

# Individual state controls temperature dependence in a butterfly (*Lasiommata maera*)

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In ectotherms there is typically a strong and positive correlation between growth rate and ambient temperature when food is not limiting. However, the exact relationship between growth rate and temperature varies among populations in many species. As a consequence, it has been suggested that selection for a rapid increase in growth rate with temperature should be stronger in populations experiencing a high degree of time-stress, compared with populations experiencing little time-stress. In the present study we take this adaptive hypothesis further and investigate if variation in time-stress among individuals of a single population may affect the relationship between growth rate and ambient temperature. Time-stress was manipulated by rearing larvae of the butterfly *Lasiommata maera* in different day-length regimes. The results show that individuals experiencing a higher degree of time-stress increase their growth rates more in higher temperatures compared with individuals under less time-stress. Hence, the adaptive hypothesis was supported and the relationship between growth rate and temperature was highly state dependent. These findings may be of general importance for understanding the evolution of life histories in seasonal environments.

**Keywords:** life-history theory; growth rate; state dependence; phenotypic plasticity; time-stress; seasonality

## 1. INTRODUCTION

State-dependent life-history theory is emerging as one of the major conceptual and theoretical tools for understanding the evolution of life histories (McNamara & Houston 1996). Life-history theory sees the scheduling of events such as growth, sexual maturation and reproduction as the result of strategic decisions over an organism's life. The major idea of the state-dependent approach is that the outcome of these decisions depends on the state of the individual organism. In any particular case the relevant individual state may include different aspects of the organism's physiological condition but also external circumstances. Hence, a life-history strategy is seen as a decision rule that specifies how an individual should respond to its internal state and its environment (Houston & McNamara 1992; McNamara & Houston 1996). State-dependent life-history theory also provides a conceptual framework for investigating adaptive plasticity in life-history traits (Houston & McNamara 1992; McNamara & Houston 1996).

Ambient temperature and its pattern of variation are major components of any natural habitat. Consequently, the role of temperature regime as a selective agent, and its effect on physiological processes, have long been issues in biology (Bergmann 1847; Alexandrov 1977; Hoffman 1985; Ratte 1985; Schmidt-Nielsen 1990; Atkinson 1994; Atkinson & Sibly 1997). One obvious example is the strong temperature dependence of growth and developmental rates in ectotherms. Within the non-harmful temperature range of a species, these rates are typically expected to increase with temperature. However, there is evidence that the exact relationship between growth rate

and temperature may vary adaptively among populations. For example, in several species of fish the rate of growth increases faster with temperature in northern populations than it does in populations from more southern latitudes (Conover & Present 1990; Schultz *et al.* 1996). The result is a rather non-intuitive pattern; when raised in a common, high-temperature environment juvenile growth rate is higher in populations from northern and colder areas compared with populations from more southern and warmer locations. Similar results from a range of ectothermic organisms have been documented (Conover & Schultz 1995; Nylin & Gotthard 1998). Conover & Present (1990) proposed an adaptive explanation for this pattern that is based on two notions: (i) ambient temperatures favourable for growth and development occur during a shorter period of the year in northern areas and, therefore, the length of the growing season declines with latitude, and (ii) high growth rates are associated with fitness costs. All other things being equal, the populations in the north will be subject to a greater time-stress if reaching a particular size or developmental stage is essential. Since fast growth is expected to carry costs, the less time-stressed southern populations will have lower optimal growth rates, especially in high but still non-harmful temperatures. Therefore, selection on the capacity to grow quickly during the period of favourable temperatures can be expected to be stronger in northern than in southern populations (Conover & Present 1990; Schultz *et al.* 1996).

In the present study we take the adaptive hypothesis presented above one step further, and investigate to what degree variation in time-stress among individuals within a single population may influence the relationship between juvenile growth rate and temperature. This strategy has the advantage of avoiding unknown differences between populations that may potentially affect the relationship between growth and temperature.

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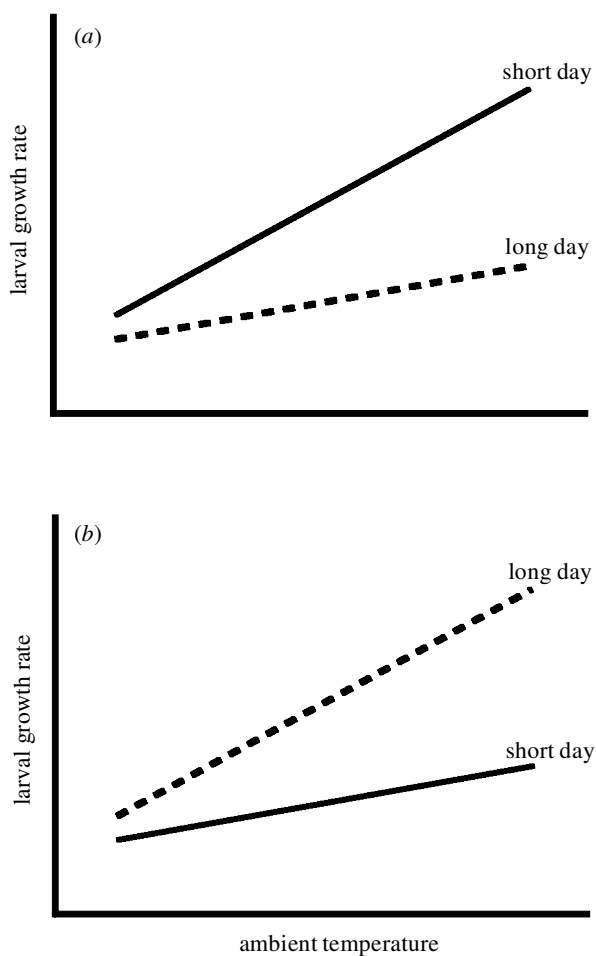


Figure 1. The predicted patterns of adaptive variation in the relationship between larval growth rate and ambient temperature in *L. maera* during (a) autumn and (b) spring. Within both seasons, individuals that experience day lengths indicating high degrees of time-stress should show a faster increase in growth rate with temperature compared with individuals experiencing less time-stress. In autumn a high degree of time-stress is indicated by a short day length, while in spring the time-stress is greater in long day lengths.

We take the approach of state-dependent life-history theory and apply it to larval growth in a temperate butterfly, *Lasiommata maera*. We treat time-stress as the state variable and derive specific predictions of how it will affect the temperature dependence of larval growth (figure 1). Variation in time-stress in these experiments refers to relative differences in how much time is left of the favourable season, before a particular developmental stage has to be reached. In *L. maera* the perceived time-stress can be experimentally manipulated by rearing larvae at different day lengths that signal early and late dates of the growing seasons (Nylin *et al.* 1996; Gotthard *et al.* 1999). As predicted by some life-history models (Abrams *et al.* 1996), larvae of *L. maera* and several of its closest relatives increase their growth rates in day lengths that signal relatively later dates of a growing season (Nylin *et al.* 1989, 1996; Gotthard 1998; Gotthard *et al.* 1999). An adaptive interpretation of this pattern is supported by results showing that high larval growth rates may carry fitness costs (Gotthard *et al.* 1994; Gotthard 1999).

In Sweden *L. maera* produces one generation per year and the winter is survived in larval diapause. Only individuals that reach the third larval instar and enter diapause before the onset of winter can survive to the next year (there are four larval instars in total). Because of the larval diapause *L. maera* has two distinct growth periods; the first in late-summer–autumn prior to diapause and the second in spring after the termination of diapause. Consequently, each larva faces two timing problems, to enter diapause at some optimal time in autumn and to break diapause, grow and pupate in a manner that allows it to emerge as an adult at an appropriate time in summer. Larvae of *L. maera* increase their growth rates in day lengths that signal relatively later dates in the season, during both these growth periods (in autumn growth is faster in short day lengths, while in spring growth is faster in long day lengths). Hence, individual *L. maera* larvae have the capacity to adequately respond to a given range of day lengths both in autumn and in spring (Gotthard *et al.* 1999).

In line with an adaptive hypothesis we expected that individual variation in the state variable, time-stress, should influence the relationship between larval growth rate ambient temperature (figure 1). Individual larvae that experience day lengths corresponding to relatively later dates of each season should show a faster increase in growth rate with increasing temperatures, compared with individuals that experience day lengths signalling earlier dates. A comparison between the two seasons will allow a stronger test of the adaptive hypothesis, since the interaction between day-length regime and rearing temperature is predicted to be qualitatively different during the two growth periods (figure 1).

## 2. MATERIAL AND METHODS

### (a) *Study organism*

*L. maera* is widely distributed throughout continental Europe up to 68° N and its primary habitats are rocky grasslands and forest edges (Higgins & Hargreaves 1983). Females oviposit and larvae feed on grasses from several different genera (Higgins & Hargreaves 1983; Wiklund 1984). In Sweden *L. maera* has one generation per year (univoltine life cycle), and in the Stockholm area (59.5° N) adults fly from the end of June to the end of July. Further south in Europe the species has two generations per year (bivoltine life cycle). Throughout the geographical range winter is survived in diapause in the third larval instar.

### (b) *Experimental procedure*

The offspring of six wild-caught females were allowed to develop from egg to larval diapause outdoors, in natural light and temperature conditions. The experiment started in winter when these diapausing larvae were brought to the laboratory (17 January 1998). They were weighed and placed individually in plastic cans (0.5 l) where a tuft of the grass *Dactylis glomerata* was cultured. Larvae from each family were split in equal numbers among the different day-length–temperature treatments. The total number of larvae per family varied and, therefore, the number of larvae per family in each treatment varied between one and four. In total we started 13 individuals in all treatments. The day-length regimes were 17 L : 7 D and 14 L : 10 D (hereafter referred to as 17 h and 14 h), and three different temperatures were used (14, 17 and 20 °C). These temperatures match the

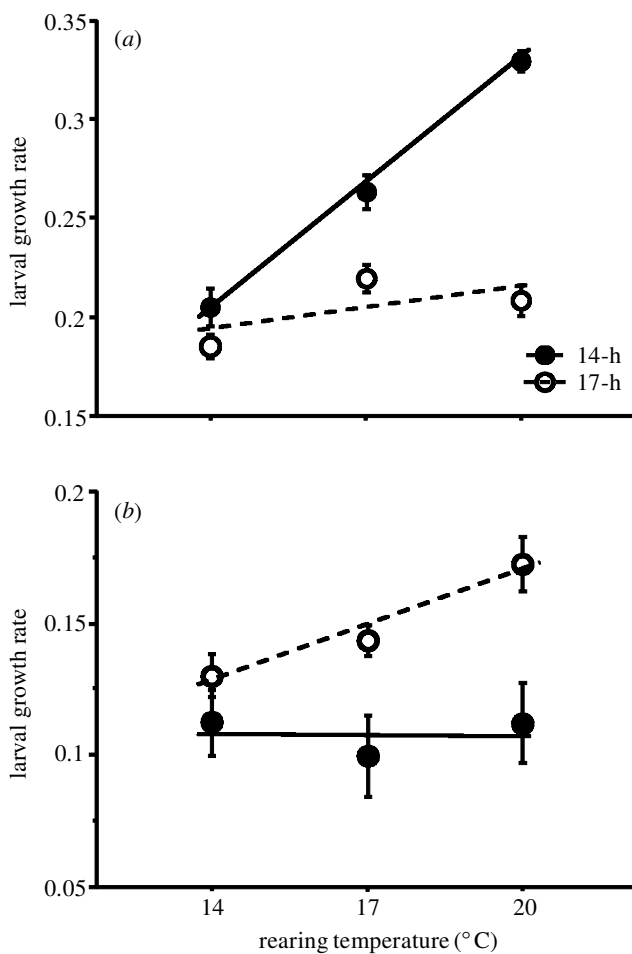


Figure 2. The relationship between larval growth rate (mean  $\pm$  1 s.e.) and rearing temperature during (a) autumn (second instar) and (b) spring (fourth instar). With the exception of the 14 h treatment in spring, all correlation coefficients were significantly different from zero. Estimated correlation coefficients in autumn: 14 h = 0.21; 17 h = 0.004; and in spring: 14 h =  $-9 \times 10^{-5}$  (non-significant); 17 h = 0.007.

natural situation in the Stockholm area and are clearly within the non-harmful temperature range for *L. maera*. All rearings were done in climate chambers. All larvae were weighed on the day of moulting to the fourth larval instar (the last) and were reweighed five days later. These two size measurements were then used to calculate individual growth rates during a period when all larvae were certain to have broken the winter diapause. We also calculated the average growth rate between the start of the experiment and the last moult. Individuals were reared to pupation and subsequent adult emergence.

All emerging butterflies were allowed to mate randomly in the laboratory and their offspring were used in the next step of the experiment, where we investigated larval growth prior to winter diapause. When larvae hatched from the eggs they were split among the same day-length-temperature treatments as described above. In each treatment we started 20 larvae. The rearing procedure was in all aspects identical to the first part of the experiment and all larvae were monitored on a daily basis. Each larva was weighed when it entered the second larval instar and five days later it was weighed again. This allowed us to calculate individual larval growth rates during a period just prior to the time of entering the third larval instar (the instar

where winter diapause can take place). Earlier studies have shown that most of the growth adjustments prior to winter diapause take place during this part of the growth trajectory (Gotthard *et al.* 1999). As a comparison we also calculated individual growth rates during the first instar. All larvae were also weighed at days 31 and 38 after egg hatch to detect the slowing down of growth that is typical of the onset of diapause.

### (c) Data management and statistical methods

All estimates of relative growth rates (RGR) were calculated according to the formula  $RGR = (\ln(w_{end}) - \ln(w_{ini})) / (t_{end} - t_{ini})$ , where  $w_{ini}$  and  $w_{end}$  are larval weights (mg) at the initial ( $t_{ini}$ ) and final ( $t_{end}$ ) days of the growth period in question. The interaction between day length and temperature was investigated by ANCOVA where day length was treated as a fixed factor and temperature as a covariate. We performed a separate analysis for each growth period.

## 3. RESULTS

### (a) Growth in autumn before diapause

Growth rate in the first larval instar showed a strong positive correlation with temperature and there was no effect of day-length treatment (ANCOVA,  $p_{day\ length} = 0.84$ ,  $p_{temperature} < 0.0001$ ,  $p_{interaction} = 0.21$ ).

In the second larval instar growth rate increased with ambient temperature in both day-length treatments (figure 2a; linear regression:  $p_{14\ h} < 0.0001$ ,  $N_{14\ h} = 52$ ,  $r^2_{14\ h} = 0.72$ ;  $p_{17\ h} < 0.026$ ,  $N_{17\ h} = 47$ ,  $r^2_{17\ h} = 0.10$ ). However, the relationship between growth rate and temperature differed between day-length treatments, and the interaction between day-length treatment and temperature was highly significant (table 1). Hence, just prior to the moult to the third larval instar (where all individuals entered winter diapause) growth rate increased significantly faster with temperature when larvae were reared with a day length indicating greater time-stress (figure 2a, faster increase in 14 h treatment than in the 17 h treatment).

### (b) Growth in spring after diapause

When the diapausing larvae were brought from outdoor conditions into the experimental treatments, 90% of the individuals were found to break diapause (70 out of 78). They subsequently started to feed and moulted into the fourth larval instar. There was no treatment bias in mortality. Prior to the moult to the fourth instar, larval growth rates were highly influenced by both temperature and day-length treatment but there was no significant effect of the interaction (ANCOVA:  $p_{day\ length} < 0.0001$ ,  $p_{temperature} < 0.0001$ ,  $p_{interaction} = 0.15$ ). On average the more time-stressed 17 h larvae grew faster in all temperatures, but within each day-length treatment growth rate increased with temperature to a similar degree.

After the moult the relationship between growth and temperature showed a different pattern (figure 2). Within the 17 h treatment there was still a significant positive correlation between growth rate and temperature, but in the 14 h treatment there was no such relationship (figure 2b, linear regression within day-length treatments:  $p_{14\ h} = 0.98$ ,  $N_{14\ h} = 35$ ,  $r^2_{14\ h} < 0.001$ ;  $p_{17\ h} = 0.0011$ ,  $N_{17\ h} = 35$ ,  $r^2_{17\ h} = 0.28$ ). The interaction between day-length treatment and temperature was, however, not quite statistically significant (table 1). Nevertheless, the overall

Table 1. Results of ANCOVAs on growth rate during the two focal growth periods

	growth in autumn (second instar)			growth in spring (fourth instar)		
	d.f.	<i>F</i>	<i>p</i>	d.f.	<i>F</i>	<i>p</i>
day length	1	26.7	< 0.001	1	1.4	0.23
temperature	1	95.2	< 0.001	1	3.1	0.08
interaction	1	43.9	< 0.001	1	3.3	0.07
error	95	—	—	66	—	—

result still indicates that also during this developmental period the increase in growth rate with temperature was greater in the more time-stressed larvae (figure 2*b*).

#### 4. DISCUSSION

The level of time-stress influenced the relationship between larval growth rate and ambient temperature during critical phases of both growth seasons. As predicted, growth rates increased faster with temperature when the day-length regime indicated a greater time-stress (figure 2). Moreover, the comparison of the two growth seasons allowed us to disentangle potential effects of day length *per se*, from the effect of time-stress. This comparison clearly indicates that it is the information on time horizons provided by the day length that is of importance for the temperature dependence of growth rate. The results, furthermore, indicate that the relationship between the temperature dependence of growth and time-stress varies throughout the growth trajectory. For example, during the first larval instar, growth rate increased with temperature but was unaffected by time-stress. During development from diapause to the last moult in spring, there were strong effects both of temperature and time-stress on growth rate but no effect of the interaction between these factors.

Within non-harmful limits, an increase in temperature accelerates most physiological processes (Schmidt-Nielsen 1990), and this is also the typical expectation in the case of growth and developmental rates in adequately fed ectotherms (Ratte 1985; Atkinson 1994; Atkinson & Sibly 1997). Potentially adaptive variation in the temperature dependence of these rates has been identified among populations of several ectothermic organisms, and has often been identified as so-called counter-gradient variation (Conover & Schultz 1995). Counter-gradient variation is present when the geographical distribution of genotypes is such that the genetic and environmental influences on the phenotype counteract one another across an environmental gradient. Hence, it indicates genetic differences between populations that may represent local adaptations. In essence, our experiment supports the adaptive hypothesis proposed by Conover & Present (1990) for explaining variation among populations in the temperature dependence of growth. However, the results from *L. maera* show that the relationship between growth rate and temperature may vary not only among genotypic categories, but also among individuals that differ in state.

Larvae of *L. maera* gather information on the prevailing time horizon and use this information to determine to

what degree a change in ambient temperature should be used for growth. As could be expected, this regulation of growth seems to be possible only within certain limits. The difference in growth rates between the two levels of time-stress was large in the highest temperatures while there was almost no difference in the lowest temperature (figure 2). This suggests that individuals experiencing little time-stress do not fully use the growth opportunity given by a rise in ambient temperature. In the lowest temperature, on the other hand, thermodynamic constraints appear to force the time-stressed larvae to grow at more or less the same rates as the individuals experiencing a lower time-stress. The type of interaction between time-stress and the temperature dependence of growth documented here may also be present in other insects. For example, in a study of the yellow dung fly Blanckenhorn (1997) found that the number of degree days needed for the completion of juvenile development decreased with the time available for growth (degree days express the developmental period as the cumulative sum of degrees above a critical lower temperature threshold where development ceases). The use of degree days is motivated by the fact that developmental processes are temperature dependent, and degree days may therefore be a better measurement of physiological time (Ratte 1985; Blanckenhorn 1997). However, if state-dependent effects on the relationship between growth and temperature are present, the use of degree days for investigating variation in development time may introduce new problems. This is because a given rise in ambient temperature may result in very different amounts of growth in individuals that differ in relevant state variables.

Earlier studies of *L. maera* and several of its relatives have suggested that the reaction norms relating larval growth rates to day length are adaptations for optimizing life histories in a seasonally changing environment (Nylin *et al.* 1989, 1996; Gotthard 1998, 1999; Gotthard *et al.* 1999). The present results provide further support for this suggestion, especially through the relatively weak relationship between growth rate and temperature in larvae experiencing low time-stress. The comparison between day-length treatments indicate that these less time-stressed larvae are 'choosing' not to increase their growth rates in higher temperatures to the degree to which they apparently can do. The fact that individuals do not grow at the highest rate possible in a given environmental setting suggests that high growth rates carry some fitness cost (Sibly & Calow 1986; Werner & Anholt 1993; Arendt 1997). Indeed, there is empirical evidence that in the butterfly species *Pararge aegeria* (a

close relative of *L. maera*) high larval growth rates may be costly both in terms of lowered starvation endurance and increased predation risks (Gotthard *et al.* 1994; Gotthard 1999). The ambient temperature regime will affect the range of growth options that are open to a given individual, and a warm environment is likely to be of very different value to individuals that are in different states of time-stress.

The growth strategies of *L. maera* and some of its relatives appear to be the result of a series of state-dependent, developmental decisions taken by each single individual, and variation among populations and species suggests that the decision rules have a genetic basis (Nylin *et al.* 1989, 1996; Gotthard 1998, 1999; Gotthard *et al.* 1999). These growth strategies can essentially be interpreted as adaptations for allowing individuals to reach some optimal body size in a situation where the time available for growth varies between individuals (or generations), and where high juvenile growth rates are costly (Gotthard 1999; Gotthard *et al.* 1999). Since these conditions are likely to be common (Tauber *et al.* 1986; Rowe & Ludwig 1991; Danks 1994; Nylin 1994; Conover & Schultz 1995; Nylin & Gotthard 1998), the findings reported here may be of general significance for the evolution of life histories in seasonal climates.

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