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Life history plasticity in the satyrine butterfly *Lasiommata petropolitana*: investigating an adaptive reaction norm

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Abstract

This study addresses the general hypothesis that insects living in seasonal environments should shorten development times at progressively later dates in the growth season, and that insects living outside equatorial areas should use daylength as a cue to determine the date. Diapause strategies and reaction norms relating the duration of larval development to daylength was investigated in a French population of the butterfly, *Lasiommata petropolitana*. The results are compared with those of an earlier study of the species in Sweden. Because of the diapausing strategy and phenology of the population, it was expected that an adaptive reaction norm relating larval time to daylength should have a positive slope, i.e. relatively shorter daylengths induce faster growth and development. This prediction was supported, and the reaction norm was qualitatively similar to the one found in Swedish populations. In the French population it was, however, shifted to a range of shorter photoperiods which corresponds to the regime of shorter daylengths in southern Europe. Shorter larval development times and high growth rates were associated with a reduction in pupal size, suggesting a trade off between time and size at pupation. There was no evidence of a trade off between growth rate and starvation endurance. The results suggests that the daylength-dependent decision of what growth trajectory an individual larva will follow, is not made continuously but rather at one or a few occasions during larval development.

It is clear that larvae of *L. petropolitana* make developmental decisions in relation to the daylength they experience during larval growth. The result is a reaction norm that agrees closely to what is predicted by some life history models, suggesting that it is an adaptation for optimising life history traits in a seasonal environment.

Introduction

Studies on the evolution of adaptive phenotypic plasticity have to a large extent focused on underlying genetic mechanisms and on how plasticity is affected by natural selection (Scheiner, 1993; Via et al., 1995). In other words they have mainly investigated how selection in progress affects phenotypic plasticity and modelled the evolutionary dynamics of this process. Another approach, however, is to study plasticity and reaction norms as evolutionary adaptations. The focus is then more on the functions of the products of natural selection, than on the actual process (Bradshaw, 1965; Newman, 1992; Gotthard and Nylin, 1995; Abrams et al., 1996, and see Grafen (1988) for a general discussion of adaptations and selection in progress). The theoretical aspect of this approach is the development of optimality models that try to predict the shape of reaction norms (Stearns and Koella, 1986; Houston and McNamara, 1992; Sibly and Atkinson, 1994; Abrams et al., 1996). Although these approaches seek answers to different questions there are obviously connections between them, one of them being the fact that adaptations are the result of selection past (Grafen, 1988). Here, I have used the functional approach to investigate plasticity in life history traits, especially development time, as a possible adaptation to seasonality.

Insects in seasonal environments typically have as a set of alternative developmental pathways allowing growth and reproduction in favourable parts of the season, and dormancy during periods of unfavourable conditions (Danks, 1994). Phenotypic plasticity in response to seasonal cues such as daylength and temperature is the most common mechanism for determining the pathway followed by a developing individual (Danks, 1994). The induction of diapause as a response to daylength is perhaps the best documented case of adaptive plasticity in insect life histories, at least in relation to a cue predicting future conditions (Beck, 1980; Tauber et al., 1986; Danks, 1994). However, seasonality may also favour plasticity in the timing of development within a given pathway (Nylin et al., 1989, 1996; Abrams et al., 1996; Leimar, 1996). This is because winter diapause typically can only take place in a single species-specific developmental stage, while the onset of development varies in time between years due to weather conditions and between individuals within a year, due to variation in time of oviposition. In order to survive, a growing insect larva must develop in such a way that it or its offspring reaches the diapausing stage, independently of when in the favourable season it finds itself (Reavey and Lawton, 1991). This is likely to constitute a strong selection pressure for plasticity in the timing of insect growth and development. In line with this, recent life history modelling shows that an individual should, if possible, shorten its development time by increasing its growth rate and/or by maturing at a smaller size as time to the optimal maturation date decreases (Abrams et al., 1996). The prediction that insects have shorter developmental times in daylengths indicating progressively later dates, has been supported in crickets (Masaki, 1978) and in several species of butterflies (Nylin et al., 1989; 1995; 1996; Nylin, 1992; Leimar, 1996).

Nylin et al. (1996) compared the plastic response in development time to daylength in two closely related species, *Lasiommata maera* and *L. petropolitana* from Sweden. Because the species feed as larvae during different parts of the season, before and after summer solstice respectively, they were predicted to interpret a given range of daylengths qualitatively different. Hence, adaptive reaction norms should be qualitatively different in the two species. This prediction was supported and plotting larval development time against daylength showed approximately linear reaction norms with a positive slope in *L. petropolitana* and a negative slope in *L. maera*. Since reaction norms of the species were crossing, a non-adaptive, mechanistic explanation involving evolutionary fixed feeding behaviour (restricted day-time or night-time feeding) seems less likely. Thus, both species speeded up development in photoperiods signalling later dates but the translation of a given daylength to a date was different and fitted the before/after solstice growth periods very well. This implies that the reaction norms are adaptations for optimising time budgets in a seasonally varying environment (Gotthard and Nylin, 1995; Nylin et al., 1996). In this study I further investigate the reaction norm of *L. petropolitana*, by testing the hypothesis of adaptation in another population originating from the French Alps.

The population in the Alps is of interest for several reasons. First, it is isolated from the populations in northern Europe (Higgins and Hargreaves, 1983), which to some degree allows independent evolution. Second, this population is reported to have a larval winter diapause (Benz et al., 1987), in contrast to the pupal diapause displayed in the north (Wiklund et al., 1983; Nylin et al., 1996). Third, a comparison between the northern and southern populations in terms of photoperiod plasticity is interesting in the light of the difference in latitudinal origin. There is a large difference in the daylength regime experienced by the populations that is not accompanied by a marked difference in seasonality, in turn due to the strict alpine occurrence of the southern *L. petropolitana*. The alpine habitat also explains why the southern *L. petropolitana* generally do not produce an additional generation compared to their northern conspecifics as is common in insects.

Given the earlier results (Nylin et al., 1996), and the possible difference in diapausing stage, the predictions were formulated as follows. (1) If larval diapause is the most common diapause strategy in the Alps, newly hatched larvae growing in short daylengths indicating late summer or autumn conditions are expected to enter larval diapause. In short daylengths this should result in a period with no development and very long larval development times. Very long daylengths, indicating constant midsummer conditions may produce direct development (Fig. 1). (2) If, pupal diapause is the predominant overwintering strategy also in the Alps, a reaction norm similar to the one found in the northern population (Nylin et al. 1996) is expected (Fig. 1). This is because *L. petropolitana* also in the Alps grow after summer solstice and short days should typically signal a late date in the season. (3) Because of its southern origin, the population from the Alps could be expected to have a diapause-direct development threshold at a daylength considerably shorter than the Swedish populations investigated by Nylin et al. (1996). At July 1, the daylength (h:min) at the latitude of Stockholm (≈60° N) excluding twilight is 18:43 and at August 1 it is 16:57. The corresponding figures at the latitude of the southern French Alps (≈45° N) are at July 1, 15:33 and at August 1, 14:44 (Beck, 1980).

On both empirical and theoretical grounds life history theory assume that short development times and high growth rates are costly (Roff, 1992; Stearns, 1992). If such costs were not present individuals should always minimise development time and maximise growth rate and there would be no selection for plasticity in the timing of metamorphosis. The most widely recognised cost of short larval development time is a reduction in size, simply because the time available for growth is shorter. Moreover, studies of other species of Lepidoptera has suggested a trade-off between growth rate and starvation endurance, where fast growing individuals are less likely to survive a period of food shortage (Stockhoff, 1991) and also loose weight at a relatively higher rate during starvation (Gotthard et al., 1994). Therefore, I investigated if the physiological trade-off between growth rate and starvation endurance seems to be present in *L. petropolitana*. Rearing and starving larvae in two different daylength regimes, indicating one early date and one late date, allowed me to experimentally induce variation in growth rate and test if it correlates with weight loss during starvation.

This experiment also allowed a more detailed study of the growth trajectories chosen by the larvae. Specifically, I investigated whether a disturbance in the growth process, such as a period of food shortage, influences the developmental decisions taken later in the larval period. In other words, are these decisions state dependent (Houston and McNamara, 1992)? It is clear that an individual larva, to be able to use the information on season provided by the daylength, at some point in development must have some "knowledge" of its intrinsic state. Such indicators of intrinsic state may be the instar a larva finds itself in, its size or its nutritional status. A period of starvation as it is applied here alters both size and nutritional

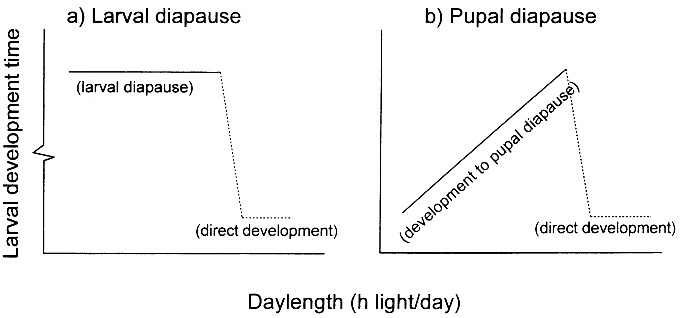

Fig. 1. Predictions of reaction norms relating larval development time to daylength in the case of a) larval diapause and b) pupal diapause, being the primary overwintering strategies respectively. Dotted lines mark intervals of daylengths where a mixture of diapause and direct development or purely direct development may occur. Direct development is uncommon in the field and therefore predictions are primarily concerned with developmental pathways leading to diapause. Note that the Y-axis is not of the same magnitude in the two separate graphs.

status at one point in development. If individuals continuously sample their external and internal environment and make developmental "decisions" based on this information, larvae that are in different "seasonal states" could be expected to react differently when the internal state is altered.

Materials and methods

Study organism

L. petropolitana (Satyrinae: Parargini) has a disjunct distribution in Europe. In the north (Fennoscandia, the Baltic states and Russia) it is found in forests at low altitudes (Higgins and Hargreaves, 1983). In southern Europe, however, it only occurs at altitudes above 500 m in the higher mountain ranges (the Alps, the Pyrenées, the Carpathians and in the Balkans). Consequently, the populations in northern Europe and the southern alpine populations are isolated from each other (Higgins and Hargreaves, 1983). The larvae feed on various grasses from the genera *Festuca*, *Poa* and *Dactylis*, and there is no indication that this differs between populations (Higgins and Hargreaves, 1983, Benz et al., 1987). In Scandinavia the species is univoltine and this is generally true also in the Alps but in extreme years, some individuals develop directly and fly in September (Higgins and Hargreaves, 1983, Benz et al., 1987).

Life history plasticity in response to daylength

Seven mated females of *L. petropolitana* were caught in July 1995 in the southern French Alps at altitudes between 1600–1800 m a.s.l, around the villages Baudinard (44° 15′ N), Crevoux (44° 30′ N) and Nevache (45° N). They were allowed to oviposit separately, and the eggs were brought back to the laboratory in Stockholm. In the main experiment, offspring from six of the females were split between environmental cabinets with daylengths ranging from 13L:11D (13 h daylength in the following) to 18L:6D (18 h daylength) with a one hour interval, which sums up to six different daylength treatments. The temperature was 17 °C in all cabinets, mimicking an average summer day temperature at an altitude of ≈1500 m a.s.l. in the Alps (HMS Stationary Office, 1972) and allowing comparison with the experiment of Nylin et al. (1996). Five larvae from each female were placed in each daylength and were reared individually on the host plant *Dactylis glomerata* cultured in plastic jars. The cabinets were checked daily to ensure that all individuals had access to fresh food. Three of the six females were fecund enough to have offspring in all six daylengths, while the smallest family is represented in the four shortest daylengths only (excluding 17 h and 18 h). Larvae from the three largest families were also grown outside on the roof of the Dept. of Zoology in Stockholm, starting at July 11. Pupae that did not hatch within 40 days were classified as diapausing.

Larvae were followed daily to pupation and larval development time (Lt), pupal weight (Pw), pupal development time, sex and the diapause/direct development status were recorded for each individual. Average larval growth rate was calculated with this data as

$$\ln(\text{growth rate}) = (\ln(\text{Pw}) - \ln(\text{hatching weight}))/\text{Lt}$$

The formula yields a value for relative growth rate such as 1.15, representing a 15% daily weight gain (cf. Nylin, 1992; Brakefield and Mazzotta, 1995).

Directly developing butterflies were used for breeding in one flying cage. Two females, known to have mated, were allowed to oviposit separately and their offspring were used in the starvation experiment. Grandoffspring from the rest of the females were used to investigate the importance of low temperatures on the diapause decision. They were given a short day (13:11) in combination with a low temperature (13 °C) and the stage of diapause was recorded.

Starvation experiment

The offspring of two females (F_1) were split in equal numbers between two daylength treatments, 13 h and 15 h daylength, with a constant temperature of 17 °C. These photoperiods were chosen because in the main experiment the 13 h treatment on average induced shorter larval development times and higher growth rates compared to the 15 h treatment. The larvae were reared individually on *Dactylis glomerata* and twice a week they were weighed and the larval instar was noted. In addition, I recorded the same life history traits as in the main experiment. At the start of the experiment half of the larvae in each family and daylength were designated to the starvation treatment, while the other half were treated as a control group and had access to fresh food continuously up to pupation. When a larva from the starvation group first reached a weight greater than 25 mg (early third instar) it was removed from the host plant and was starved for three days. During this period I weighed the larvae more frequently (five to seven times per individual) to get a good estimate of the weight loss function.

Butterfly growth can usually be well described as exponential (Nylin et al. 1989; Wickman et al., 1990; Leimar, 1996). Consequently, relative growth rates and weight loss rates were taken to be approximately equal to the slope of the regression lines for ln(weight) as a function of larval age or days of starvation (Gotthard et al., 1994). This way of estimating growth rate is preferred before using the formula above, since it estimates growth rate independent of pupal weight and larval development time. The two types of estimates are numerically different but there is a strong correlation between them (Gotthard et al., 1994). Larval development in the starvation treatment was divided into three periods, growth before starving, weight loss during starvation and growth after starving. When estimating relative growth rates for the controls, comparable to growth before and after starvation, I used the average weight at the start of starvation as "endpoint" of the early growth, and the average weight at the end of starvation as "starting point" of the late growth, respectively. This allowed me to compare the growth rate of the "starvation group" with the unstarved controls, before and after starvation. I also estimated larval growth rates in the fourth instar only and compared them in the same way.

Statistical treatment

All individuals within an environmental cabinet shared, in addition to daylength, unknown and potentially varying factors. Thus, using individuals as independent observations when testing the effect of the only manipulation (daylength) might produce false significances. In the main experiment, therefore, I used a more conservative approach and tested the effect of daylength and sex on cabinet averages in an analysis of covariance, with daylength as a covariate and sex as a fixed factor. If the effect of the manipulation was significant in this test I could feel quite confident that it was true. After this I used the individual values in an ANCOVA to test if there were any differences between families while controlling for the effect of daylength. Again daylength was used as a covariate while sex and family was treated as fixed factors. This two-step approach was taken to be sure that I tested each factor at a reasonable level of independence. When an interaction between a covariate and a fixed factor was insignificant in an ANCOVA it was removed from the statistical model and the model was retested. To improve on homogeneity of variances, larval development time was 1/X-transformed in the main experiment. This variable has a clear biological interpretation as developmental rate, which, however, should not be confused with growth rate.

In the starvation experiment individuals were used as independent observations. The rate of weight loss was compared between the two daylengths by a one-way ANOVA. Larval development time, pupal weight and growth rate was analysed in two-way ANOVA:s with daylength and starvation treatment as fixed factors. This allowed me to compare the two daylength treatments (13 h and 15 h) and the two starvation treatments (starved and non-starved), but most important, to assess the interaction between these factors. If this interaction is significant it would tell us that an equal change in internal state (starvation) alters developmental decisions differently in the two daylength treatments, indicating that these decisions depend on the "seasonal state" of larvae. All analyses were performed with SYSTAT (Wilkinson et al., 1992).

Results

Diapause strategies

Contrary to expectations, *L. petropolitana* from the southern French Alps primarily uses the pupal stage for winter diapause. In the main experiment the three shortest daylengths (13 h, 14 h, 15 h) induced a pupal diapause in all individ-

Table 1. Propensity of pupal diapause/direct development and life history data (means ± SE) of *L. petropolitana* in six experimental daylength treatments and one outdoor rearing. In treatments with both diapausing and directly developing individuals, the categories are presented separately.

Daylength treatment	13 h	14 h	15 h	16 h		17 h	18 h	Outdoor	
Pupal diapause/ Direct development	22/0	22/0	22/0	17/11		0/17	0/12	5/7	
Females				*diapause*	*direct*			*diapause*	*direct*
Larval time (days)	40.4 ± 0.3	43.4 ± 0.7	49.0 ± 0.7	66.7 ± 6.1	62.2 ± 2.0	86.0 ± 5.4	70.2 ± 5.0	55.5 ± 8.5	45.0 ± 0.0
Pupal weight (mg)	146.4 ± 4.2	162.4 ± 3.5	170.7 ± 3.4	163.6 ± 4.4	164.1 ± 6.2	179.3 ± 5.1	173.9 ± 4.1	161.2 ± 14.6	159.4 ± 2.6
Growth rate (%/day)	15.5 ± 0.2	14.7 ± 0.3	13.1 ± 0.2	9.7 ± 0.8	10.1 ± 0.3	7.6 ± 0.4	9.3 ± 0.5	11.6 ± 1.7	14.1 ± 0.0
Pupal time (days)	–	–	–	–	23.8 ± 0.9	25.8 ± 0.9	24.7 ± 0.6	–	34.0 ± 0.0
N	7	12	12	6	5	11	9	2	2
Males									
Larval time (days)	39.9 ± 1.0	44.4 ± 0.7	50.6 ± 1.3	74.4 ± 4.9	78.5 ± 3.8	90.2 ± 6.1	61.0 ± 4.6	82.3 ± 14.2	45.2 ± 5.3
Pupal weight (days)	141.0 ± 2.8	146.7 ± 2.8	148.2 ± 6.6	159.1 ± 4.1	157.6 ± 3.9	160.2 ± 6.5	170.0 ± 11.0	158.6 ± 1.8	145.1 ± 8.0
Growth rate (%/day)	15.8 ± 0.4	14.1 ± 0.2	12.3 ± 0.3	8.7 ± 0.6	7.9 ± 0.4	7.0 ± 0.6	10.5 ± 0.9	8.1 ± 1.8	13.9 ± 0.6
Pupal time (days)	–	–	–	–	24.0 ± 1.8	22.8 ± 0.9	23.0 ± 0.6	–	27.4 ± 4.5
N	15	10	10	11	6	6	3	3	5

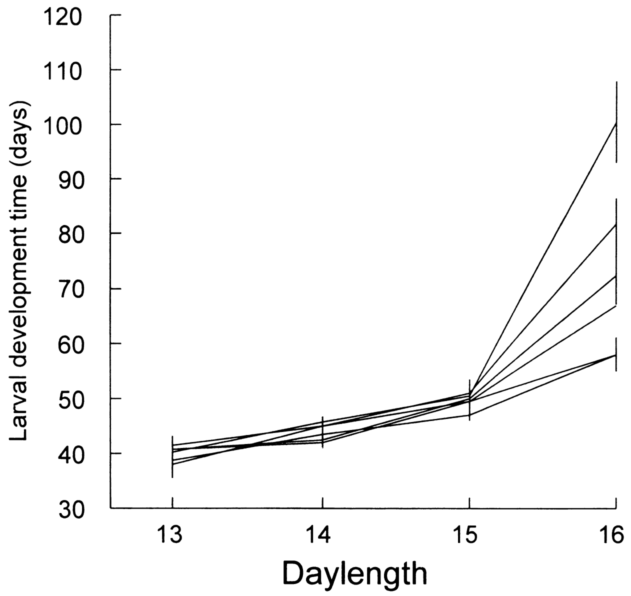

Fig. 2. Family level reaction norms describing larval development time in response to daylength. Only individuals that entered pupal diapause are included. ± 1 SE is given for each family and there is plenty of overlap.

uals, while in 16 h there was a mixed response leading either to pupal diapause or direct development (Tab. 1). In the two longest daylengths (17 h, 18 h) all individuals developed directly. This pattern is very similar to the pattern found in the Swedish populations although the diapause-direct development threshold is shifted towards shorter daylengths (Nylin et al., 1996). The group of larvae that was reared outdoors showed a mixed response of pupal diapause and direct development (Tab. 1). Furthermore, in the short day (13 h), low temperature (13 °C) condition, all surviving individuals (17 out of 20) grew to the pupa and entered diapause in that stage.

Plasticity in time and size at pupation

Larval development time was clearly plastic in relation to the daylengths used in the main experiment and development times were shorter in shorter days (Tab. 1, Fig. 2). Variation in development time was significantly smaller in the three shortest daylengths compared to 16 h and longer days, therefore developmental rate (1/larval development time) was used in subsequent analyses and figures. Since predictions of the shape of the reaction norms are concerned with diapause development I only included individuals that entered pupal diapause in figures and analyses.

Within the developmental pathway leading to diapause, there was a significant effect of daylength on the rate of development, on pupal weight and on average growth rate (Tab. 2). The correlation between developmental rate and daylength was negative and this was true also for growth rate while pupal weight was positively correlated with daylength (Tab. 1, Fig. 3a and b). Back-transforming the result on developmental rate gives a positive relationship between larval development time and daylength. Thus, in shorter days the larvae achieved a smaller size, had shorter larval development times and higher growth rates. The more sensitive test using individual values as independent observations confirmed that sexes differed in pupal weight but not in developmental rate or average growth rate. Furthermore, it showed that there were significant differences between families in developmental rate and pupal weight but not in growth rates (Tab. 3). A closer inspection of Figure 2, however, suggests that the differences between families is mainly due to the 16 h treatment. If this daylength is excluded from the analysis the family effect is still significant on pupal weight but insignificant on developmental rate ($P = 0.673$). In both cases the effect of daylength was still highly significant ($P < 0.01$ in both cases).

Starvation experiment

As in the first experiment, daylength treatment affected larval growth rate, development time and final size of the pupae (Tab. 4). In contrast, the starvation treatment only affected the duration of larval development (Tab. 4), which on average was longer in the starvation group (Tab. 5). Contrary to predictions there was no indication that the faster growth of the short day larvae is traded off against a higher weight loss rate during food shortage (Fig. 4). There was no significant effect of daylength on relative weight loss rate (Tab. 4). Testing the families separately did not change any of the results. On average a larva lost 14.6 ± 0.5% of its weight during the three day period. In absolute figures the average size at the start of the starvation was 34.3 ± 1.3 mg and 29.3 ± 1.1 mg after three day period (mean ± SE). Mortality was not higher in the starvation treatment, only two individuals, one from each daylength, died at some point after starvation.

Table 2. Results of ANCOVA:s on developmental rate, pupal weight and average growth rate, with daylength as a covariate and sex as a fixed factor. Means of sex and daylength treatment are used as independent observations. The interaction was non-significant in all cases and r^2 are for the model without interaction.

Dependent variable	Daylength	Sex	r^2
Developmental rate (day^{-1})	$\mathbf{P < 0.001}$	$P = 0.541$	0.940
Pupal weight (mg)	$\mathbf{P = 0.020}$	$\mathbf{P = 0.027}$	0.807
Growth rate (%/day)	$\mathbf{P = 0.020}$	$P = 0.968$	0.693

df for daylength, sex and error are 1, 1 and 5 respectively in all cases.

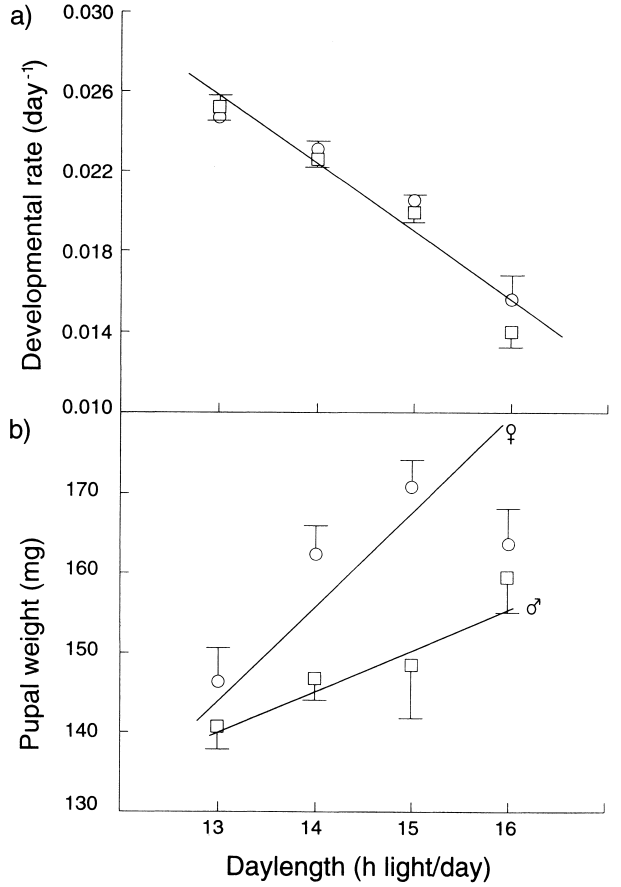

Fig. 3. The effect of daylength on a) developmental rate and b) pupal weight. Circles are female means and squares represent means of males; bars are ±1 SE. Functions are linear regressions and since sexes differed significantly in pupal weight, regression lines are given separately. Note, however, that the difference in slopes is not significant (Tab. 2).

Despite the weight loss, the effect of starvation on the growth trajectories was in general very small. Three days with no food seems just to have transported the larvae along the time axis approximately 3–5 days. After that, larvae picked up on the same growth trajectory they had been following before starvation, reaching full mass about 3–5 days later than the control. Figure 5 shows that within daylengths the slopes of the growth curves is very similar, although shifted to the right for the starvation group. It is quite obvious that the starved larvae did not compensate for the time loss by growing faster (Fig. 5).

Growth rates differed significantly between daylengths already before the start of the starvation treatment (Tab. 4). The total growth trajectories of the controls,

Table 3. Results of ANCOVA:s on developmental rate, pupal weight and average growth rate with daylength as a covariate and family and sex as a fixed factors. Individual measurements are used as independent observations.

Dependent variable	Daylength	Sex	Family	Family × Sex	r^2
Developmental rate (day^{-1})	**$P < 0.001$**	$P = 0.446$	**$P = 0.040$**	$P = 0.485$	0.815
Pupal weight (mg)	**$P < 0.001$**	**$P < 0.001$**	**$P < 0.001$**	$P = 0.968$	0.554
Growth rate (%/day)	**$P < 0.001$**	$P = 0.204$	$P = 0.057$	$P = 0.487$	0.794

df for daylength, sex, family, the interaction and error are 1, 1, 5, 5 and 70 respectively in all cases.

indicate that already after the first moult there are some individuals in the long day treatment that follow a more shallow growth curve (Fig. 6). Most of the differences, however, seems to accumulate later in development, especially in the third and fourth larval instars.

Discussion

All individuals in this study either entered pupal diapause, or developed directly and there was no evidence of larval diapause in *L. petropolitana* from the French Alps. This was counter to expectations based on observations in Switzerland (Benz et al., 1987), but similar to what is known of this species in northern Europe (Wiklund et al., 1983; Nylin et al., 1996). Given the realistic range of daylengths used in the experiments and the fact that the outdoor as well as the low temperature rearing gave the same result, pupal diapause is likely to be the most common diapausing strategy also in the field. It seems, however, probable that in the Alps, the phenology and, thus, the time of reproduction and egg laying depends strongly on

Table 4. Results of the starvation experiment. Statistics from ANOVA:s with daylength and starvation as factors.

Dependent variable	Daylength	Starvation	Starvation × Daylength	r^2
Growth rate before starvation	**$P < 0.001$**	$P = 0.937$	$P = 0.606$	0.433
Growth rate after starvation	**$P < 0.001$**	$P = 0.405$	$P = 0.227$	0.608
Growth rate in the 4th instar	**$P < 0.001$**	$P = 0.527$	$P = 0.798$	0.383
Development time	**$P < 0.001$**	**$P < 0.001$**	$P = 0.541$	0.773
Pupal weight	**$P = 0.003$**	$P = 0.405$	$P = 0.790$	0.181
Weight loss rate	$P = 0.14$			0.090

df for daylength, starvation, the interaction and error are 1, 1, 1 and 47 respectively for all of the five first cases. *df* for weight loss rate are 1 for daylength and 23 for the error term.

Table 5. Means ± SE of larval development times, pupal weights and relative growth rates in the starvation experiment. Total larval growth rate is not given for larvae that have been starved since their growth was interrupted by the starvation treatment.

Daylength treatment	13 h		15 h	
Starvation treatment	Control (N = 13)	Starved (N = 11)	Control N = 13)	Starved (N = 14)
Development time (days)	37.8 ± 1.2	41.3 ± 1.3	51.7 ± 1.2	56.7 ± 1.2
Pupal weight (mg)	155.3 ± 5.3	150.0 ± 4.1	168.7 ± 2.9	166.1 ± 5.4
Relative growth rate	0.197 ± 0.003	–	0.138 ± 0.005	–

altitude. This might explain the observation of larval diapause and the very long flying period (from late April to the middle of July) of *L. petropolitana* documented in other parts of the Alps (Benz et al., 1987).

The mode of diapause together with the expected appearance of larvae in the field (after solstice) predicted that an adaptive reaction norm, relating development time to daylength should have a positive slope (Fig. 1). This prediction was clearly supported here. The reaction norm found was qualitatively very similar to what is known from Sweden (Nylin et al., 1996) but in terms of the diapause-direct

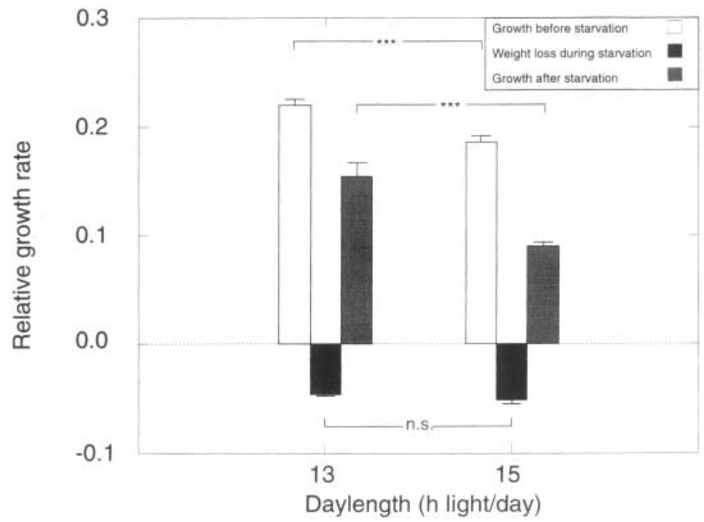

Fig. 4. Results of the starvation experiment including only individuals that was starved in the third larval instar. The two daylength treatments are compared in terms of growth rate (positive) and weight loss rate (negative). Mean ± 1 SE of relative growth rates before starvation (white), weight loss during starvation (black) and growth rate after starvation (grey). *** = $P < 0.001$ and n.s. = non significant differences in a one way ANOVA; N-values are 11 and 14 for the 13 h and 15 h treatments, respectively.

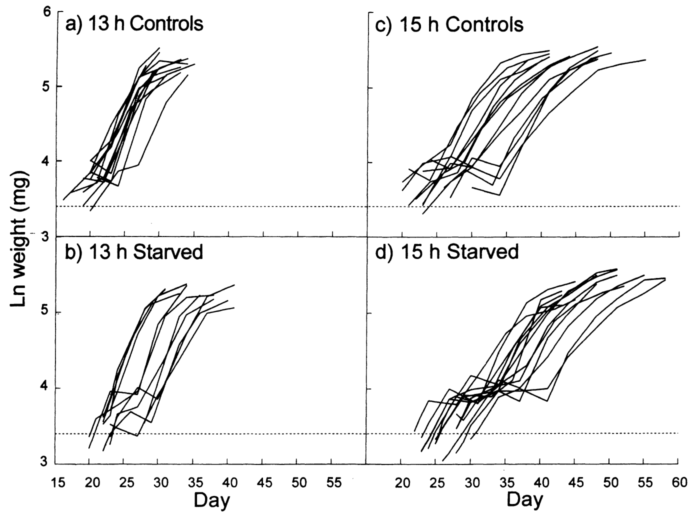

Fig. 5. Individual growth curves for growth after starvation, excluding the weight loss prior to pupation. The two upper graphs (a, c) show the unstarved controls in each daylength. The two lower graphs (b, d) depict individuals that have been starved. The horizontal dotted line show the average larval weight at the end of three days of starvation.

development threshold it was quantitatively different. In the population investigated here there was a clear threshold at 16 h light per day, which was the only daylength in the experiment that induced a mixed response (Fig. 2, Tab. 1). In the Swedish populations the shortest day to induce direct development was 19 h. Latitudinal clines in photoperiod thresholds, determining the diapause-direct developmental decision, is a quite well known phenomenon in insects and is good evidence of local adaptation (Danilevskii, 1965; Bradshaw, 1976, 1990; Masaki, 1978; Blanckenhorn and Fairbairn, 1995; Bradford and Roff, 1995).

It should be noted that *L. petropolitana* in Sweden probably never develops directly in the field. The situation in the Alps seems to be very similar although a few individuals may develop directly in exceptional years (Benz et al. 1987). However, it is not the ability to "choose" alternative developmental pathways that constitutes a local adaptation. This ability is most likely an adaptation at a much higher level in the phylogeny of the butterflies. Indeed, the adaptive value of the threshold in Sweden and probably in most of the Alps is that it totally prevents individuals from developing directly, which in most cases would mean that no offspring will survive. The pattern described here may reflect how natural selection has pushed the daylength threshold into a range of daylengths that hardly ever is experienced at a given location when the diapause-direct development decision is taken. At that point it no longer affects the phenotypic expression and escapes selection. Interestingly, the variation between families in development time showed a sudden increase in 16 h (Fig. 2) and longer daylengths (not shown) which also indicate that selection is very weak if present at all, in that range of daylengths.

Because of the difference in daylength regimes in northern and southern Europe the threshold is "hidden" from selection at a much shorter day in the Alps than in Scandinavia. Thus, this quantitative difference between the populations in "fine-tuning" of the photoperiod threshold can be viewed as being adaptive and reflecting the action of natural selection.

The model of Abrams et al. (1996) investigates the optimal response of size, development time and growth rate to changes in the amount of time available for completion of the life cycle. It assumes that fitness depends on adult size, juvenile survival and the timing of reproduction or diapause in respect to seasonality. Unlike most similar models it also assumes that individuals can adaptively vary their growth rates by balancing it against juvenile mortality. All these assumptions seem reasonable in the case of *L. petropolitana*. The model predicted that an adaptive decrease in larval development time typically ought to be associated both with an increase in growth rate and with a decrease in size at metamorphosis. In the French *L. petropolitana*, short larval developmental periods were correlated with low pupal weights and high growth rates (Tab. 1. and Fig. 3a, b). The starvation experiment confirmed this and in the shortest day (13 h) growth was faster during the whole larval period (Tab. 5. and Fig. 6). Thus, it seems as if the population of *L. petropolitana* investigated here confirm the predictions. Individuals in short days have short larval developmental times at the expense of a smaller size, but probably also at the expense of being forced to adopt more risky foraging strategies that can support the faster growth (Werner and Anholt, 1993; Abrams et al., 1996). The trade-off between growth rate and starvation endurance that has been shown in other species of Lepidoptera (Stockhoff, 1991; Gotthard et al., 1994) was, however, not found here. This could perhaps be explained by methodological differences

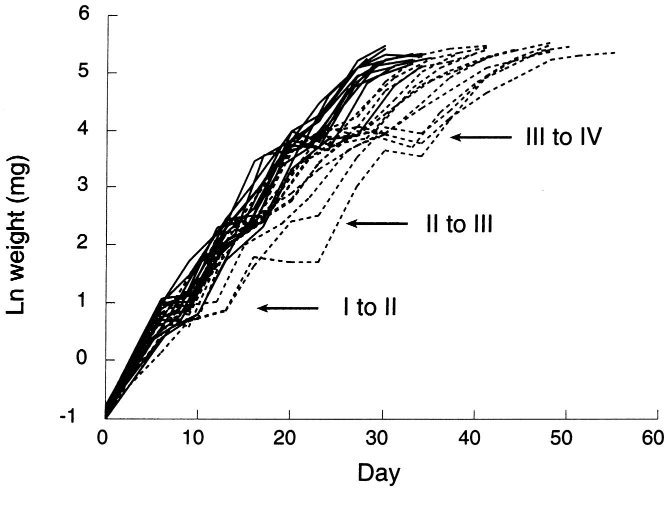

Fig. 6. Individual growth curves for the unstarved controls, showing the whole larval period except for the weight loss prior to pupation. Heavy lines mark larvae from the 13 h treatment and dotted lines represent the 15 h treatment. Arrows indicate where moulting to new instars occurred.

since the period of starvation was at least two times as long (6 days) in these studies. The results are largely in good agreement with what was found in the two Swedish populations of *L. petropolitana* (Nylin et al., 1996). Interestingly, not only the diapause-direct development threshold but the whole reaction norm is shifted towards daylengths that are relevant to southern Europe. The similarity between populations in plasticity supports the notion that this reaction norm in general is an adaptation to seasonality. Moreover, the population differences indicate that this adaptation is locally adjusted to fit the cues of seasonal change at each location.

The results of the starvation experiment suggest that a three day period with total lack of food in principle does not alter any developmental decisions in *L. petropolitana*. The treatment obviously altered the intrinsic status of the larvae in terms of weight and nutritional status and on average delayed them between 3.5 and 5 days in time to pupation. A comparison of this difference in development time with the ones induced by daylength in the main experiment show that it is approximately equal to the time difference of the two shortest days (Tab. 1. 13 h and 14 h).

If one assumes that the time lost due to the starvation would represent a real cost in a natural situation it is surprising that the starved larvae did not compensate by growing faster when they were allowed to feed again. The fact that post-starvation growth rate on average was much faster in the short daylength than in the long daylength (Fig. 4, Tab. 4), shows that at least the long day larvae could have grown faster. It appears as if the larvae are not physiologically constrained from growing faster, unless they quite early in development "decide" what growth trajectory to follow and after this are unable to change their growth rates. Evidence from a closely related species, however, indicate that this is not the case (Nylin et al., 1989). More likely, my results suggest that in this particular experimental situation larvae were constrained in the retrieval of information. In the absence an internal clock the larvae must depend on the daylength and some indicator of intrinsic state (i.e., larval instar or size) for estimating how much time they have left before they need to pupate. Daylengths in these experiments have been held constant within daylength treatments and consequently all larvae, starved or unstarved, were provided with the same external information. Hence, a new decision could only be taken on the basis of the internal parameter. A 14% weight loss was evidently not a big enough manipulation of internal state to alter any developmental decisions after starvation but it is possible that a larger weight loss would. On the other hand, if larval instar is the internal indicator of developmental progression we should not expect larvae experiencing the conditions given here to compensate, because they simply do not know that they have lost time. In nature, obviously, daylengths are not constant and if larvae take in information late in the larval development, a larva that for any reason has lost time would know from the daylength alone.

Given that the time loss is not trivial in a natural situation these results further emphasises that *L. petropolitana* larvae rely heavily on the daylength for taking developmental decisions. To what degree these decisions are state dependent is not entirely clear, but a growth model assuming that larvae continuously sample their internal status and more or less immediately adjust growth accordingly seems unlikely. It is possible that in an environment with very large seasonal variation, some quite crude aspect of internal state, such as larval instar, together with good information of date at one or a few occasions during growth is enough for all developmental decisions a growing butterfly larva has to make.

In addition to constant daylengths, all rearings reported here were done in constant temperatures. There is evidence from a tropical butterfly that fluctuating temperatures per se can have and effect on life history traits (Brakefield and Mazzotta, 1995). It seems, however, not to be a serious problem here because the animals that were reared outdoors in fluctuating temperatures were quite similar to the laboratory reared butterflies that also grew in very long daylengths (Tab. 1).

The general prediction tested in this study is that insects living in seasonal environments should shorten development time when the amount of time available for growth decreases, and that they should use daylength as a cue to determine the date of the season. This prediction has been suggested based on both verbal (Nylin et al., 1989, 1996; Nylin, 1994; Leimar, 1996) and more formal, mathematical optimality models (Abrams et al., 1996) and in this study it was corroborated. Environmental changes between seasons are typically both predictable and large and can be expected to select for plasticity in life history traits. Therefore, plasticity in relation to different aspects of seasonal change is a promising field for investigating the adaptive significance of reaction norms (Nylin, 1994; Gotthard and Nylin, 1995).

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References

Abrams, P. A., O. Leimar, S. Nylin and C. Wiklund. 1996. The effect of flexible growth rates on optimal sizes and development times in a seasonal environment. Am. Nat. 147: 381–395.

Beck, S. D. 1980. Insect Photoperiodism. (2nd ed.). Academic Press, New York and London.

Benz, E., R. Bryner, H. Buser, W. Ettmüller, W. Geiger, R. Joos, B. Jost, D. Jutzeler, E. Pleisch, L. Reser, H. Schiess-Bühler, J. Schmid, P. Sonderegger, E. Stierli, H.-P. Wymann and H. Ziegler. 1987. Tagfalter und Ihre Lebensräume. Schweizerischer Bund für Naturschutz, Basel.

Blanckenhorn, W. U. and D. J. Fairbairn. 1995. Life history adaptation along a latitudinal cline in the water strider Aquarius remigis (Heteroptera: Gerridae). J. evol. biol. 8: 21–41.

Bradford, M. J. and D. A. Roff. 1995. Genetic and phenotypic sources of life history variation along a cline in voltinism in the cricket allonemobius socius. Oecologia 103: 319–326.

Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics 13: 115–155.

Bradshaw, W. E. 1976. Geography of photoperiodic response in a diapausing mosquito. Nature 262: 384–385.

Bradshaw, W. E. 1990. The present and future of insect life-cycle evolution, pp 243–251. *In* F. Gilbert (Ed.), Insect Life Cycles. Springer-Verlag, London.

Brakefield, P. M. and V. Mazzotta. 1995. Matching field and laboratory environments: effects of neglecting daily temperature variation on insect reaction norms. J. evol. biol. 8: 559–573.

Danilevskii, A. S. 1965. Photoperiodism and Seasonal Development of Insects. Oliver & Boyd, London.

Danks, H. V. 1994. Diversity and integration of life-cycle controls in insects, pp 5–40. *In* H. V. Danks (Ed.), Insect Life-cycle Polymorphisms. Kluwer Academic Publishers, Dordrecht.

Gotthard, K. and S. Nylin. 1995. Adaptive plasticity and plasticity as an adaptation: A selective review of plasticity in animal morphology and life history. Oikos 74: 3–17.

Gotthard, K., S. Nylin and C. Wiklund. 1994. Adaptive variation in growth rate – life history costs and consequences in the speckled wood butterfly, *Pararge aegeria*. Oecologia 99: 281–289.

Grafen, A. 1988. On the uses of data on lifetime reproductive success, pp 454–471. *In* T. H. Clutton-Brock (Ed.), Reproductive Success. The University of Chicago Press, Chicago.

Higgins, L. G. and B. Hargreaves. 1983. The Butterflies of Britain and Europe. William Collins Sons, London, England.

H.M.S. Stationary Office. 1972. Tables of temperature, relative humidity and precipitation for the world. III Europe and the Azores.

Houston, A. I. and J. M. McNamara. 1992. Phenotypic plasticity as a state-dependent life-history decision. Evol. Ecol. 6: 243-253.

Leimar, O. 1996. Life history plasticity: influence of photoperiod on growth and development in the common blue butterfly. Oikos 76: 228–234.

Masaki, S. 1978. Seasonal and latitudinal adaptations in the life cycles of crickets, pp 72–100. *In* H. Dingle (Ed.), Evolution of Insect Migration and Diapause. Springer-Verlag, New York.

Newman, R. A. 1992. Adaptive plasticity in amphibian metamorphosis. BioScience 42: 671–678.

Nylin, S. 1992. Seasonal plasticity in life history traits: growth and development in *Polyonia c-album* (Leipidoptera: Nymphalidae). Biol. J. Linn. Soc. 47: 301–323.

Nylin, S. 1994. Seasonal plasticity and life-cycle adaptations in butterflies. pp 41–67. *In* H. V. Danks (Ed.), Insect Life-cycle Polymorphism. Kluwer Academic Publishers.

Nylin, S., K. Gotthard and C. Wiklund. 1996. Reaction norms for age and size at maturity in Lasiommata butterflies: predictions and tests. Evolution 50: 1351–1358.

Nylin, S., P.-O. Wickman and C. Wiklund. 1989. Seasonal plasticity in growth and development of the speckled wood butterfly, *Pararge aegeria* (Satyrinae). Biol. J. Linn. Soc. 38: 155–171.

Nylin, S., P. O. Wickman and C. Wiklund. 1995. Life-cycle regulation and life history plasticity in the speckled wood butterfly: Are reaction norms predictable? Biol. J. Linn. Soc. 55: 143–157.

Reavey, D. and J. H. Lawton. 1991. Larval contribution to fitness in leaf-eating insects, pp 293–329. *In* W. J. Bailey, J. Ridsdill-Smith (Eds.), Reproductive Behaviour of Insects. Chapman & Hall, London.

Roff, D. A. 1992. The Evolution of Life Histories. Chapman & Hall, New York.

Scheiner, S. M. 1993. Genetics and evolution of phenotypic plasticity. Ann. Rev. Ecol. Syst. 24: 35–68.

Sibly, R. M. and D. Atkinson. 1994. How rearing temperature affects optimal adult size in ectotherms. Funct. Ecol. 8: 486–493.

Stearns, S. C. 1992. The Evolution of Life Histories (1st ed.). Oxford University Press, Oxford.

Stearns, S. C. and J. C. Koella. 1986. The evolution of phenotypic plasticity in life-history traits: predictions of reaction norms for age and size at maturity. Evolution 40: 893–913.

Stockhoff, B. A. 1991. Starvation resistance of gypsy moth, *Lymantria dispar* (L.) (Leipidoptera: Lymantriidae): tradeoffs among growth, body size, and survival. Oecologia 88: 422–429.

Tauber, M. J., C. A. Tauber and S. Masaki. 1986. Seasonal Adaptations of Insects. Oxford University Press, Oxford.

Via, S., R. Gomulkiewicz, G. Dejong, S. M. Scheiner, C. D. Schlichting and P. H. Van Tienderen. 1995. Adaptive phenotypic plasticity: Consensus and controversy. Trend. Ecol. Evolut. 10: 212–217.

Werner, E. E. and B. R. Anholt. 1993. Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. 142: 242–272.

Wickman, P.-O., C. Wiklund and B. Karlsson. 1990. Comparative phenology of four satyrine butterflies inhabiting dry grasslands in Sweden. Holarc Ecol. 13: 238–346.

Wiklund, C. and J. Forsberg. 1991. Sexual size dimorphism in relation to female polygamy and protandry in butterflies: a comparative study of Swedish Pieridae and Satyridae. Oikos 60: 373–381.

Wiklund, C., A. Persson and P.-O. Wickman. 1983. Larval aestivation and direct development as alternative strategies in the speckled wood butterfly, *Pararge aegeria*, in Sweden. Ecol. Entomol. 8. 233–238.

Wilkinson, L., M. Hill, S. Miceli, P. Howe and W. Vang. 1992. SYSTAT. 5.2. SYSTAT, Inc., Evenston, Illinois.

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