

To replicate the same genetic plant individuals (genets) over all trials, we used clonal replicates in the experiment. In June–July 2013, we clonally propagated plants from 55 genets by potting pinnules from rosette leaves. Plants were sampled within an area of approximately 45 km² in Ludgo parish, southern Sweden (58°56′ N, 17°09′ E). Propagations produced at least nine genetically identical plants (ramets) from each of the 55 mother plants. Pinnules from all genets were rooted during a couple of weeks. Plants were kept in a common garden at Stockholm University during the autumn and winter. In late April 2014, we transported the plants to Tovetorp Research station, located approximately 100 km south west of Stockholm (58°56′ N, 17°09′ E), and kept them outdoors until they were used in the experiment. To reduce possible effects of differences in microenvironment, we shifted the positions of the plants regularly.

### Rearing of butterflies

The butterflies used in the experiment originated from eggs laid by females from Ljusterö Island, southern Sweden (59°30′ N, 18°35′ E) in 2012. The larvae from these eggs were reared to pupation on *Alliaria petiolata* (Brassicaceae) and allowed to overwinter in a cold room at 2°C. After hibernation in the pupal stage, the emerged adults of this parental generation were mated in laboratory cages in spring 2013. The butterflies were then allowed to lay eggs on *Alliaria petiolata* (Brassicaceae), on which we reared a second generation of larvae until pupation. To produce newly emerged and mated females of this second generation for experimental trials at different dates, we brought the pupae to warm conditions (23°C and a 22 : 2 light:dark period) sequentially from late April until the end of May 2014. The experimental trials were performed between May 16 and June 10 in that same year. During this period, 5–10 male and an equal number of female pupae were brought to warm conditions at approximately 5 d intervals. In total, we mated 76 females between May 7 and June 8. Matings were performed in cages (0.8 m long, 0.8 m wide and 0.5 m high) that were placed near big windows and under 400 W HQIL lamps. We brought the mated female butterflies to a cold room maintained at 8°C before transporting them to Tovetorp Research Station, where they were kept individually in 180 mL cups in a refrigerator (5–10°C) until they were used in the experiment. No adult females thus had any experience of the host plant species used for the experiment, or of any Brassicaceae species as adults. The butterfly females had not laid any eggs before they were introduced into the experimental trials.

### Experimental design

The overall logic of the experimental design was to keep plant-butterfly synchrony under experimental control by allowing plants to develop freely under ambient temperatures throughout the series of experimental trials, while introducing naïve, newly eclosed and recently mated females, i.e., of the same developmental stage, in all trials.

For the experimental trials, we used a large outdoor cage (20 m long, 6 m wide and 4 m high), located at Tovetorp Research station (58°56′ N, 17°05′ E). Prior to the first experiment, we installed ground level stands for the plant pots. We arranged the plants in a grid (five rows with 11 individuals), so that no plant should have <1 m to the nearest plant during the trials. We used stratified randomization to distribute the plant ramets among experimental populations, with the aim of creating nine groups of *C. pratensis* individuals with the same composition of genets. Within the experimental populations, we gave each ramet a random position in the grid. In total, we thus used 495 plant ramets (i.e., 9 experimental trials × 55 ramets). All plants used in the experiment had visible flower buds, and the variation in floral development stage among plants within experimental trials was similar to the variation observed in the field during different phases of the flowering period. The interval between trials ranged from 1 to 7 d and was largely determined by weather conditions, as the butterflies are only active on sunny days and at temperatures over approximately 17°C (C. Wiklund and M. Olofsson, personal observation), (Appendix S1: Fig. S1). In each experimental trial, we introduced three recently hatched and mated *A. cardamines* females to the experimental plant population and monitored the activity of the females. Three persons were observing the butterflies throughout each experimental trial, following one butterfly each. The experimental trials continued until the butterflies had oviposited on about 50% of the plants, at what stage they tended to lay their eggs more often on ramets already oviposited upon than on remaining plants that had not been oviposited on, or when weather conditions became unsuitable for the butterflies (trial duration: mean,
29.1–50.9%). The mean proportion of plants oviposited on in the trials was 44.0% (range = 29.1–50.9%).

Data collection

We recorded floral development stage of all ramets every second or third day from the beginning of May until all flowers had opened in late June, and at the start of each experimental trial. At each recording, we counted the number of reproductive organs in each of six stages: buds (small, medium or large), flowers (open, wilted) and siliques. We calculated the temporal developmental stage of each ramet as the proportion of open reproductive organs that were in the three later developmental stages (open and wilted flowers, and siliques), on the day it was included in an experimental trial. Mean first flowering day (calendar day) of genets was calculated over all ramets from each genet (first flowering day of genets: mean, range = 143.4, 138.2–148.2). First flowering day for each ramet was derived from the recordings of floral development described above. When a ramet initiated flowering between two recording days, we assigned the ramet first flowering day based on the size of the largest bud at the last recording before flowering. The measure of mean first flowering date of genets was thus a trait of the genet that was constant over all experimental trials and the same for all nine ramets of each genet.

During the experiments, we recorded the time for each visit to a plant and whether oviposition occurred at this visit. A visit was defined as when a butterfly landed on a plant. At the end of each experimental trial, the plants were again checked for eggs and the final egg count was cross-referenced with the recorded oviposition events. Using this information, we derived two measures of butterfly preference for plant phenotype. First, we compared ramets oviposited on during the course of the experiment with plants not oviposited on (a total 218 of the 495 ramets were oviposited on in the nine experimental trials). Second, for ramets oviposited on, we compared the time in seconds until first oviposition (mean, range = 3,790, 100–12,678). We analyzed incidence of oviposition separately, since not all ramets are suitable for oviposition in the sense that they will be oviposited on given much longer exposure to butterfly females. This could be due to that plants with no or few flowers are not visible to the butterfly or that butterfly females discriminate against plants that are too small (Arvanitis et al. 2008). In the experiment, this was evident from that butterflies tended to start laying eggs on ramets previously oviposited on at some stage of the trial. We used time to oviposition as an additional response variable as, in our experiment, all suitable host plants might be oviposited on because the butterfly females were given a limited number of hosts in a restricted space. However, under more natural conditions the choice among potentially suitable host plants by butterfly females should be important if the number of butterfly females per plant is lower or the time spent by butterflies in a plant patch is shorter, and the least preferred of the suitable hosts escape predation.

To characterize differences in plant development among experimental trials, and thus relative synchrony at each trial, we used the cumulative heat sum (growing degree-days) at each trial date. We expected mean plant developmental stage to be more strongly correlated with growing degree-days than Julian dates. We calculated the summed growing degree-days from January 1 for each experimental trial day, using the maximum daily temperatures measured by the weather monitoring station at Tovetorp, located 2.8 km from the cages. An experiment by Toftegaard et al. (2016), suggests that little or no development occurs in temperatures below 5°C, and we thus used this temperature as the baseline temperature for calculation of growing degree-days.

Analyses

We performed all analyses using R version 3.2.4 (R Core Team 2016). To obtain an estimate of the genotype component to flowering phenology (including possible non-genetic transgenerational effects), we calculated the proportion of the total variance in first flowering day that was explained by genet. We extracted the genet variance component from a linear random effects model with untransformed measures of first flowering day as the response and with genet as a random factor (function: lmer, package: lme4, Bates et al. 2015), and divided it by the total variance.

Our experimental protocol provided information about butterfly preference both in terms of whether plants were oviposited on or not and in terms of time to oviposition in plants oviposited on, and the results were analyzed in two steps. First, we estimated the probability of a ramet being oviposited on using generalized linear mixed effect models with binomial response. Second, we estimated the effect of the same predictors on time to oviposition with linear mixed effect models for the non-zero part of the data. Time to oviposition was In-transformed to improve residual heterogeneity, and all predictor variables were standardized to have zero mean and a variance of 1 using the scale function in R (package: base).

Our first prediction was that butterfly phenotypic preference for host plant floral development stage will remain constant and unimodal during the flowering season, regardless of the relative timing of butterfly development to plant floral development. To test this prediction, we used floral development stage (the proportion of the total number of flowers produced by a ramet that had opened at the time of the experiment) as a predictor in the models on incidence of oviposition and time to oviposition for plants oviposited on, respectively. We also included the quadratic term of floral development stage in the models to test for non-linear effects. To test for differences in phenotype preference over the flowering period, growing degree-days, and the interaction terms floral development
stage × growing degree-days, and floral development stage^2 × growing degree-days, were included in the models. The statistical tests of effects of these interaction terms were, however, likely hampered by the limited overlap of floral developmental stages of ramets between the earliest and the latest trials (Appendix S1: Fig. S1). We therefore examined interaction effects also in models that included only trials 5–8, which covered the broadest ranges of ramet floral developmental stage. These models yielded similar results for floral development stage × growing-degree days and floral development stage^2 × growing-degree days (P > 0.05 in all cases) as the models including all trials, and only the latter are presented in the results section. In all models, we also included the total number of flowers produced by each ramet as a covariate. Lastly, to control for effects of plant position in the cage, position was included as a fixed factor with two categories, edge and core. Experimental trial was included as random factor to control for possible variation specific for the conditions during each experimental trial, e.g., weather conditions and individual female behavior. Models on incidence of oviposition and time to oviposition were made using the lme4 (Bates et al. 2015) and lmerTest (Kuznetsova et al. 2016) packages in R, respectively. To find the best fitting models, we performed stepwise deletion of parameters based on the Akaike Information Criterion (AIC), as described by Zuur et al. (2009).

Our second prediction was that a constant preference for a given floral development stage will translate into differential selection of early vs. late flowering plant genotypes, depending on the timing of butterfly oviposition relative to the mean phenology of the host plant, i.e., result in a significant effect of the interaction between genet flowering time and relative timing. To test this prediction, we ran models of incidence on oviposition and time to oviposition for plants oviposited on, that included genotype mean values of first flowering day and number of flowers as predictors. To test for non-linear effects of genet phenology, we included the quadratic term of genet mean flowering day and relative timing. To test for variation in butterfly preference of plant genotype over the flowering period, we included growing degree-days, and the interaction terms genet mean first flowering day × growing degree-days and genet mean first flowering day^2 × growing degree-days, in the full models. Lastly, position was included as a fixed factor in the models, and experimental trial and genet as random factors.

### Results

Genet identity explained 48.5% of the total variation in first flowering day of ramets, indicating that genet level for variation in flowering initiation was important, but that there was also considerable variation among ramets originating from the same genet (First flowering day: \( \sigma^2_{\text{among}} = 3.01; \sigma^2_{\text{within}} = 3.19 \)).

<table>
<thead>
<tr>
<th></th>
<th>( \beta )</th>
<th>( \text{SE} )</th>
<th>( Z )</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Floral development stage</td>
<td>0.456</td>
<td>0.176</td>
<td>2.594</td>
<td>0.009</td>
</tr>
<tr>
<td>Floral development stage^2</td>
<td>-0.960</td>
<td>0.231</td>
<td>-4.154</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ramet total number of flowers</td>
<td>0.292</td>
<td>0.103</td>
<td>2.827</td>
<td>0.005</td>
</tr>
<tr>
<td>Position: Core vs. edge</td>
<td>1.568</td>
<td>0.208</td>
<td>7.530</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Note:** All predictor variables were standardized before analyses.

**Butterfly preference for plant floral development stage**

The probability of a ramet being oviposited on was affected by ramet floral development stage and this effect did not vary with synchrony, i.e., butterfly preference was highest for plants in intermediate developmental stages throughout the season. Both the linear and quadratic terms of ramet floral development stage had significant effects on probability of oviposition and were included in the best-fitting model (Table 1). In the preferred floral development stages, flowering had been initiated but all flowers had not opened (Fig. 2). There was no interaction between development stage and growing-degree days; butterfly preference of plant floral development stage was constant over the experimental period, and thus independent of the phenology of butterflies relative to plants. The probability of oviposition increased with number of flowers, and plants located close to the edges of the cage

**Fig. 2.** Barplot of the proportion of *Cardamine pratensis* ramets (n = 495, nine ramets from each of 55 genets), in different stages of floral development, that were oviposited on by the butterfly *Anthocharis cardamines*. The line represents the probability of oviposition by the butterfly *A. cardamines* on *C. pratensis* as a function of plant floral development stage (measured as the cumulative proportion of flowers open at the experimental trial), from a generalized linear model of untransformed data over nine experimental trials.
were oviposited on more often than plants in the center of the cages (Table 1; for correlations of ramet and genet phenology within each trial, see Appendix S2: Table S1, and for full model see Table S2). Differences in time to oviposition for plants oviposited on was not affected by floral development stage and the best-fitting model contained only cage position as predictor variable (Appendix S2: Tables S3 and S4).

**Table 2.** Effects of genet mean first flowering day, growing degree-days at experiment, genet mean number of flowers and position on the probability of *Cardamine pratensis* ramets being oviposited on by * Anthocharis cardamines* butterflies, from a generalized mixed model with genet and experiment as random factors.

<table>
<thead>
<tr>
<th></th>
<th>β</th>
<th>SE</th>
<th>Z</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>First flowering day</td>
<td>−0.046</td>
<td>0.103</td>
<td>−0.449</td>
<td>0.654</td>
</tr>
<tr>
<td>Growing degree-days</td>
<td>−0.035</td>
<td>0.117</td>
<td>−0.302</td>
<td>0.763</td>
</tr>
<tr>
<td>Genet mean number of flowers</td>
<td>0.310</td>
<td>0.104</td>
<td>2.992</td>
<td>0.003</td>
</tr>
<tr>
<td>Position: Core vs. edge</td>
<td>1.483</td>
<td>0.203</td>
<td>7.295</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First flowering day × Growing degree-days</td>
<td>0.340</td>
<td>0.108</td>
<td>3.139</td>
<td>0.002</td>
</tr>
</tbody>
</table>

*Note:* All predictor variables were standardized before analyses.

**Effect of butterfly preference on plant genotypes**

At the genotype level, butterfly preferences were influenced by synchrony with the plant, i.e., there was a significant effect of the interaction growing degree-days × first flowering day on the probability of oviposition. In experimental trials where the butterfly was introduced at earlier stages of plant development, they preferred earlier flowering genets, while in trials when they were introduced at later stages of plant development, they preferred later flowering genets (Table 2, Fig. 3). The quadratic term of genet mean first flowering day had no effect. The probability of oviposition also increased with the mean number of flowers, and was affected by plant position in

**Fig. 3.** Probability of oviposition by the butterfly *Anthocharis cardamines* on *Cardamine pratensis* as a function of first flowering day of plant genets (standardized), from a generalized mixed model over nine experimental trials. Relationships from the nine experimental trials are presented in time order from left to right, top to bottom. The number above each graph is the accumulated number of growing degree-days at the trial date.
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the cage (Table 2; for full model see Appendix S2: Table S5). Differences in time to oviposition for plants oviposited on was not affected by first flowering day of genets and the best-fitting model contained only position as a predictor variable (Appendix S2: Tables S6 and S7).

DISCUSSION

Our manipulations of butterfly timing, while allowing plants to develop freely, made it possible to assess how the outcome of plant–butterfly interactions at the phenotype and genotype level depends on their relative phenology. The conditions examined in the experiment are likely to represent conditions that are similar to those actually experienced by plants and butterflies in the field in different years. Development rates of our two model species have been shown to differ in their response to spring temperature (Phillimore et al. 2012, Posledovich 2015). This implies that relative phenology of A. cardamines and its host plants should differ among years in response to differences in spring temperatures, which is in accordance with field observations from Southern Sweden (König et al. 2015).

Our results are consistent with that butterflies preferred plants at an intermediate flowering stage, regardless of the timing of adult butterfly emergence relative to flowering onset of the population. As a result of the preference for plant phenotypes in intermediate flowering stages, butterfly selection acting on flowering phenology of genotypes varied with the timing of butterflies relative to the mean developmental stage of plants. Early flowering genets were preferred in experimental trials early in the season, while late flowering individuals were preferred later in the season.

Butterfly preference for plant floral development stage

One possible explanation for consistent preferences of butterflies for plants in intermediate flowering stages in this system is that the white flowers and maturing buds of plants in an intermediate stage of flowering provide a stronger visual cue for the butterflies than plants that are in the bud stage, or flowers that are starting to wilt, as A. cardamines are attracted to the light colored flowers of their host plants (Wiklund and Åhrberg 1978). It is also likely that plants in intermediate flowering stages provide the best combination of food quality and quantity for the developing larvae. In plants at too early developmental stages, floral parts might be consumed completely by the larva, leading to host plant switch or starvation. At the other end, plants oviposited on in a late flowering stage might mature fruits and become unsuitable as food before the larva has completed development. Indeed, Posledovich et al. (2015) showed that C. pratensis plants that were oviposited on in late flowering stages were the least likely to allow completion of larval development. Although we found no evidence of that butterfly preferences for floral development stage change with synchrony, tests for effects of interactions between floral development and synchrony in this experiment were hampered by that the ranges of floral developmental stages differed among trials. Still, also analyses including only the trials covering broad ranges of ramet floral developmental stage failed to detect any such effects. Preferences for a given plant development stage have been shown also in other systems. For example, Phengaris alcon primarily oviposit on Gentiana pneumonanthe plants that have many young buds (Arnaldo et al. 2014, Wynhoff et al. 2015), and larval survival is strongly related to host plant phenology and size (Arnaldo et al. 2014). Moreover, hatching before leaf budburst in Quercus robur may cause larval starvation in Opeopthera brumata (Visser and Holleman 2001). Taken together, the results of our and other studies suggest that when host recognition, or offspring performance, is dependent on developmental stage of the host, we should expect consumer preferences of developmental stage, as well as selection mediated by consumers, to be independent of timing relative to the mean phenology of the host plants.

Effect of butterfly preferences on selection acting on plant genotypes

The butterflies primarily oviposited on early flowering genets early in the season, and on late flowering genets later in the season. This pattern was the result of that butterfly preferences for floral developmental stage did not change significantly over the season in combination with differences in the timing of the butterfly relative to plant development. Early flowering genets were more often in the preferred stage of floral development early in the season, and late flowering plant individuals were more often in the most preferred stage at the end of the season. This implies that in years when the butterfly is early relative to the mean flowering phenology of plants, the earliest flowering individuals will be preferred for oviposition, while in years when the butterfly is late, late-flowering plants will be more attacked. In this scenario, synchronization of butterfly flight time and plant flowering time might also potentially lead to disruptive selection on plant flowering time, favouring plants flowering early or late relative to the mean of the plant population. However, in our experiment we were not able to detect any evidence of disruptive selection. Although no formal analysis of heritability was made, the large part of variation in flowering time explained by genet suggests that timing of flowering is heritable in C. pratensis, and that a large fraction of the observed variation in flowering phenology among ramets occurred at the genet level. The observed variation in flowering phenology among genets of C. pratensis thus strongly suggests that different genotypes should be in the preferred stage of floral development in years differing in spring temperature. Given that larval herbivory has strong negative effects on plant fitness (König et al. 2014), differences in flowering phenology among genets, in combination with differences in thermal reaction...
norms between butterflies and plants, should translate into temperature-driven variation in herbivore-mediated genotypic selection on plant flowering phenology. In our study system, experiments suggest that the plant shifts its phenology faster than their butterfly herbivore in response to increasing temperatures (Posledovich 2015). This would imply that in warm springs, butterfly flight time should be late relative to flowering time, while in cold springs butterflies should be early relative to flowering. Variation in phenological synchrony has also been found to result in variation in strength and outcome of interactions in other systems. For example, the egg loads of the weevil Rhinocyllus conicus on Cirsium canescens flower heads increased with increasing phenological synchrony (Leland Russell and Louda 2004). Also, the intensity of interactions between the plant Armeria velutina and its butterfly herbivore Cyaniris semiargus varied among patches differing in ground water level, as water availability affected the phenology of the plant, but not that of the butterfly (Rodriguez et al. 1994). Taken together, the result of our and other studies thus suggest that environmental variation over time and space in combination with differences in the sensitivity of development rates to environmental factors may often lead to differences in synchrony and natural selection.

CONCLUSION

Identifying the mechanisms underlying variation in selection on timing of reproduction is a key objective in studies of life histories. Such knowledge is also fundamental to make predictions about the outcome of interactions and selective regimes in changing environments. Although potential effects of climate variation in combination with differences in sensitivity to temperature between interacting organisms has often been discussed (e.g., Visser and Both 2005, van Asch and Visser 2007, Forrest and Miller-Rushing 2010, Gienapp et al. 2014, Ehrlén 2015), few studies have yet attempted to separate the effects of synchrony, genetic variation and phenotype preferences on selection on flowering phenology (but see Austen and Weis 2015). We suggest that the pattern shown in our experiment, where constant phenotypic preferences translated into variation at the genotype level as a result of differences in relative phenology, constitute a potentially important mechanism for generating both temporal and spatial variation in selection. Differences in reaction norms of development between interacting species, in combination with environmental variation, might thus often lead to differences in phenological synchrony and variation in natural selection, also when phenotype preferences are constant.

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LITERATURE CITED


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