



# Temperature and the synchrony of plant-insect interactions

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# Abstract

Increasing temperatures resulting from climate change have within recent years been shown to advance phenological events in a large number of species worldwide. Species can differ in their response to increasing temperatures, and understanding the mechanisms that determine the response is therefore of great importance in order to understand and predict how a warming climate can influence both individual species, but also their interactions with each other and the environment. Understanding the mechanisms behind responses to increasing temperatures are however largely unexplored.

The selected study system consisting of host plant species of the Brassicaceae family and their herbivore *Anthocharis cardamines*, is assumed to be especially vulnerable to climatic variations. Through the use of this study system, the aim of this thesis is to study differences in the effect of temperature on development to start of flowering within host plant species from different latitudinal regions (study I), and among host plant species (study II). We also investigate whether different developmental phases leading up to flowering differ in sensitivity to temperature (study II), and if small-scale climatic variation in spring temperature influence flowering phenology and interactions with *A. cardamines* (study III). Finally, we investigate if differences in the timing of *A. cardamines* relative to its host plants influence host species use and the selection of host individuals differing in phenology within populations (study IV).

Our results showed that thermal reaction norms differ among regions along a latitudinal gradient, with the host plant species showing a mixture of co-, counter- and mixed gradient patterns (study I). We also showed that observed differences in the host plant species order of flowering among regions and years might be caused by both differences in the distribution of warm days during development and differences in the sensitivity to temperature in different phases of development (study II). In addition, we showed that small-scale variations in temperature led to variation in flowering phenology among and within populations of *C. pratensis*, impacting the interactions with the butterfly herbivore *A. cardamines*. Another result was that the less the mean plant development stage of a given plant species in the field deviated from the stage preferred by the butterfly for oviposition, the more used was the species as a host by the butterfly (study IV). Finally, we

showed that the later seasonal appearance of the butterflies relative to their host plants, the higher butterfly preference for host plant individuals with a later phenology, corresponding to a preference for host plants in earlier development stages (study IV).

For our study system, this thesis suggest that climate change will lead to changes in the interactions between host plants and herbivore, but that differences in phenology among host plants combined with changes in host species use of the herbivore might buffer the herbivore against negative effects of climate change. Our work highlights the need to understand the mechanisms behind differences in the responses of developmental rates to temperature between interacting species, as well as the need to account for differences in temperature response for interacting organisms from different latitudinal origins and during different developmental phases in order to understand and predict the consequences of climate change.

*Keywords:* Brassicaceae, *Anthocharis cardamines*, climate change, development, oviposition, phenology, species interactions, herbivory, synchrony, timing, phenotypic plasticity, local adaptation

# List of papers

This thesis is based on the following papers, referred to by their roman numerals in the text:

- I. Toftegaard, T., Posledovich, D., Navarro-Cano, J. A., Wiklund, C., Gotthard, K., & Ehrlén, J. (2016) Variation in plant thermal reaction norms along a latitudinal gradient—more than adaptation to season length. *Oikos*. 125, 622-628.
- II. Toftegaard, T., Posledovich, D., Navarro-Cano, J. A., Wiklund, C., Gotthard, K., & Ehrlén, J. Effect of spring temperature on flower development differs among closely related plants. *In review with Oecologia*
- III. Toftegaard, T., Posledovich, D., König, M.A.E., Wiklund, C., Gotthard, K., & Ehrlén, J. Linking small-scale temperature variations to plant phenology and plant-herbivore interactions. *Manuscript*
- IV. Toftegaard, T., Posledovich, D., Navarro-Cano, J. A., Wiklund, C., Gotthard, K., & Ehrlén, J. Timing of a butterfly influences host plant species use and the relationship between plant phenology and butterfly attack. *Manuscript*

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# Introduction

Within recent years, studies have shown a general pattern where a warming climate has led to advancements of phenological events (Parmesan & Yohe 2003, Visser & Both 2005, Parmesan 2006). However, responses to increasing temperatures might differ among interacting species, and this can lead to mismatches in terms of differences in response to climate change across several trophic levels (Both et al. 2009). It is therefore of great importance to understand what mechanisms that underlies differences in species' responses if we are going to predict how a warming climate can influence species interactions. The mechanisms behind increasing temperatures for species interactions are however largely unexplored.

Phenological responses to changes in temperature can be caused by either genetic changes in response to selection (Visser 2008, Merilä 2012) or phenotypic plasticity, e.g. where a genotype in different environments produce phenotypes with different phenology (Bradshaw 1965, Pigliucci 2001, Whitman and Agrawal 2009, Wilczek et al. 2009). Both types of responses are important for the understanding of the effect of temperature on phenology. It is believed, that at a short to medium time scale, phenological responses to climate change will mainly be due to phenotypic plasticity (changes along a given reaction norm), while at the long time scale, phenological responses might at a higher extent be due to genetic changes (Merilä & Hendry 2014).

## **Box 1. Phenology**

Phenology is “the study of cyclic and seasonal natural phenomena, especially in relation to climate and plant and animal life”

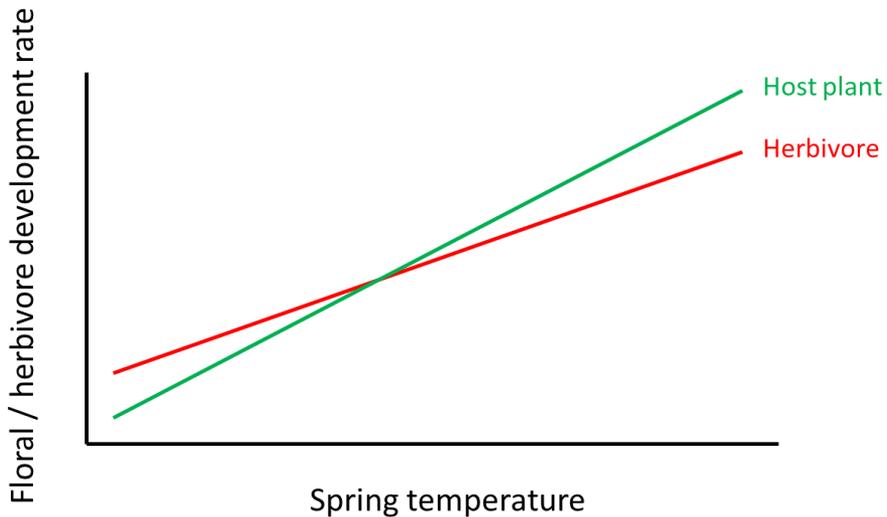
(<http://www.oxforddictionaries.com/definition/english/phenology>)

Differences in species responses to changes in temperature can be the result of species reacting to different cues, such as temperature or photoperiod (Visser & Both 2005, Wilczek et al. 2009, Lessard-Therrien 2014), but it can also be the result of differences in sensitivity to the same cues (Post et al. 2008, Gugger et al. 2015). Several studies have for example shown how increasing temperatures lead to faster development of insect herbivores compared to their host plants (Bale et al. 2002, Berggren et al. 2009, O'Connor 2009). Moreover, species might differ with regards to the sensitivity to a particular cue at different phases of development, highlighting the need of studies investigating variation in an environmental variable, such as temperature, at different times in a species life cycle.

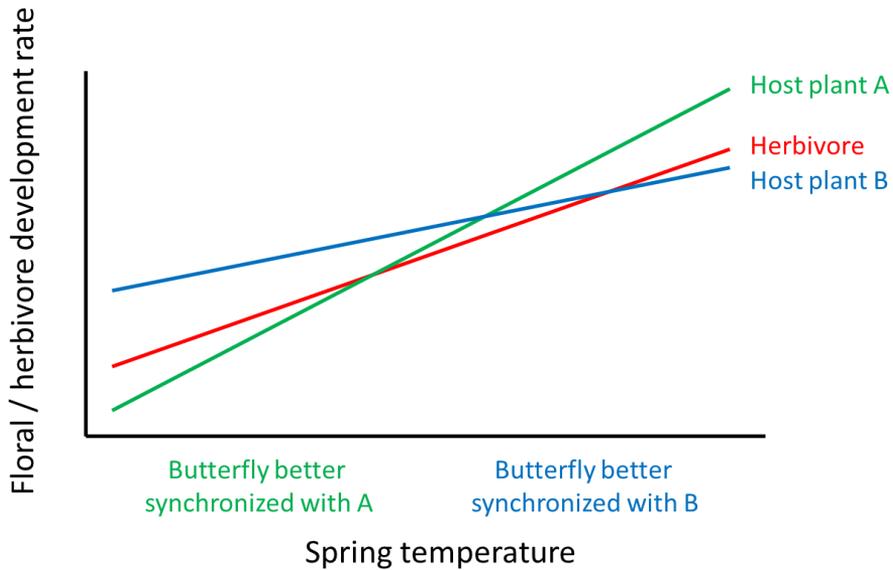
If different species use different cues or are differently sensitive to a given environmental factor, the phenology might be influenced to a different extent either by variation among years or by a warming climate. When studying the effects of a given temperature on the phenology of an individual, this can be described by thermal reaction norms. The slope of the reaction norm thus represents the sensitivity of developmental rates to temperature and the elevation of the reaction norm represents the phenology in the average environment (Kluehn & Brommer 2013). If phenological shifts in interacting species change in opposite directions or differ in strength, this can lead to changes in plant-herbivore interactions. One scenario is when differences in thermal reaction norms between a host plant and a herbivore might lead to the development rate of the herbivore being higher than that of the host plant at colder spring temperatures, which could mean that the herbivore would appear before the host plant in years with a cold spring, but lower than that of the host plant at warmer spring temperatures, which could mean that the host plant would appear before the herbivore in years with a warm spring (Fig. 1). Differences in thermal reaction norms between interacting species (Fig. 1) could also mean that individuals within a population of the same species would be differently influenced by temperature variation; affecting selection of host individuals within populations. Another scenario is when different host plants of a herbivore show differences in thermal reaction norms, leading to the development rate of one host plant species being lower than that of another host plant at colder temperatures but higher at warmer temperatures (Fig. 2). This could mean that the average phenological stage of the two host plant species would differ in a cold or warm spring (Fig 2). In other words, temperature variations can lead to changes in the relative timing of interacting species (e.g. Both et al. 2009, Singer & Parmesan 2010, Thomson 2010), which might influence species interactions (Visser & Both 2005, Altermatt 2010, Singer & Parmesan 2010). The consequences of such differences in shifts between interacting species can be changes in host plant choice for generalist herbivores. The synchronization of plant herbivore interactions might for example be changed if phenological shifts in interacting

species change in opposite directions or differ in strength (Root et al. 2003, Visser & Both 2005, Cook et al. 2012).

Few studies have however experimentally investigated the potential consequences of differences in thermal reaction norms between species. For plants for example, a review by Franks et al. (2014) only found four studies which investigated more than one plant species (two species showing both evolutionary and plastic responses and two species showing either evolutionary or plastic responses). Only a few studies have investigated how differences in the use of multiple host plant species by herbivores are related to differences in synchrony by the use of artificial warming (Liu et al. 2011, Warren et al. 2011), and to our knowledge, no studies have carried out such investigations under natural conditions. The implications of differences in the relative timing of interacting species are therefore poorly known, especially for interactions depending strongly on phenology.



**Fig. 1** Example of reaction norms of development rates to spring temperature for a host plant (green line) and a herbivore (red line). Both the host plant and the herbivore have positive slopes, which mean that the warmer it gets the higher development rates. The host plant has a steeper positive slope than the herbivore, which means that at colder spring temperatures, the development rate of the herbivore is higher than that of the host plant whereas at warmer spring temperatures, the development rate of the host plant is higher than that of the herbivore.



**Fig. 2** Example of reaction norms of development rates to spring temperature for host plant A (green line), host plant B (blue line) and a herbivore (red line). Both host plants and the herbivore have positive slopes, which mean that the warmer it gets the higher development rates. Host plant A has the steepest positive slope, which means that at colder spring temperatures, the development rate of host plant A is lower than that of host plant B and the herbivore whereas at warmer spring temperatures, the development rate of host plant A is higher than that of host plant B and the herbivore. Host plant B has the gentlest positive slope, which results in the opposite pattern than that of host plant A.

Not only is the response of species to changes in the average temperature over the season important, but also differences in sensitivity to temperature among different developmental phases might be important. Yet, such responses have received very little attention in plants (Post et al. 2008), despite the fact that climate change often results in uneven temperature changes across seasons (Visser et al. 1998, Inouye et al. 2000, Walther et al. 2002), and since many interactions are closely associated with particular developmental phases (Boege & Marquis 2005, Posledovich et al. 2015).

Lastly, genetic and environmental influences on a phenotype can differ along latitudinal and altitudinal gradients, which can lead to local adaptation in response to temperature. Such differences can follow a co-gradient pattern, where genetic and environmental influences on a phenotype act in the same direction, or a counter-gradient pattern where they act in opposite directions, or show no latitudinal or altitudinal pattern (Levins 1969).

In conclusion, a better knowledge of the mechanisms underlying species' and population responses to climate change and studies examining thermal reactions norms for both interacting species and closely related species at multiple developmental stages is a key component of understanding and predicting how individual species as well as species interactions will respond to climate change.

# Aim of the thesis

The selected study system consisting of the following host plant species of the Brassicaceae family: *Arabidopsis thaliana*, *Arabis glabra*, *Arabis hirsuta*, *Capsella bursa-pastoris*, *Cardamine pratensis* and *Thlaspi caerulescens* together with their herbivore, the univoltine orange tip butterfly *Anthocharis cardamines*, is assumed to be especially vulnerable to climatic variations. Through the use of this study system, the aim of this thesis is to investigate differences in the effect of temperature on development to start of flowering both within and among host plant species of *A. cardamines*, if different developmental phases leading up to flowering differ in sensitivity to temperature, if small-scale climatic variation in spring temperature influence flowering phenology and interactions with *A. cardamines* and if differences in the timing of *A. cardamines* relative to its host plants influence host species use and the selection of host individuals differing in phenology within populations. For paper **I** and **II**, we investigate differences in thermal reaction norms for the five plant species *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris* and *C. pratensis* among three regions along a latitudinal gradient in Sweden. In paper **I**, we examine if there is differences in the effect of temperature on development to start of flowering within host plant species from different latitudinal regions, and if such variation is mainly the result of differences during early (start of experiment to bud) versus late (bud to flower) phases of development. In paper **II**, we examine if sensitivity to temperature of development to flowering differ among host plant species, whether different developmental phases differ in sensitivity to temperature and how such differences contribute to overall differences in flowering time among species. In paper **III**, we examine whether small-scale climatic variation in spring temperature influence the flowering phenology of two ploidy levels of *C. pratensis* and its interaction with *A. cardamines*. Finally, in paper **IV**, we examine how differences in the timing of *A. cardamines* relative to the host plant species *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris*, *C. pratensis* and *T. caerulescens* influence host species use and the selection of host individuals differing in phenology within populations.

# Methods

## Study system

For this thesis, we used a study system consisting of one or several plant species of the Brassicaceae family (*C. pratensis* in paper III, *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris* and *C. pratensis* in paper I and II and *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris*, *C. pratensis* and *T. caerulescens* in IV) together with the pierid herbivorous butterfly, *A. cardamines* (Lepidoptera: Pieridae, paper III and IV). All of the plant species used for this thesis are known host plants of *A. cardamines* (Wiklund & Åhrberg 1978). The studies were carried out at one region (paper III) or several regions along a latitudinal gradient in Sweden (paper I, II, IV). The reason for choosing this study system for this thesis and a parallel thesis (Posledovich 2015) was that this study system is believed to be especially vulnerable to climatic variations. This is because: (1) the interaction between the host plants and the herbivore happens in a short seasonal time window (see details below) (2) *A. cardamines* is one of the species of butterflies which have advanced its spring time emergence the most over the previous 20-30 years (Karlsson 2014, Navarro-Cano et al. 2015) (3) *A. cardamines* is a univoltine species, which is thought to make it more vulnerable to variations in climate compared to bi- or multivoltine species (Post & Forchhammer 2008, Bale & Hayward 2010).

The six host plant species used for this thesis all have a flowering period that coincides with the flight time of *A. cardamines* (Wiklund & Åhrberg 1978). *Arabis hirsuta* (L) Scop and *Cardamine pratensis* (L) are perennial species with a flowering time from May to June. In the south-eastern part of the Södermanland province in central Sweden (study site of study III), both tetraploid and octoploid cytotypes of *C. pratensis* are commonly found. Flowering time differs between the two ploidy levels; the tetraploids having an earlier start to flowering by a few days compared to the octoploids (König et al. 2015). *Arabis glabra* (L) Bernh is a monocarpic perennial plant with a flowering time from June to July. *Arabidopsis thaliana* (L) Heynh, *Thlaspi caerulescens* (J. Presl & C. Presl) and *Capsella bursa-pastoris* (L) Medik are all annual species with a flowering time from April to June, April to May and April to October respectively (Mossberg & Stenberg 2010).

*Anthocharis cardamines* overwinters at the pupal life stage and hatches in late April to early May in southern Sweden and late May to early June in northern Sweden (Navarro-Cano et al. 2015). *Anthocharis cardamines* females hatch as butterflies a few days later than the males (Wiklund & Åhrberg 1978). The flight season lasts three to four weeks in Sweden, with the majority of the eggs (~80%) being laid within the first half of the flight season. In most cases, the female *A. cardamines* lays a single egg per inflorescence, although multiple eggs laid on the same host plant is common in *A. glabra* (Wiklund & Åhrberg 1978, Wiklund & Friberg 2009).

*Anthocharis cardamines* is a phenological specialist, and studies have shown that this is likely to cause strong selection for the butterfly to match oviposition to host plant phenology (Posledovich 2015, Dempster 1997). The butterfly has a very early flight period compared to other univoltine butterflies in Sweden, and this is thought to be a result of the butterflies dependence on early flowering host plants (Karlsson 2014, Navarro-Cano et al. 2015). Females have a strong preference for ovipositing on host plants at late bud or early flowering stages, and when the eggs hatch after 7-10 days, the young larvae can only feed on flowers or newly developed seed pods. Since this stage of development of the host plant only lasts a few weeks on average, this makes *A. cardamines* a phenological specialist (Wiklund & Åhrberg 1978, Courtney & Duggan 1983, Wiklund & Friberg 2009). An exception is *A. glabra*, where the newly hatched larvae feed on the leaves, followed by the consumption of seed pods when the larvae are half grown (Wiklund & Friberg 2009). Every year, host plants of *A. cardamines* are suitable for oviposition around 4 weeks, which means that the interaction between the host plants and the herbivore happens in a short seasonal time window (Wiklund & Åhrberg 1978, Wiklund & Friberg 2009). An attack by *A. cardamines* can lead to the fitness of the host plant being markedly reduced for all host plant species, since as the larva continues to grow, it consumes all reproductive parts and at times even vegetative parts of its host (Wiklund & Friberg 2009, König et al. 2014). Variation in abundance of the plant species and attack rates of *A. cardamines* have been shown among study species (Wiklund & Åhrberg 1978), regions (Navarro-Cano et al. 2015) and years (Wiklund & Friberg 2009). *Anthocharis cardamines* attack rates also differ between the ploidy levels; octoploids experiencing lower attack rates than tetraploids (Arvanitis et al. 2007). This difference in attack rate is likely to be caused by environmental factors, since the two cytotypes show no difference in attack rate when grown under controlled conditions (Arvanitis et al. 2007).

## Data collection and analysis

### *Study I (Variation in plant thermal reaction norms along a latitudinal gradient—more than adaptation to season length)*

The aims of this study was to examine differences in the effect of temperature on development to start of flowering within host plant species from different latitudinal regions, and if such variation is mainly the result of differences during early (start of experiment to bud) versus late (bud to flower) phases of development.

In order to investigate those aims, plant material was collected from the southern (eastern part of the Skåne province), central (eastern part of the Uppland province) and northern (eastern part of the Ångermanland province) regions of Sweden during the spring of 2011. From the southern region, we collected plant material from *A. thaliana*, *A. hirsuta*, *C. pratensis* and *C. bursa-pastoris*. From the central region, we collected plant material from *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. pratensis* and *C. bursa-pastoris*. From the northern region, we collected plant material from *A. thaliana*, *A. glabra*, *C. pratensis* and *C. bursa-pastoris*. From this plant material, plants were propagated in a greenhouse during August and September 2011. Here they were kept for 2-3 weeks until rosettes had formed, and were then moved to a common garden until the start of the experiment on the 21<sup>st</sup> of March 2012, when the plants were randomly placed in climate chambers in one of three temperature treatments (~13, 15 and 17 °C) with a constant photoperiod (15L:9D). Recordings of plant development, first bud and first open flower were carried out every second day.

R statistical software ver. 3.1.3 was used for all statistical analysis (R core team 2015). Linear mixed-effect models for each species (LMM; function lmer) (Bates et al. 2015) were carried out in order to investigate differences in the following response variables: time (days) from the start of the experiment to the appearance of the first bud (start of experiment to bud), time from appearance of the first bud until opening of the first flower (bud to flower), time from the start of the experiment until opening of the first flower (start of experiment to flower) and time from the start of the experiment until the appearance of the first seed pod (start of experiment to pod). The models contained the following explanatory variables: temperature, region and plant size at the start of the experiment. In all models, climate room was included as random effect. Any effect of region was regarded as evidence for differences in the mean response (elevation of the reaction norms) and effect

of temperature  $\times$  region was regarded as evidence for differences in sensitivity to temperature (slope of the reaction norms) among regions. The three-way interaction temperature  $\times$  region  $\times$  size and the two-way interaction temperature  $\times$  size were not incorporated as preliminary analysis showed that they had small effects. Initial models for all species included the interaction temperature  $\times$  region, and if this interaction was not significant, we carried on with models without the interaction effects. If the interaction was significant, we carried on with separate models for each region. Normality of residuals and equality of variances were graphically explored for each model. For each species at each region, we examined the relative contributions of variation in the number of days from start to first bud and from first bud to first flower, respectively, to variation in the number of days from start to flowering among regions separately by regressing the total time for development from start to first flower on each of its two components. The regression coefficients for the two periods will sum to unity.

### *Study II (Effect of spring temperature on flower development differs among closely related plants)*

The aims of this study was to examine if sensitivity to temperature of development to flowering differ among host plant species, whether different developmental phases differ in sensitivity to temperature and how such differences contribute to overall differences in flowering time among species.

In order to investigate those aims, the same data collection was used as for study I. R statistical software version 3.1.3 was used in order to carry out Linear mixed-effect models (LMM; function lmer) (Bates et al. 2014) studying the effects of temperature, region, plant species and initial plant size on development time (days) from the start of the experiment until opening of the first flower (start of experiment to flower), as well as from the start of the experiment to the appearance of the first bud (start of experiment to bud) and from appearance of the first bud until opening of the first flower (bud to flower). Initial models were run for all regions combined including all two- and three-way interactions between region, species and temperature. Those models revealed significant effects of the interaction region  $\times$  species  $\times$  temperature on time from the start of the experiment to the appearance of the first bud and of the interaction region  $\times$  temperature on time from appearance of the first bud until opening of the first flower. Due to the significant effects involving temperature and region in both development phases, we carried on running separate models for each region. In all models, climate chamber identity was included as random effect. In our statistical models, the elevation of the reaction norm corresponds to the central intercept of species and the slope of the reaction norm corresponds to the slope of the

regression of development time on temperature. We calculated elevation species-wise as the central intercept, i.e. development time at mean temperatures during each of the three developmental periods (15.8 °C from start of the experiment to bud, 15.4 °C from bud to flower and 15.5 °C from start of the experiment to flower). In order to examine differences in sensitivity of development time among species, the effects of the interaction temperature × species was tested. We did not incorporate the three-way interaction temperature × species × size and the two-way interactions temperature × size and species × size, because those interactions were not needed in order to test our hypotheses and since preliminary analyses showed that they had small effects. The interaction temperature × species was included in all initial models since it represented differences in sensitivity of development to temperature among species. In the event of a non-significant interaction for temperature × species, this interaction was removed and the models were rerun. In the event of a significant interaction, separate models of sensitivity of development to temperature were carried out for each species. All models were explored graphically and normality of residuals and equality of variances was found in all cases.

### *Study III (Linking small-scale temperature variations to plant phenology and plant-herbivore interactions)*

The aims of this study was to examine whether small-scale climatic variation in spring temperature influence the flowering phenology of two ploidy levels of *C. pratensis* and its interaction with *A. cardamines*.

In order to investigate those aims, the phenology of individual plants of *C. pratensis* was followed throughout their flowering season of 2012 (21<sup>st</sup> of May 2012 - 3<sup>rd</sup> of July 2012) in 15 populations (8 tetraploid and 7 octoploid) within 10 km of each other in the south-eastern part of the Södermanland province in central Sweden. On the 5<sup>th</sup> of June 2012, the plant individuals had the highest number of open flowers and seed pods on average combined over all populations. This date was therefore selected as the peak of flowering, and on this day, buds, flowers and seed pods for up to 30 randomly chosen plant individuals per population were recorded (total of 410 individuals). In addition, the incidence of oviposition of *A. cardamines* on the *C. pratensis* individuals was recorded on a weekly basis throughout the flowering season. In each population, the temperature was monitored continuously at 1 hour intervals at 5-8 position from the start of the growing season until the peak of flowering (4<sup>th</sup> of April 2012 until the 5<sup>th</sup> of June 2012). Individual plant temperatures were calculated by using the following formula:  $((1 / D_1) \times T_1) + (1 / D_2) \times T_2 \dots (1 / D_n) \times T_n) / ((1 / D_1) + (1 / D_2) \dots + (1 / D_n))$ , where D = Distance from a given plant individual to each of the temperature loggers

from 1 to n within a given population (meters), and T = Temperatures of each logger within a given population (°C). Based on these estimated individual temperature data, we calculated growing degree hours from the start of the growing season until the peak of flowering which was used as a measure for heat accumulation for each plant individual. For this measure we used hourly temperatures above 5 °C since this is the threshold for initiation of plant development in *C. pratensis* (study I).

In order to characterize the developmental stage of each plant individual, we calculated a phenological index by use of the following formula:  $(0 \times \text{number of buds} + 0.5 \times \text{number of flowers} + 1 \times \text{number of pods}) / (\text{number of buds} + \text{number of flowers} + \text{number of pods})$ . R statistical software version 3.2.2 was used for all statistical analyses. A linear mixed-effect model (LMM; function `lmer`, Bates et al. 2015) was used to examine differences in heat accumulation within populations, with individual plant phenology index as response variable, individual heat accumulation as fixed effect and population as a random effect. A generalized linear model was used to examine differences in heat accumulation among populations, with average population phenology as response variable and average population heat accumulation and ploidy level (individuals of each population had only one ploidy level) as explanatory variables. Both the direct effects of heat accumulation on probability of *A. cardamines* oviposition, and the indirect effects through host plant phenology within populations were investigated by the use of generalized linear mixed models (GLMM, function `glmer`, Bates et al. 2015). The models included incidence of oviposition as the binomial response variable, heat accumulation, plant phenology and total number of open flowers (as a proxy for plant size) as fixed effects, and population as a random factor. Data from all populations were pooled for those analyses of oviposition since oviposition incidence was too low for population-wise analysis. All fixed effects were standardised. Initial models were carried out with all variables and two-way interactions. Stepwise removal of terms based on the Akaike Information Criterion was then carried out in order to obtain the best model (Bolker et al. 2009). Visual examination showed normality of residuals and equality of variances in all models.

#### *Study IV (Timing of a butterfly influences host plant species use and the relationship between plant phenology and butterfly attack)*

The aims of this study was to examine how differences in the timing of *A. cardamines* relative to the host plant species *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris*, *C. pratensis* and *T. caerulea* influence host species

use as well as selection of host individuals differing in phenology within populations.

In order to investigate those aims, recordings of reproductive phenology (buds, flowers and seed pods) was recorded for *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. pratensis*, *C. bursa-pastoris* and *T. caerulea* during the years 2010-2013, together with recordings of incidence of oviposition by *A. cardamines*. The observations were carried out soon after the peak of flight for *A. cardamines*. In the southern region of Sweden (eastern part of the Skåne province), this resulted in recordings between May 17 and May 22, in a central part of Sweden (eastern part of the Uppland province) between May 28 and June 4 and in a northern part of Sweden (eastern part of the Ångermanland province) between June 10 and June 17. The aim was to record the reproductive phenology for a total of 300 individuals of each host plant species over three patches in each region and year (we succeeded with this in 39 of 60 cases). We aimed at recording the same patches of plants each year in each region, but if a patch did not have any plants, we tried to find a substitute patch within the same region. During the years 2011-2013, we inspected 200 additional individuals of each species in each region for presence of *A. cardamines* eggs. If an egg was present, we recorded the reproductive phenology and height of the attacked plant, and followed this same procedure for a non-attacked reference plant.

R 3.2.2 statistical software was used for all analyses (R Core Team 2015). A development index for each plant individual was calculated in order to assess the host plant reproductive development:  $((0.5 \times \text{number of flowers}) + (1 \times \text{number of pods})) / (\text{total number of buds, flowers and pods})$ . By using this developmental index, the stage of development of plant reproductive structures at the time of recording was found, where differences in the value of the index reflect differences in reproductive phenology. In order to estimate the degree of synchrony between the butterflies and each of the host plant species in each region in each year, the deviation from the preferred developmental stage for oviposition was calculated:  $(\text{preferred developmental stage for oviposition}) - (\text{mean development stage recorded in the field})$ . Data from a previous preference experiment (Posledovich 2015) was used in order to assess the preferred development stage for oviposition by *A. cardamines*. The deviation of the mean development stage recorded in the field from the estimate of the preferred stage indicates how closely plant development in a given species, location and year matched the preferred development stage for butterfly oviposition for that particular species.

In order to examine how variation in butterfly use of different host plant species was related to synchrony, we calculated the proportion of the total number of eggs recorded on all host species in each year and region that

were laid on each host plant species. We then examined how these values were related to the deviation of the mean host developmental stage observed in the field from the preferred stage through statistical analysis. Initially, we ran a generalized linear model (GLM) including only the linear and quadratic effect of development difference on host use. We then performed a GLM including also the factors species, year and region as explanatory variables in order to examine how this overall relationship depended on those additional factors. To avoid overfitting as a result of few observations, two-way or higher order interactions were not included.

To investigate if butterflies have a stronger preference for host plant individuals with an earlier phenology when peak butterfly flight occurs early relative to mean plant development, we calculated estimates of the strength of butterfly preferences for plant individuals differing in phenology for each species in each region in each year. The strength of butterfly preference for individuals with an early phenology was estimated as the slope (beta values) of the regression of incidence of oviposition (0 or 1) on development index of individuals (as described above), using generalized linear mixed models (GLMM, function `glmer`, Bates et al. 2015). These estimates of butterfly phenology preference were then regressed on estimates of how much mean development in the field deviated from the preferred stage, using a linear model (`lm`) of the overall relationship between beta values and deviation from preferred stage. We also carried out a GLM with the factors species year and region as explanatory variables to examine how the overall relationship was related to differences among those additional factors. Due to a strong species effect in these analyses, we carried out additional species-wise models for the relationship between butterfly phenology preferences and the deviation of mean development in the field from the preferred stage.

# Results and discussion

## *Study I (Variation in plant thermal reaction norms along a latitudinal gradient—more than adaptation to season length)*

For the development period from start of the experiment to flowering, the mean response (elevation of thermal reaction norms) differed among regions for all investigated plant species; three plant species followed a co-gradient pattern, one followed a counter-gradient pattern and one followed a mixed pattern. The sensitivity (slopes of the thermal reaction norms) only differed among regions for *C. pratensis*, the differences in developmental rate among regions being largest at high temperatures. Thermal reaction norms for the two different developmental periods, start to bud and bud to flower, were very similar to the patterns for the whole developmental period start to flower.

The two development periods, start to bud and bud to flower, differed in their contribution to differences in flowering time among regions and within species. In one species, differences among regions in time to flowering were explained mainly by the time from start of the experiment to bud appearance, in two species, differences were explained mainly by the time from bud appearance to first open flower and in two species, both phases contributed equally to the differences in flowering time. Within regions, variation in time to flowering among individuals of a species was explained mainly or to some extent by the time from start of the experiment to first bud in three species, whereas the time from first bud until opening of the first flower was explaining most of the variation in two species.

Overall, this study suggests genetically based differences in thermal reaction norms of flower development among regions along a latitudinal gradient for all species. Our results also showed a mixed results with regards to co and counter-gradient, a pattern which is in agreement with previous studies which compared developmental rates of plant species from different regions or environments under controlled conditions (Heslop-Harrison 1964, Langlet 1971, Stinchcombe et al. 2004, Hoffmann et al. 2005, Vitasse et al. 2009). If the growing season is the only factor which underlies selection on timing of flowering, counter-gradient patterns would have been expected for all spe-

cies. We therefore suggest that other factors influence selection on timing of flowering, e.g. intensity of herbivory.

Our study also showed differences among species regarding the extent to which variation in time to flowering was explained by variation in time from start of the experiment to bud appearance vs. time from bud appearance to flowering. The observed pattern suggests that timing of onset of development is relatively more important in annual species, and rate of development more important in perennial species. This is in agreement with previous research (Reader 1982, Rathcke & Lacey 1985).

The results of study I showing differences in latitudinal patterns of thermal reaction norms among species are very important in order to identify agents of selection and to predict how species will respond to both short- and long-term increases in temperature.

### *Study II (Effect of spring temperature on flower development differs among closely related plants)*

The sensitivity of development time to temperature (slope of the reaction norm) did not differ among species for the total time from start of the experiment to flowering. However, the sensitivity for time from the start of the experiment to bud formation differed among plant species from the central region, and sensitivity for development time from buds to flowering differed among plant species from all regions. The average time for development from start of the experiment to flowering (elevation of the reaction norm), differed among species in all three regions. We found a short total developmental time to flowering for the annual species, which was largely the result of a short development time from bud to flower. Long development time to flowering was seen for two perennials due to long developmental times both for development to the bud stage and for development from bud to flower. The perennial species *C. pratensis*, had however the shortest time for development to the bud stage in all regions, but longer development time from bud to flower.

Overall, this study showed that both the average total time for development from start of the experiment to flowering, and the average time for development during the two investigated phases leading up to flowering, differed among species for all regions. Such differences in optimal development rate for different developmental phases can be influenced by many factors such as competition, nutrient and water availability, but also by the phenology of pollinators and herbivores (reviewed by Rathcke & Lacey

1985). In the case of our five study species, they are likely to influence the timing both relative to each other and to the butterfly herbivore *A. cardamines* since this butterfly is a phenological specialist which oviposits on the plant species that are in the most favourable developmental stage at the time of butterfly flight (Study IV, Posledovich 2015, Stålhandske et al. 2016).

This study showed that sensitivity to temperature of total development time to flowering did not differ among species. This suggests that increasing mean spring temperatures will not change the order of flowering among the host plant species included in this experiment. Our study did, however, find that sensitivity to temperature in each of two investigated phases of development differed among species. Fitter and Fitter (2002) found first flowering date in early spring flowering species to be more sensitive to temperature increase than in later flowering species. Post et al. (2008) found how warming increased development rate and affected total development time. These results suggest that the phenology of species relative to each other might still be influenced by increasing temperatures and that not only average seasonal temperatures play a role in the timing of individual species and phenological matching of interacting species, but that the distribution of warm and cold days over the season is also important. This study therefore shows that in order to understand and predict species' responses to climate change, studies which integrate temperature responses over different phases of development are needed.

### *Study III (Linking small-scale temperature variations to plant phenology and plant-herbivore interactions)*

Heat accumulation significantly affected differences in phenology among individuals of *C. pratensis* within populations. The more growing degree hours a plant had accumulated at the peak of flowering, the more advanced was the development of reproductive structures. A higher mean heat accumulation in a population was linked to a more advanced mean development stage of buds, flowers and seed pods. Oviposition probability by *A. cardamines* was not directly affected by heat accumulation over the flowering season at the location of the plant. Oviposition probability did, however, increase with more advanced host plant phenology. Our results also showed that there was a difference in heat accumulation between the two ploidy levels, the habitats of tetraploid populations on average having a higher heat accumulation than the habitats of the octoploid populations. However, at similar levels of heat accumulation, octoploid populations had a more advanced phenology than tetraploid populations.

Our study showed that the more growing degree hours individual plants within a population had accumulated, the more advanced was the reproductive structures. This illustrates, that small-scale temperature variation can influence seasonal timing of flowering among individuals within populations. Our finding agrees with Dahlgren et al. (2007) who showed how soil temperature was closely correlated with timing of flowering within populations of *Actaea spicata*. Taken together, this suggests that small scale variation in temperatures is part of the explanation for among-individual differences in flowering phenology. Our study also showed that differences in the heat accumulation in spring were associated with differences in the mean phenology among populations of *C. pratensis*. This finding agrees with Jackson (1966) and Dunne et al. (2003) who showed, that warming led to earlier start of flowering for several plant species. Taken together, this suggests that differences in heat accumulation explain some of the differences in developmental rates of plants among populations. Such differences could be important both for abiotic environmental conditions such as day length and for interactions with competitors, herbivores and pollinators. In our study, we found that heat accumulation did not directly influence oviposition probability of female *A. cardamines* among individuals, but an indirect effect through plant development was found. This is in contrast to Davies et al. (2006) who found that temperature influenced oviposition site selection in the silver-spotted skipper *Hesperia comma*. Another finding of our study was that similar heat accumulation led to octoploid populations of *C. pratensis* having a more advanced phenology compared to tetraploid populations despite the fact that tetraploids start flowering earlier than octoploids under field conditions (Arvanitis et al. 2007). This is likely to be caused by the tetraploids growing in dry open meadows that are warmer than the damp shady habitats of the octoploids.

The results of study III showing that a higher rate of heat accumulation was related to an earlier flowering of individuals within populations and an earlier average flowering among populations together with the fact that the probability of oviposition by *A. cardamines* on *C. pratensis* increased with more advanced host plant phenology shows that small-scale variation in climate is important for phenology both within and among-populations in single species but also for the synchrony of interacting species and the intensity of species interactions and should be integrated into studies investigating the response of species interactions to climate change.

*Study IV (Timing of a butterfly influences host plant species use and the relationship between plant phenology and butterfly attack)*

The overall use of a given host plant of *A. thaliana*, *A. glabra*, *A. hirsuta*, *C. bursa-pastoris*, *C. pratensis* and *T. caerulea* by the herbivore butterfly *A. cardamines* was associated with plant phenology. A smaller mean deviation from the preferred mean development stage under natural conditions was correlated with a greater use of a host plant species. Within host plant species, the strength of butterfly preferences for plant phenology was significantly affected by the mean deviation of plant individuals from the preferred developmental stage. More specifically, the later flight time of the butterfly in comparison to plant development, the stronger preference for individual host plants with a later phenology, meaning host plants in earlier development stages at the time of butterfly flight.

Our findings that the less the mean development stage of a host plant species deviated from the butterflies preferred developmental stage for oviposition, the higher host use of this species suggest that host species use by the butterfly is influenced by synchrony with each host species. A few studies have found that artificially increased temperatures influenced differences in the relative timing of interacting species (Liu et al. 2011, Warren et al. 2011). A combination of this study and previous studies therefore indicate that differences in the relative timing of interacting species can be mediated by temperature, which can be important both for spatial and temporal variation in host use and interaction intensities. Although this study did not demonstrate the causal relationship between phenology and host use, a previous study (Stålhandske et al. in press) have shown that relative timing of butterfly flight to phenological stage of host plants is more important than host species identity for butterfly oviposition choice. Our study also showed that populations in later phenology and thus in earlier developmental stages always were more used by the butterfly. These results, in combination with findings that the performance of *A. cardamines* larvae is favored by oviposition on early stages of plant reproductive structures (Posledovich et al. 2015), suggest that host plant selection based on phenology becomes increasingly important the further the hosts plants have developed on average, at the time of butterfly flight.

The results of study IV showing how butterfly use of a given host species was associated with its mean developmental stage and how within host plant species, the deviation from the preferred stage influenced butterfly selection of host plant individuals for oviposition suggest that temperature differences and differences in thermal reaction norms between interacting species can cause differences in host species use and that this can cause herbivore-

mediated selection on plant reproductive phenology. These findings are important for the understanding of how species ecological and evolutionary interactions are influenced by increasing temperatures.

# Conclusion

The findings of the controlled experiments (study I and II) established that thermal reaction norms differed within and among all investigated host plant species. The response to temperature variations differed also between different phases of development leading up to flowering, implying that not only average seasonal temperatures play a role in the timing of individual species and phenological matching of interacting species, but that also the distribution of warm and cold days over the season is important in order to understand and predict species' responses to climate change. A parallel thesis by Posledovich (2015) showed that the thermal reaction norms of the herbivorous butterfly *A. cardamines* were less steep than the thermal reaction norms of the plants. An implication of these findings is that the phenological synchrony depends on temperatures, the number of days between butterfly emergence and plants flowering and fruiting decreasing at higher temperatures. Those differences in reaction norms between the butterfly and the host plant species in combination with the butterfly being a phenological specialist (Wiklund & Åhrberg 1978, Courtney & Duggan 1983, Wiklund & Friberg 2009) for which developmental stage of the host plant is more important than species identity for oviposition choice (study IV, Navarro-Cano et al. 2015, Posledovich 2015), leads to the prediction that the realized use of different hosts in the field depend on temperature-induced differences in the relative timing of host plants and the butterfly herbivore. Observed differences in host species use for *A. cardamines* among years and regions (Wiklund & Friberg 2009, Navarro-Cano et al. 2015), might thus be caused by differences in synchrony between the butterfly and the different host plant species, which in turn are likely to be the results of differences in temperature and differences in thermal reaction norms among interacting species (Study II, Posledovich et al. 2014). Study III, showing that temperature differences among populations and individuals within populations lead to differences in phenology and attack rates, strongly suggest that these mechanisms also act at relatively small spatial scale.

Moreover, the differences in reaction norms between the butterfly and its host plants also have evolutionary implications and the results of thesis suggest that differences in temperature will lead to differences in selection of host plant individuals differing in phenology within populations, and thus phenotypic selection. In study IV we showed that the later the butterflies

appear in the season relative to their host plants, the higher preference they have for host plant individuals with a later phenology, meaning preference for host plants in earlier development stages at the time of butterfly flight. This should lead to temperature-mediated variation in natural selection on plant phenology via butterfly herbivory.

The results from the controlled experiments in study I together with the results of the parallel thesis by Posledovich (2015) shows that thermal reaction norms of both butterflies and host plants vary among latitudinal regions. This suggests that local adaptation is important, but the mixed pattern of co- and counter-gradient patterns suggest that the variation is not only driven by differences in season length. An effect of these latitudinal differences in reaction norms is that the effects of a given change in temperature on the outcome of plant-butterfly interactions will be region-specific.

The results of this thesis and the parallel thesis (Posledovich 2015) predict that for our study system, climate change in combination with differences in thermal reaction norms will lead to changes in species interactions between the host plants and the herbivore *A. cardamines*, but that differences in phenology among host plants and changes in host species use of *A. cardamines* are likely to buffer the herbivore against strong negative effects of increasing temperatures resulting from climate changes. The results are important to predict responses of plants and plant-herbivore interactions to a warming climate, and the results are relevant both in an ecological – in terms of mean interaction intensities – and evolutionary – in terms of effects on selection of individuals within populations – perspective.

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# Populärvetenskaplig sammanfattning

Forskning under de senaste årtiondena har visat att merparten av jordens ekosystem påverkas av klimatförändringar. För att kunna förutspå hur ekosystem kommer att påverkas av klimatförändringar i framtiden är det viktigt att studera hur enskilda individer svarar på förändringar i det lokala klimatet. Ett vanligt sätt att undersöka hur individer svarar på förändringar i klimat är att studera om, och i så fall hur, förändringar i temperatur påverkar individens fenologi. Fenologi är tiden på året då viktiga händelser sker i en individs livshistoria. För en växt kan sådana händelser till exempel vara lövsprickning och blomning, och växtens blomningsfenologi är den tid på året som blommorna slår ut. Fenologistudier görs ofta på individens reaktionsnormer, det vill säga på hur en karaktär uttrycks i olika miljöer. Ett exempel på en reaktionsnorm kan vara förändringen i blomningstid hos en växt över olika temperaturer, eller i hur tiden för äggläggning hos fåglar förändras med förändringar i temperatur. Reaktionsnormen utgör alltså en funktion som beskriver hur en karaktär förändras över en miljögradient. Reaktionsnormens höjdriktning representerar organismens fenologi i en genomsnittlig miljö, och lutningen representerar hur känslig organismens utvecklingshastighet är för förändringar i en miljöfaktor.

Förändringar i fenologi som svar på ändringar i temperatur kan orsakas av genetiska ändringar till följd av selektion eller fenotypisk plasticitet (dvs. att en genetisk individ kan ha olika fenologi i olika miljöer). Genetiska faktorer och miljöfaktorers påverkan på karaktärens uttryck kan skilja sig över latitudinella och altitudinella gradienter. På lång sikt kan sådana skillnader leda till anpassning till det lokala klimatet. Variationen som uppstår kan följa ett medgradientmönster, där både genetiska faktorer och miljöfaktorer påverkar karaktären i samma riktning, ett motgradientmönster där de verkar i motsatt riktning, eller inte följa något latitudinellt eller altitudinellt mönster.

De flesta studier som har undersökt hur individens fenologi svarar på temperaturförändringar har visat att tiden för viktiga händelser i individens livshistoria, såsom fåglars häckningstid och lövsprickning hos växter, inträffar allt tidigare på säsongen. Studier av fåglar, växter, insekter och däggdjur visar också på att omfattningen på förändringar i karaktärer, som svar på förändringar i olika miljöfaktorer, kan skilja sig åt både mellan individer och mellan arter. Skillnader i hur olika arter svarar på temperaturförändringar kan bero på att de svarar på olika signaler från den abiotiska miljön, såsom

temperatur eller fotoperiod, men det kan också bero på att olika arter är olika känsliga för samma signal. Endast ett fåtal studier har undersökt mellanartsvariation i temperaturkänslighet experimentellt, men de studier som finns visar till exempel på att ökad temperatur leder till snabbare utveckling hos växtätande insekter än hos deras värdväxter. När arter påverkas olika av variation i temperatur kan skillnader uppstå i deras relativa timing och det kan i sin tur påverka artinteraktioner. Till exempel kan synkroni mellan växtätare och värdväxter rubbas om arterna skiftar sin fenologi i olika utsträckning eller riktningar. En konsekvens av sådana förändringar i synkroni mellan interagerande arter skulle kunna vara att växtätare som är generalister byter värdväxt. Så vitt vi vet har inga studier gjorts som undersöker om skillnader i utnyttjandet av flera värdväxtarter kan kopplas till skillnader i synkroni under naturliga omständigheter. Vi vet alltså mycket litet om vilka följder som skillnader i relativ timing kan medföra, speciellt vad gäller artinteraktioner som är starkt beroende av fenologi.

Fokus för denna avhandling är ett studiesystem bestående av flera korsblommiga växtarter, Backtrav (*Arabidopsis thaliana* L. Heynh.), Rockentrav (*Arabis glabra* L.), Lundtrav (*Arabis hirsuta* L.), Lomme (*Capsella bursa-pastoris* L. Medik.), Ängsbräsma (*Cardamine pratensis* L.) samt Backskärvrö (*Thlaspi caerulescens* J. Presl & C. Presl), som alla används som värdväxter av Aurorafjärilen (*Anthocharis cardamines* L.). Genom att använda detta studiesystem syftar avhandlingen till att undersöka skillnader i effekten av temperatur på utvecklingshastighet fram till blomningstid, både inom (studie I) och mellan (studie II) arter, om växternas temperaturkänslighet varierar mellan olika utvecklingsfaser innan blomning (studie II), om småskalig variation i vårtemperatur påverkar blomningstid och interaktioner med aurorafjärilen (studie III), samt om skillnader i synkroni mellan fjärilen och dess värdväxter påverkar valet av värdväxter, med avseende både på val av art och individer inom samma population som skiljer sig i blomningstid (studie IV).

Anledningen till att använda just det här studiesystemet för avhandlingen, är att det kan vara speciellt känsligt för klimatförändringar. Detta på grund av följande orsaker: (1) interaktionen mellan aurorafjärilen och dess värdväxter sker under en kort period under säsongen, (2) aurorafjärilen är en av de fjärilsarter som tidigareläggd den tid den är aktiv under våren allra mest under de senaste 20-30 åren och (3) aurorafjärilen har bara en generation per säsong och är därför känsligare för variationer i klimatet i jämförelse med fjärilsarter som har två eller flera generationer per säsong.

Samtliga sex värdväxtarter som användes blommar under tiden på säsongen då aurorafjärilen är aktiv. Lundtrav och Ängsbräsma är perenner och blommar från maj till juni. Det finns två vanliga underarter av Ängsbräsma i Sverige, Äkta ängsbräsman (ssp. *pratensis*) och Kärrbräsma (ssp. *paludosa*).

Äkta ängsbräsma blommar något tidigare än Kärrbräsman. I sydöstra Södermanland (där studie III utfördes) är de båda underarterna lika vanliga. Rockentrav blommar från juni till juli. Den är monokarp vilket innebär att den endast blommar en gång och dör efter frösättning, även om den är perenn och alltså behöver mer än en säsong för att växa till sig och blomma. Backtrav, Backskärvfrö och Lomme är alla annueller. Backtrav blommar från april till juni, Backskärvfrö från april till maj och Lomme från april till oktober. Aurorafjärilen övervintrar som puppa och kläcks tidigt i maj i södra Sverige och sent i maj eller tidigt i juni i norra Sverige. Fjärilen är sedan aktiv i tre till fyra veckor. Fjärilshonorna föredrar att lägga sina ägg på värdväxter som är i ett sent knoppstadium eller tidigt blomningsstadium och de unga larverna kan bara konsumera blommor eller nybildade fröskidor (med undantag för Lundtrav). Man kan alltså säga att Aurorafjärilen är en fenologisk specialist.

Växtmaterial för studie I och II samlades in från tre regioner i Sverige, en sydlig (östra Skåne), en mellan- (östra Uppland) och en nordlig (östra Ångermanland), under våren 2011. Växtmaterialet användes för att driva upp nya plantor i växthus under augusti och september 2011. Den 21:a mars 2012 placerades plantorna slumpmässigt i klimatkammare och utsattes för en av tre temperaturbehandlingar (~13, 15 och 17 °C) med en konstant fotoperiod (15L:9D). Växternas utveckling, datum för första knopp och datum för första öppna blomma dokumenterades varannan dag.

För studie III följdes fenologin hos Ängsbräsmeindivider under hela blomningssäsongen 2012 (21:a maj till den 3:e juni 2012) i 15 populationer (8 populationer med Äkta ängsbräsma och 7 populationer med Kärrbräsma), belägna inom 10 km från varandra i sydöstra Södermanland. Varje vecka dokumenterades antalet knoppar, blommor och skidor, samt förekomsten av aurorafjärilsägg, hos upp till 30 slumpmässigt utvalda individer från varje population (totalt 410 individer). Det högsta medelantalet öppna blommor och frukter över samtliga populationer uppmättes den 5:e juni 2012 och värdena från detta datum användes senare i analyserna. Temperaturen mättes kontinuerligt, varje timme, på 5 till 8 ställen i varje population. Temperaturmätningarna pågick från början av växtsäsongen tills växterna blommade maximalt (4:e april till 5:e juni 2012). Timme grader från växtsäsongens början till maximal blomning användes som mått på värmeackumulering.

För studie IV, som utfördes under åren 2010-2013, dokumenterades reproduktiv fenologi (knoppar, blommor och skidor) och förekomst av aurorafjärilsägg hos Backtrav, Rockentrav, Lundtrav, Ängsbräsma, Lomme och Backskärvfrö. Observationerna gjordes den 17:e till den 22:a maj i den södra regionen, den 28:e maj till den 4:e juni i mellanregionen och från den 10:e till den 17:e juni i den norra regionen. Målsättningen var att dokumentera

reproduktiv fenologi för 300 individer av varje värdväxtart (målet uppnåddes i 39 av 60 populationer).

Resultaten av studie I visade att höjdriktningen av reaktionsnormerna för blomningstid skiljde sig mellan de tre regionerna för samtliga växtarter. Det vill säga att blomningstiden för samtliga arter varierade mellan de olika regionerna. Endast en art visade variation i temperaturkänslighet (reaktionsnormens lutning) över de olika regionerna. Skillnaderna i höjdriktning av reaktionsnormerna visar en blandning av medgradient-, motgradient- och ett blandat gradientmönster. Detta indikerar att blomningstid inte bara påverkas av temperaturskillnader och växtsäsongens längd, utan också av artspecifik selektion och selektion medierad av lokala faktorer (exempelvis herbivori). Skillnader i utvecklingshastighet inom arter förklarades i första hand av variation i tidiga knoppbildningsstadier hos några arter, men av sena knoppbildningsstadier hos andra arter. Mellanartsskillnaderna i latitudinella mönster av reaktionsnormer över temperatur i denna studie är viktiga, både för att identifiera vilka faktorer som medierar selektion på växternas fenologi, och för att förutspå hur växter kommer svara på klimatförändringar på både kort och lång sikt.

Resultaten av studie II visade att arterna inte skilde sig i temperaturkänslighet (reaktionsnormens lutning) under den totala tiden från experimentets början till slut, men när vi studerade de olika tillväxtfaserna innan blomning var för sig observerades mellanartsskillnader för alla tre regioner. Den genomsnittliga tiden från experimentets start till blomning (höjdskillnad), skiljde sig mellan arter inom alla tre regionerna. Kombinationen av skillnader i utvecklingstidens känslighet för temperatur (reaktionsnormens lutning) och av skillnader i genomsnittlig utvecklingstid (höjdskillnad), är troligtvis ett resultat av hur varma dagar är fördelade under växternas utveckling och mellanartsskillnader i temperaturkänslighet under olika faser av utvecklingen. Mer generellt innebär resultaten av den här studien att vi måste ta hänsyn till hur arter svarar på temperatur under olika faser av utvecklingen för att kunna förstå, och förutspå, hur olika arter svarar på klimatförändringar.

Resultaten av studie III visade att småskalig variation i temperatur medförde variation i blomningstid både mellan och inom populationer av Ängsbräsma. Kärrbräsman tidigarelade blomningstiden som svar på värmeackumulering i större utsträckning än Äkta ängsbräsma, medan värmeackumulering skedde snabbare i lokalerna där Äkta ängsbräsma växte. Sannolikheten att Aurorafjärilen skulle lägga ägg på en växt påverkades inte direkt av värmeackumulering, men den ökade med tidigare blomningsfenologi. Resultaten visar att småskalig variation i temperatur delvis kan förklara variation i blomningstid mellan och inom populationer av Ängsbräsma, och att detta i sin tur har viktiga följder för interaktioner med Aurorafjärilen. Detta indike-

rar att småskalig variation i temperatur kan vara en viktig orsak till att skillnader i fenologi uppkommer både inom och mellan populationer av samma art, och att det därför är troligt att sådan variation påverkar synkronin mellan interagerande arter såväl som intensiteten av mellanartsinteraktioner.

Resultaten av studie IV visade att fjärilens användning av en given värdväxt var associerad med värdväxtens genomsnittliga utvecklingsstadium. Ju mindre avvikelser från det stadium fjärilen föredrar för att lägga ägg, desto mer användes värdväxtarten. Inom värdsväxtarter, blev fjärilens selektion av värdväxtindivider för äggläggning påverkade av avvikelser från det stadium fjärilen föredrar. Ju mer utvecklade växterna var i genomsnitt i tiden när fjärilen flög, desto starkare var fjärilarna preferens för värdväxtindivider med senare fenologi, dvs. växter i tidigare utvecklingsstadier. Resultaten indikerar att skillnader i temperatur, i kombination med att de interagerande arterna skiljer sig i reaktionsnormer, kan leda till skillnader i värdväxtval såväl som herbivormedierad selektion på växters reproduktiva fenologi.

Sammanfattningsvis tyder resultaten från denna avhandling på att klimatförändringar kommer att leda till förändringar i interaktionerna mellan värdväxterna och herbivoren i detta system, men att kombinationen av skillnader i fenologi mellan de olika värdväxtarterna och förändringar i fjärilens värdväxtval skulle kunna buffra herbivoren mot negativa effekter av klimatförändringar. Denna avhandling belyser vikten av att undersöka hur interagerande arter svarar på temperatur, samt hur viktigt det är att undersöka temperaturrespons hos arter med olika latitudinell härkomst, under olika utvecklingsstadier för att kunna förstå och förutspå konsekvenserna av klimatförändringar.

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