Compensatory responses in lepidopteran larvae: a test of growth rate maximisation

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It is often claimed that some organisms maximise individual growth rates (within limits set by physiological and environmental constraints) whereas others do not. Adequate experimental protocol to test for these hypotheses has not been available. We proceeded from the idea that a maximiser should be unable to further improve its growth performance, i.e. there should be no adaptive plasticity in relative growth rates. In the case of an insect larva always maximising its weight gain, we should thus expect no increase in growth rate following a short-term environmental perturbation. Another prediction is that initial and final weights of a larval instar should be highly correlated. We applied this approach to test for the growth rate maximiser status of the geometrid moth *Epirrita autumnata*. Based on various lines of ecological evidence it had previously been suggested that this spring-feeding species is strongly selected for high growth rates, and may be growing at its maximum physiological potential. Contrary to expectations, the larvae responded to starvation treatments by a subsequent compensatory increase in their relative growth rates. Such a plasticity may indicate that the rates of gaining weight are not maximised even in a 'classical' example a time-constrained organism. This observation supports the idea that endogenous regulation of growth rates is widespread, and it remains to be shown if true maximisation ever exists in nature. In contrast, possibilities to compensate for adverse conditions experienced early in larval development appeared to be limited. This was indicated by the strong correlation between initial weight in the last instar, and pupal weight. Though consistent with our expectations, such an inability to compensate can hardly be ascribed to growth rate maximisation, as indicated by quantitatively similar patterns found in an unrelated species with different ecology, the butterfly *Polygonia c-album*.

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Much of the theory of life-history evolution is concerned with body size, and the time schedule of attaining size (Roff 1992, Stearns 1992). Thus, the growth rate of an individual is of central importance in life history theory. Rather unexpectedly, however, this trait may not have received the treatment it deserves (Arendt 1997). In fact, some of the most influential life history models have viewed individual growth rate as a purely environmental variable, thereby assuming that a growing individual always maximises its weight gain within the limits of environmental and physiological constraints (Stearns and Koella 1986, Kawecki and Stearns 1993). However, growth rate plasticity may also be endogenous, governed by genetically determined norms of reaction that have been favoured by selection. It has been shown that models allowing for such adaptive plasticity in growth

rates may lead to very different predictions regarding, for instance, the response in life history traits to increased time stress (Abrams et al. 1996). However, the limits of applicability of such models remain unclear both in terms of organisms, and the range of the plastic responses themselves.

There is no doubt that many organisms typically grow slower than their physiological potential and can increase their growth rates adaptively in situations where this is called for (Arendt 1997, Gotthard 2001). Likely examples include fish (Conover and Present 1990, Metcalfe 1998, Metcalfe et al. 2002), amphibians (Berven 1982a, b, Newman 1992), humans (Mace 2000), and insects (Nylin and Gotthard 1998, Margraf et al. 2003). Potential costs of rapid growth, able to explain the phenomenon of submaximal growth rates, remain insufficiently known (Arendt 1997). In some environments, low growth rates may appear adaptive even in the absence of costs of high growth rates per se. In particular, short-lived organisms in seasonal environments are often faced with the 'extra time' problem (Reavey and Lawton 1991, Tammaru et al. 2001); since the adverse season can often be spent only in a particular developmental stage, adapted for diapause, it may just be necessary for the organism to 'wait' in some earlier stage of development.

On the other hand, there are environments in which selection for high growth rates can hardly be questioned. Along with the vulnerable nestlings of altricial birds (Case 1978, Ricklefs 1984), and plants experiencing intense competition (Grime and Hunt 1975), the so-called spring-feeding insects provide a well-known example that has attracted attention from various perspectives (Ayres and MacLean 1987, Kause et al. 1999, Hunter and Elkinton 2000). Larvae of these insects (typically, various moths and sawflies feeding on deciduous trees) are faced with severe time stress caused by rapid deterioration of food quality, the deterioration being caused by phenological changes in host foliage (Feeny 1970, Riipi et al. 2002, Haukioja 2003). However, it is not unequivocally clear if selection for rapid growth has always, or even sometimes, led to maximisation of growth rates within physiological limits (Konarzewski et al. 1996, Arendt 1997). The rapid accumulation of evidence for endogenous plasticity in individual growth rates prompts us rather to ask if there ever are any true growth rate maximisers, i.e organisms void of the ability to control growth rates endogenously. A rigorous answer to this question would require availability of an adequate experimental protocol. One possible approach to testing maximisation could be to show that growth performance cannot further be improved (Konarzewski et al. 1996, Mace 2000); i.e. to document the inability to display compensatory responses in growth rates (Metcalfe and Monaghan 2001).

In the present paper, we apply this approach to test for the proposed growth rate maximiser status of the geometrid moth *Epirrita autumnata* Borkhausen. Several lines of evidence suggest that larvae of this spring-feeding insect are subjected to permanent selection for rapid development and high relative growth rates. Only young, developing leaves, available during a limited time period, can ensure the attainment of high pupal weights (Ayres and MacLean 1987, Kaitaniemi et al. 1997). The phenological stage of the host foliage is not only important for newly hatched larvae but has a considerable impact also later in larval life (Tammaru 1998). Attaining high pupal weights is, in turn, critical due to a strong correlation between female body weight and fecundity (Tammaru et al. 1996a); the relationship is less clear for males (Tammaru et al. 1996b). The larvae are thus selected to become large quickly, a task which can only be accomplished via high growth rates. Moreover, the larval stage is clearly the least safe one in terms of mortality rates (Teder et al. 2000, Tanhuanpää et al. 2001, Teder and Tammaru 2001), and has to be passed quickly. In contrast, the knowledge accumulated during more than 30 years of intensive study of this outbreaking species (Haukioja et al. 1988, Ruohomäki et al. 2000) does not allow us to propose any ecological factors selecting against high larval growth rates.

The data on the species' ecology have thus led to a view that growth rates of *E. autumnata* may be driven to a physiologically attainable maximum. Consistently, results of several studies have suggested close-to-zero heritabilities of relative growth rates (Ayres et al. 1987, Tammaru 1998, Kause et al. 1999), a situation indicative of a long-lasting directional selection on this trait (Mousseau and Roff 1987, Falconer and Mckay 1996). Moreover, relative growth rates of this species are close to the maximal found among tree-feeding insects (Ayres and MacLean 1987).

We made some predictions which should hold if *E. autumnata* were a true growth rate maximiser. Firstly, the larvae of this species should lack the ability for compensatory growth in response to transient environmental perturbations because they should not be able to improve their growth performance. The inability to compensate should also be reflected in high correlations between the values of growth rate, and pupal weights. Secondly, initial weights in the last larval instar and pupal weights should be highly correlated. This is because of the expected lack of any form of compensatory growth, but also because an organism under severe time stress is not expected to compensate for low initial weight by prolonging its growth period considerably. For the same reason, starvation treatments can be expected to affect pupal weight rather than the duration of larval period. These predictions were tested by studying the effects of short-term starvation treatments on pupal weight and larval period, as well as examining correla-

tions between different parameters of larval growth schedule. In addition to the correlative approach, in one experiment, the initial weight of last instar was manipulated by applying different temperature treatments during early larval development.

For comparison, we also present empirical data from starvation treatments on another lepidopteran, the comma butterfly *Polygonia c-album* Linnaeus (Lepidoptera, Nymphalidae). There are reasons to believe that this species is subjected to weaker selection for high larval growth rates than is the case for *E. autumnata*. In particular, there is no strong dependence of larval performance on plant phenology (Nylin 1988), or any evidence of other ecological factors causing severe developmental time stress. This is particularly true for the studied Stockholm population, which has ample time for its single generation per year (Nylin 1988, Janz et al. 1994). Moreover, the aposematic spiny larva appears to be relatively well protected from natural enemies (Nylin et al. 2001). Additionally, there are reasons to believe that the correlation between realized fecundity and body weight may be weaker in the income-breeding *Polygonia* (both due to the importance of adult feeding, and nuptial gifts, Wedell 1996) than in the capital-breeding *Epirrita* (Tammaru and Haukioja 1996). If so, we can also assume weaker selection for attaining high pupal weights. We thus predicted that this species might show compensatory growth (or, alternatively, prolong its growth period after starvation). This should also lead to weak correlations between the values of growth rate, and pupal weights. Moreover, we expected weaker correlations between initial and final weights than in *E. autumnata*. This could happen because of compensatory increases in growth rate and/or because of compensatory prolongation of the growth period when initial weight is low. In the latter case we should also see strong negative correlations between initial weight and last instar duration. Finally, in this species we expected starvation treatments to affect larval duration rather than pupal weight.

Methods

Experimental design

The principal approach was to subject larvae to short-term starvation periods (=perturbations), and to record any compensatory changes in relative growth rates following the starvation period. Moreover, the effect of the perturbations on developmental periods and pupal weights was of interest. Additionally, the resulting data sets allowed us to examine relationships between different parameters of the growth curve correlatively. We first present methodical details common to all experiments, and will describe particular trials thereafter.

In each of the experiments, the larvae were reared in identical but uncontrolled conditions until they were near the end of their fourth (penultimate) instar. The development of the larvae was then synchronised by varying the temperature regime of the rearing on an individual basis. As a result, the larvae moulted into the last instar synchronously. Larvae were weighed immediately after moult to determine initial weight of the last instar. Thereafter, the insects were randomly assigned to different experimental treatments.

On the first day of their development in the last instar, the larvae were allowed to feed normally on the foliage of their preferred host plants – birch (*Betula pendula* Roth, in Estonia, or *B. pubescens* Ehrh. in Finland, for *E. autumnata*) and wych elm (*Ulmus glabra* Huds., for *P. c-album*). In each experiment (with the exception of the year 1994 trial, below), the treatment group larvae were let to starve for a nine hour period on the second (and, additionally – in some trials – on the third and fourth) day of the last instar. The control larvae had an unlimited access to their food. Food was removed from the starved larvae in the mornings; the larvae were allowed to resume feeding after nine hours. Larvae were weighed daily in the mornings, i.e. prior to the application of the starvation treatment and 15h after its termination. Such a delayed timing of post-treatment weight determination was chosen to avoid the possibility that the larvae were weighed with guts being empty following starvation. This was important to exclude the possibility that the 'plain' effect of filling the gut was confused with true compensatory growth. Weighings were terminated two days after the application of the perturbations. Leaves of the host plant were renewed daily during the period when the larvae were weighed. Thereafter, the larvae were reared on ad libitum food to pupation, they were checked daily to record the duration of larval period. Pupae were weighed and sexed when their cuticulae were sclerotised.

In each particular experiment, the leaves that were fed to the larvae were collected from one tree individual and stored at +1°C. Such a procedure was chosen to avoid phenological changes in host foliage, thereby ensuring constant food quality during the course the experiment (Tammaru 1998). All larvae were reared individually in 50 ml plastic vials at +14°C. Insects belonging to different broods (=progeny of an individual female) were divided equally between treatments. Positions of the vials were randomised with respect to treatment, brood and population (where applicable).

In the experiment of 1999 with *E. autumnata,* the insects originated from the Turku area in southwestern Finland (60°N, 22°E, the southern population, hereafter) and were reared in Tartu, Estonia. The larvae represented nine unrelated broods. The starvation treatment was applied on the second day of the last instar.

Experiment of 2000 with *E. autumnata* was prompted by the intention to test for the generality of the effects recorded in the year 1999 experiment. Accordingly, to test for consistency across geographical populations, insects representing populations of two substantially different climatic, and vegetation zones were reared together under a 'common garden' design. Again, the southern population refers to the Turku area (mixed heminemoral forests) whereas the northern insects originated from northernmost Fennoscandia (70°N, 27°E, wooded tundra). There were five unrelated broods from both populations. Moreover, to study the dependence of the compensatory responses on food quality, the larvae were reared on two different host quality levels. Two individual birch trees with contrastingly high and low quality foliage were selected on the basis of pilot experiments. All four combinations of these factors (2 populations × 2 food quality levels) were equally represented. At both levels of food quality, in addition to control, two starvation regimes were applied. In one of the treatments, the larvae were forced to starve only on the second day of the last instar (the 'milder' treatment). In another (the 'extreme') treatment, the starvation procedure was applied both on the second and the third day of the last instar. The experiment was performed at Kevo Sub-arctic Research Station, northernmost Finland.

Analogously, in the experiment of 2000 with *P. c-album* two different starvation regimes were applied. In the milder treatment, the larvae were starved for nine hours on two subsequent days (second and third). In the more extreme treatment, the starvation procedure was applied for three days (second to fourth day). Using a starvation regime different than that in *E. autumnata* was motivated by the longer within-instar developmental period in *P. c-album*. Since the between-species difference in instar duration is about twofold, a two-day starvation treatment in *P. c-album* could be seen as approximately corresponding to the one-day starvation in *E. autumnata*. The insects represented eight broods from the Stockholm area (59°N, 18°E), Sweden, and were reared in Stockholm.

In the experiment of 1994 with *E. autumnata*, initial weight of the last instar larvae (representing the northern population, 9 broods) was manipulated by rearing the 4th (penultimate) instar larvae under two different temperatures, +10° and +14°C (lower temperatures resulting in higher weights, as it is typical for invertebrates, Atkinson 1994). During the last instar, larvae were divided between two treatments differing in the quality of birch foliage, no starvation treatments were applied. This experiment, performed at Kevo, is complementary to the starvation trials as it allowed us to study the ability to compensate for environmental effects experienced earlier in the course of larval development. Though the initial/ final weight correlations were ob-

tained from all of the trials, the advantage of this dataset is in its manipulative nature which allows us to isolate the environmental component (unconfounded by possible genetical correlations) of the relationship.

Data analysis

Relative growth rates (RGR) were calculated as log(final weight/ initial weight)/time (days) separately for each day of the last instar. To test for the presence of compensatory growth, RGR values on the day immediately following the application of the starvation treatment were compared to those of the control group, sex was used as an additional factor in the ANOVAs (SAS PROC MIXED, with brood as a random factor). Additionally, initial weight of the respective day was included in the models as a covariate. This was necessary because there is a within-instar dependence of RGR on body size in *E. autumnata* (Tammaru 1998, but see Lytle 2001), and, naturally, larvae starved in the preceding day had lower average initial weights. Our objective was thus to detect any compensatory increase in RGR that was not solely attributable to lower weights caused by starvation. For the purpose of illustration, the *Epirrita* experiment of the year 2000 was analysed in three alternative ways: separately by sexes, populations, and host quality levels (Table 1). All the data were, however, analysed jointly when testing for sex × treatment, population × treatment and host × treatment interactions (Table 2). A joint analysis was also necessary to avoid statistical problems related to multiple testing which appeared when the data were analysed separately by different subsets.

Analogous ANOVA approaches were applied to study the effect of the treatments on the duration of the last larval instar (Table 3), and pupal weights. In these analyses, initial weight of the last instar was used as a covariate. In some of the ANOVAs, one or a few outlying observations caused the distributions of residuals to deviate from normality. Omissions of these outliers had only limited effects on the relevant statistics, and in no case led to different conclusions; therefore, tables present analyses that were performed with the full data-set.

In addition to the manipulative approach, the sensitivity of pupal weight and larval period to environmental conditions was studied correlatively (Table 4, Introduction). ANOVAs with initial weight of the instar, and the daily RGR values as independent variables were run to explain the variance in pupal weights and developmental periods. We admit that a quantitative interpretation of these correlative analyses may not be straightforward. However, a qualitative comparison of such tables constructed for different variables, populations and species, was found to be informative and illustrative. The treatments were pooled in this analysis.

Table 1. Responses of the relative growth rate to environmental perturbations. Larvae were subjected to 9h starvation on the 2nd day of their last instar (*Epirrita autumnata*), or for two to three days in the beginning of the last instar (*Polygonia c-album*); the 'extreme' treatments (Table 3) are not included in this analysis; sample sizes refer to all treatments combined. Responses in the RGR were recorded both in the days in which the treatments were applied (current change), and in days immediately following application of the treatments (compensatory responses). Statistics refer to treatment vs. control comparisons on the day when compensatory responses were recorded.

Experiment	Grouping variable	Grouping variable value	Relative growth rate (day^{-1})				Variance explained by compensation**	p***
			current change	day after adverse treatment*		relative compensatory effect		
				control	treatment			
Epirrita 1999 (N =170)								
	–	–	−24.6%	0.202 ± 0.006	0.241 ± 0.006	+19.3%	9.3%	<0.0001
Epirrita 2000 (N =372)****								
	sex	male	−26.1%	0.288 ± 0.009	0.296 ± 0.008	+9.9%	3.0%	0.020
		female	−23.5%	0.262 ± 0.008	0.292 ± 0.008	+11.4%	4.3%	0.012
	population	southern	−27.3%	0.263 ± 0.008	0.292 ± 0.008	+11.1%	4.0%	0.008
		northern	−25.1%	0.261 ± 0.007	0.288 ± 0.008	+10.3%	3.3%	0.016
	host quality	high	−24.0%	0.280 ± 0.007	0.310 ± 0.007	+10.7%	4.7%	0.0017
		low	−25.3%	0.244 ± 0.008	0.272 ± 0.008	+11.9%	4.4%	0.023
Polygonia 2000 (N =131)								
	starvation days	2nd to 3rd	−19.8%	0.238 ± 0.007	0.241 ± 0.008	+1.3%	0.1%	0.69
		2nd to 4th	−32.6%	0.214 ± 0.007	0.231 ± 0.006	+7.9%	2.8%	0.051

* – all mean values (±SE) of RGR are those with the effects of initial weight, sex and brood (as well as host or population, for *Epirrita* in 2000) statistically removed (SAS GLM LSMEAN option);
** – R^2 values are calculated as SS(effect)/SS(total), type III SS, for the effect of treatment;
*** – statistical tests are those of ANOVAs for treatment vs control comparisons with sex and brood (random factor) as additional factors, and initial weight of the respective day as a continuous covariate;
**** – this experiment was analysed in three different ways: separately by sexes, populations, and host qualities.

The importance of the initial weight of the last instar as a determinant of pupal weight was visualised by presenting regressions among these variables (Fig. 1). To make the results directly comparable among species and experiments, both variables were divided by the mean values of the respective samples. The slope of these regressions was treated as a measure of the strength of the dependence of pupal weights on initial weights of last instar. For the year 1994 experiment, it was possible to test for the effect of the initial weight manipulation (ANOVA with sex and brood as additional factors) on

Table 2. ANOVA results (SAS PROC MIXED, with brood as a random factor; the year 2000 experiment) for the effect of the starvation treatment on *E. autumnata* growth rates one day after its application. The effect of treatment reflects the compensatory increase in RGR.

Effect	F*	p
treatment	14.0	0.0002
initial weight	36.5	<0.0001
sex	5.3	0.022
host	60.0	<0.0001
population	2.6	0.11
treatment × host	0.51	0.48
treatment × sex	0.01	0.96
treatment × population	0.01	0.93

* – ndf =1, ddf =229.

final weight. Moreover, in this analysis, a simultaneous incorporation of the treatment and initial weight to the ANOVA model allowed us to test if the purely environmental influence on the initial weight (treatment) had an effect distinguishable from the overall one.

Results

Responses of relative growth rates to starvation treatments: compensation for current perturbations

Unexpectedly, larvae of *E. autumnata* consistently displayed a compensatory increase in the relative growth rate (RGR) on the day following the day when the starvation treatment was applied. This effect was not solely attributable to differences in initial weights of the post-treatment day, as this variable was included in the respective ANOVA designs (Table 1, 2). There were no differences in this compensatory response among the studied populations, sexes and food qualities, as indicated by very weak and non-significant interactions of treatment with these variables (Table 2). In *P. c-album*, the increase in RGR on the day following starvation was somewhat lower and remained marginally non-significant (Table 1).

Table 3. Responses of larval period (duration of last larval instar) to environmental perturbations. See Table 1 for more details.

Experiment	Grouping variable	Grouping variable value	Larval period, days				R^2	p***
			control	'milder'* treatment	extreme* treatment	absolute effect** (hours)		
Epirrita 1999								
	–	–	6.42±0.09	6.62±0.09	–	+4.8	1.1%	0.13
Epirrita 2000								
	sex	male	7.43±0.09	7.44±0.10	7.73±0.10	+9.6	1.6%	0.038
		female	8.04±0.08	8.12±0.08	8.32±0.08	+6.7	1.3%	0.029 (0.0072)
	population	southern	7.95±0.09	8.18±0.09	8.31±0.09	+8.6	2.3%	0.0082 (0.0033)
		northern	7.33±0.10	7.56±0.10	7.79±0.10	+11.0	3.1%	0.0041 (0.0014)
	host quality	high	7.08±0.07	7.28±0.07	7.49±0.07	+9.8	4.1%	0.0004 (0.0001)
		low	8.17±0.11	8.40±0.11	8.67±0.11	+12.0	4.0%	0.010 (0.0024)
Polygonia 2000								
	–	–	12.33±0.18	12.78±0.18	12.82±0.19	+11.8	3.2%	0.090 (0.031)

* – 'milder' treatment was the one with the lower number of starvation days (see text), the extreme one with the higher (year 2000 only);
** – the absolute effect of the starvation treatment on larval period is expressed as the difference of the 'extreme' treatment and control; summed duration of starvation was 18h in the extreme treatments with *Epirrita* and 27h for *Polygonia*.
*** – R^2 and p-values are those of an ANOVA with both starvation treatments and the control included (sex and brood as additional effects, initial weight of the last instar as a covariate); p-values in parentheses refer to extreme treatment vs control comparisons (presented if lower than the p value from ANOVA with three treatment groups).

Responses of developmental periods and pupal weights

When subjected to the starvation treatments, the larvae prolonged their development time in the last larval instar. The extra time spent in the last instar was, however, consistently shorter than the summed duration of the applied starvation periods (Table 3). The effect did not differ between populations, host qualities, and sexes in *E. autumnata* ($F_{2,345} < 0.2$, $p > 0.8$ for all two-way interaction terms of treatment, in an ANOVA for larval period analogous to the one in Table 2).

Starvation treatments slightly ($\sim 2\%$) reduced pupal weights in the year 2000 experiment with *E. autumnata*

Table 4. Results of ANOVAs for pupal weights and larval periods (duration of last instar) of *E. autumnata* as dependent on RGR values (reflecting current growth conditions) and initial weights of the last instar (reflecting early larval history). Proportions of variance explained ($R^2 =$ SS(effect)/SS(total), type III SS) and significance levels$ are presented. Treatments were pooled.

	Experiment year and data sub-sets$$					
	1999	2000 N, low$$$	2000, S, low	2000, N, high	2000, S, high	*Polygonia* 2000
pupal weight						
initial weight$$$$	29.8***	31.7***	11.7***	22.8***	23.8***	25.2***
sex	4.5***	0.0	2.7*	5.9***	11.3***	0.5
brood	3.0*	4.9+	3.7	5.0*	3.2+	3.1
RGR, 1st day	0.9*	5.3**	8.2***	4.0*	5.5***	0.2
RGR, 2nd day	3.9***	0.7	9.1***	0.7	4.6**	0.1
RGR, 3rd day	3.4***	1.2+	9.4***	1.2+	2.3*	0.9
larval period						
initial weight	8.2***	26.1***	4.0**	6.7**	1.9+	3.4**
sex	21.1***	12.5***	8.7***	26.7***	35.3***	0.4
brood	3.6	1.1	1.0	2.6	6.8*	12.1***
RGR, 1st day	24.9***	17.3***	16.1***	0.0	9.1***	18.2***
RGR, 2nd day	4.9***	5.5**	16.7***	18.8***	0.8	12.6***
RGR, 3rd day	0.0	7.8***	8.3***	0.2	0.1	0.2

$ – levels of statistical significance: + – $p < 0.1$; * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$;
$$ – data for the year 2000 experiment are presented by subsets defined by combinations of population of origin (northern and southern), and host quality, see Methods;
$$$ – omitting three outlying observations (the smallest larvae with very long development) periods reduced the atypically high R^2 for larval period to 9.7% whereas the corresponding value for pupal weight dropped to 23.0%;
$$$$ – initial weight of the last larval instar.
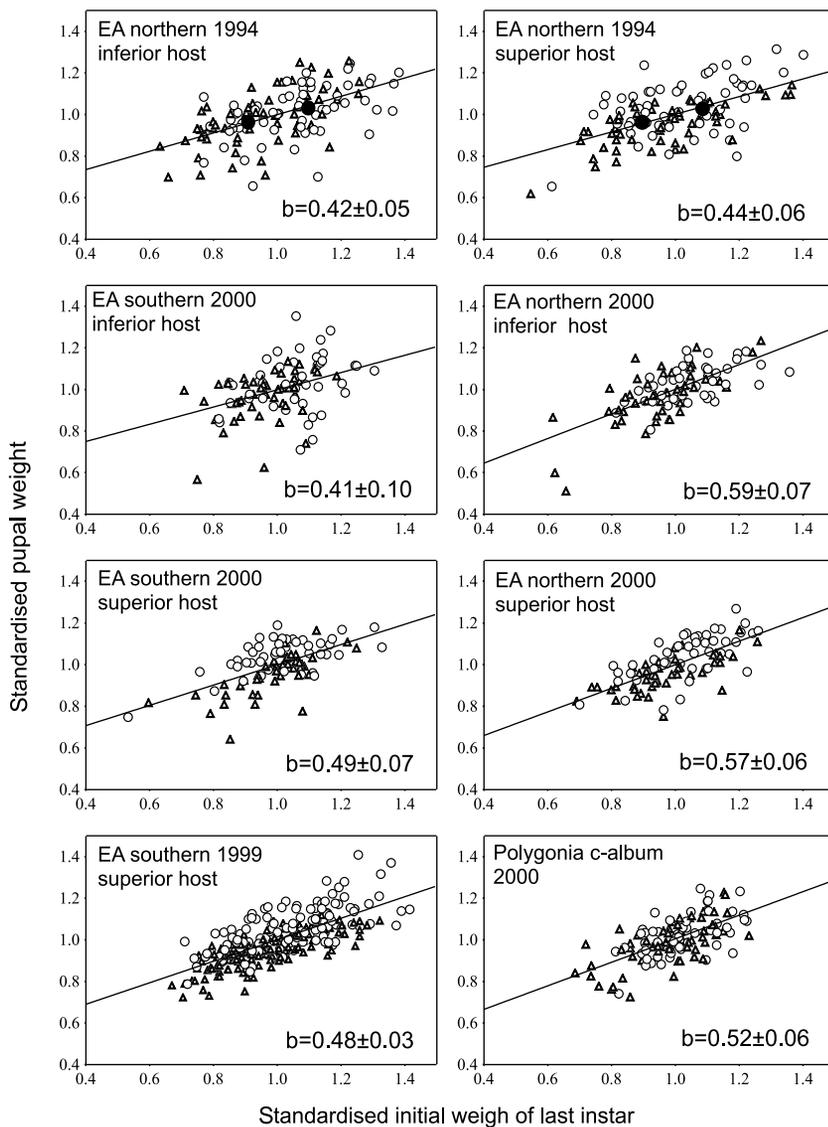
Fig. 1. Relationship between initial weight of the last instar and pupal weight in different (sub)experiments (=cases), both variables being divided by the mean values of respective samples; EA – *Epirrita autumnata*. Triangles stand for males, circles for females, values of slope ($\pm$SE) are indicated. Solid circles in the two upper panels designate bivariate means of treatment groups in which initial weights of the last instar were manipulated. The values of slopes in the 8 cases were not significantly different from each other ($F_{1,993}=1.08$, p = 0.37, for the initial weight $\times$ case interaction).

($F_{2,333}=3.24$, p =0.040, in an ANOVA approach analogous to that of Table 2), but did not attain significance in the year 1999 trial. The response in pupal weight in *P. c-album* (−1, 4%) was not significant.

The correlative analysis supported the results of the manipulative approach (Table 4): as a general pattern, daily RGR values affected pupal weights more weakly (though always significantly) than was their effect on developmental periods (note the values of R^2). This pattern was broadly consistent over the species and populations studied. This result hints at a high sensitivity of developmental period to environmental conditions experienced by the larvae. Apparently, such a sensitivity

can largely (but not completely) buffer pupal weights against short-term environmental perturbations.

Dependence of developmental variables on initial weight: compensation for adverse events in history

Initial weight of the last instar was a strong and consistent determinant of the attained pupal weight (Table 4). This relationship was remarkably similar in different species and populations, and was not affected by food quality (Fig. 1). The effect of initial weight on developmental period was consistently weaker (Table 4,

as indicated by the values of R^2), although generally significant. Thus, the larvae appeared to have limited abilities to compensate for low initial weights by prolonging their development times in the last instar.

The experiment (*Epirrita* 1994) in which initial weights of the last instar were manipulated confirmed the pattern emerging from the correlative results. The manipulation of initial weights by temperature (an about 1.2-fold difference was achieved, Fig. 1, two upper panels) of the last instar had a clear effect on pupal weights ($F_{1,241} = 18.8$, p < 0.0001). Moreover, the effect of the manipulation lost its significance ($F_{1,240} = 0.97$, p = 0.32) when initial weight was included in the respective ANOVA model as a continuous variable. The effect of the manipulation was thus mediated by initial weights of the last instar, and limited to it. This is visualised by the pattern in Fig. 1 (two upper panels) – the bivariate means of the treatments lay exactly on the overall regression line, irrespective of the food quality experienced by the last instar larvae. These results indicate that the environmental component in the initial/ final weight correlation is indistinguishable from the overall relationship (which may involve genetic correlations between body sizes in different instars). This encourages us to interpret the correlative patterns of other experiments (Fig. 1.) as describing the influence of environmental effects on initial weights of the last instar.

Discussion

A suggested 'maximiser' is capable of compensatory growth

Larvae of *Epirrita autumnata* – a suggested growth rate maximiser – were unexpectedly found capable of increasing their growth rates following short-term environmental perturbations experienced in the course of their last instar. The used approach allows us to interpret the observed increase in growth rates as representing a 'true' compensation. In particular, the experimental protocol excluded the possibility that the increase were attributable to a plain gut-filling effect. Furthermore, the observed response was not merely caused by lower weights of the starved larvae: the latter effect was controlled for statistically. Notably, the compensatory responses were not affected by food quality (cf. Flanagin et al. 2000): the ability to compensate in studied species is thus not a prerogative of favourable environments.

An ability to display compensatory growth is inconsistent with the predictions that were made for a growth rate maximiser. Such a result may be interpreted as indicating that growth rates are normally below the physiological potential even in a very likely candidate for maximising, a spring-feeding lepidopteran (cf. Margraf

et al. 2003). Our results are consistent with those of analogous studies on another group of expected maximisers, altricial birds: compensatory growth has also been found in experiments with these animals (Wiggins 1990, Kunz and Ekman 2000), though not universally (Konarzewski et al. 1996).

It remains thus unclear why the larvae do not grow faster under normal conditions if they apparently are capable of doing so. Faster growth may entail trade-offs that could not be detected in the present study (Arendt 1997, Gotthard 2000, Metcalfe and Monaghan 2001). An alternative interpretation of our result, however, is a purely physiological mechanism. The possibility cannot be excluded that, in one way or another, the post-starvation increase in growth rate is based on a mechanism that cannot be realized without starvation. If this were the case, the observed plastic increase in growth rate could well reflect a phenomenon different from those an evolutionary increase could rely upon. An ability to display a compensatory response may then not necessarily question the RGR maximiser status of *E. autumnata* in the microevolutionary sense. Specific attention to the details of growth physiology (e.g. consumption rate, digestive efficiency, energy allocation, Perrin et al. 1990, Bradley et al. 1991) may provide an answer.

Irrespective of the physiological background, however, our results indicate that the ability to display compensatory growth per se cannot be used as an (inverse) measure of selection for high growth rates. In particular, we have little doubt that *E. autumnata* is selected for high growth rates by all ecological factors that we are able to evaluate, but the larvae did not 'behave as predicted'. Illustratively, the presumably non-maximising *P. c-album* displayed a weaker compensatory response in RGR than *E. autumnata*. This is, however, not necessarily surprising. In particular, it may be the case that for a species developing under a limited time stress there is no need to display compensatory responses even if these were physiologically possible.

It remains thus to be shown if a 'true' growth rate maximisation ever exists in nature, and how it could be detected experimentally. In any case, the ability to control growth rates endogenously appears to be remarkably widespread: even the most unlikely candidates appear to have a potential for such a plasticity. Life-history models allowing for adaptive flexibility in growth rates should thus rather be treated as the general, not a special case.

Compensation for current and past perturbations is different

Compensatory responses available to the larvae were not limited to the increase in RGR: the larvae that were

subjected to short-term starvation periods prolonged their developmental periods. In combination with the increase in RGR, this response largely eliminated the effects of short-term perturbations on pupal weights. The analysis of correlative patterns strengthened this message. The ability to compensate for current perturbations by prolonging larval periods is thus considerable, a somewhat unexpected result in the case of an organism growing under time stress, such as *E. autumnata*.

Responses to adverse environmental effects experienced earlier during the larval development were remarkably different from the responses to perturbations experienced during the last instar. In particular, weight of the larvae in the beginning of the last instar was a major determinant of the final pupal weights attained. We interpret this relationship as reflecting responses to environmental effects rather than genetic correlations. This is because a manipulative study showed that the environmental component of this correlation does not deviate from the overall relationship. Compared to pupal weights, development periods were more weakly affected by initial weights. These patterns are thus qualitatively different from the responses to perturbations experienced during the last instar: the ability of larvae to compensate for lower initial weights by means of prolonging their growth periods is limited.

Limited abilities to compensate for adverse environments experienced earlier in the life can, however, hardly be ascribed to the suggested growth-rate-maximiser status of E. autumnata, neither be used to test for such a status. This is because the patterns were notably similar in the presumably non-maximising 'reference' species *P. c-album,* and a strong correlation between initial and final size of an instar may be a widespread phenomenon among arthropods in general (Nijhout 1975, 1994, Hutchinson et al. 1997, Lytle 2001).

Some aspects of the growth schedules may be conservative

The potential for compensatory growth present in *E. autumnata* could be interpreted as evidence of evolutionary conservatism regarding some aspects of larval growth schedules. If selection for maximal growth rates has indeed taken place in *E. autumnata*, as seems likely, this has either failed to produce true maximisation (indicating evolutionary constraints on growth rates and perhaps conservatism of compensatory responses) or else the supposed compensatory growth in fact represents some other physiological phenomenon. If the latter is true, it seems likely that such an unknown phenomenon, producing faster growth as a direct result of starvation

(and hence a growth rate increase which is not possible without starvation, and thus not subject to general directional selection) would be even more evolutionary conservative than growth responses that are adapted to the local situation.

Various other results of this study may be seen as leading to a hypothesis that some parameters of the larval growth curve are evolutionarily conservative, i.e. not rapidly responding to changes in selective environment. In particular, there were remarkably limited differences in the studied variables between sexes and populations of *E. autumnata*, and even a distantly related species with different ecology displayed considerably similar patterns (note the results of the correlative analyses, Table 4). Naturally, with only two species involved, any of the between-species comparisons should be interpreted with extreme caution. However, we find the interspecific similarity in the studied patterns highly remarkable (the species were chosen as supposedly very dissimilar!) and thereby of some value at least as providing a cue for further comparative research.

As was the case regarding the compensatory changes in RGR, no between-population or sex-related differences were detected in the responses in the length of the larval period. Such a constancy is surprising considering the most likely presence of sex-related differences in fitness consequences of body size (Tammaru et al. 1996a, b), as well as the likely differences in developmental time stress between the geographical populations studied. Although the latter difference may be hard to quantify, 'no difference' should be considered a highly unlikely possibility. This is because there are obvious dissimilarities in the host plants used (much broader diets in the 'south'), contrastive climatic conditions, and perhaps also differences in predation pressure. Strong gene flow preventing regional adaptation in *E. autumnata* is unlikely (cf. Tammaru et al. 1999, for geographical patterns in pupal period).

Though the patterns of compensation for low initial weights were different from the responses to current perturbations (i.e. starvation treatments applied late in development), the invariability is similar in the two types of responses. In particular, the dependence of final weight on initial weight did not differ among populations (Fig. 1) whereas the absence of sex-related differences is reported in an earlier study (Tammaru 1998). The similarity of the initial/ final weight relationship even in *P. c-album* is particularly striking (Fig. 1). Moreover, Nijhout's (1975) data on a strong initial weight/ critical weight correlation in a sphingid moth are (even quantitatively) well consistent with the patterns found in the present study. Once again, it seems unlikely that there are no differences in selective pressures. Rather, the invariability might be based on physiological constraints inherent to the processes of larval growth.

Relative conservatism of some aspects of attaining larval weight might contribute to explaining why we failed to detect any special, 'expected' features of life history plasticity in the species presumably selected for growth rate maximisation.

It must be emphasised, however, that the hypothesis of evolutionary conservatism cannot apply to all aspects of lepidopteran growth schedules. For instance, there are numerous reports of among-population and within-species differences in growth rates, as well as in the number of larval instars (Nagasawa 1988, Nylin 1994, Fischer and Fiedler 2000, 2001). The proposed conservatism appears thus to apply primarily to reaction norms for final weight of an instar, given the initial weight and growth rate (Tammaru 1998). Obviously, however, a broader comparative study is needed to test this hypothesis.

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