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The effects of environmental variation on a mechanism that controls insect body size

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ABSTRACT

Adult body size in animals is determined by the duration of the growth period and the amount of mass gained during that period. Few models of body size regulation distinguish between these two components or explicitly address the mechanisms that control the duration of the growth period. Body size in the tobacco hornworm *Manduca sexta* is controlled by three underlying physiological factors: growth rate; timing of the onset of juvenile hormone decay (which initiates the processes leading to pupation), as measured by the critical weight; and the timing of prothoracicotropic hormone (PTTH, which stimulates ecdysteroid secretion) and ecdysteroid secretion, as measured by the interval to cessation of growth (ICG, the time interval between the attainment of the critical weight and entry into the pre-pupal wandering stage). The critical weight and the ICG determine the duration of the growth period, while growth rate determines how much mass accumulates during that period. We studied how phenotypic plasticity of body size in *M. sexta*, in response to variation in temperature and diet quality, is affected by phenotypic plasticity of these three physiological determinants of body size. We show that plasticity of size in response to diet quality is regulated by variation in growth rate and critical weight, while plasticity of size in response to temperature is regulated by variation in growth rate and the ICG. These results demonstrate the importance of the timing of hormonal events in the regulation of phenotypic plasticity. We suggest that the differential sensitivity of the physiological processes that regulate body size may enable insects to adjust adult body size in response to simultaneous variation in multiple types of environmental stimuli.

Key words: body size, critical weight, endocrine regulation, growth rate, interval to cessation of growth, *Manduca sexta*.

INTRODUCTION

The ecology and evolution of body size in animals has been of interest to biologists because of the dramatic effects that body size has on fitness and its correlates (Calder, 1984; Schmidt-Nielsen, 1984; Stearns, 1992; Roff, 1992; and references therein). Phenotypic plasticity of body size is thought to be an important mechanism by which organisms can

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increase fitness in response to short-term environmental variation. As a consequence, much work has been done to understand the ecological and evolutionary implications of plasticity of body size (Bradshaw, 1965; Schlichting, 1986; Scheiner, 1993; Schlichting and Pigliucci, 1998; Pigliucci, 2001; Roff, 2002) and which has been found to be adaptive in several studies (e.g. Pfennig, 1992; Blanckenhorn, 1998; Gotthard, 1998). Yet, we do not have a clear understanding of the mechanisms by which organisms translate environmental signals into either a particular body size or into a reaction norm of body size (Stern, 2001).

For organisms with determinate growth, final body size is determined by two factors: (1) the duration of the period available for growth and (2) the growth rate, which determines the mass (or linear size) gained during this period. Much previous work on understanding body size regulation has focused on growth rate (e.g. Simpson and Raubenheimer, 1995; Abrams *et al.*, 1996; Kingsolver and Woods, 1997, 1998).

To understand how growth rate affects size, it is necessary to distinguish between the rate at which cells differentiate to produce distinct tissues (differentiation rate) and the rate at which individual cells within a tissue grow (growth rate) (Arendt, 1997). Van der Have and de Jong (1996) proposed that the difference in temperature coefficients of growth rate and differentiation rate can explain the effects of temperature on ectotherm body size. Genetic studies have demonstrated that the regulation of cell size and of cell number can be caused by mutations in the insulin signalling pathway (Oldham *et al.*, 2000; Brogiolo *et al.*, 2001), suggesting that insect body size is regulated by insulin signalling. Nijhout (2003b) proposed a mechanism by which tissues simultaneously secrete a growth activator (such as an insulin-like molecule) as well as a growth inhibitor. Final tissue size is controlled by the relative rates of activator and inhibitor synthesis and breakdown and the rate of growth-activator stimulated growth. Unspecified cellular mechanisms, in which body size is controlled by the independent regulation of the size of cells and the number of cells in a tissue, have been proposed by Partridge *et al.* (1994), Van Voorhies (1996), De Moed *et al.* (1997) and Sibly and Atkinson (1994).

Knowing the rates of growth and differentiation is not sufficient, however, to understand how body size is regulated. An individual with a particular growth rate may become either large or small, depending on how long the individual grows: a slow-growing individual may become very large given enough time, and a rapidly growing individual may be small if its growth period is truncated (e.g. Blanckenhorn, 1999). Therefore, understanding body size regulation also requires an understanding of the mechanism that regulates the termination of the growth period. For many organisms with determinate growth, the duration of the growth period ends with the onset of the adult stage or with first reproduction. For example, growth in holometabolous insects ceases with pupation and metamorphosis (Chapman, 1998) and individual size in monocarpic plants reaches its peak at the time of bolting or flowering (Harper, 1977).

Recently, D'Amico *et al.* (2001) demonstrated that the evolution of body size (a 50% increase over 230 generations) in a laboratory colony of the tobacco hornworm, *Manduca sexta*, could be attributed to changes in three of the underlying physiological factors that affect growth rate and the timing of the cessation of growth at the end of larval life. Evolutionary changes in growth rate, in the critical weight (the size at which juvenile hormone synthesis ceases) and in the interval to cessation of growth (ICG) – the interval between the critical weight and the secretion of the prothoracicotropic hormone (which terminates growth and induces entry into the pre-pupal wandering stage) – accounted for over 95% of this evolutionary increase in body size. The critical weight and the ICG

together determine the endpoint of the growth period of the caterpillar and thus the duration of the growth period.

Davidowitz *et al.* (2003) examined one of these three components, the critical weight, which controls the onset of juvenile hormone decay and the cascade of hormonal events that culminate in the cessation of larval growth and metamorphosis (see below). They showed that the critical weight changed in response to diet quality but not in response to temperature, indicating that different environmental stimuli may affect phenotypic plasticity through different mechanisms. Although there was significant genetic variation for body size, critical weight and plasticity of body size, there was no genetic variation for *plasticity* of critical weight. Thus, the critical weight, while playing a key role in the regulation of body size, may at the same time act as a constraint on the evolution of plasticity of body size.

Insects generally grow to smaller sizes at higher temperatures (Atkinson, 1994) and on lower quality diets (Chapman, 1998). Yet, how these environmental signals alter the developmental program to produce smaller individuals is still unknown. It is also unclear how environmental variation in temperature and diet quality is translated by the physiological and developmental program into phenotypic plasticity of body size. Here, we address these issues by building on the work of D'Amico *et al.* (2001) and Davidowitz *et al.* (2003). We explore how variation in temperature and diet quality changes the three factors that control body size, and how variation in these factors affects the plasticity of body size.

Background: the control of the timing of metamorphosis and body size in *Manduca sexta*

Manduca sexta is a large hawkmoth that inhabits a diversity of habitats, including the deciduous forests of Central America, the Southwestern North American desert washes and farmlands in the Eastern United States and southern Canada. As with other insects, adult *M. sexta* do not grow. In *M. sexta*, total development time (from hatching to the pre-pupal stage) under standard laboratory rearing conditions is 18.98 ± 0.08 days (mean ± standard error). Of this, 5 days (4.97 ± 0.03) are spent in the final (fifth) instar. The colony with which we worked is described below in the Methods section. During the fifth instar, larvae grow from about 1.2 to 12 g, so that 90% of the increase in mass occurs during this last larval instar (D'Amico *et al.*, 2001; Davidowitz *et al.*, 2003). After reaching the 12 g peak mass, larvae purge their gut contents, enter the wandering stage and form a pupa about 6 days later. The pupae weigh, on average, 54% of the peak larval mass.

In fifth instar larvae, there is a close causal association between somatic growth and the timing of various endocrine events that induce the onset of metamorphosis (Nijhout and Williams, 1974a,b; Nijhout, 1981). During the fifth instar, secretion of prothoracicotropic hormone (PTTH) and ecdysteroids is inhibited by the presence of juvenile hormone (Nijhout and Williams, 1974b; Rountree and Bollenbacher, 1986). The circulating concentration of juvenile hormone is high during the first few days of the instar, but drops precipitously when the larva reaches a specific critical weight (Nijhout and Williams, 1974b). Attainment of a critical weight causes the corpora allata, the glands that synthesize and secrete juvenile hormone, to switch off. During this period, the activity of juvenile hormone esterase in the haemolymph increases and enhances the rate of degradation of juvenile hormone. A few days after larvae pass the critical weight, their juvenile hormone is fully cleared from the haemolymph and secretion of PTTH and ecdysteroids is disinhibited

(Nijhout and Williams, 1974b; Rountree and Bollenbacher, 1986). The timing of secretion of PTTH is governed by a photoperiodic clock and can occur only during a well-defined window of time, called a photoperiodic gate, that recurs each day at the same time. Secretion of PTTH occurs during the first photoperiodic gate that follows after complete clearance of juvenile hormone (Truman, 1972; Truman and Riddiford, 1974). The secretion of PTTH and ecdysone leads to an immediate cessation of growth and initiates a pre-pupal wandering behaviour during which the larva seeks an appropriate site for pupation. The photogate is thought to ensure that the insects wander at night, presumably to avoid predators (Chapman, 1998). We term the time interval between the attainment of the critical weight and the secretion of PTTH, the interval to cessation of growth (ICG).

Prothoracicotropic hormone stimulates the secretion of ecdysteroids; these cause the larva to stop feeding, and induce the switchover to pupal commitment and, a few days later, the metamorphic moult (Riddiford, 1985; Nijhout, 1994). Larval growth thus stops when the sequence of events initiated by the critical weight culminates in secretion of ecdysteroids. The final size of the larva is determined by how the critical weight and the delay time required for the clearance of juvenile hormone interact with the growth rate of the larva. The growth rate of the larva after the critical weight has been reached determines how much bigger the larva will grow before PTTH is secreted (under standard rearing conditions, larvae nearly double their weight during this period).

It would appear that three factors control the timing of the gut purge in *M. sexta* and the size that a larva has attained at that time: (1) The *growth rate* of the last instar larva, which determines how much weight an individual can gain in a given time period. (2) The *critical weight*, which is the mass at which juvenile hormone secretion stops and sets in motion the events that lead to disinhibition of PTTH secretion. (3) The *interval to cessation of growth* (ICG) after the critical weight. The length of this interval is determined by two factors, the time required to eliminate traces of juvenile hormone, plus the subsequent time required until the opening of the next photoperiodic gate. The time required to eliminate traces of juvenile hormone is determined in part by the activity of juvenile hormone esterase (Browder *et al.*, 2001). The photoperiodic gate for PTTH release only occurs during a specific window of time each day, defined by the light : dark cycle of photoperiod. The timing and duration of this photoperiodic gate for PTTH secretion determines when this hormone is secreted. During the ICG, the larva continues to grow and therefore variation in the ICG can have a great effect on the final size the larva attains (the larva almost doubles in mass during this period).

Evolutionary changes in these three factors accounted for over 95% of the increase in body size in response to inadvertent laboratory selection for increased body size (D'Amico *et al.*, 2001). In the present study, we focus on how changes in these three factors in response to environmental variation in diet quality and temperature affect phenotypic plasticity in body size.

MATERIALS AND METHODS

The study colony of *M. sexta* was created by outcrossing laboratory colonies obtained from the University of Washington, the University of Arizona and North Carolina State University. The colony from the University of Washington was that used by D'Amico *et al.* (2001). To minimize maternal effects, all experiments were conducted a minimum of eight generations after the study colony was established. We tested how much of the variation in

adult size can be attributed to peak larval size by regressing the wet mass of newly eclosed moths on their peak larval weight before pupation. This regression was done for males and females separately because of sexual dimorphism in size (male moths are 8% lighter than females).

Diet and temperature treatments

Larvae were maintained at a 16 : 8 light : dark photoperiod and a constant temperature of 25°C (standard rearing conditions) and fed on the standard diet (Davidowitz *et al.*, 2003), except where noted. Larvae used in this study were taken at random from the stock population. Genetic variances and correlations between size and the three factors will be reported elsewhere. All larvae were kept in these conditions from hatching through the fourth instar and placed in the relevant experimental treatment for the fifth (last) instar only. For each treatment we measured body size, critical weight, ICG and growth rate (see below). To estimate the effect of temperature, we measured each of the four traits at three temperatures: 20°C, 25°C and 30°C. All last instar larvae in these experiments were fed the standard rearing diet *ad libitum*. To estimate the effect of food quality, fifth instar larvae were reared at 25°C and fed 100% (which is the standard rearing diet: Davidowitz *et al.*, 2003), 60% or 40% diets *ad libitum*. The nutritional components of these diets were reduced by the appropriate percentages, so that a 60% diet contained 60% of the nutrients per gram of diet, with the remaining bulk made up by adding 50% cellulose (Alphacel, ICN) as non-nutritive bulk. The vitamin, antibacterial and antifungal components were identical in all three diets. Diet formulae are given in Davidowitz *et al.* (2003). Each diet and temperature experiment was conducted independently of all others, except that the 25°C and 100% diet experiments are the same data set. We tested for significant differences between diets and between temperatures independently for body size, ICG and growth rate using a one-way analysis of variance (ANOVA). Pairwise comparisons were tested using a Tukey-Kramer *post-hoc* pairwise comparison test.

Measurements

When larvae reach the critical weight, an irrevocable series of physiological events is set in motion that determine the timing of ecdysone secretion and therefore the timing of metamorphosis. This sequence of events is independent of further growth or nutrition. The critical weight is therefore operationally defined as the minimal weight at which further growth is not necessary for a normal *time course* to metamorphosis. Data for critical weight were taken from Davidowitz *et al.* (2003). All other data reported here were taken concurrently with those reported in Davidowitz *et al.* (2003).

The interval to cessation of growth is the time interval from when the larva attains its critical weight until the secretion of PTTH and ecdysteroids. It is measured in the critical weight experiment as the number of days to PTTH release *at* the critical weight (termed the PTTH delay time by D'Amico *et al.*, 2001, Davidowitz *et al.*, 2003).

Growth rate was measured as grams per day during the last 2 days of growth before the secretion of PTTH. During this time, growth is linear. During the fifth instar, we measured growth rates at nine temperatures between 10°C and 30°C and found that the relationship between growth rate and temperature was linear ($y = -0.89 + 0.13x$; $n = 9$, $r^2 = 0.98$, $F = 416.4$, $P < 0.0001$) over this range. The proportional relationship between temperature

and growth rate (*gr*) can therefore be expressed as: $gr = a^*t - b$ or $gr = a^*(t - b/a)$, where a and b are constants and t is temperature. The constant b/a is the x-intercept (i.e. the temperature axis intercept) of the regression of growth rate on temperature, and represents the hypothetical temperature at which growth rate would be zero. In this paper, we refer to the constant b/a as T_0, the threshold temperature, because it is the highest temperature at which growth rate is expected to be zero.

Some authors determine T_0 from the x-intercept of the regression of development *rate* on temperature (Berven *et al.*, 1979; Pritchard *et al.*, 1996; Chapman, 1998; Blanckenhorn, 1999). In so far as development rate is difficult to define and quantify, it is typically approximated by the inverse of development time. In the present case, development *time* (*dt*) is the time required to complete growth in the fifth instar, and can also be described as $dt = K/(t - T_0)$, where K is a constant. Rearranging this equation we obtain $K = dt^*(t - T_0)$, indicating that the product of development time and the amount to which ambient temperature is above threshold is a constant. K is referred to as the 'thermal constant' with units of degree-days (Wigglesworth, 1972). Because of the linearity of the relationship between growth rate and temperature, development will take a fixed number of degree-days (given by the value of K), independent of the temperature at which the animal is reared. We estimated T_0 by measuring the growth rates of 12 fifth instar larvae at each of six temperatures between 10°C and 17°C and, using the regression equation of growth rate on temperature, calculated T_0. Differences in the thermal constants at different temperatures were determined using a one-way ANOVA. Pairwise comparisons were tested using a Tukey-Kramer *post-hoc* pairwise comparison test.

RESULTS

As is the case in other insects, the larvae of *M. sexta* grew significantly larger at lower temperatures and on higher quality diets (Fig. 1, Table 1). Davidowitz *et al.* (2003) showed that the critical weight was lower on lower quality diets and was apparently no longer functional in larvae reared on a very poor (40%) diet, as there was no weight at which the fed and starved treatments diverged (Fig. 2). In such slow-growing larvae, the timing of PTTH secretion was presumably regulated by the same (yet unknown) physiological processes that control PTTH secretion in larvae starved below the critical weight.

The critical weight did not change with temperature, but remained constant at 7.0 g across the 10°C range (Fig. 2). The ICG increased significantly with decreasing temperatures but did not change with changes in diet (Fig. 3, Table 1).

Growth rate varied significantly with variation in both diet or temperature and was higher at higher temperatures and lower on lower quality diets. (Fig. 4, Table 1). The minimal threshold temperature for growth (T_0) was 7.8°C ($y = -1.23 + 0.16x$; $n = 6$, $r^2 = 0.99$, $F = 377.2$, $P < 0.0001$).

The thermal constant was 97.08 ± 2.7 degree-days at 20°C, 105.92 ± 0.9 degree-days at 25°C and 105.99 ± 1.3 degree-days at 30°C. The one-way ANOVA showed significant differences in the thermal constants (ANOVA: d.f. = 165, $F = 8.57$, $P = 0.0003$) and the *post-hoc* test showed that the thermal constant at 20°C differed significantly from those at 25°C and 30°C, which did not differ from each other. Regression analysis revealed that variation in peak larval mass explained 64% of the variation of female moth size ($y = 0.24 + 0.22x$; $n = 30$, $r^2 = 0.64$, $P < 0.0001$), but only 33% of the variation in male moth size ($y = -0.77 + 0.33x$; $n = 32$, $r^2 = 0.33$, $P = 0.0006$).

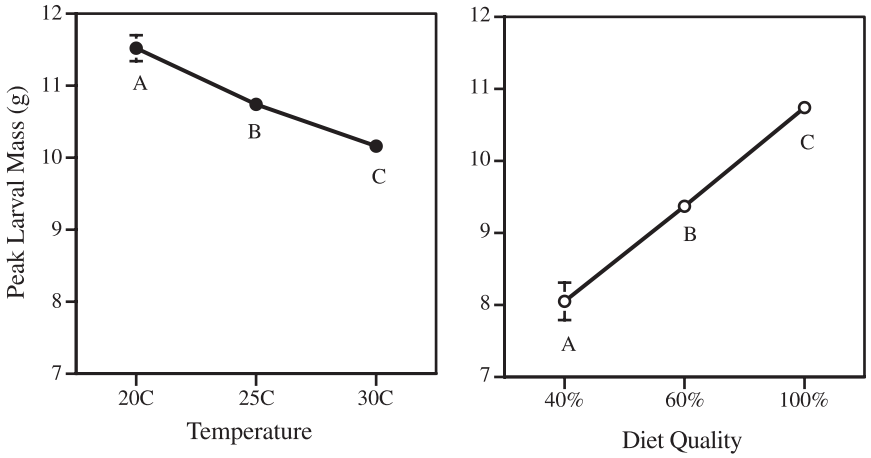

Fig. 1. Reaction norms of peak larval mass of last instar *Manduca sexta* larvae reared under different temperatures and diets. All larvae were reared on standard rearing diet (100%) at 25°C from hatching until the moult to the last instar. Error bars are one standard error of the mean and in some cases are smaller than the symbol. Different letters indicate treatments that differ significantly at $\alpha = 0.05$ (Tukey-Kramer pairwise comparison). Redrawn after Davidowitz *et al.* (2003).

Table 1. Results of one-way ANOVA of body size, interval to cessation of growth (ICG) and growth rate for each of the diet and temperature treatments (see text)

Trait	Treatment	d.f.	MS	*F*	*P*
Body size	diet	124	48.47	46.45	<0.0001
	temperature	165	24.99	20.18	<0.0001
ICG	diet	82	1.83	1.70	0.1955
	temperature	109	46.18	39.33	<0.0001
Growth rate	diet	124	25.53	232.76	<0.0001
	temperature	165	22.31	127.90	<0.0001

Note: Each analysis is independent of all others. The analysis for diet of the ICG includes only the 60% and 100% diets (see text).

DISCUSSION

It is a truism that body size is the product of the amount of time available for growth and the rate at which mass (or linear size) is accumulated during that period. This point has widely been noted (e.g. Atkinson, 1994; Partridge and French, 1996; Blanckenhorn, 1998; Gotthard, 1998; Stern, 2001; Nijhout, 2003b) and underlies one of the most important life-history trade-offs, that between age and size at maturity (Roff, 1992, Stearns, 1992). Most work in the past has focused on the regulation of the growth rate, while much less attention has been paid to the mechanisms that control the duration of the growth period.

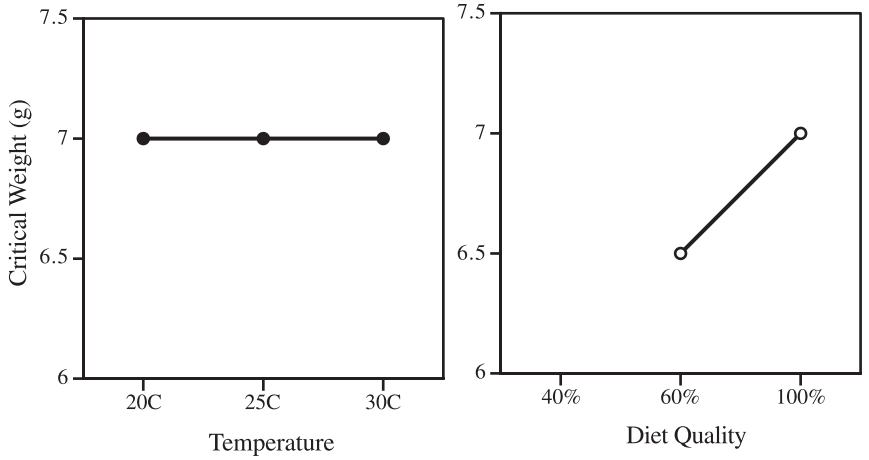

Fig. 2. Reaction norms of critical weight of last instar *Manduca sexta* larvae reared under different temperatures and diets. On very poor diets (40%) the critical weight is not functional (see text). Redrawn after Davidowitz *et al.* (2003).

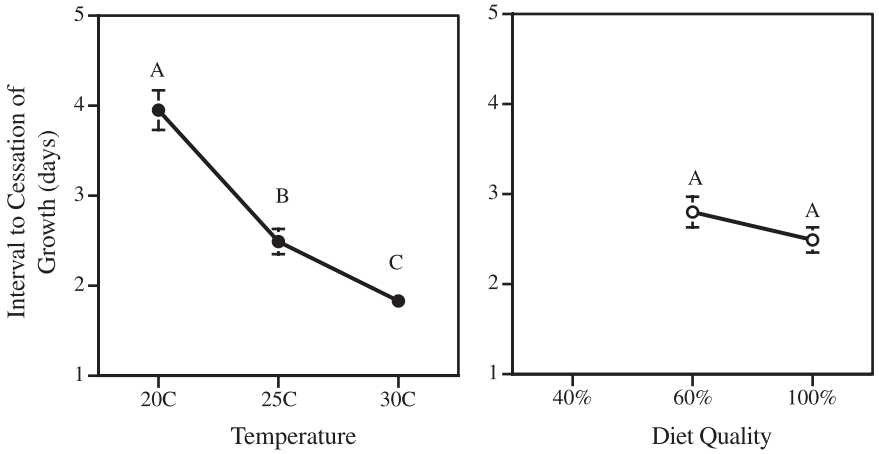

Fig. 3. Reaction norms of the interval to cessation of growth of last instar *Manduca sexta* larvae reared under different temperatures and diets. Error bars are one standard error of the mean and in some cases are smaller than the symbol. Different letters indicate treatments that differ significantly at $\alpha = 0.05$ (Tukey-Kramer pairwise comparison).

For instance, theoretical models of body size regulation have focused either on the rates of growth and differentiation (Van der Have and de Jong, 1996) or on the total size accumulated, irrespective of the duration of the growth period (Partridge *et al.*, 1994; Sibly and Atkinson, 1994; Van Voorhies, 1996; De Moed *et al.*, 1997; Oldham *et al.*, 2000; Brogiolo *et al.*, 2001). The physiological mechanism we describe here (see also Nijhout, 1981, 1994; D'Amico *et al.*, 2001; Davidowitz *et al.*, 2003) specifically addresses the control of the duration of the growth period as well as the rate at which size changes during this

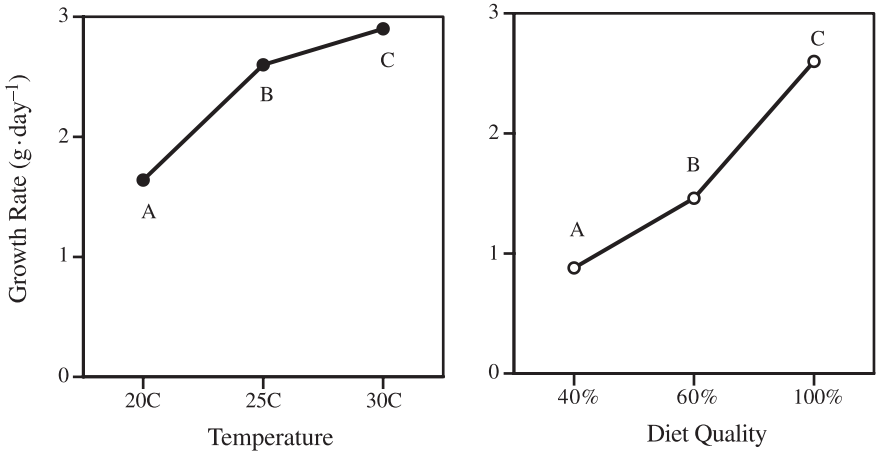

Fig. 4. Reaction norms of growth rate of last instar *Manduca sexta* larvae reared under different temperatures and diets. Error bars are one standard error of the mean and in all cases are smaller than the symbol. Different letters indicate treatments that differ significantly at $\alpha = 0.05$ (Tukey-Kramer pairwise comparison).

period. The duration of the growth period is ultimately determined by the mechanism that terminates the growth period.

The termination of the growth period is a complex trait that differs with life-history strategies and taxon. In general, organisms with determinate growth end their growth period at the adult stage or at first reproduction. Hemimetabolous arthropods attain their maximal size at eclosion to the adult stage (Chapman, 1998). Holometabolous insects cease growth with the initiation of pupation and metamorphosis (see above). Monocarpic (semilparous) plants end their vegetative growth period with bolting or flowering, while iteroparous (polycarpic) plants often have indeterminate growth and increase in size throughout their reproductive period (Harper, 1977). Semilparous animals, such as insects, generally attain their maximal size at the adult stage, while iteroparous mammals and reptiles attain maximal growth with the fusion of the epiphyses of the long bones (Hildebrand, 1995). It is clear, then, that the regulation of the termination of the growth period is of primary interest in understanding how body size is controlled and how development time (the duration of the growth period) interacts with body size. The number of species in which the mechanism that controls the termination of the growth period is known is, at present, still small and generally restricted to a few model organisms.

A few model organisms provide evidence for the mechanisms involved in the cessation of the growth period. In *Arabidopsis thaliana*, either five or two QTL on either 3 or 2 chromosomes (for two RI lines, respectively) are involved in determining bolting time (Ungerer *et al.*, 2002). Thyroid hormone secretion triggers cessation of the tadpole life stage and initiates metamorphosis in anurans (Denver, 1996). In insects in general, the timing of ecdysteroid secretion determines when larval growth ceases and pupation or the adult stage begins (Nijhout, 1994, 2003b). As described above, ecdysteroids are secreted in response to PTTH. The actual stimulus for PTTH secretion is not well known except for in a few hemipeterans. In these bugs, abdominal stretch receptors, activated by distention of the abdomen with food, stimulate PTTH secretion (Wigglesworth, 1934; Nijhout, 1979, 1984;

Chiang and Davey, 1988). This stimulus appears to be unique to that order of insects (Nijhout, 2003b).

Here we have shown that the mechanism that controls body size in *Manduca sexta* also controls phenotypic plasticity of body size. Plasticity of body size is controlled by the interaction of three factors: growth rate and the timing of two hormonal events – initiation of juvenile hormone decay and ecdysteroid secretion. Ecdysteroid secretion is the final step in the process and effectively causes a larva to stop feeding and initiate metamorphosis. Adult size is thus largely determined by the size the larva has achieved at the time of ecdysteroid secretion, and plasticity of body size must thus be due to plasticity in this underlying endocrine control mechanism.

Hormonal mediation of phenotypic plasticity is well known and has received much recent attention (Nijhout, 1994, 1999; Zera and Zhang, 1995; Zera *et al.*, 1996; Roff and Fairbairn, 1999; Stern and Emlen, 1999; Zera and Huang, 1999; Emlen and Nijhout, 2000; Gilbert, 2001; Pigliucci, 2001; Zera and Harshman, 2001; Dufty *et al.*, 2002). Hormones can affect molecular and developmental processes by activating transcription factors that initiate new patterns of gene expression (as is the case with steroid hormones like ecdysone), or by activating protein kinase signalling cascades (as is the case with the peptide hormone PTTH). In addition, the translation of environmental signals received by the nervous system into an appropriate developmental or physiological response in the whole organism is carried out largely by hormones (Nijhout, 1994, 1999; Dufty *et al.*, 2002). Thus, hormones are involved in both the gene (G) and the environment (E) components of phenotypic variation. As a consequence, they are of primary importance in regulating the $G \times E$ component of phenotypic variation (Pigliucci, 2001). Genetic variation in the $G \times E$ component enables the evolution of phenotypic plasticity.

The time required to attain the critical weight and the ICG both determine the development time of the fifth instar larvae. The effect of temperature on development time may be explained by its effects on the general metabolism of poikilothermic animals (Gillooly *et al.*, 2001, 2002). At higher temperatures, biochemical reaction rates are higher. This may be the ultimate cause of shorter development times, when measured on a strictly chronological time-scale (Wigglesworth, 1972; Higley *et al.*, 1986). Our data show that larvae required the same number of degree-days for development at 25°C and 30°C, suggesting that in this range the effect of temperature on development time is most likely a simple function of altered biochemical reaction rates (Wigglesworth, 1972; Roff, 1992; Chapman, 1998). Larvae reared at 20°C, by contrast, required fewer degree-days to develop than larvae reared at higher temperatures. This finding suggests that at low temperatures, an additional, but as yet unknown, mechanism comes into play by which larvae decrease the time required for growth.

Organisms are also typically exposed to multiple types of environmental variation simultaneously (the 'fundamental niche' of Hutchinson, 1957), such as variation in temperature, nutrient availability, predation and crowding, and many traits exhibit plasticity in response to different types of environmental stimuli (Bradshaw, 1965; Schlichting, 1986; Scheiner, 1993; Schlichting and Pigliucci, 1998; Pigliucci, 2001). For example, plasticity in the timing of metamorphosis of spadefoot toads (*Scaphiopus couchii*) is affected by environmental variation in pond duration, diet and temperature (Newman, 1989). Various measures of plant size in the herbaceous plant *Phlox drummondii* respond plastically to environmental variation in nutrients, water and pot size (Schlichting and Levin, 1990); body size in *Manduca sexta* exhibits phenotypic plasticity to both diet and temperature (Fig. 1).

Recent advances in developmental genetics has shown that each of the discrete phenotypes of polyphenisms involve an alternative pattern of gene expression (Evans and Wheeler, 2000, 2001; Nijhout, 2003a). Of major import to evolutionary ecologists is to understand how these different genetic pathways enable an organism to respond to the different and simultaneous environmental stimuli it experiences in its fundamental niche. Genetic studies on flowering time in *A. thaliana* suggest one such mechanism; our study here suggests another. Mouradov *et al.* (2002) recently reviewed the genetic regulation of flowering time in *A. thaliana*. Flowering is regulated by distinct genetic pathways that are responsive to different types of environmental signals. All of these separate genetic pathways converge to regulate the expression of the same downstream genes that control flowering. They propose that this convergence of distinct genetic pathways on a common set of genes enables *A. thaliana* to produce a coordinated flowering response to the multiple and simultaneous environmental stimuli it experiences (Mouradov *et al.*, 2002).

Our study suggests another mechanism by which a trait such as body size may respond to different types of environmental stimuli simultaneously – namely, through the *differential sensitivity* of the components of the same mechanism that control the trait to the different types of environmental stimuli. In *M. sexta*, body size plasticity in response to diet quality is due to plastic variation in critical weight and growth rate, while plasticity in response to temperature is due to plastic responses in ICG and growth rate (Figs 1–4). Thus, critical weight is sensitive to variation in diet but not temperature, ICG is sensitive to variation in temperature but not diet and growth rate is sensitive to both types of environmental variation. This differential sensitivity to the different types of environmental stimuli provides a mechanism by which the caterpillars should be able to respond simultaneously to multiple types of environmental variation. The final size of the larva is a result of a balance between these sensitivities and their responses.

The general mechanism described here should apply to any organism with determinate growth. The processes that lead to cessation of growth entail two steps. The first step is the activation of a mechanism that commits the organism to the adult stage (for example, pupate or bolt, in holometabolous insects and annual plants, respectively). Once committed, there is no turning back, the organism will, at some point, stop growing. The second step is the time interval between when the commitment to the adult stage is made and when growth actually stops. The duration of this step is species-specific and depends on the physiological/endocrine/genetic mechanism that actually terminates the growth period. In *M. sexta*, the mechanism is primarily endocrine: disinhibition of PTTH following juvenile hormone decay. Partitioning the plasticity response to pre- and post-commitment phases may better elucidate the sources and mechanisms of phenotypic plasticity.

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