Variation in two phases of post-winter development of a butterfly

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Anthocharis cardamines; countergradient variation; local adaptation; orange tip butterfly; phenology; post-winter development; ramping up.

Abstract
The temporal aspects of life cycle characteristics, such as diapause development, are under strong selection in seasonal environments. Fine-tuning of the life cycle may be particularly important to match the phenology of potential mates and resources as well as for optimizing abiotic conditions at eclosion. Here, we experimentally study the spring phenology of the orange tip butterfly, Anthocharis cardamines, by analysing post-winter pupal development in three populations along a latitudinal cline in each of Sweden and the United Kingdom. These countries differ substantially in their seasonal temperature profile. By repeatedly recording pupal weights, we established that post-winter development has two separate phases, with a more rapid weight loss in the second phase than in the first, likely corresponding to a ramping up of the rate of development. Variation in the duration of the first phase contributed more strongly than the second phase to the differences in phenology between the localities and sexes. We found that insects from Sweden had a faster overall rate of development than those from the United Kingdom, which is consistent with countergradient variation, as Sweden is colder during the spring than the United Kingdom. Similar trends were not observed at the within-country scale, however. A cogradient pattern was found within Sweden, with populations from the north developing more slowly, and there was no clear latitudinal trend within the United Kingdom. In all localities, males developed faster than females. Our results point to the importance of variation in the progression of post-winter development for spring phenology.

Introduction
Variation in environmental suitability for survival, growth and reproduction over the year has given rise to intricate adaptations in the timing of life history events of temperate flora and fauna (Tauber et al., 1986). The phenological regulation of life cycle traits is important for maximizing fitness and affects the spatial and temporal extents of a species’ biotic and abiotic niche (Ragland & Kingsolver, 2007; Chuine, 2010). Many such traits have a heritable component (Etterson & Shaw, 2001; Winterhalter & Mousseau, 2007; Demont & Blanckenhorn, 2008) and show geographical trends within and among species (Tauber et al., 1986; Gienapp et al., 2010). Intraspecific clines in life cycle traits often follow environmental gradients, such as temperature gradients along latitude (Bradford & Roff, 1995; Masaki, 1999; Gaston et al., 2008). The degree to which genes and environments, and their interaction, contribute to the geographic phenological pattern influences how species and populations respond to short-term environmental fluctuations and long-term ecological changes (Blanckenhorn & Fairbairn, 1995; Gienapp et al., 2008; Phillimore et al., 2010; Pöykkö & Tammaru, 2010).

Countergradient variation occurs when the inherent relationship between phenotype and environment given by a reaction norm is reduced or removed through genetic adaption across an environmental gradient (Conover & Schultz, 1995). The phenomenon has been described for a number of life history traits of a wide variety of taxa (Conover et al., 2009) and has often been observed for growth rate in relation to a
limitation in the length of the growing season at higher latitudes and altitudes (Blanckenhorn & Fairbairn, 1995). Populations that experience a shorter growing season or lower temperatures may compensate for this by a faster growth rate at any given temperature (Yamahira & Conover, 2002). Typically, countergradient variation has been studied in terms of geographic variation where seasonal averages of an environmental variable show a latitudinal or altitudinal gradient to which a plastic phenotypic response can be expected. However, if the pattern of seasonal change in the environmental variable differs geographically, the seasonal averages may give an incomplete picture of the actual selective conditions. How the influence of latitudinal variation on seasonal profiles of environmental variables may influence life cycle adaptations has hardly been explored. In addition, temperature variation early and late in development may have different effects on the phenological outcome for the individual (Clark et al., 2014). Taking the interplay between developmental physiology and seasonal progression into consideration can improve the understanding of the ecology and evolution of phenological traits.

In this study, we examined geographic variation in the development from overwintering pupa to adult in the orange tip butterfly, *Anthocharis cardamines*, from three latitudinally separate localities in the United Kingdom and three latitudinally separate localities in Sweden (Fig. 1a). As is generally the case for spring species in temperate regions, higher latitudes are associated with colder temperatures for our populations. In addition, the temperature profile over the season is different in the different localities. In particular, the spring temperature profiles of Sweden and the United Kingdom, in relation to the date of *A. cardamines* adult emergence, show strikingly divergent patterns (Fig. 1b). Early spring in Central Sweden is considerably colder than in Central United Kingdom, but the increase in temperature is more rapid in Sweden, resulting in temperature conditions around the time of emergence that are similar, with even somewhat higher temperatures in Sweden (Fig. 1b). Such geographic and seasonal variation in temperature profiles is likely to be common, and it is our aim to investigate and interpret *A. cardamines* post-winter development in relation to this kind of environmental variation.

*Anthocharis cardamines* has one generation per year and the post-winter development is constrained by the need to reach the adult stage in time for egg laying on the buds and flowers of its early-flowering host plants. *Anthocharis cardamines* is thus a phenological specialist, in which larval survival and growth is affected by host plant phenology as well as by species (Wiklund & Åhrberg, 1978; Wiklund & Friberg, 2009). Females are selected to emerge in time to match a specific phenological stage of their host plants (Posledovich et al., 2014) and males to match the female emergence. The timing of the spring emergence can depend both on the duration of winter diapause and on the rate of post-diapause development. Studying diapause phenology involves phenomena such as diapause duration, diapause termination, post-diapause quiescence and post-diapause development (Tauber & Tauber, 1976; Kostal, 2006; Wadsworth et al., 2013), and their precise characteristics for different organisms may sometimes be unclear. Here, we focus on the development that occurs after winter conditions that we call post-winter development, and we leave open the question of which terminology is most suitable for these processes. As is generally the case (Tauber et al., 1986), the rate of development in the post-winter stage is influenced by temperature. An advantage of such temperature dependence is that it permits a matching to host plant flowering, given that the latter is sensitive to year-to-year variation in spring conditions (see Valtonen et al., 2011 for a general argument about this).

Based on a study of the post-winter development of larvae of the spruce budworm, Régnière (1990) proposed that synchrony in emergence to suitable conditions can be achieved by an increased temperature sensitivity towards the end of the post-winter period. This type of ramping up of development, which Régnière (1990) discovered, has subsequently been observed in other insects (e.g. Gray et al., 1995; Gray, 2009; Ragland et al., 2009; Wadsworth et al., 2013) and might be a widespread phenomenon. By keeping

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**Fig. 1** Temperature profiles of the United Kingdom and Sweden. (a) Locations of Northern, Central and Southern populations in the United Kingdom and Sweden. (b) Mean daily temperature in Central United Kingdom (red) and Central Sweden (blue) is shown by solid lines. Daily maximum and minimum temperature limits are shown by the transparent colours. All temperature data are averaged over 1995–2013 (E-OBS) for years where phenological observations were available. Temperature noted from 60 days prior to first phenological observation of *Anthocharis cardamines* in the specified area (0.5° longitude by 0.5° latitude square).
post-winter pupae in different temperatures and recording their changes in weight, we looked for such a pattern also in A. cardamines. We examined how geographic and latitudinal variation in the temperature sensitivity throughout post-winter development contributed to differences among geographical localities, as well as to differences between the sexes. In general, our geographic sampling and experimental protocol was designed to be able to detect a multifaceted geographic pattern of post-winter development in our study organism.

Materials and methods

Study species

The orange tip butterfly, A. cardamines, is a charismatic spring species with a pan-European distribution that extends into Asia and North Africa (Courtney & Duggan, 1983). It is oligophagous and oviposits on young inflorescences of several species of Brassicaceae (Wiklund & Åhrberg, 1978). Despite the existence of a preference hierarchy of host plant species, the phenological state of a plant strongly influences oviposition choices (Wiklund & Åhrberg, 1978; Courtney, 1981). The larvae mainly consume the protein-rich seed pods of the host plants, but they may also eat the leafy parts if necessary during later larval stages. The eggs are mostly laid singly on a plant as the larvae are cannibalistic. Anthocharis cardamines is obligately univoltine throughout its range (Courtney, 1980).

Field collection and laboratory rearing

To study population differentiation in phenology, A. cardamines eggs (or sometimes young larvae) were collected from three latitudinally separate localities each in the United Kingdom and Sweden (Fig. 1a) in May of 2012. British localities: Bournemouth (Lat 50.72N, Lon 1.88W, N = 154 eggs, 71 females, 83 males), Cambridge (Lat 52.20N, Lon 0.12E, N = 190 eggs, 92 females, 98 males), and Durham (Lat 54.68N, Lon 1.57W, N = 156 eggs, 87 females, 69 males); Swedish localities: Skåne (Lat 55.49N, Lon 14.05E, N = 129 eggs, 66 females, 63 males), Ljusstð (Lat 59.30N, Lon 18.35E, N = 147 eggs, 63 females, 84 males), and Angermanland (Lat 63.03N, Lon 18.19E, N = 85 eggs, 42 females, 43 males).

The larvae hatched and were reared to pupation in a common garden environment in the laboratory at the Department of Zoology at Stockholm University during the early summer of 2012. All larvae had ad libitum access to garlic mustard, Alliaria petiolata, and were kept at low density (three in a 500-mL rearing cup). The pupae were kept singly in plastic cups in a climate controlled room (17 °C and 12L:12D) until late autumn 2012. To simulate winter conditions, the pupae were put into 2 °C and 0L:24D for 5 months.

Development experiment

After 5 months in cold treatment, the pupae were moved to different but constant temperature treatments (T1: 11 °C, T2: 13 °C, T3: 15 °C and T4: 17 °C). The temperatures in the cabinets were logged using two thermal data loggers (Maxim's iButtons) in each cabinet, placed on the top and bottom shelves. The actual mean temperature recorded in each cabinet was used for later statistical analyses (T1: 11.02 °C, T2: 13.50 °C, T3: 15.07 °C and T4: 16.80 °C). They were exposed to long day conditions (16L: 8D), similar to the day length in southern Sweden or northern United Kingdom in May. For each population, equal numbers of individuals were put into each of the four treatments (in each treatment: Southern United Kingdom: 38, Central United Kingdom: 47, Northern United Kingdom: 39, Southern Sweden: 32, Central Sweden: 36, Northern Sweden: 21). The positions in the cabinet were randomized and the shelves were shifted one vertical step daily to minimize position effects. After the initial allocation to treatments, new ID numbers were assigned to the pupae, and for the remainder of the experiment, the population of origin of each individual was unknown to the experimenter.

The developmental rate in the different temperature treatments was monitored by weighting pupae on a Precisa 205A SCS electrobalance (±0.0002 g). Weights were recorded in units of 0.0001 g. The weight of each pupa was measured every 48 h. The rate of weight loss was used as a proxy for the rate of development (Posledovich et al., 2014). The number of days from introduction to the treatment temperatures until hatching was recorded, and the sex of an individual was determined by adult wing morphology.

Data analyses

All statistical tests were performed using the statistical software R, version 3.0.2 (R Core Team, 2013). For the fitting of models of post-winter pupal weight loss, we used the Bayesian MCMC software Stan (Stan Development Team, 2014). Most of the statistical analyses were performed on female butterflies, because they are the ones selected to emerge in time to match the flowering of host plants. For males, we examined the extent of protandry, that is the timing of male emergence in relation to female emergence for the different localities.

Rate of post-winter development

Using the day a pupa was taken out of winter storage as the zero point, the post-winter development time tₚ was measured as the day of adult eclosion. The overall rate of post-winter development was defined as r = 1/tₚ. The effect of the two factors temperature treatment and locality on this variable, log-transformed to achieve homogeneous variances, was studied using a linear
model. We used pupal weight as a covariate in the model, to control for possible locality differences in pupal weight, which in turn could influence development times.

To further partition the variation in developmental rate between the six localities, we performed planned comparisons in the form of five orthogonal contrasts, as follows: (i) Sweden vs. the United Kingdom; (ii) Central Sweden vs. Northern and Southern Sweden; (iii) Southern vs. Northern Sweden; (iv) Central United Kingdom vs. Northern and Southern United Kingdom; and (v) Northern vs. Southern United Kingdom. In this way, we could test for differences between the countries as well as for latitudinal differences within each country. We examined protandry by comparing the development rates of males and females for each of the localities through t-tests.

**Post-winter weight loss**

We compared three models of pupal weight loss as a function of time: (i) linear; (ii) quadratic; and (iii) two-phase, that is two linear phases of weight loss, joined smoothly together, with an increase in the rate of weight loss from the first phase to the second phase. Further details about these models and our procedures for fitting the model parameters appear in the Supporting Information online. To judge which model (linear, quadratic or two-phase) provided the best fit, $R^2$ values of each of the three model fitted weight loss curves were computed for individual female and male pupae from different temperature treatments.

Here, we give an overview of the two-phase model, which turned out to give the best fit. The model has four parameters: $w_0$ is the weight on day 0, $l_1$ is the weight loss per day (g/d) in the first phase, $l_2$ is the weight loss per day in the second phase, and $t_f$ is the time (d) of the shift between the phases. The proportion of development time spent in the first phase is then $p_1 = t_f / t_p$. We assume that the rates $r_1$ and $r_2$ of development in the two phases are proportional to the rates of weight loss, based on the idea that weight loss would be proportional to metabolism. From this assumption, we get that

$$r_1 / r_2 = l_1 / l_2.$$  

The overall rate of development, $r$, should be the average of the rates in the two phases, weighted by the time spent in the phases, which leads to the equation

$$r_1 p_1 + r_2 (1 - p_1) = r.$$  

From the fitting of the parameters $l_1$, $l_2$ and $t_f$ to the weights for individual pupae, and using the post-winter development time $t_p$, we can use equations (1) and (2) to compute the developmental rates $r_1$ and $r_2$ in the two phases. Variation between localities and temperature treatments in these rates, as well as in the proportion $p_1$, was analysed in the same way as for the overall developmental rate $r$.

The parameters of the two-phase model were fitted using Bayesian MCMC estimation. See the Supporting Information for details about the model fitting. Finally, to disentangle which of the two phases of post-winter development contributed most to variation in development time, we partitioned the variance of $t_p$ into two components, corresponding to the development times $t_1$ and $t_2$ of the two phases. The proportion of the total variation explained by variation in the first phase was measured as the sum of the variance of $t_1$ and the covariance of $t_1$ and $t_2$, divided by the variance of $t_p$ (see the Supporting information).

**Seasonal temperature profile in Sweden and the United Kingdom**

Using freely available gridded daily temperature data from z-oss version 10.0 (Haylock et al., 2008) with 0.5 by 0.5 degree resolution, the temperature profiles of the Central Swedish and British localities were averaged over the years 1995–2013, for the years where phenological data were available. The temperature profiles were calculated from *A. cardamines* first observation, in the relevant locations (0.5 × 0.5 degree grid).

**Results**

**Population differences in post-winter development**

Overall post-winter developmental rate in *A. cardamines* varied between the six localities and across the four temperature treatments (Table 1, Fig. 2a), with

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Effect</th>
<th>d.f.</th>
<th>$F$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall, $r$</td>
<td>Locality</td>
<td>5</td>
<td>16.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>3</td>
<td>1514.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Initial pupal weight</td>
<td>1</td>
<td>12.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>First phase, $r_1$</td>
<td>Locality</td>
<td>5</td>
<td>14.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>3</td>
<td>516.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Initial pupal weight</td>
<td>1</td>
<td>2.96</td>
<td>0.086</td>
</tr>
<tr>
<td>Second phase, $r_2$</td>
<td>Locality</td>
<td>5</td>
<td>13.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>3</td>
<td>1045.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Initial pupal weight</td>
<td>1</td>
<td>38.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion, $p_1$</td>
<td>Locality</td>
<td>5</td>
<td>4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>3</td>
<td>60.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Locality × Temperature</td>
<td>15</td>
<td>2.6</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Initial pupal weight</td>
<td>1</td>
<td>25.7</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Significant values are shown in bold. Residual degrees of freedom for tests for rate of development: 419, residual degrees of freedom for test for proportion of development spent in the first phase: 404. Residual mean squares: $r$: 0.00843; $r_1$: 0.0320; $r_2$: 0.0124; $p_1$: 0.00192.
statistically significant main effects of locality and temperature, but with no statistically significant locality by temperature interaction, making it reasonable to perform planned comparisons for the locality main effect. From these, Swedish and British butterflies differed in overall developmental rate (Table 2), such that Swedish butterflies had a higher developmental rate across temperature treatments (Fig. 2a). The between-country comparison therefore showed a countergradient pattern, as Sweden is colder than the United Kingdom during most of the spring (Fig. 1b). However, such a countergradient trend was not found when comparing populations within each country. A latitudinal trend was apparent in Sweden, but it was consistent with cogradient rather than countergradient variation. The southernmost population had a significantly higher developmental rate than the northernmost population, and the Central population had an intermediate rate of development and did not differ significantly from the northern and southern populations pooled (Table 2). In the United Kingdom, no simple latitudinal trend was observed. After controlling for the temperature treatment, the Central population had a significantly lower developmental rate than both the southern and the northern populations and the southern population had a significantly higher rate than the northern one (Table 2, Fig. 2a).

The pupal weight covariate had a statistically significant effect on the overall rate of development (Table 1). Because British pupae on average weighed more than Swedish pupae ($t_{427} = 8.41, P < 0.001$), it is conceivable that this could explain the lower overall developmental rate of UK populations. However, the effect size of initial weight on developmental rate was rather small, such that the effect of weight would result in a 5-h difference in developmental time between the largest and smallest pupae in the experiment. In fact, all our qualitative results on geographic differences in rates of development remain the same also when the pupal weight covariate is removed from the statistical models.

**Post-winter weight loss**

Of the three different weight loss models (Fig. 3), the two-phase model produced the best fit (Table S1). See the Supporting Information online for a description of the models and our Bayesian MCMC approach to estimating model parameters from pupal weight data. The fitted weight loss curves for the two-phase had an average coefficient $R^2$ of determination of 0.993 over the entire material, which was significantly higher than for the other two models, both for the temperature treatment T1 with the greatest number of weight data points.

![Graphs showing developmental rate and weight loss](image-url)

**Fig. 2** Properties of post-winter development of *Anthocharis cardamines*. (a) Reaction norms of overall developmental rate (1/days in development) for the six populations. (b) Reaction norms of developmental rate of first phase of post-winter development ($r_1$). (c) Reaction norms of developmental rate of second phase of post-winter development ($r_2$). (d) Proportion of post-winter development (in time) at which the switch between the first and second phase of development occurs for each of the six populations. All error bars show 95% confidence intervals.
we computed the developmental rates of individual pupae using a linear mixed effects model (see Materials and Methods and Supporting information for further explanation), showing the two linear phases of weight loss with a ramped up rate of from the first to the second. The first phase of development is shown by a light grey horizontal bar and the second phase of development by a dark grey horizontal bar. The vertical dashed line marks where the shift between the two phases occurs. Linear and a quadratic fitted weight loss models are plotted in thin grey lines. The individual was from Southern Sweden and the temperature treatment was 11 °C.

Table 2 Summary of planned comparisons of post-winter developmental rate, \( r \), and the treatment T4 with the lowest number of weight data points (Table S1).

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Comparison</th>
<th>Effect (log developmental rate)</th>
<th>t</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>UK – Swe</td>
<td>(-0.0186)</td>
<td>(-3.705)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>developmental rate, ( r )</td>
<td>C – N &amp; S Swe</td>
<td>(-0.0026)</td>
<td>(-0.528)</td>
<td>0.598</td>
</tr>
<tr>
<td></td>
<td>N – S Swe</td>
<td>(-0.0335)</td>
<td>(-3.682)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C – N &amp; S UK</td>
<td>(-0.0246)</td>
<td>(-5.852)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>N – S UK</td>
<td>(-0.0194)</td>
<td>(-2.656)</td>
<td>0.008</td>
</tr>
<tr>
<td>First phase, ( r_1 )</td>
<td>UK – Swe</td>
<td>(-0.0532)</td>
<td>(-5.451)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C – N &amp; S Swe</td>
<td>(-0.0023)</td>
<td>(-0.237)</td>
<td>0.813</td>
</tr>
<tr>
<td></td>
<td>N – S Swe</td>
<td>(-0.0444)</td>
<td>(-2.504)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>C – N &amp; S UK</td>
<td>(-0.0067)</td>
<td>(-4.491)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>N – S UK</td>
<td>0.0069</td>
<td>0.489</td>
<td>0.625</td>
</tr>
<tr>
<td>Second phase, ( r_2 )</td>
<td>UK – Swe</td>
<td>0.0194</td>
<td>3.194</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>C – N &amp; S Swe</td>
<td>(-0.0003)</td>
<td>(-0.055)</td>
<td>0.956</td>
</tr>
<tr>
<td></td>
<td>N – S Swe</td>
<td>(-0.0371)</td>
<td>(-3.360)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>C – N &amp; S UK</td>
<td>(-0.0238)</td>
<td>(-4.655)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>N – S UK</td>
<td>(-0.0478)</td>
<td>(-5.401)</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Significant values are shown in bold.

Fig. 3 Example of weight loss of an individual pupa. The solid light grey line shows a model fitted two-phased development (see Materials and Methods and Supporting information for further explanation), showing the two linear phases of weight loss with a ramped up rate of from the first to the second. The first phase of development is shown by a light grey horizontal bar and the second phase of development by a dark grey horizontal bar. Linear and a quadratic fitted weight loss models are plotted in thin grey lines. The individual was from Southern Sweden and the temperature treatment was 11 °C.

Points per individuals and the treatment T4 with the lowest number of weight data points (Table S1).

From the fitted parameters of the two-phase model, we computed the developmental rates \( r_1 \), \( r_2 \) and the proportion \( p_1 \) of developmental time spent in the first phase for females from all localities (Fig. 2b–d). The first phase constituted around 70% of the total time in development (Fig. 2d) and was followed by, on average, a fourfold increase in the rate of weight loss in the second phase. Most of the variation in overall post-winter development time was caused by variation in the first phase of development. In the total material (all individuals, all temperatures), 73.6% of the variation in \( t_p \) can be attributed to variation in the duration of the first phase, leaving 26.4% of the variance in developmental time to reflect variation in the second phase. This qualitative pattern holds true for the entire material as well as for females only for all temperature treatments (Table 3).

Population-level differences were observed in each of \( r_1 \), \( r_2 \) and the proportion \( p_1 \) (Table 1, Fig. 2b–d). For \( r_1 \), the results were similar to those for the overall developmental rate \( r \), the main quantitative difference being that a lower United Kingdom than Swedish rate was even more pronounced for \( r_2 \) than for \( r \) (Table 2). The second phase of development, on the other hand, was faster in the United Kingdom than in Sweden (Table 2). The higher overall rate of development in Sweden was thus a result of a dominating contribution from the first phase. This pattern of variation of \( r_1 \) and \( r_2 \) also shows that the ramping up of development was more marked in the United Kingdom than in Sweden.

The proportion \( p_1 \) of time in the first phase showed statistically significant variation between populations and across temperatures (Table 1, Fig. 2d). The magnitude of the variation was, however, relatively small (Fig. 2d) and might be of small ecological significance. It could also be that the slightly higher values of \( p_1 \) at the 17 °C treatment (T4) reflect a lower number of weight data points per pupa for this treatment, by making it harder for our Bayesian method to produce unbiased estimates.

**Protandry**

Analyses of male development revealed similar geographic patterns as for females (Fig. S1). *Anthocharis*
cardamines exhibits protandry in the field (Davies & Saccheri, 2013), and consistent with this, developmental rate was higher for males than females in all populations \( (t_{23} = 13.9, P < 0.001) \). The effect was mainly driven by males having a shorter first phase \( (t_{23} = 19.9, P < 0.001) \) as no difference was observed between males and females in duration of the second phase \( (t_{23} = 0.639, P = 0.529) \) (Fig. 4).

**Discussion**

Our discovery of the two phases of post-winter development revealed an intricate pattern of life cycle regulation of spring phenology in *A. cardamines*. The comparisons between and within the United Kingdom and Sweden suggest adaptation to particular local environmental conditions (Tables 1 and 2, Fig. 2a–c). Swedish pupae had an overall faster rate of development compared with British pupae (Table 2), differing by around 2% across all temperature treatments. From the colder Swedish spring temperatures (Fig. 1b), this is consistent with countergradient variation. The regional scale patterns were different, however, with cogradient variation within Sweden (in agreement with Posledovich *et al.*, 2014) and a lack of a latitudinal trend in the United Kingdom. The latter seems to be at variance with previous findings of countergradient variation in *A. cardamines* phenology in the field within the United Kingdom (Phillimore *et al.*, 2012).

Countergradient variation in life history traits is often discussed in connection with temporal pressure to complete the life cycle at higher altitudes or latitudes where the benign season is shorter (Baumann & Conover, 2011; Välimäki *et al.*, 2012). Although *A. cardamines* is univoltine and under no such pressure, the species is highly dependent on the phenology of its host plants. Selection to phenologically match the local host plants should therefore be strong (Wiklund & Friberg, 2009; Posledovich *et al.*, 2014) and may well underlie the patterns in post-winter development we have detected.

The progression of spring looks quite different in Sweden and the United Kingdom, and the relationship between phenology and temperature may therefore also differ (Fig. 1b). The Swedish spring arrives later and temperature increases more rapidly, even slightly surpassing the British spring temperatures (note that the day of the temperatures in Fig. 1b is relative to the adult emergence of *A. cardamines* and is not the same day of the year). In Central United Kingdom, the first emergence of *A. cardamines* for the relevant years occurs from end of March to late April, and in Central Sweden, the first emergence is from end of April to mid-late May.
The observed phenology in the field will be affected by post-winter development but perhaps also by the duration of diapause (Posledovich et al., 2014; the latter alternative was found by Wadsworth et al., 2013). The timing of adult eclosion could thus depend on when an individual becomes sensitive to temperature cues, but the geographic trends in the current study only concern post-winter development. Because we did not take a possible geographic variation in diapause duration or intensity into account, our results might only partially reflect phenological variation in the field. Diapause intensity and duration may be linked with the risk of untimely termination of diapause and can be important in the control of diapause phenology (Masaki, 1999).

According to Kostal (2006), post-diapause development is the resumption of direct development from a state of post-diapause quiescence. In post-diapause quiescence, the individual is sensitive to external cues for the resumption of development. The 5 months in winter conditions experienced by the individuals in this study should be sufficient for all six populations to have terminated diapause before the beginning of the experiment, so the individuals should have been in post-diapause quiescence and ready to start developing when moved to the warm treatments. This assumption seems reasonable, considering that the cold period in our study was substantially longer than the normal winter duration in most of the localities. As *A. cardamines* is obligately univoltine, the definition of post-diapause development as potential resumption of direct development is not directly applicable to our system. Nevertheless, we suggest that the developmental phenomena we studied can reasonably be regarded as part of post-diapause development.

**Two phases of post-winter development**

We found that post-winter development is heterogeneous and consists of two separate phases, differing in the rate of pupal weight loss (Figs 3 and 4). It is of interest to know whether the rate of weight loss we observed in the first phase differs from the rate for pupae that are known to be in diapause, but are otherwise measured under the same environmental conditions. Using data from another, more recent experiment, we can make such a comparison. The weight loss observed during the first 10 days after pupae in the current experiment were taken out of cold storage was higher and had a nonoverlapping distribution compared with the corresponding weight loss of pupae from the other experiment, which were known to be in diapause (Fig. S2). We can thus conclude that the first phase, as identified here, is a distinct process from full diapause.

The differences observed in overall time in post-winter development between Sweden and the United Kingdom were mainly due to variation in the duration of the first phase, for which we found a between-country difference of around 5% (Table 2, Fig. 2b). In fact, most of the variation in overall developmental time is due to variation in the first phase (73.6%, Table 3), including male–female differences. During the first phase, the temperature differences in the field are also likely to be the largest. The temperature in Central Sweden would be close to 0 °C and therefore below the temperature threshold for development. 2 months before adult *A. cardamines* emergence, whereas temperatures in the United Kingdom could be near 10 °C at this time (Fig. 1b). A weaker response to temperature early on may prevent development to advance unnecessarily or untimely in the British populations. The fact that population differences, that is local adaptation, is more pronounced in this phase could be due to physiological constraints in development, genetic or phylogenetic constraints on adaptation or due to advantages of fine-tuning of the spring phenology through a less energetically expensive phase of post-winter development.

This last point is similar to the reasoning by Wadsworth et al. (2013) that varying in the duration of diapause, when metabolism is depressed, is an energetically efficient way of adjusting the timing of spring emergence.

The increase in the rate of weight loss from the first to the second phase, or ramping up, was more pronounced in the British populations and highest in the southernmost locality (Fig. 2b,c). Because the temperature in the British localities does not increase very much during the relevant time, a more pronounced ramping up may enhance synchronization among conspecifics. This reasoning could also explain why the highest degree of ramping up is observed in the location with the flattest temperature profile (southern United Kingdom). In Sweden, the spring signal comes later and with a stronger effect, perhaps reducing the need for such a pronounced ramping up. Further studies of the effects of ramping up on the timing of emergence are, however, needed to resolve this issue.

Similar changes in developmental rate with physiological age have previously been described for forest pest species (Régnière, 1990; Gray, 2009), as well as agricultural pest species (Ragland et al., 2009). The lower developmental rates observed during the first phase make the individuals less sensitive to temperature fluctuations early in the spring. Having an increased rate of development nearer hatching may be highly beneficial as it allows for fine-tuning of phenology to host plants, conspecifics and good weather.

Whereas females may be under pressure to phenologically match the host plants, males are selected to temporally match the females. *Anthocharis cardamines* exhibits protandry, and males actively search for females at the edges of forests and on meadows (Courtney & Duggan, 1983). The use of ramping up of
post-diapause development as a tuning tool for phenology could well be important for matching the life cycle events to conspecifics, as the likelihood of finding a mate at a local geographical scale greatly increases if temperature variation late in development is more important. Microhabitat and microclimate effects at a local geographical scale may be reduced by the phenomenon of ramping up. For local weather, short-term variation might be smaller than long-term variation, such that when good weather (a high pressure) arrives, it is likely to stay for a few days. Ramping up of development would then lead to a higher probability of emerging during such a period. Emerging during a spell of good weather has numerous benefits, including increased likelihood for the ambient temperature being sufficiently high for flight and allowing for longer search time to find mates and host plants.

Diapause phenology is an important component of life cycle adaptations to seasonality. We have shown here that post-winter development is locally adapted in several aspects in Sweden and the United Kingdom. The observed differences in developmental time within and between countries are mainly caused by variation in the first phase of post-winter development, where temperature differences between sites are most marked. Our results show a need for an understanding of the progression of development in combination with seasonal profiles in phenological studies. We have highlighted how selection can act on specific aspects of post-winter development and lead to local adaptation in diapause phenology.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Properties of post-winter development of male A. cardamines.

Figure S2 Weight loss of A. cardamines during the first 10 days following removal from cold treatment.

Appendix S1 Modelling post-winter weight loss.

Table S1 Proportion of variance explained by the different models for different subsets of individuals.

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