



Latitudinal variation in thermal reaction norms of post-winter pupal development in two butterflies differing in phenological specialization

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Latitudinal clines in thermal reaction norms of development are a common phenomenon in temperate insects. Populations from higher latitudes often develop faster throughout the range of relevant temperatures (i.e. countergradient variation) because they must be able to complete their life cycle within a shorter seasonal time window compared to populations at lower latitudes. In the present study, we experimentally demonstrate that two species of butterflies *Anthocharis cardamines* (L.) and *Pieris napi* (L.) instead show a cogradient variation in thermal reaction norms of post-winter pupal development so that lower latitude populations develop faster than higher latitude populations. The two species share host plants but differ in the degree of phenological specialization, as well as in the patterns of voltinism. We suggest that the pattern in *A. cardamines*, a univoltine phenological specialist feeding exclusively on flowers and seedpods, is the result of selection for matching to the phenological pattern of its local host plants. The other species, *P. napi*, is a phenological generalist feeding on the leaves of the hosts and it shows a latitudinal cline in voltinism. Because the latitudinal pattern in *P. napi* was an effect of slow development in a fraction of the pupae from the most northern population, we hypothesize that this population may include both bivoltine and univoltine genotypes. Consequently, although the two species both showed cogradient patterns in thermal reaction norms, it appears likely that this was for different reasons. © 2014 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2014, ••, ••–••.

ADDITIONAL KEYWORDS: *Anthocharis cardamines* – cogradient – degree days – local adaptation – *Pieris napi*.

INTRODUCTION

The life cycles of most organisms need to be well synchronized with seasonal occurrence of favourable environmental conditions, food availability, and interactions with prey/host species (Tauber, Tauber & Masaki, 1986). Temporal and spatial variation of these important aspects of seasonality have engendered a variety of adaptations in the timing of life-history events (i.e. phenology), which can be the

result of either genetic differences between populations (local adaptations) or phenotypic plasticity in phenological traits in response to environmental variables. Latitudinal clines in local adaptations in phenology (including growth rate, number of generations, morphological traits) are a common phenomenon in insects (Bradshaw & Lounibos, 1972; Arnett & Gotelli, 1999; Demont & Blanckenhorn, 2008). These geographical patterns were argued to be an adaptive consequence of variation in the length of the growing season (Kipyatkov & Lopatina, 2010).

Differences in phenology are often the result of differences in thermal reaction norms of developmental rates, which is the most commonly measured characteristic that correlates with fitness (Yamahira

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& Conover, 2002). Intraspecific geographical adaptation in development often involves an increase of developmental rate with latitude throughout the relevant temperature range (Danilevskii, 1957; Holzapfel & Bradshaw, 1976; Dingle & Mousseau, 1994; Pöykkö & Tammaru, 2010) rather than shifts in the optimum of the thermal reaction norm along the temperature gradient (Yamahira & Conover, 2002). Because development is positively temperature-dependent and mean temperatures become cooler with higher latitude, this form of local adaptation implies that genetic and environmental effects on the phenotype oppose each other across an environmental gradient and it is therefore known as a countergradient variation (Conover & Schultz, 1995). This pattern is commonly found in species for which the period of food availability is strongly correlated with the length of the growing season (Yamahira & Conover, 2002).

The absence of countergradient variation in developmental rate is less common (Trimble & Smith, 1978; Angilletta, 2009) and may be present in species where selection as a result of the length of the season is relaxed. This occurs, for example, when the number of generations per season (voltinism) decreases at more northern latitudes, creating a so-called 'saw-tooth' cline in developmental rate (Roff, 1980; Nygren, Bergstrom & Nylin, 2008; Välimäki *et al.*, 2013). However, if the number of generations is constant, seasonal constraints would favour the selection of higher development rates in populations from higher latitudes.

In temperate insects, the phenological timing of spring emergence is strongly dependent on developmental processes subsequent to the termination of winter diapause. Local selection on post-diapause developmental rate is likely to be dependent not only on the season length, but also on the exact seasonal timing of important resources such as larval host plants or even a particular phenological stage of these hosts. The typical expectation of countergradient variation is primarily the result of differences in season length, which would lead to high latitude populations developing faster than low latitude ones in the relevant range of temperatures because they need to produce the same number of generations during a shorter period of time. However, to what degree species that are strongly dependent on the seasonal emergence of a particular phenological stage of their host plants are expected to show countergradient variation is not well studied.

In the present study, we experimentally explored latitudinal variation in thermal reaction norms of post-diapause pupal development in two butterfly species with similar geographical and host range but different in the patterns of voltinism. *Pieris napi*,

the green-veined white, feeds preferably on green leaves of various Brassicaceae species and, within the sampled area, is known to have two generations per season (Petersen, 1949). The other species, the orange tip butterfly *Anthocharis cardamines* is a phenological specialist because its larvae feed almost exclusively on flowers and growing siliqua of Brassicaceae species and often prefer to leave the plant when all the seed pods have been eaten rather than consume the leaves. *Anthocharis cardamines* is univoltine throughout the range, finishing its larval development and entering diapause in the beginning of summer/midsummer. We investigated whether post-diapause pupal development differs between three populations of the butterfly species after 5 months of winter diapause, which is a common length of winter in the area. In addition, we also explored which aspect of post-diapause development is more important for explaining variation in adult emergence. To do this, we monitored pupal weight loss rates during post-diapause as a proxy of development and tested whether variation among groups is a result of differences in the early period of diapause termination or the developmental rate towards the end of post-diapause development.

MATERIAL AND METHODS

The experiments on thermal reaction norms of pupal development rate were repeated in two consecutive years: in spring 2011 with only *A. cardamines* (at the Botany Department, Stockholm University (SU)) and 2012 with both butterfly species (at Tovetorp field station, Zoology Department, SU). Eggs, larvae, and adult females of *A. cardamines* and *P. napi* were collected 1 year before the experiments (in 2010 and 2011, respectively) along the Eastern coast of Sweden from three locations, ranging 900 km from the South to the North: Skåne province in the South, Uppland province (Ljusterö island for *A. cardamines*, Stockholm area for *P. napi*) in the Centre, and Ångermanland province in the North (Fig. 1). For *A. cardamines*, all experimental individuals were consequently wild collected as eggs or larvae and are likely to represent the genetic variation of the respective populations. For *P. napi*, we used the same procedure but this sample was further supplemented with larvae obtained from population cages that received eggs from multiple wild collected females (seven to 10 females/population).

Larvae from wild collected eggs and those laid by the wild-caught females were reared until pupation in a climate controlled room (LD 12 : 12 h photoperiod, 17 °C). *Anthocharis cardamines* larvae were fed with Garlic mustard (*Alliaria petiolata*) seedpods and kept in pairs in rearing cups to avoid larvae preying

on each other. *P. napi* were reared on horseradish (*Armoracia rusticana*) leaves in a single container (80 × 50 × 40 cm; one per population of approximately 150–180 individuals). The diapausing pupae of both species were placed in cold conditions in late October or November. In 2010, *A. cardamines* pupae were placed outside of the Zoology Department, SU on 20 October. In 2011, both *A. cardamines* and *P. napi*

pupae were kept in a refrigerator (+1 °C, constant darkness) from 17 October, except for the Skåne population of *P. napi*, which pupated later and was placed into the refrigerator one month later on 21 November.

The sample size (after accounting for mortality) of the pupae tested in the treatments is given in Table 1.

EXPERIMENTAL SET-UP

After 5 months of diapause under cold conditions, all the pupae were randomly divided among four treatment groups: three different indoor temperatures (t_1 – t_3) and one outdoor treatment as a control (t_4) to verify that the indoor findings in the constant temperatures are relevant and can be extrapolated to the real conditions with varying temperatures in nature. Pupae from the southern (Skåne) population of *P. napi*, which became available to us much later, were placed into the cold 1 month later than other pupae and were added to the indoor treatments 1 month later so that all the pupae had the same amount of time in cold. However, in the outdoor treatment (t_4), they were placed at the same time as other populations (i.e. only after 4 months in the cold) so that all the pupae could experience exactly the same spring temperatures.

The indoor treatments were performed in a greenhouse at SU in 2011 and in thermally controlled rooms at Tovetorp research station in 2012. The mean indoor treatment temperatures during the experimental period were (mean ± SD; t_1 , t_2 , and t_3 , respectively) 15.7 ± 3.5 °C, 16.8 ± 2.8 °C and 22.8 ± 4 °C in 2011; and 13.5 ± 1.6 °C, 15.8 ± 1.8 °C and 17.6 ± 2 °C in 2012. For the control outdoor conditions (t_4), the pupae were placed outdoors, shaded from direct sunlight. The mean t_4 temperatures were: 9.1 ± 4.8 °C (with minimum = –0.8 °C and maximum = 18.3 °C) in 2011; and 9.5 ± 4.6 °C (with minimum = –0.02 °C and maximum = 18.9 °C) in 2012. In both years, the actual experimental temperatures were recorded

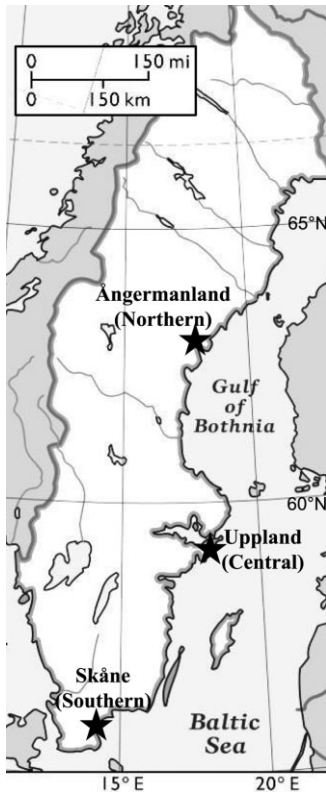


Figure 1. The three sampling areas of the butterflies' populations: Southern (Skåne province), Central (Uppland province), and Northern (Ångermanland province) along the eastern coast of Sweden.

Table 1. Number of female/male pupae eclosed in each of the four treatments in 2011–2012, after accounting for mortality

	Species	Population	t_1 f/m	t_2 f/m	t_3 f/m	t_4 f/m	Unbroken diapause
2011	<i>Anthocharis cardamines</i>	Skåne	22/22	22/25	22/30	16/27	–
		Ljusterö	6/10	8/10	11/6	15/16	–
		Ångermanland	3/6	3/6	5/3	6/8	–
2012	<i>Anthocharis cardamines</i>	Skåne	11/6	9/10	8/9	4/10	–
		Ljusterö	16/21	15/23	15/24	18/18	–
		Ångermanland	10/10	10/10	7/13	11/6	–
	<i>Pieris napi</i>	Skåne	19/19	20/20	19/20	25/14	1
		Stockholm	18/17	16/14	7/27	6/9	6
		Ångermanland	8/17	9/16	6/18	17/21	37

with the help of temperature loggers every 30 min (Tinytag Plus2 data loggers in 2011, one per treatment; DS1921G Thermochron iButtons in 2012, three to four buttons per room). Pupal developmental time, from the moment the pupae were placed into one of the treatments and until they hatched, was recorded for each individual. Because the developmental period was quite extended and the temperatures varied during the course of development (especially in t_4), we calculated the mean individual developmental temperature for each pupa, from the day it was placed into the treatment and until the day it hatched.

PUPAL DEVELOPMENTAL PERIODS

In 2011, the adult butterflies were weighed after eclosion. In 2012, during the post-winter period, all pupae were weighed to the nearest 0.01 mg with a precision balance (Precisa XB 102A) three times a week. The sample sizes of the survived pupae tested in the treatments are provided in Table 1.

Pupal weighing at regular intervals during the post-winter period allowed the time when the actual post-diapause pupal development began to be pinpointed. Because a pupa represents a nonfeeding stage and is totally dependent on energy reserves stored prior to pupation, pupal weight loss rate was used as an approximation of metabolic rate (Forsberg & Wiklund, 1988), which is known to increase when diapause is terminated (Schneiderman & Williams, 1953; Hahn & Denlinger, 2007; Ragland *et al.*, 2009).

In 2012, for each individual pupa, we calculated a relative weight loss per day (change in weight between two consecutive measurements as a percentage of the earlier measurement), which allowed us to assess the typical pattern of weight loss for pupae that did develop after the cold period, as well as pupae that stayed in diapause throughout the whole period of warm temperature treatment.

A regression of relative daily weight loss against time of pupae that did not develop when moved to the warm conditions was performed separately for each of the temperature treatment groups. The upper bounds of 95% confidence interval of the slopes were between 0.15% and 0.18% in *P. napi* pupae depending on the treatment temperature. The same procedure was repeated for *A. cardamines*. However, because all the pupae developed during the warm treatment, we used nine pupae that did not hatch in one of our other experiments. The upper bound of 95% confidence interval for these individuals was 0.09%. We rounded the values and chose the critical limit of the daily weight loss, beyond which the pupae were considered to have started their development, of 0.2% for *P. napi* and 0.1% for *A. cardamines*.

By applying these limits, the total developmental period of each pupa (i.e. from the moment it was placed in the favourable warm conditions and until an adult eclosed) was divided into two periods: the early period of quiescence (the early developmental phase; EDP) during which the pupae were considered to be still in quiescence; and the actual post-diapause development when a pupa is clearly out of dormancy and commences morphogenesis (which we further call the late developmental phase; LDP). Because the early phase may include a period of late diapause (sensitive to diapause terminating conditions) or quiescence, during which the processes of dormancy completion and switching to tissue transformation are likely to take place, we can regard the EDP as a sort of lag-phase or a waiting period during which there is no active development. Thus, the units of measurement for the EDP were the number of days from the moment a pupa was placed in the favourable warm conditions until the point at which it began morphogenesis. During the LDP, which is the time from the start of pupal morphogenesis to eclosion, the actual pupal development occurred; therefore, pupal developmental rate (1/number of days) was used as a unit of measurement. The total developmental period (TDP) was also expressed as a rate, calculated as the inverse of the number of days from the moment a pupa was placed in the favourable warm conditions until eclosion.

STATISTICAL ANALYSIS

Because the butterfly pupae were treated somewhat different during the overwintering and experimental periods in 2011 than in 2012, the data from each year were analyzed separately. The analyses were conducted with R, version 2.15.1 (R Development Core Team, 2012). In all the models population of origin, mean individual temperature (continuous variable), sex, and adult (in 2011)/pupal (in 2012) weight (continuous) were used as explanatory variables. Because the sample sizes used were not particularly large, the initial models included the explanatory variables and all their two-way interactions. The best model structure was determined by gradually removing nonsignificant terms according to the principle of hierarchy by using Akaike information criterion. Goodness-of-fit of the final models was evaluated by visual investigation of the residual plots. In cases when some violations of the assumptions were observed, a proper transformation of response variable was determined with the help of Box-Cox transformation (see below for the analysis-specific transformation).

Linear models (LM) were fitted to developmental rate (DR) data (both during the TDP and LDP) of both butterfly species. In 2011, DR of *A. cardamines*

in the TDP was inverse of fourth root-transformed; in 2012, DR in the LDP was log-transformed for *A. cardamines* and inverse of cubic root-transformed for *P. napi*.

Generalized linear models with a gamma error distribution and a log link function were fitted to the data on pupal developmental time in the EDP t_1 – t_3 2011–2012 of both butterflies.

General least squares models fitted by restricted maximum likelihood (function `gls`; Pinheiro *et al.*, 2013) were applied to DR in the TDP from the outdoor treatment (t_4) of both butterfly species in 2012. Because all the pupae were subjected to the same conditions during development in the outside t_4 treatments, temperature was not included as a factor in the analysis. The variance function `varIdent` (Pinheiro *et al.*, 2013) was used for modelling heteroscedasticity between different variances for each level of population and sex in *P. napi* 2012 data. The variance function `varPower` with different variance parameters for each level of sex was applied to *A. cardamines* 2012 data. For *A. cardamines* 2011 data, LM were applied.

We also used the data to calculate population- and species-specific threshold temperatures (t_0) and degree-days for post-winter pupal development, which is presented in the the Supporting information (Appendix S1).

RESULTS

ANTHOCHARIS CARDAMINES

Both in 2011 and 2012, pupal DR in the TDP of *A. cardamines* in the three indoor treatments (t_1 – t_3) showed a significantly positive relationship with treatment temperature, as well as significant effects of population and sex. Pupal DRs were higher in more southern populations [Figs 2A, 3A; Table 2 (2011) and

Table 3 (TDP)]. On average, from all the model estimates, males of *A. cardamines* developed faster and eclosed from 2.9 days (at 13 °C) to 1.03 days (at 17 °C) earlier than females (Figs 2A, 3A). Because the sex effect was strongly pronounced in most of the analyses, with the results with males being invariably faster than females, only one sex (females) is shown in the regression figures for clarity. Both sexes are shown in the plots of means, where adding of more elements did not compromise their clarity. In the outdoor treatment (t_4), the effects of population and sex (a mean of 2.5 days earlier for males compared to females) on DR in the TDP were significant in both years and agreed with the results from indoor treatments (Figs 2B, 4A).

The length of the pupal EDP in the 2012 experiment was affected by sex (males had a shorter early phase) and treatment temperature (the early phase was increasingly likely to shorten in warmer treatments) but not by population (Fig. 3A, Table 3; EDP). The significant effect of initial pupal weight was the result of an individual outlier that had a very long extended early phase. When removed, the pupal weight term had no significant influence on the duration of the EDP.

Population had a strong effect on pupal DR in the LDP, as well as sex and treatment temperature (Fig. 3A, Table 3; LDP). A significant effect of the population \times temperature interaction both in the TDP and LDP indicates that the differences between the populations were more pronounced as the treatment temperature increased [Figs 2A, 3A, Table 2 (2011) and Table 3 (TDP)].

PIERIS NAPI

Similar to *A. cardamines*, DR values in the TDP of *P. napi* in the indoor treatments 2012 (t_1 – t_3) were

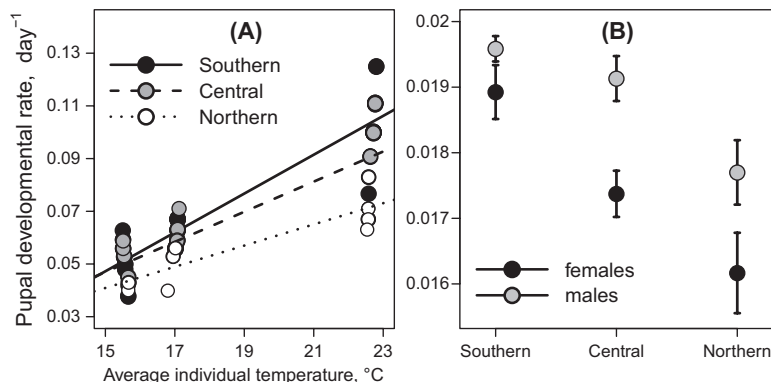


Figure 2. Pupal developmental rate in the total developmental period (TDP) of the southern (black), central (grey) and northern (white) populations of *Anthocharis cardamines* populations in (A) the indoor treatments t_1 – t_3 (females only) and (B) outdoor t_4 treatment (both sexes) in 2011. Values presented in (B) are the means \pm 95% confidence interval.

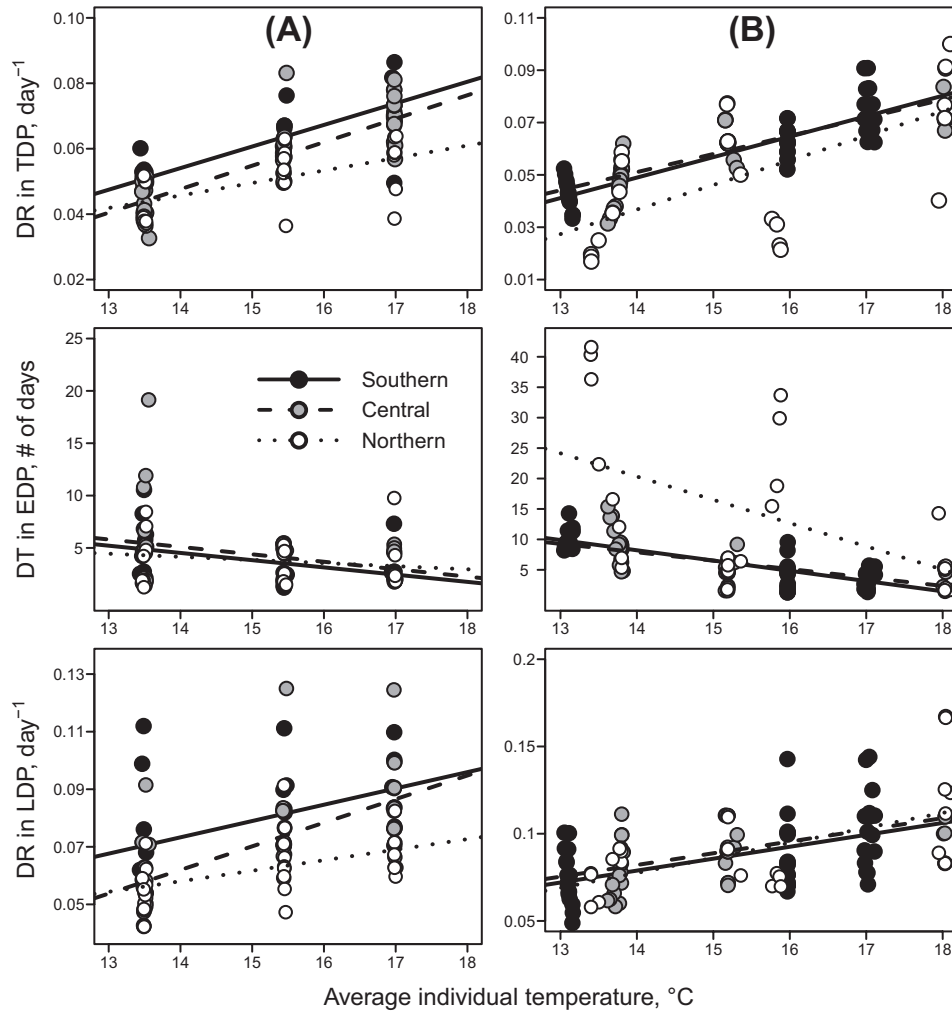


Figure 3. Pupal developmental rate (DR) in the total (TDP) and late (LDP) developmental periods, and developmental time (DT) in the early developmental phase (EDP) of the southern (black), central (grey) and northern (white) populations of *Anthocharis cardamines* (A) and *Pieris napi* (B) within the three indoor treatments (t_1 – t_3) in 2012. Only females are presented.

significantly affected by treatment temperature, population, and sex (Table 3; TDP). The southern and the central populations had similar DRs and developed quicker than the northern population (Fig. 3B). Similar to *A. cardamines*, males of *P. napi* eclosed from 2.4 days (at 13 °C) to 0.8 days (at 17 °C) earlier than females in the indoor treatments. The results from the outdoor treatment (t_4) reflected those of the indoor treatment (Fig. 4B, Table 2). From the model estimate, the males eclosed 2.3 days earlier than the females.

The effect of population on the length the EDP and DR in the LDP was opposite to *A. cardamines*: population differences in the TDP of *P. napi* were entirely a result of variation in length of the early phase (Fig. 3B, Table 3).

DISCUSSION

Overall, in both *A. cardamines* and *P. napi*, more southern populations developed faster than more northern ones under all thermal conditions, revealing a cogradient pattern in pupal developmental rates. In both species, the rank order of adult emergence in the control outdoor groups generally confirmed the estimation of thermal reaction norms in the controlled thermal conditions. The cogradient variation in the emergence time was primarily a result of the unequal length of the early developmental phase of quiescence in *P. napi* (Fig. 3B) and the late phase of active morphogenesis in *A. cardamines* (Fig. 3A). The pattern of degree days (DD) accumulation by *A. cardamines* and *P. napi* populations, being in

Table 2. Linear models (2011) and general least squares models (2012) for pupal post-winter development in TDP of *A. cardamines* and *P. napi* in the indoor (t_1 – t_3) and outdoor (t_4) treatments in 2011 and 2012

	Species	Treatment	Parameter	d.f.	<i>F</i>	<i>P</i>
2011	<i>Anthocharis cardamines</i>	t_1 – t_3	Population	2	63.9	$< 2.2 \times 10^{-16}$
			Sex	1	51.9	9×10^{-12}
			Temperature	1	1395.2	$< 2.2 \times 10^{-16}$
			Pupal weight	1	4.5	0.035
			Population \times Temperature	2	3.4	0.036
			Residuals	212		
	<i>Anthocharis cardamines</i>	t_4	Population	2	59.6	$< 2.2 \times 10^{-16}$
			Sex	1	66.2	3×10^{-12}
			Population \times Sex	2	6.2	0.0032
			Residuals	82		
2012	<i>Anthocharis cardamines</i>	t_4	Intercept	1	98525	< 0.0001
			Population	2	11.9	< 0.0001
			Sex	1	27.9	< 0.0001
			Residuals	63		
	<i>Pieris napi</i>	t_4	Intercept	1	79466	< 0.0001
			Population	2	13.6	< 0.0001
			Sex	1	18.9	< 0.0001
			Residuals	88		

Table 3. Linear models (total developmental period, TDP; late developmental phase, LDP) and generalized linear models (early developmental phase = EDP) for pupal post-winter development of *A. cardamines* and *P. napi* in the indoor treatments (t_1 – t_3) in 2012

<i>Anthocharis cardamines</i>					<i>Pieris napi</i>			
	Parameter	d.f.	<i>F</i>	<i>P</i>	Parameter	d.f.	<i>F</i>	<i>P</i>
TDP	Population	2	45.3	$< 2 \times 10^{-16}$	Population	2	29.5	2×10^{-12}
	Sex	1	71.3	4×10^{-15}	Sex	1	12.3	0.00053
	Temperature	1	385.6	$< 2 \times 10^{-16}$	Temperature	1	465.7	$< 2 \times 10^{-16}$
	Population \times Temperature	2	15.7	4×10^{-7}	Residuals	281		
	Residuals	220						
EDP			χ^2				χ^2	
	Sex	1	41.95	9×10^{-11}	Population	2	102.6	$< 2 \times 10^{-16}$
	Temperature	1	24.40	7×10^{-7}	Temperature	1	166.4	$< 2 \times 10^{-16}$
	Pupal weight	1	4.96	0.026	Pupal weight	1	2.78	0.096
LDP			<i>F</i>				<i>F</i>	
	Population	2	27.4	2×10^{-11}	Sex	1	7.4	0.0069
	Sex	1	10.6	0.0013	Temperature	1	133.3	$< 2 \times 10^{-16}$
	Temperature	1	200.1	$< 2 \times 10^{-16}$	Residuals	283		
	Pupal weight	1	5.8	0.016				
	Population \times Temperature	2	8.9	0.00019				
Residuals	219							

mathematical dependence with slope of DR (Honěk & Kocourek, 1988), gave the same result as the pattern in DR (see Supporting information, Appendix S1).

The cogradient pattern of adult emergence of *A. cardamines* in Sweden is likely to be partly a consequence of a lack of selection from the length of the

vegetative season. The species is univoltine across its whole distribution, and its larval developmental period is short relative to the growth period both in central Sweden, where the first pupae were observed already in the end of June (Wiklund & Åhrberg, 1978), and in Britain, where the larvae were shown to complete their

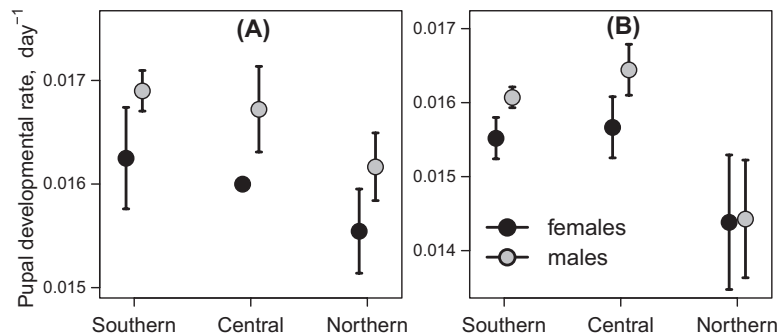


Figure 4. Pupal developmental rate in the total developmental period (TDP) of the three *Anthocharis cardamines* (A) and *Pieris napi* (B) populations in the outdoor treatment (t_4) in 2012. Values presented are means \pm 95% confidence interval.

development by mid-July in the north-east (Courtney, 1981). In comparison, *P. napi*, with a similar developmental time, manages to produce two to three adult generations per season at the same latitudes. Thus, the phenology of *A. cardamines* is unlikely to be limited by the duration of the warm season *per se*. Instead, the most likely limitation is the length of the period when flowers and seedpods of its host plants are available. This dependence on a specific developmental plant stage is likely to be the main reason of the obligatory *A. cardamines* univoltinism because mass flowering of its Brassicaceae host plants sufficient for larval maintenance, occurs only once in late spring to early summer (Wiklund & Friberg, 2009). A potential second generation would find only sparse flowering individuals of late flowering annuals (e.g. *Capsella bursa-pastoris* or *Arabidopsis thaliana*) that are usually poorer in seedpod yield at this time of the season (i.e. productivity of annuals drops in the second half of the summer as a result of rapid maturation at high temperatures and decreasing photoperiod; Atkinson & Porter, 1996). Hence, the adults are expected to avoid emerging too early, before the host plants are suitable for development, and emerging too late, when insufficient time remains to complete larval development before host plant resources are exhausted as a result of the plant developmental stage. Thus, the timing of phenological events of the host plant species in spring should be the main selection pressure acting on thermal reaction norms of post-winter pupal development in *A. cardamines*. The cogradient pattern in the butterflies could thus be a result of matching their emergence to a cogradient pattern of reproductive stage of the preferred host plant(s). Another host-related explanation for the observed cogradient pattern is a shift in host use at more northern latitudes (Williams, 1983; Sword & Dopman, 1999), reflecting differences in plant availability from southern populations (Hengeveld & Haeck, 1982; Brown, 1984). Climatic differences and natural enemies can act as nonhost-mediated factors that shift the balancing of

the trade-off between host plant availability and other factors (Porter, 1983; Mira & Bernays, 2002; Wiklund & Friberg, 2009). It may also be speculated that the generally warmer climate of southern Sweden leads to both an earlier start and a shorter duration of flowering and seed set in the host plants of *A. cardamines* (Primack, 1985).

A temperature \times population interaction in the DRs of the three *A. cardamines* populations suggests that the DR of the northern population exceeds that of the most southern one at temperatures lower than those used in the indoor treatments (Fig. 3A). This type of interaction between temperature and populations from different latitudes has been found in several other studies (Heron, 1972; Calvin *et al.*, 1991) and has been given an adaptive interpretation: faster development at relatively cold spring conditions should be more strongly favoured at higher latitudes compared to low latitudes (Trudgill & Perry, 1994; Trudgill, 1995; Honěk, 1996). However, even if this had been the case in our study system and a more northern population truly exceeded a more southern one in developmental rate at temperatures below 13 °C, we would have expected the control outside groups to have a reversed pattern, with the northern pupae developing more quickly because most of the spring mean daily temperatures in 2011 and 2012 were below 10 °C for a majority of the developmental period. However, we observed a cogradient pattern in DR in the outdoor controls in both years, suggesting that, even if the thermal reaction norms do cross, they do so only at temperatures close to the developmental threshold, t_0 .

Given our results, it is interesting that Phillimore *et al.* (2012) found the opposite pattern of counter-gradient variation based on field observations for *A. cardamines* in the UK. It appears unlikely that the length of the vegetative season is the selective force that shaped these patterns in the UK. First, because Sweden is located further north, the season length is shorter there compared to the UK. Thus, if there is

selection mediated by the length of the vegetation period for the countergradient pattern, it should be even stronger for Swedish *A. cardamines*. Second, Phillimore *et al.* (2012) found no evidence that the pattern of adult emergence of *A. cardamines* in the UK in spring is a result of coadaptation with the flowering phenology of its main hosts *Alliaria petiolata* and *Cardamine pratensis*. Moreover, unlike *A. cardamines*, these plant species showed no local adaptations in the flowering phenology in south–north range within the UK. Phillimore *et al.* (2012) argue that the temporal and spatial interactions between the herbivore and the hosts are maintained by the plastic response in the butterflies, which have overlapping thermal cues with their host plants. This lack of apparent coadaptation in the species interaction may arise from the fact that, in the UK, the southern population of *A. cardamines* is primarily associated with *A. petiolata*, whereas the northern one is primarily using *C. pratensis* (Courtney & Duggan, 1983; Davies & Saccheri, 2013) and, thus, the populations could be more adapted to the thermal reaction norms of the prevailing host species at their own locations. Differences in the phenological timing of *A. petiolata* in the south and *C. pratensis* in the north may create the observed countergradient pattern in Britain. As for Swedish *A. cardamines*, all the populations throughout the sampled area utilize *C. pratensis* to a greater extent. In this case, the difference in host use across the latitudes (see above) is a less important driver and, if *C. pratensis* has a cogradient pattern of flowering times in Sweden, the butterfly spring emergence might follow this pattern as a result of coadaptation. Another potential reason for the difference in the latitudinal patterns between the two geographical regions could be that, in Sweden, *A. cardamines* do not depend only on the mentioned pair of host plant species but have an ability to switch to a wider range of other Brassicaceae species at suitable phenological stage that are available at the period of their flight time. Wiklund & Åhrberg (1978) and Wiklund & Friberg (2009) demonstrated that, by the time when the newly emerged *A. cardamines* females start their oviposition, some suitable host plant species had already finished their flowering and seed set and were disregarded by the females, suggesting that there are host plants available to the butterflies before their emergence. Thus, thermal reactions of pupal development might be adapted to track not only phenological events of a few hosts, but also the combined flowering period of all the suitable plant species in the region. Variation in *A. cardamines* pupal development may reflect host species composition and latitudinal pattern differences between the UK and Sweden. Thus, further investigations of the host species thermal reaction norms should help to identify some of the important selection factors.

The presence of a ‘security buffer’ consisting of earlier flowering host species and the fact that the butterflies do not fully use it by emerging even earlier in spring suggest that there might be factors other than the host species phenology that determine the butterflies’ development. For example, these could be unsuitable weather conditions for the adult *A. cardamines*’ flight earlier in the season or internal developmental costs of starting post-diapause development and maintaining high developmental rate at lower temperatures (Trudgill *et al.*, 2005).

As for *P. napi*, instead of the expected countergradient, we found a partially cogradient pattern: pupal developmental rates of both the southern and central populations were similar to one another and higher than those of the northern population. In the northern population, it appears that a small group of the tested pupae had substantially lower developmental rates than the majority, which resulted in a mean population reaction norm with a lower intercept (Fig. 3B) and contributed to the observed cogradient pattern in *P. napi*. Such a low developmental rate of the northern population could also lead to a substantial increase in the estimated t_0 . A possible explanation may be that the Ångermanland region is the northern border where *P. napi* still maintains two generations per season and is situated in the transition zone where a portion of the butterflies (as a consequence of either genetic variation or plastic response) produces only one generation (Species Gateway, 2012). The univoltine fraction of the population experiences significantly lower seasonal time limitations and may start the post-diapause development later, at higher temperatures. Hence, the pattern of divergent developmental rates and threshold values in this northern population of *P. napi* may be the result of a mixture of uni- and bivoltine genotypes; however, this hypothesis needs testing further.

The cogradient pattern of adult emergence was a result of variation in pupal developmental rate in the late phase of post-diapause development of *A. cardamines*, when rapid morphogenesis occurred. All the tested populations of *P. napi*, however, were similar at this phase but differed in the duration of the early post-winter phase. These differences may be a result of different evolutionary histories between the two butterfly species. Host plants of *A. cardamines* flower relatively early in spring (Duggan, 1985) and the eclosing adults are unable to respond directly to this. Therefore, the timing must be achieved indirectly through the response to temperature, which is assumed to be the main environmental driver of development in this species (Lees, 1980). Hence, *A. cardamines* is likely to be under particularly strong selection to be temperature

sensitive early in the season to track the period of flowering and to be able to respond to an increase in temperature at early developmental period. On the other hand, more cold-adapted northern populations of *P. napi* may have higher chilling requirements before pupal development can begin, which the laboratory winter did not fulfill.

CONCLUSIONS

Both *A. cardamines* and *P. napi* showed a cogradient pattern in the pupal post-winter developmental thermal reaction norms. However, for *P. napi*, this pattern was mainly a result of the much lower developmental rate of the northern population, which itself could be a result of the population being only partially bivoltine.

For *A. cardamines*, it is likely that the start and duration of the adult period is shaped by the length of the flowering time of the primary host plant or full the range of host species available in a given region. Therefore, there is potential for intraspecific local adaptation given the heterogeneous distribution of host plant availability across latitudes. On the other hand, it is still possible that some butterfly populations preferably use and are mostly adapted to match the flowering time of one host species, whereas some populations follow another host plant species (Phillimore *et al.*, 2012). A joint experimental testing of developmental patterns of both the butterfly and a range of its host plant species in controlled thermal conditions should clarify whether the observed reactions are formed by the need for synchronization with plants (i.e. coevolution), by temperature only or a combined effect of local differences in spring temperatures and shifts in host use at different latitudes.

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SUPPORTING INFORMATION

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Appendix S1. Population-specific parameters of *Anthocharis cardamines* and *Pieris napi* in relation to post-winter pupal developmental rate, threshold temperature, and number of degree days for adult hatching.