

The developmental race between maturing host plants and their butterfly herbivore – the influence of phenological matching and temperature

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Summary

1. Interactions between herbivorous insects and their host plants that are limited in time are widespread. Therefore, many insect–plant interactions result in a developmental race, where herbivores need to complete their development before plants become unsuitable, while plants strive to minimize damage from herbivores by outgrowing them.

2. When spring phenologies of interacting species change asymmetrically in response to climate warming, there will be a change in the developmental state of host plants at the time of insect herbivore emergence. In combination with altered temperatures during the subsequent developmental period, this is likely to affect interaction strength as well as fitness of interacting species.

3. Here, we experimentally explore whether the combined effect of phenological matching and thermal conditions influence the outcome of an insect–host interaction. We manipulated both developmental stages of the host plants at the start of the interaction and temperature during the subsequent developmental period in a model system of a herbivorous butterfly, *Anthocharis cardamines*, and five of its Brassicaceae host plant species.

4. Larval performance characteristics were favoured by earlier stages of host plants at oviposition as well as by higher developmental temperatures on most of the host species. The probability of a larva needing a second host plant covered the full range from no influence of either phenological matching or temperature to strong effects of both factors, and complex interactions between them. The probability of a plant outgrowing a larva was dependent only on the species identity.

5. This study demonstrates that climatic variation can influence the outcome of consumer–resource interactions in multiple ways and that its effects differ among host plant species. Therefore, climate warming is likely to change the temporal match between larval and plant development in some plant species, but not in the others. This is likely to have important implications for host plant use and possibly influence competitive relationships.

Key-words: climate change, insect–plant interaction, orange tip butterfly, phenological matching, synchronization

Introduction

In seasonal environments, the strength of species interactions is determined by the degree of temporal overlap between species and depends on the timing of life cycle events (i.e. phenology) in each of the interacting species

(Harrington, Woiwod & Sparks 1999; Russell & Louda 2004; Høye *et al.* 2013). In herbivorous insects, temporal matching with host plants, that are suitable as food only for a short period of time, is widespread (e.g. van Asch & Visser 2007; Aide & Londoño 2008; Singer & Parmesan 2010). In many cases, this sets the stage for a developmental race between insect larvae and the developing plants, that is the insects need to finalize larval growth before the plants become unsuitable as food, and plants will be

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selected to develop in a way that minimizes damage from the herbivores.

Temperatures, and thus climate change, can influence the outcome of such consumer–resource interactions via differential effects on the development of the respective species. This may occur in at least two ways. First, early spring temperatures determine plant developmental rates and the timing of insect emergence before the onset of the interaction. Changes in the degree of developmental progression and timing of co-occurrence are normally the focus of phenological synchrony studies (Harrington, Woiwod & Sparks 1999; Russell & Louda 2004; van Asch & Visser 2007; Forkner *et al.* 2008; Rafferty *et al.* 2013), as climate change has been shown to cause alterations in phenologies of many species (Walther *et al.* 2002; Parmesan 2006; Walther 2010). Secondly, temperature may have differential effects of the species development during the period of direct interaction (e.g. Hill & Hodkinson 1992). These two types of effects might be counteracting or reinforcing, and the outcome of the insect–host plant race can sometimes be reversed by developmental temperature depending on the relative thermal sensitivities between insects and plants (Hill & Hodkinson 1992).

Both these aspects of temperature impact – on phenological synchrony and relative developmental rates – have been demonstrated to influence the outcome of insect–plant relationships. Phenology of host plants is often a key aspect of insect–plant interactions, as nutrient content and profile of defensive compounds often differ between developmental stages of plants. Consequently, in many insect species, larval performance and survivorship are affected by the degree of synchrony with plant phenology (Hill & Hodkinson 1992; Jordano & Gomariz 1994; Parry, Spence & Volney 1998; Forister 2005; Aide & Londoño 2008). For example, early egg hatching in winter moth (*Operophtera brumata*) and several species of psyllids (Hemiptera) before budburst/catkin formation would cause larval starvation, while individuals that start development very late instead face high content of leaf tannins and matured catkins (Feeny 1970; Hill & Hodkinson 1992; Visser & Holleman 2001). As a result, the ability to synchronize larval feeding with appropriate stages of host plant development will have a large impact on survival, reproductive success and population dynamics in many herbivorous insects (Feeny 1970; Cappuccino & Kareiva 1985; Stamp & Deane 1990; Cushman *et al.* 1994; Jordano & Gomariz 1994; Awmack & Leather 2002; Yukawa & Akimoto 2006). It is typically the case that earlier plant phenology is associated with higher larval performance. The other aspect – thermal conditions during the subsequent developmental period – has been shown to influence herbivore fitness by accelerating senescence and changing nutritional quality of host plants (Stamp & Deane 1990; Hill & Hodkinson 1992; Nava-Camberos, Riley & Harris 2001; Hellmann 2002; Kingsolver *et al.* 2006; Braschler & Hill 2007; Pelini *et al.* 2009). In many of these studies, the net effect on herbivore fitness was due to interactions

between temperature and larval diet. This suggests that changes in climatic conditions have the potential to modify host utilization patterns (Liu *et al.* 2011). Although variation both in phenological synchrony and thermal conditions is likely to take place simultaneously under the changing spring conditions, the combined influence of the two factors on the outcome of insect–host interactions has not yet been experimentally explored.

In this study, we examine how the simultaneous effects of host plants being in different developmental stages at the onset of the interaction may affect the outcome of the insect–host plant race under a range of thermal conditions during development in a system of a butterfly and its host plant species. Larvae of the orange tip butterfly, *Anthocharis cardamines*, feed on reproductive organs (flowers and seedpods) of their Brassicaceae host plants that only occur during a very limited period of time, and they need to complete larval development before the seedpods mature, dry out and become inedible (Wiklund & Åhrberg 1978; Courtney 1981; Dempster 1997). Hence, the interaction is temporally constrained and occurs only in spring. As larvae feed on the reproductive organs of their hosts and can destroy the entire inflorescence (Dempster 1997; Arvanitis, Wiklund & Ehrlén 2007), they can have strong effects on fitness of their host plants. Therefore, the host species benefit if they can escape from being oviposited on at early reproductive stages and by completing development before the larva becomes fully grown. Hence, the growing larvae and the developing plants compete via their developmental rates – the insects to complete development – and the plants to reproduce successfully. The study system of *A. cardamines* and its host plants, therefore, is highly relevant for estimating the outcome of such a race for both insects and plants. Within this framework, we explored the combined effects of the degree of phenological synchronization at the beginning of the interaction (i.e. at oviposition) and thermal conditions during the subsequent developmental period on a series of important aspects of the species interaction. We investigated these effects on larval development in several butterfly populations along a latitudinal cline to explore possible local adaptations in larval and host species development. These butterfly populations are genetically differentiated with respect to post-winter pupal development (Posledovich *et al.* 2014). We specifically ask whether these two aspects – phenological synchrony and thermal conditions – influence the suitability of different host plant species for butterfly development and survival, and to what degree host plant species differ in their capacity to outrace the developing larvae and successfully produce seeds.

Materials and methods

STUDY SPECIES

The orange tip butterfly, *Anthocharis cardamines*, is a widespread and common pierid butterfly, occurring across Europe and in

most temperate areas of Asia (Eliasson, Ryrholm & Gärdenfors 2005). The species is obligatory univoltine throughout its distribution and overwinters as a pupa. Adult butterflies emerge in late April–early May in the south of Sweden and late May–early June in northern Sweden. The females oviposit on a range of Brassicaceae species and larval survival seems to vary little between plant species; in central Sweden, a five-year study showed that all of the 16 available plant species were oviposited on and larval survival, under laboratory conditions, exceeded 50% on all but one of these (Wiklund 1982; Wiklund & Friberg 2009). The females usually lay a single egg in the inflorescence, choosing large young flower heads. The larvae feed on flower buds, flowers and growing seedpods, and prefer to leave a plant, when all its reproductive parts are consumed, rather than feed on vegetative parts (Wiklund & Åhrberg 1978; Courtney 1981, 1982; Dempster 1997; Wiklund & Friberg 2009). *Arabis glabra* seems to be the only exception, where the females choose to oviposit on green leaves of the stem before the plant flowers and the larvae can establish their development by feeding on the leaves (Wiklund & Åhrberg 1978). However, at later stages, the larvae typically move to the inflorescences in order to complete development (pers. obs.). Larvae can feed on more than one host species, but as they are not particularly mobile and because hosts are sparsely distributed, they usually fail to find another suitable plant (Dempster 1997).

Thus, *Anthocharis cardamines* and its host plants represent an example of a temporally constrained plant–herbivore interaction during spring, which are considered to be particularly vulnerable to phenological mismatches, and therefore most sensitive effects of climate change (Menzel *et al.* 2006; van Asch & Visser 2007; Forkner *et al.* 2008b; Singer & Parmesan 2010).

We chose five host plant species that are the most abundant in the sampled areas and most commonly utilized by the butterflies in the field (Wiklund & Friberg 2009) and therefore considered to be important for fitness of *A. cardamines*. Among them were two perennial species – *A. hirsuta* (Arhi) and *Cardamine pratensis* (Capr), two annuals – *Arabidopsis thaliana* (Arth) and *Capsella bursa-pastoris* (Cabu), and one biennial *Arabis glabra* (Argl). There is a succession in the flowering time between the plant species with partial temporal overlap between them, which varies among years but with annuals typically being earlier (Wiklund & Åhrberg 1978; Wiklund & Friberg 2009). Since these plant species represent a spectrum from capital to income breeders, they have different possibilities to adjust developmental rate in response to herbivory with the capital breeders expected to be more flexible (Stephens *et al.* 2009).

EXPERIMENTAL DESIGN

The animals were collected 1 year earlier as eggs or first instar larvae from three locations along the eastern coast of Sweden. The southern location is situated in Skåne Province (55°49'N, 14°05'E), the central – in Uppland Province (59°30'N, 18°35'E), and the northern – in Ångermanland Province (63°03'N, 18°19'E). The collected individuals were reared on one of their natural host plants (*Alliaria petiolata*) until pupation and kept in a climate controlled room at +17 °C (12L: 12D) until the end of October, when they were transferred to cold conditions. The pupae overwintered in a climate chamber at +2 °C conditions and constant darkness for 5 months. In spring, pupae were incubated in warm temperatures to induce eclosion and the emerged females were mated, individually marked and allowed to oviposit

on potted host plants of five commonly used species that were in different phenological states. Each plant was presented singly to a butterfly female until it received an egg. The total number of oviposited plants varied from 83 in *A. hirsuta* to 154 in *C. pratensis*, and these ended up with the numbers used in the analyses and shown in the paper below. The plants, each bearing a single newly oviposited egg, were randomly assigned between six climate rooms with three temperature treatments (two replicates per treatment) where we aimed at 13 °C (13.2 ± 0.2 ; 13.6 ± 0.5 in the two rooms, respectively; mean \pm SD), 15 °C (15.4 ± 0.7 ; 16.2 ± 0.3) and 17 °C (17.5 ± 0.4 ; 17.5 ± 1). Average field temperatures during the period of larval development is about 11–13 °C although varying within a range substantially wider than our experimental conditions. The photoperiod was 15L: 9D in all treatments (Osram FQ 54w/865 HO T5 Constant). The experiment continued until the animals pupated. After accounting for larvae that failed to establish (213 individuals), altogether 310 *A. cardamines* individuals pupated: southern population – 3 families with 102 individuals, central population – 13 families with 146 individuals, and northern population – 9 families with 63 individuals.

The different phenological states of the host plants included vegetative stage not long before bud formation (mostly *A. glabra* and *A. hirsuta*, which were the species *A. cardamines* females accepted for oviposition at this stage), bud stage (inflorescences consisted only of buds), flower stage (both buds and flowers were present) and seedpod stage (buds (if any), flowers (if any) and seedpods were present). The exact number of buds, flowers and seedpods was counted for each host plant immediately after it was oviposited upon, and phenological index was calculated as $\#buds/(\#buds+\#flowers+\#seedpods)$. Thus, the phenological index varied between 1 and 0 with earlier developmental stages closer to 1 and later stages closer to 0. The phenological index was set to 1 also for those plants that were at a vegetative stage with no reproductive organs yet formed at the time of oviposition. This allowed us to use host plant data from the vegetative stage at the same scale of phenological index.

LARVAL PERFORMANCE

In order to estimate larval performance on the different host species in relation to their initial phenological state and temperature, the following parameters were measured: (i) larval establishment as the probability of an individual to start feeding; (ii) development time (in days), defined as time between oviposition and pupation; (iii) pupal mass (in grams), measured 30 days after pupation; (iv) larval growth rate, measured as $\log(\text{pupal weight})/\text{development time}$; and (v) survival until pupation and survival until eclosion (after 5 months of overwintering conditions at +2 °C).

HERBIVORE–HOST PLANT DEVELOPMENT RACE

In order to estimate the development race between larvae and host plants in relation to the host's initial phenological state and temperature, we provided larvae with as many host plant individuals as needed to pupate successfully. The second plant was phenologically advanced, all of them being at the seedpod stage, to imitate what larvae would find in nature when changing to another host individual. Larvae were checked every second day, and as soon as all reproductive organs of a host were consumed,

they were transferred to another host of the same species. After larval pupation, each host plant was inspected to determine whether larvae had fed on it after the transfer to make sure that the second plant was really required to complete larval development. The final number of buds, flowers and seedpods of the initial hosts was also recorded. The outcome of the race was measured as follows:

- the probability of a larva needing a second host plant in order to reach pupation and
- the probability of the initial host plant (that was oviposited upon) to outgrow the larva and form mature seedpods that were not suitable for larval feeding.

STATISTICAL ANALYSES

Statistical analyses were conducted with R 3.1.0 statistical software (R Core Team 2014). Linear mixed-effect (LMM; function *lmer*) and generalized linear mixed-effect (GLMM; function *glmer*) models (Bates *et al.* 2014), fitted with the maximum likelihood (ML) method, were used for the data analyses. In all mixed models, climate room and butterfly family identity were treated as random effects.

Larval performance

To investigate how larval performance depended on host species, host phenological state and temperature treatment, each larval performance characteristic was set as a response variable in a separate model – larval establishment (GLMM), development time (LMM), pupal mass (LMM), growth rate (LMM), survival until pupation (GLMM) and survival until eclosion (GLMM) – with host plant species, host plant phenological index, temperature treatment and butterfly population as fixed effects. Since larval establishment and survival until both pupation and eclosion were defined as 1 (established/survived) and 0 (did not establish/survive), the GLMMs were fitted with a binomial error distribution.

Herbivore–host plant development race

One of the two development race characteristics – the probability of needing a second plant individual for a larva to reach pupation (1/0) – was used as a response variable in GLMM with a binomial error distribution. The other characteristic of the development race – the probability of the initial host plant outgrowing the larva (1/0) – was used as a response variable in generalized linear model (GLM) with a binomial error distribution. This was done because there was no variation in the response of *C. bursa-pastoris* and *A. thaliana* (all the plants produced matured seedpods) and the GLMM failed to calculate likelihood estimates. In addition, only the initial hosts that had to be replaced with a second plant were used in the analysis of the probability of a host plant outgrowing larvae, that is plants that had not been exchanged during the experiment (Table 3) were not included as data on seed production from them were not available. These plants obviously had not been able to outgrow the larva, while the majority of these plants did produce seeds as they would have been replaced if there were no seedpods left on them. *Arabis glabra* was not included in this analysis as it hardly ever needed to be exchanged and therefore its response variable was represented only by zeros. In all the models, host plant species, host plant phenological index and temperature treatment were set as

explanatory variables; in the GLM, climate room and butterfly family identity were set as explanatory variables in the initial full model as well. Since larval performance traits – developmental time, pupal mass, growth rate and survival – showed no differences between the three populations, all the individuals were pooled and population identity was not used as a factor in these analyses.

Results

LARVAL PERFORMANCE

There were no significant differences among the butterfly populations in any of the larval performance characteristics.

Larval establishment also did not differ between host species, temperature treatments or plant phenological state (null model: z value = 0.90, P value = 0.37). The mean probability of larval establishment based on the model estimate was 0.58.

Larval developmental time was affected by host species as well as by temperature (Fig. 1a; Table 1a). Development time was shortest on *C. pratensis* and longest on *A. glabra* and, as expected, a higher temperature accelerated larval development (Fig. 1a; Table 1a). A significant effect of the host species \times phenological index interaction was due to extended larval development on early stages of *A. hirsuta* compared to its later stages, while the other species showed the opposite pattern. When *A. hirsuta* was excluded from the analysis, larval development time was negatively correlated with phenological index at oviposition on all other plant species (Fig. 1b; Table 1), with development being on average 2.1 days faster on younger than on older inflorescences (phenological index 1 compared to 0.3) across host species.

Pupal mass also differed between host species and temperature treatments, with pupae becoming heavier on *A. glabra* compared to the other host species, as well as in warmer treatments (Fig. 1c, Table 1b). An increase in temperature from 13 °C to 17 °C resulted in 9–10% gain in pupal mass across plant species, which corresponds to about 2.5% increase per 1 °C. Although larvae received a second plant (of quite late phenological state) after they consumed all reproductive organs suitable for feeding of the first plant, the effect of phenological state of the original plant on pupal mass was still significant with animals growing larger on plants of earlier initial phenology (Fig. 1d; Table 1b). The difference in pupal mass between bud stage of the initial plant (phenological index = 1) and later flower stage (phenological index = 0.3) was about 7%.

Larval growth rate was higher on *C. pratensis* than on all other host species. The influence of temperature on larval growth rate was similar to that on larval development time (above), while host phenological state had no effect. The effect of the interaction host species \times phenological index was close to being significant again due to extended larval development on early stages of *A. hirsuta* (Table 1c).

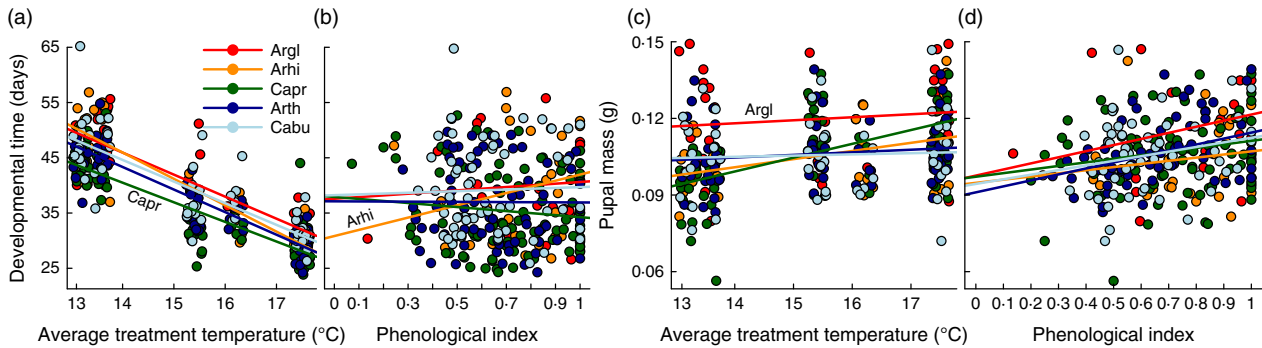


Fig. 1. Larval performance characteristics on the five host species: developmental time (days) in relation to (a) temperature treatment and (b) plant phenological index at oviposition; and pupal mass (g) in relation to (c) temperature treatment and (d) plant phenological index. Abbreviations of plant species are as follows: Argl – *Arabis glabra*, Arhi – *A. hirsuta*, Capr – *C. pratensis*, Arth – *A. thaliana*, Cabu – *C. bursa-pastoris*. In figures with temperature on the *x*-axis, data points are jittered around the mean temperatures of the climate rooms.

Table 1. Linear mixed-effects of larval performance characteristics of *A. cardamines*

		Parameter	Chi-square	d.f.	<i>P</i>
(a)	Larval development time	Host species	52.49	4	1.1×10^{-10}
		Phenological index	2.77	1	0.096
		Temperature	165.47	1	$<2.2 \times 10^{-16}$
		Host species \times phenological index	9.84	4	0.043
		Host species	53.58	3	1.4×10^{-11}
(b)	Larval development time (<i>A. hirsuta</i> excluded)	Phenological index	5.66	1	0.017
		Temperature	144.25	1	$<2.2 \times 10^{-16}$
		Host species	28.45	4	10^{-5}
		Phenological index	6.85	1	0.0089
		Temperature	22.61	1	1.9×10^{-6}
(c)	Pupal mass	Host species	43.3	4	1.4×10^{-8}
		Phenological index	2.9	1	0.089
		Temperature	176.71	1	$<2.2 \times 10^{-16}$
		Host species \times phenological index	8.18	4	0.085
		Host species	4.06	4	0.39
(d)	Survival until pupation	Phenological index	4.68	1	0.031
		Temperature	0.28	1	0.59
		Host species \times temperature	1.45	4	0.84

Although larvae received a second plant and were not allowed to starve to death, their survival until pupation still was affected by the host phenological state at oviposition (Table 1d), varying from 98% at bud stage with phenological index 1–85% at late stages with phenological index 0.3. Butterfly survival until adult eclosion after winter diapause the following spring was not affected by any of the factors during larval development (intercept estimate = 1.81, *z* value = 8.73, *P* value = $<2 \times 10^{-16}$) with average survival of 86% once they had reached the pupal stage.

HERBIVORE–HOST PLANT DEVELOPMENT RACE

The probability of needing a second plant for a larva varied between host plant species. Less than 5% of larvae needed a second plant when feeding on *A. glabra*, while on all other species 40–55% of larvae required more than

one plant individual to reach the pupal stage (Fig. 2; Tables 2a and 3). Since the analysis with all plant species indicated significant plant species \times phenological index and a close to significance effect of plant species \times temperature interactions, we performed a separate analysis for each of the host species to examine the effects of phenological state and temperature. Neither phenological state nor temperature influenced the probability of needing a second plant of *A. hirsuta* (intercept estimate = -0.18 , *z* value = -0.49 , *P* value = 0.63), and the model estimate for the mean probability of needing a second host was 46%. In *C. bursa-pastoris*, there was no significant effect of temperature but phenological index influenced the probability of needing a second plant with earlier phenological states having a lower probability (17% at phenological index 1 and 86% at phenological index 0.3) (Table 4c). Finally, the probability of needing a second individual of *C. pratensis* and *A. thaliana* was

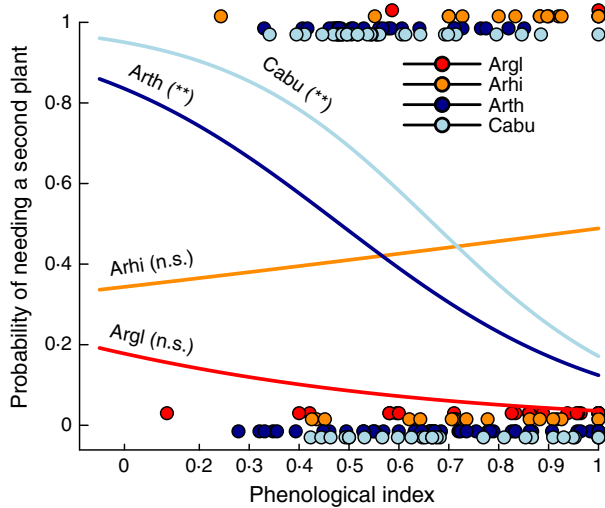


Fig. 2. Development race outcome for the butterfly. Probability of a larva needing a second host plant in relation to plant phenological index at oviposition. Argl – *Arabis glabra*, Arhi – *A. hirsuta*, Arth – *A. thaliana*, Cabu – *C. bursa-pastoris*. *C. pratensis* is not plotted since the interaction between phenological index and temperature was significant.

Table 2. Generalized linear mixed-effects and GLM of herbivore–host plant development race characteristics of *A. cardamines* and its host species

	Parameter	Chi-square	d.f.	P
(a) Probability of needing a second plant	Host species	7.21	3	0.065
	Phenological index	3.8	1	0.051
	Temperature	0.52	1	0.47
	Host species × phenological index	11.46	3	0.0095
	Host species × temperature	7.66	3	0.054
	Phenological index × temperature	3.45	1	0.063
	Residual deviance			
(b) Probability of host outgrowing larva	Deviance	216.9		
	Host species	117.7	3	$<2.2 \times 10^{-16}$
	NULL			

Table 3. The number of the first host species individuals that were exchanged depending on the cause: all the seedpods matured or no seedpods were left for feeding. ‘No exchange’ is for the number of plants on which the larvae finished their development

Host plant species	Seedpods matured	No seedpods left	No exchange
<i>A. glabra</i>	2	0	42
<i>A. hirsuta</i>	5	13	21
<i>A. thaliana</i>	27	0	49
<i>C. pratensis</i>	11	42	40
<i>C. bursa-pastoris</i>	32	0	27

Table 4. Generalized linear mixed-effects of the probability of a larva needing a second plant, separately for each of the host species. *Arabis glabra* is not included, as the larvae hardly ever needed the initial plant to be exchanged. None of the factors affected probability of needing a second plant of *A. hirsuta*

	Parameter	Chi-square	d.f.	P
(a) <i>C. pratensis</i>	Phenological index	0.0018	1	0.97
	Temperature	0.43	1	0.51
	Phenological index × temperature	4.08	1	0.043
	Residual deviance			
(b) <i>A. thaliana</i>	Phenological index	8.5	1	0.0035
	Temperature	6.2	1	0.013
(c) <i>C. bursa-pastoris</i>	Phenological index	7.57	1	0.0059

influenced both by phenological index and temperature (Table 4a,b). For *C. pratensis*, the interaction of these factors affected the probability so that it increased with temperature when plants were at early phenological state, but decreased with temperature when the hosts were at late phenological state (Fig. 3a). In contrast, in *A. thaliana*, the probability of needing a second plant increased abruptly from earlier to later phenological state of the host by 75%, while the increase in temperature caused a relatively modest decrease in the probability by about 18% (Fig. 3b).

It is important to note that there were two reasons why larvae needed a second plant: (i) the host plant completed its development and all the seedpods matured and became unsuitable for feeding; (ii) a larva consumed all the reproductive parts of the host. The relative importance of these

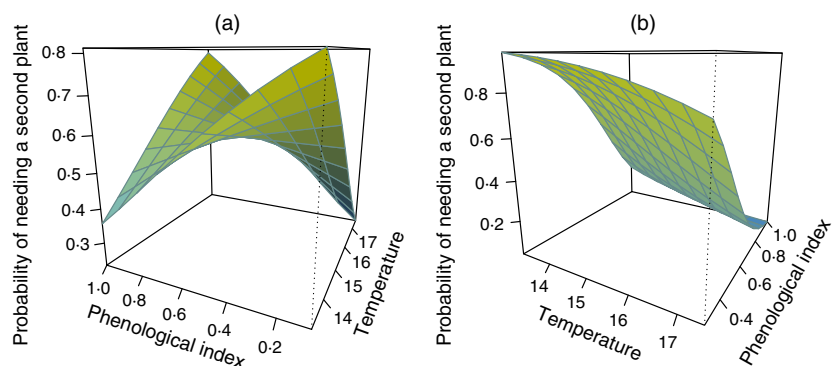


Fig. 3. Probability of a larva needing a second plant when developing on (a) *C. pratensis* and (b) *A. thaliana* in relation to plant phenological index at oviposition and temperature.

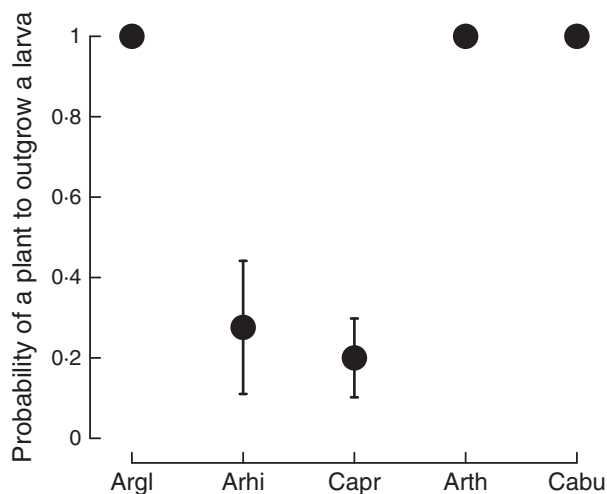


Fig. 4. Development race outcome for the host plant species as the probability of the first host plant outgrowing the larva and forming mature seedpods.

two causes for needing a new plant varied among the host species. For the annuals *A. thaliana* and *C. bursa-pastoris*, larvae were transferred exclusively because the plants had matured all seedpods, while for the perennials *A. hirsuta* and *C. pratensis*, the vast majority of cases was due to larvae consuming all reproductive parts of the plants. Finally, the majority of larvae needed only one individual of the biennial *A. glabra* to complete their development (Table 3).

The probability of the initial host plant outgrowing a larva and forming mature seedpods (vs. not forming them at all) varied between the host plant species, where *Arabidopsis thaliana*, *C. bursa-pastoris* and *A. glabra* were invariably better at outgrowing the larvae independently of temperature or phenological index (Fig. 4; Tables 2b and 3).

Discussion

This study demonstrates that the influence of phenological matching and thermal conditions on the developmental race between an insect herbivore and its host plants is highly species specific. The plant-specific responses covered the full range from no influence of either phenological matching or temperature to strong effects of both factors, including complex interaction between them. On the other hand, these two factors had no effect on the probability of the host plants outgrowing the herbivore. Instead, the capacity to produce seeds while being attacked by the butterfly varied greatly among the plant species. Overall, host plant species and their phenological stage had significant effects on butterfly life-history traits that are likely to influence fitness of the herbivore. It thus appears that natural selection should favour butterfly oviposition on early developmental stages in most of the investigated host plant species.

LARVAL PERFORMANCE

Phenological state of the host species did not affect larval establishment. This is in contrast to previous work showing that the neonate larvae have difficulties in attacking tougher seedpod walls of older hosts (Wiklund & Åhrberg 1978; Courtney 1981; Dempster 1997). The majority of these observations were done in the field, suggesting that host plant suitability may not be a direct function of ageing, or species identity, but due to the growth conditions of the hosts in the natural environment. The average larval establishment in the present experiment was still quite low (58% across all the host species) indicating that all plant species were fairly successful in their resistance to attacks of young larvae independent of their phenological state or temperature.

Other larval performance characteristics – development time, pupal mass and survival until pupation – indicate that larvae benefited from developing on host plants of earlier phenological state at oviposition (all species but *A. hirsuta*). Higher temperatures further contributed to even shorter development time and larger pupal mass, which are known to be positively correlated with female fecundity (Courtney 1981) and decreasing exposure to predators/parasitoids (Berger & Gotthard 2008). This agrees well with the observations that females of *A. cardamines* demonstrate a strong preference for flower heads of *C. pratensis* in the first half of development (Wiklund & Åhrberg 1978; Dempster 1997). Unlike the other host species, earlier phenology of *A. hirsuta* had a negative influence on larval performance by extending development of the larvae. In our experiment, females of *A. cardamines* willingly laid eggs on pre-reproductive and early bud stages of both *A. hirsuta* and *A. glabra*. However, in nature, this oviposition behaviour of females has only been observed in *A. glabra* where female butterflies frequently lay eggs on the green leaves of the plant (pers. obs.). The results presented here suggest that this difference in oviposition on the two *Arabidopsis* species in the field is indeed adaptive for the butterfly. In conclusion, earlier reproductive stages of the plants contributed to a better larval performance on the majority of species, but the effect differed between the host species.

Larval performance of individuals from different populations was affected by host phenology and treatment temperature in a similar way, and there was no latitudinal variation in larval development times. It is interesting to note that post-winter pupal development of these *A. cardamines* populations shows a cogradient latitudinal pattern, with pupae from the southern population developing faster than pupae from the northern population throughout a relevant temperature range (Posledovich et al. 2014, 2015a; Stålhandske et al. 2014; Navarro-Cano et al. 2015). Local adaptations in pupal development are likely to be engendered by the need of females to match emergence to the flowering period of their host plants. The fact that thermal reaction norms of larval develop-

ment showed no variation between the populations suggests that selection on developmental rates of larvae is less variable compared to selection on pupal development for seasonal timing of adult eclosion.

HERBIVORE–HOST PLANT DEVELOPMENT RACE

The effect of temperature and host phenology on the outcome of the development race differed for the herbivore and its host plant species. The optimal outcome for a herbivore is to complete its entire development on a single host individual, since the chance of finding another plant in the vicinity is highly uncertain (Dempster 1997; Wiklund & Friberg 2009). Moreover, when larvae are moving to search for a new host, they are likely to be more exposed to predation (Lima & Dill 1990; Bernays 1997; Gotthard 2000). The effect of host phenological state and temperature on the probability of a larva needing a second plant varied between the host species. These two environmental factors had no effect on the likelihood of needing a second plant when larvae developed on *A. glabra* and *A. hirsuta*. On *A. glabra*, practically all larvae managed to finish their development on a single plant, while on *A. hirsuta*, they needed a second plant in 46% of the cases. In contrast, the probability of completing the entire larval development on the fast-growing annuals of *C. bursa-pastoris* and *A. thaliana* increased as eggs were laid on earlier reproductive stages of the hosts because of plant rapid maturation. In addition, an increase in temperature increased the likelihood that the larvae could keep up their development rate with that of *A. thaliana*, the fastest developing species in this pair of annuals. As for *C. pratensis*, the outcome of the development race resulted from an interaction between both temperature and plant phenological stage. The larvae were largely unable to finish their development on a single plant both when the plant was in the earliest phenological stage when the temperature was high, and in the latest phenological stage when the temperature was low. One likely reason for this complex interaction comes from the fact that *C. pratensis* has the smallest number of reproductive parts of all the host species tested. Therefore, it is likely to provide less food for the larvae, especially at very early reproductive stages when only few buds are available. In addition, the relative thermal sensitivity of larval and plant development also seemed to differ. When eggs were laid on a plant in an early reproductive stage, the probability of larvae needing a second host increased with temperature, suggesting that the thermal sensitivity of the growing larvae is higher than that of the host plant. Hence, in higher temperatures, larvae quickly consumed all reproductive parts of *C. pratensis* of early stages and needed a new plant to reach pupation. In contrast, when eggs were laid on a plant in a late phenological stage, the need of a second host plant decreased with temperature. Hence, when plants were in later stage, a colder environment allowed

the host plant to outgrow the larvae and form mature seedpods that larvae could not eat. In the warmer conditions, larvae developed faster relative to their hosts and managed to finalize development before the seedpods became inedible. Therefore, the complicated interaction between phenology of *C. pratensis* and temperature on the probability of a larva needing a second plant was both due to the generally small size of the host plant, as well as to the relative thermal sensitivity of larval and plant development. As a result, the herbivore could be expected to benefit from ovipositing on *C. pratensis* of earlier reproductive stages in colder microhabitats and later stages in warmer locations. With respect to plant size, it should be noted that even though *A. thaliana* and *C. bursa-pastoris* show considerable variation in size in the field, *A. cardamines* typically lay eggs on the largest individuals of these species that have plenty of reproductive organs (pers. observation). In our experiment, we used plants with sizes similar to those naturally used by the butterflies and small specimens of *A. thaliana* and *C. bursa-pastoris* were therefore not included in the experiments.

The outcome of the development race for the host plant species was ambiguous. First of all, the probability of a plant outgrowing a larva and forming mature seedpods was dependent only on the species identity. The annuals *A. thaliana* and *C. bursa-pastoris* were invariantly better at completing their life cycle and producing mature seedpods than the perennials *A. hirsuta* or *C. pratensis* (Fig. 4; Table 3). This did not meet the prior expectation that perennials, being capital breeders and possessing nutrient reserves from previous seasons, should be superior to annual income breeders in outgrowing larvae. Instead, the annuals demonstrated a 'sprint strategy', which may be due to that the trade-off between growth and reproduction differs between annual and perennial strategies (Pianka 1976; Jönsson 1997). In annuals, stronger selection for fast growth and early reproduction can reduce the risk of mortality before the single reproductive event, compared to what is typically the case in perennial plants, like *A. hirsuta* or *C. pratensis*. Despite the fact that the probability of plants outgrowing larvae and forming mature seedpods was not dependent on phenological state and temperature, it is still possible that mean host plant fitness, in terms of the number of viable seeds produces, is affected by the two factors.

The potential effect of climate warming on a time-limited interaction of herbivores and their host species can be expected to act in several ways. It may act through a change in spring phenologies of the hosts (Visser & Holleman 2001; van Asch *et al.* 2007; Thackeray *et al.* 2010) at the time of a herbivore's emergence/attack and through the direct impact of temperature. Temperatures, in turn, may alter host quality (Kingsolver *et al.* 2006; Braschler & Hill 2007; Pelini *et al.* 2009), as well as affect developmental rates of insects and plants unequally (Visser, Both & Gienapp 2006).

The findings of this study demonstrate that *A. cardamines* in general benefits from oviposition and development on host species in earlier phenological states. Perhaps even more interesting, oviposition on relatively young plants appears to decrease risks associated with starvation and searching for another host as the larval capacity to complete development on the same individual of the annuals *A. thaliana* and *C. bursa-pastoris* increases. It appears that annual species generally are more responsive to climatic change than perennials (Fitter & Fitter 2002), and an increase in environmental temperature may lead to a greater advancement in flowering period of *A. thaliana* and *C. bursa-pastoris* compared to the other hosts. If this is the case, the butterfly females may encounter these species only during later reproductive developmental stages. This is likely to have a negative consequence for butterfly fitness and could possibly influence host utilization patterns. Obviously, advancement in flowering may be advantageous for the two annuals, allowing them to escape predation pressure in time, if attacked at later phenological stages by the larvae. The consequences for the biennial and perennial plant species will depend on the relative change in the plant flowering time and the butterfly spring emergence. This is especially the case for *C. pratensis*, where the outcome of the developmental race for both the plant species and the butterfly is likely to be a consequence of the combined effects of developmental temperature and phenological advancement of the host plant by the time of the butterfly's flight period (Fig. 3a).

High temperatures had positive effects on the herbivore larval performance. It also influenced the probability of larvae completing development on a single plant individual in two out of five host species tested. Therefore, it is possible that climate warming will alter synchrony between larval and plant development in some, but not all, of the host plant species. Overall, the results of the present study suggest that temperatures, and thus climate change, can influence the outcome of consumer–resource interactions via differential effects on development rates up to the start of the feeding as well as during the period of feeding. Because the observed effects were largely plant species specific, such changes in climate may lead to changes in insect–host use. The broader implication of this is that mechanisms, underlying responses of interactions to temperature and climate change, are more complex than commonly anticipated, and therefore, a better knowledge of all relevant mechanisms is needed to predict effects of climate change on species interactions.

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Data accessibility

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.g064k> (Posledovich et al. 2015b).

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