The growth strategy of an alpine beetle: maximization or individual growth adjustment in relation to seasonal time horizons?

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Summary

1. Life-history theory typically predicts that juvenile growth rate should be maximized and consequently juvenile period minimized. However, in several examples it has been shown that insect larvae do not always grow as fast as they can and this has been explained by costs of high growth rate, typically higher juvenile mortality rate. Hence, some insect larvae have the ability to adaptively adjust growth rate to catch up if development is delayed.
2. The presence of such ability was tested for in the alpine beetle *Oreina elongata* Suffrian. In this species, the favourable period for development is relatively short and of unpredictable length, and individuals are chemically defended against predation; factors that could affect the balance between the benefits and costs of high growth rate.
3. Here it is shown that when time stressed, *O. elongata* larvae were able to increase growth rate, accelerate development and reach the normal final weight.
4. Hence, individual growth rate adjustment was present in a situation where its adaptive value appeared to be relatively weak, which supports the notion that flexible growth strategies are a common phenomenon in temperate insects.

Key-words: Growth rate, life history, phenotypic plasticity, photoperiod, seasonality

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Introduction

A short juvenile period is generally believed to be advantageous mainly because this reduces the time window for juvenile mortality (Roff 1992; Stearns 1992). It is also frequently observed that a large adult size increases fitness (Roff 1992; Stearns 1992). Therefore, life-history models typically predict that development time should be minimized and concurrently that juvenile growth rate should be maximized. However, theoretical and empirical investigations suggest that under a range of conditions it may be favourable for an individual to grow more slowly although this leads to a longer juvenile period or a smaller adult size (Abrams *et al*. 1996). The reasons for this are that high growth rates are often associated with fitness costs and there will be a balance between the benefits and costs of a high growth rate (Arendt 1997; Gotthard 2000). Hence, for any organism there may be an optimal growth rate that is not necessarily the maximum. The optimal balance between the costs and benefits is likely

to be influenced by environmental factors such as predation rate and time stress (Nylin, Gotthard & Wiklund 1996; Gotthard 2000). In temperate regions, where part of the year is unsuitable for growth and development, most insects have to reach a certain size or developmental stage before the onset of winter in order to survive. Hence, temperate insects typically face time horizons that limit their development, and variation in climatic variables such as temperature and photoperiod are often used as cues to estimate the remaining time available (Nylin & Gotthard 1998). Time is an important resource for developing juveniles and there should be strong selection for its efficient use. In a seasonally changing environment, it may then be beneficial for an individual to be able to adjust its growth rate in response to the amount of time left for development as well as other environmental factors that affect the balance between the costs and the benefits of fast growth (Abrams *et al*. 1996).

Several temperate butterfly species have been shown to have plastic growth that allows individuals to grow more slowly or to speed up development in relation to seasonal time stress (Nylin, Wickman & Wiklund 1989; Leimar 1996; Gotthard 1998; Gotthard, Nylin & Wiklund 1999). Individuals experiencing 'end of the favourable season' conditions are able to increase growth

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rate, speed up development and finish at the normal size rather than at a smaller size, which might otherwise result in lower adult fitness.

In this study, we wanted to test if the predictions of a plastic growth strategy hold for *Oreina elongata* Suffrian (Coleoptera: Chrysomelidae), a beetle with life-history characteristics that are distinctly different from the butterflies mentioned above. This species lives at high altitudes in the Alps (2000–2200 m). The snow-free period is about 3 months in summer and the growth season for the host plants of *O. elongata* is even shorter, which leaves little time for growth and development within a single season. Moreover, weather conditions at such high altitudes are highly unpredictable and even in the middle of summer, temperatures can drop below zero and snowfall can occur (N. Margraf, K. Gotthard & M. Rahier personal observation). Hence, growing larvae constantly face a high risk of being interrupted in their development and this risk cannot be readily predicted from cues of seasonal change (e.g. photoperiod or temperature). These factors are probably the main reasons why *O. elongata* have a 2-year juvenile period including two winter diapauses, which guarantees sufficient time for development. Furthermore, *O. elongata* larvae are to some degree chemically defended owing either to sequestration of plant defensive compounds or to endogenously produced compounds (Dobler & Rowell-Rahier 1994a). An increased predation risk is one of the major costs of both a higher growth rate and a long juvenile development and it seems likely that the chemical defence may reduce the effect of these costs and that it will affect the optimal growth rate. Despite specific studies on potential parasitoids of *O. elongata*, there has not been a single observation of parasitoid emergence from eggs or larvae, suggesting that this is a minor mortality factor. Finally, in contrast to the butterflies, *O. elongata* is more of an income breeder (Stearns 1992; Jonsson 1997). Resources for reproduction are accumulated not only throughout the juvenile period (as in capital breeders) but also during the adult stage. Since adults are long-lived, an individual may reproduce during several consecutive summers (D. Conconi, personal communication). This may imply a less direct impact of size at maturity on adult fitness. It seems likely that the short and unpredictable season increases the benefit of growth maximization while the chemical defence lowers the cost of fast growth and together they suggest that a flexible growth strategy would be less advantageous for *O. elongata* than for most other temperate insects. The effect of income breeding is less obvious but it does suggest that variation in size at maturity has weaker fitness consequences and that selection for an ability to reduce such variation through a plastic growth strategy may be weak.

The conditions outlined above suggest that selection for a flexible growth strategy should be relatively weak in *O. elongata* larvae and that they should typically maximize growth rate to be able to finish larval growth during the first summer (first winter diapause is in the prepupal stage), independently of what time horizons they are experiencing (Abrams *et al.* 1996). Since day-length is a more accurate predictor of date than temperature (Nylin & Gotthard 1998), we assumed it would be the most important cue used by the beetle larvae to gauge the actual time left for development. These predictions were tested in a laboratory experiment where day-length conditions were systematically manipulated. To provide a general picture of the seasonal growth strategy we also included several populations and host plants in the experimental set-up.

Materials and methods

STUDY ORGANISM

Oreina elongata is an alpine species found in the Alps and Apennines. Both larvae and adults feed on the leaves of three host plants from different tribes of the Asteraceae: *Adenostyles alliariae* (Gouan) Kerner, *A. glabra* (Miller) DC (both Senecioneae) and *Cirsium spinosissimum* (L.) Scop. (Cardueae). *Adenostyles alliariae* and *A. glabra* contain pyrrolizidine alkaloids (hereafter abbreviated to PAs) that *O. elongata* larvae and adults can sequester for their own defence (Dobler & Rowell-Rahier 1994b). *Cirsium spinosissimum* lacks PAs and beetles feeding on this species synthesize cardenolides endogenously (Dobler & Rowell-Rahier 1994b). Some populations of *O. elongata* encounter only one of the three host plants whereas in other sites both *A. alliariae* and *C. spinosissimum* are present and are used by the beetles. Earlier studies indicate that *O. elongata* females from all populations have a strong oviposition preference for *C. spinosissimum* (K. Gotthard, N. Margraf & M. Rahier, unpublished data).

The development of *O. elongata* extends over 2 years. Eggs are laid during the earliest part of the short alpine summer season (July) and hatch after 2–3 weeks, after which the larvae grow through four instars before digging down in the soil to overwinter and pupate. Surviving adults emerge in the second part of the next summer, feed and again dig down into the soil for a second winter diapause. The second year, reproductive adults emerge early in the season (mid-June), mate and oviposit. Adults can survive and reproduce up to three consecutive seasons (D. Conconi, personal communication).

STUDY POPULATIONS

In the first week of July 2000 we collected beetles from three populations in the Alps: from Petit Saint-Bernard (PSB) on the border between France and Italy (45°40′20·6″ N, 6°52′39·4″ E, WGS84, altitude 2188 m) where insects can be found on both *A. alliariae* and *C. spinosissimum* in mixed patches (beetles collected in equal numbers from both plants); from Mattmark (MTT) in the Swiss Alps (46°1′56·4″ N, 7°57′25·9″ E,
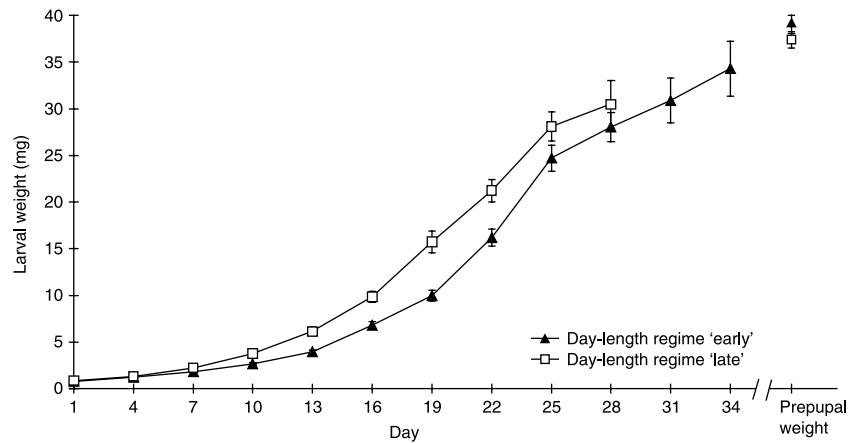
Fig. 1. Mean growth trajectory and mean prepupal weight of *Oreina elongata* larvae under two day-length regimes, mimicking early and late light conditions.

WGS84, altitude 2200 m) where beetles feed only on *C. spinosissimum* (the only plant available at MTT); from Lautaret (LAU) in the Vanoise region of France (45°0′39·0″ N, 6°22′19·3″ E, WGS84, altitude 2058 m) where *O. elongata* is present only on *A. glabra* (the only plant available at LAU).

EXPERIMENTAL DESIGN

We performed a larval growth experiment using a full factorial design with three factors: population (three levels), host plant (two levels) and day-length treatment (two levels). We reared 15 larvae in each of the 12 combinations. Field-collected females from each population were given *A. alliariae* and *C. spinosissimum* for oviposition in plastic jars. Eggs were collected daily and placed in transparent plastic Petri dishes. To provide the necessary humidity, the bottom of each dish was filled with a layer of moistened plaster covered with filter paper. Sixty newly hatched larvae from each population were weighed, and placed individually in Petri dishes of the type described above, in incubators set at constant temperature cycles and one of two day-length treatments. The incubators were programmed to follow the natural decrease in photoperiod and the first treatment was chosen to mimic the first part of the summer (start 5 July) and the second to mimic the end of the summer season (start 14 August). The temperature cycle was identical for both day-length treatments: 17 °C for 16 h and 7 °C for 8 h, more or less corresponding to the day–night cycles. Larvae were randomly assigned to one of the two hosts plants (*A. alliariae* or *C. spinosissimum*) and every third day larvae were weighed and new food was provided in excess until they reached the fourth instar (~25 mg). At that time they were transferred to larger plastic boxes where they were provided with a 2-cm layer of soil for pupation. Their food was renewed and the soil moistened every third day. The boxes were placed in an unheated room where the temperature fluctuated between 7 and 17 °C (values that fall within the range of daily temperature fluctuations

in the field). The day of disappearance of the larvae into the soil was recorded and on 14 September all prepupae were dug out and weighed.

STATISTICAL ANALYSES

After log-transformation of the weight data, regression lines were fitted to the individual growth trajectories (Fig. 1). The slope of each line was used as a measure of the growth rate of each individual larva. Development time was calculated as the period from the beginning of the experiment to the day the larvae disappeared into the soil. The effects of day-length, diet and population and their interactions on growth rate, development time and prepupal weight were tested with analyses of variance (ANOVA). Analyses were carried out using the Splus statistical package with type III sums of squares and all effects were considered as fixed factors. Because the three-way interactions were non-significant in all analyses, we removed them and recalculated the ANOVAs.

Results

Growth rate differed significantly between day-length treatments and populations (Table 1). Except for the Mattmark beetles reared on *A. alliariae*, all populations increased growth rate when reared in end of the season light conditions (Fig. 2). However, Mattmark larvae generally grew faster than larvae from the two other populations. There was a significant interaction between plant and day-length treatment, which indicates that depending on the host plant on which they were reared, *Oreina elongata* larvae responded differently to time stress.

As for growth rate, development time was significantly affected by population and day-length treatment (Table 2). Larvae had a shorter development time under late season light conditions, with the exception of larvae from Mattmark reared on *A. alliariae* (Fig. 2). There was again a significant interaction
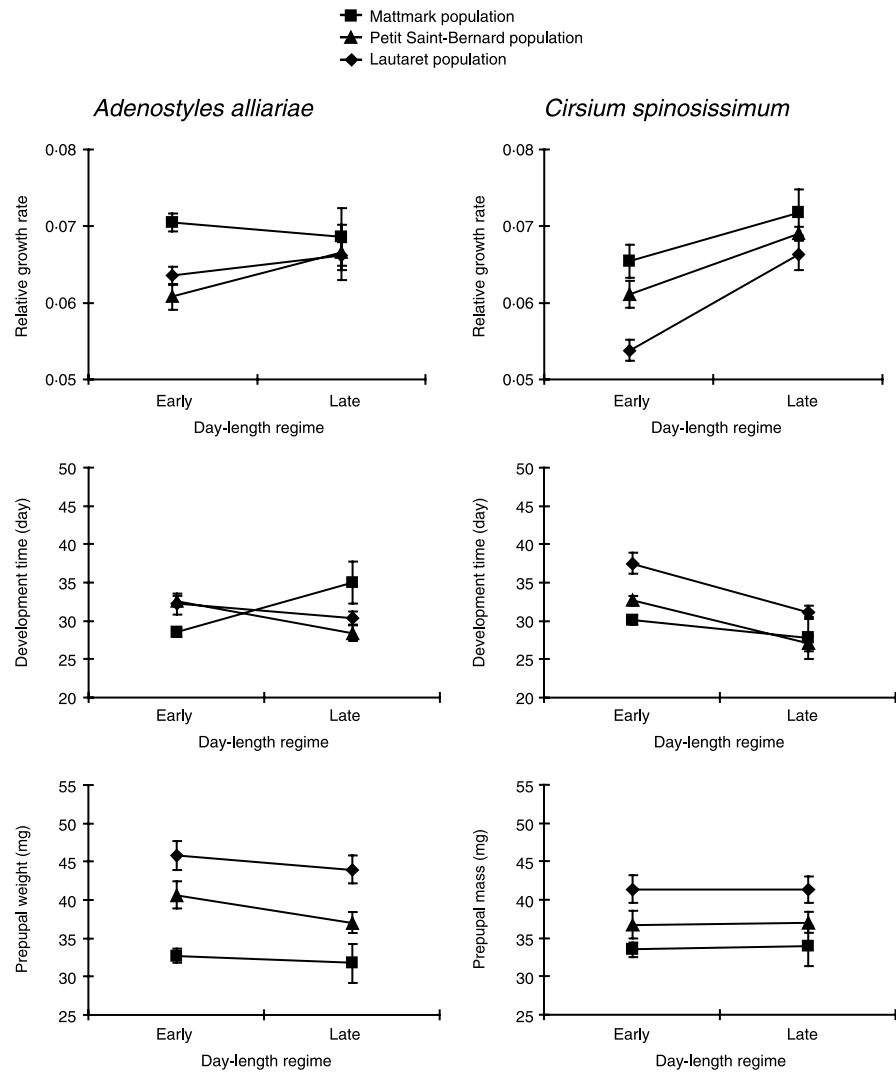
Fig. 2. Interaction plots of the three fitness parameters: relative growth rate, development time and prepupal weight, showing mean and standard error, split by host plant, day-length regime and populations.

Table 1. ANOVA for relative growth rate

Source of variation	d.f.	SS	F	P
Population	2	0·001022370	10·52878	0·0000703
Host plant	1	0·000011418	0·23518	0·6287592
Day-length treatment	1	0·000940000	19·36099	0·0000269
Population × Host plant	2	0·000237246	2·44326	0·0919978
Population × Day-length treatment	2	0·000097433	1·00341	0·3702531
Host plant × Day-length treatment	1	0·000344031	7·08594	0·0090406
Residuals	101	0·004903672		

between plant and day-length regime but also between population and day-length treatment. In other words, instead of shortening their development time under late season conditions as did the other populations, Mattmark larvae reared on *A. alliariae* increased their developmental period.

Prepupal weight was unaffected by day-length treatment and host plant. Only population had a significant effect on prepupal weight (Table 3). Mattmark prepupae were lighter than other populations, Petit Saint-

Bernard prepupae were intermediate, and Lautaret larvae ended with the highest prepupal weight, independent of light conditions or host plant (Fig. 2).

Discussion

Our results indicate that larvae of *Oreina elongata* from all the tested populations are able to adjust their growth rate in response to seasonal time stress. However, this ability varies, according to which host plant

Table 2. ANOVA for development time

Source of variation	d.f.	SS	F	P
Population	2	131·663	3·220223	0·0441231
Host plant	1	11·513	0·563166	0·4547495
Day-length treatment	1	140·662	6·880641	0·0100769
Population × Host plant	2	177·722	4·346735	0·0154820
Population × Day-length treatment	2	263·256	6·438715	0·0023423
Host plant × Day-length treatment	1	153·817	7·524112	0·0072155
Residuals	100	2044·321		

Table 3. ANOVA for prepupal weight

Source of variation	d.f.	SS	F	P
Population	2	1611·428	37·37200	0·0000000
Host plant	1	54·008	2·50510	0·1173755
Day-length treatment	1	17·529	0·81304	0·3698969
Population × Host plant	2	120·957	2·80522	0·0663814
Population × Day-length treatment	2	3·791	0·08791	0·9159310
Host plant × Day-length treatment	1	26·785	1·24241	0·2683036
Residuals	81	1746·303		

the beetles are feeding on. On *Cirsium spinosissimum*, all populations increased their growth rate when experiencing the photoperiod typical of late in the season. On *Adenostyles alliariae* the pattern was the same, with the exception of beetles from Mattmark. Development time followed the inverse pattern; an increase in larval growth rate led to a shorter development time. As a consequence, prepupal weight was not affected by day-length regime, varying only between populations.

It seems that beetles from Mattmark have a different life history from the other populations, with a higher larval growth rate and smaller prepupal weight. This confirms previous results (Ballabeni *et al.* 2003) suggesting that local selection on the life cycle may be different in Mattmark from the two other populations. Moreover, potential explanations for the relatively limited degree of local adaptation to variation in host plant use have also been examined elsewhere (Ballabeni *et al.* 2003). The focus of the present experiment was plasticity in relation to seasonal cues and the Mattmark population feeding on *A. alliariae* provided the only observation against the general pattern of increasing growth and decreasing development time in late season conditions (Fig. 2). The main difference between the populations was that the Mattmark larvae grew very fast in the early season conditions whereas larvae of the two other populations displayed reduced growth rates. Indeed, it seems as if the Mattmark larvae are maximizing growth independently of seasonal cues, which would lend some support to our original expectations. However, in light of the rest of the results it seems more likely that this is a consequence of the Mattmark population never encountering *Adenostyles* plants in the field and that feeding on this novel host plant somehow disrupts the seasonal adjustment of

growth (the other two populations do use *Adenostyles* in the field). In all treatments where larvae are feeding on native hosts the trend is that larvae grow and develop faster in late season conditions (Fig. 2).

In contrast to the expectations outlined in the Introduction, it appears that selection favours an ability to adjust growth in response to time stress in a chemically defended beetle that is restricted to an alpine habitat where the length of the favourable season is highly unpredictable. This ability may allow larvae to catch up after weather-induced delays in developmental schedules, a pattern found in a range of ecothermic animals (Gotthard 2001). The results of the late season treatment suggest that for *O. elongata* larvae the costs of being small at the time of diapause exceed the costs of growing fast. Larvae that finish at a reduced size may, for example, suffer from higher winter mortality in addition to any costs of smaller adult size. On the other hand, the reduction in growth of larvae in the early treatment suggests that the benefit of an even larger size is typically lower than the cost of maximizing growth rate. These results indicate that both size at diapause and larval growth rate in *O. elongata* are under stabilizing selection and that a plastic growth strategy rather than growth rate maximization seems to be beneficial in this insect.

The combination of a short and unpredictable period available for larval growth, chemically protected larvae and an income breeding strategy suggest to us that *O. elongata* larvae should be likely to experience selection for growth rate maximization and it is of interest to examine why this expectation was not supported. It seems very likely that the two-year development period of *O. elongata* is an adaptation for coping with the short alpine season and it may thus reduce the effect of time limitation on juvenile

development even if larval growth has to completed in the first summer. Hence, the time constraints on larval development may be less severe than we expected. There is also considerable variation among individuals in the start of larval growth as oviposition goes on for at least 1 month, and therefore it may be beneficial to adjust growth in relation to seasonal time horizons. However, this potential benefit depends on the presence of costs of high growth rates, which we assumed to be relatively low because of the chemical defence. Field studies, performed subsequent to this experiment, do suggest that despite their chemical defence predation rates of *O. elongata* larvae are substantial (K. Gotthard, S. Rasmann, N. Margraf & M. Rahier, unpublished data). Predators that have been observed predating on *O. elongata* larvae include the commonly occurring Harvestman *Mitopus morio* and sawflies of the family Tenthredinidae (Hymenoptera), and it is quite likely that fast growth and increased feeding effort may be associated with a higher risk of detection by these predators. Increased growth may also occur at the expense of investment in defence.

Finally, we expected that the income breeding of *O. elongata* was likely to weaken the correlation between size at metamorphosis and adult fitness. However, in *O. elongata* the size at metamorphosis also coincides with the size at winter diapause and it is possible that below a certain size there is indeed a tight correlation between weight and over-wintering survival. Unfortunately there are no data on the relationship between winter mortality and the size at winter diapause in this species but increased winter survival with increased size at diapause has been found in other insects (Gotthard *et al.* 1999). Such a situation could help to explain why larvae increase growth rate if they have fallen behind their developmental schedule. On the other hand, the finding that larvae reduce their growth in early season conditions suggests that becoming a very large over-wintering larva or reaching a large adult size does not pay off in terms of total fitness. This pattern might be expected from an income breeding strategy in that above a given threshold size, determined by the chance of surviving winter diapause, the relationship between final size and fitness shows a strong diminishing returns pattern. Even so it is fascinating that in the time-constrained growth conditions of *O. elongata* there seems to be room for fine-tuning of growth by means of a plastic developmental strategy.

In conclusion, this study shows that in an insect species whose life-cycle characteristics and habitat suggest a very high degree of time limitation of the larval period, larvae do not grow at their maximal rate unless they experience late season conditions. Hence, individual growth rate adjustment was present in a situation where many of the theoretical prerequisites for it to be adaptive appeared to be relatively weak. Therefore juvenile growth rate adjustment in relation to time

horizons in insects may be a more common phenomenon than previously thought.

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