The Physiological Basis of Reaction Norms: The Interaction Among Growth Rate, the Duration of Growth and Body Size[1]

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Synopsis.   The general effects of temperature and nutritional quality on growth rate and body size are well known. We know little, however, about the physiological mechanisms by which an organism translates variation in diet and temperature into reaction norms of body size or development time. We outline an endocrine-based physiological mechanism that helps explain how this translation occurs in the holometabolous insect *Manduca sexta* (Sphingidae). Body size and development time are controlled by three factors: (i) growth rate, (ii) the timing of the cessation of juvenile hormone secretion (measured by the critical weight) and (iii) the timing of ecdysteroid secretion leading to pupation (the interval to cessation of growth [ICG] after reaching the critical weight). Thermal reaction norms of body size and development time are a function of how these three factors interact with temperature. Body size is smaller at higher temperatures, because the higher growth rate decreases the ICG, thereby reducing the amount of mass that can accumulate. Development time is shorter at higher temperatures because the higher growth rate decreases the time required to attain the critical weight and, independently, controls the duration of the ICG. Life history evolution along altitudinal, latitudinal and seasonal gradients may occur through differential selection on growth rate and the duration of the two independently controlled determinants of the growth period.

INTRODUCTION

Growth and development of ectotherms are determined in part by their thermal environment. In general, ectotherms are smaller at higher temperatures (Atkinson, 1994) but develop faster. Body size and development time are important components of fitness (Roff, 1992; Stearns, 1992), and there has been a long-standing interest in the effects of temperature on growth and development (Johnston and Bennett, 1996; Atkinson and Sibly, 1997).

A number of models have been proposed to explain the changes in ectotherm body size in response to the thermal environment. For example, van der Have and de Jong (1996) proposed that the difference in temperature coefficients between growth rate and differentiation rate could explain the temperature effects on ectotherm body size. Using a muscle fiber model, Arendt (2000) demonstrated that size could be a function of the interaction between cellular growth rate and differentiation rate. Other studies suggest that reaction norms of size in response to temperature can be explained by the correlation between the growth coefficient of the Bertalanffy equation and asymptotic size. (Berrigan and Charnov, 1994; Atkinson and Sibly, 1997).

Such models typically describe the response of growth to the thermal environment as varying functions of growth rate and temperature. Growth rate alone, however, cannot fully explain body size, as body size is the product of growth rate and the duration of the growth period (Atkinson, 1994; Partridge and French, 1996; Blanckenhorn, 1998; Gotthard, 2001; Stern, 2001; Davidowitz et al., 2004). 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). The duration of the growth period is ultimately determined by the mechanism that terminates growth (Davidowitz et al., 2004).

Here we present an endocrine-based, physiological mechanism for the control of body size and development time. We show how this mechanism can explain the observed variation of body size and development time along a thermal gradient.

THE MECHANISM

The general scheme of hormone mediated postembryonic development in insects has been known since the 1950s. Briefly, the prothoracicotropic hormone (PTTH) is secreted from the brain and stimulates the prothoracic glands to secrete ecdysteroids. The ecdysteroids induce either a larval or metamorphic molt depending on the titer of juvenile hormone, secreted by the corpora allata. If juvenile hormone titers are high, ecdysteroids induce a larval-larval molt. In the absence of juvenile hormone there are two pulses of ecdysteroid secretion. The first smaller pulse induces switch-
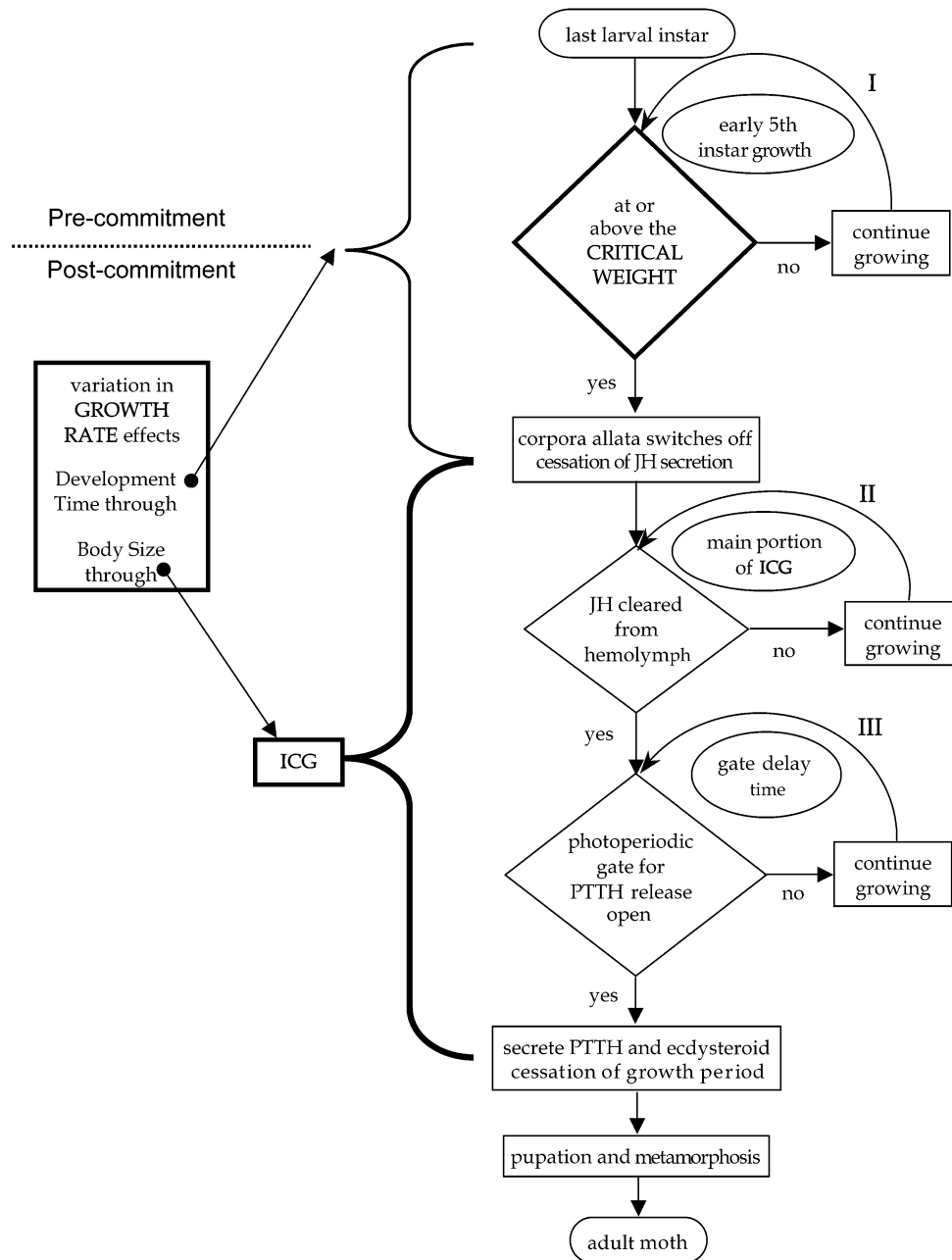
FIG. 1. Summary flow diagram of an endocrine-based physiological mechanism of the control of body size and development time in *Manduca sexta*. The right-hand part of the model describes the regulation of the duration of the growth period. The left-hand portion shows how growth rate interacts with the growth period to determine final body size and development time. The boxes in bold indicate the three main factors (critical weight, interval to cessation of growth, and growth rate) that control body size and development time. The roman numerals I, II, III indicate the three regulatory loops. Diamonds indicate decisions, rectangles indicate processes, ellipses are descriptors, and rounded rectangles indicate end stages. ICG—interval to cessation of growth. The dotted line above the growth rate box distinguishes between the pre-commitment and post-commitment phases of growth.

over from larval to pupal commitment, and the second much larger pulse induces the pupal molt (Nijhout, 1994, 1999). Understanding how environmental variation effects this endocrine cascade can help determine the physiological control of phenotypic plasticity in body size and development time (Davidowitz *et al.*, 2004).

The study of this endocrine cascade has focused pri-

marily on understanding the regulation of molting and metamorphosis (see Gilbert *et al.* [1996] and Nijhout [1994, 1999] for recent overviews). Here we show how this endocrine-based physiological mechanism can account for the control of body size and development time (Fig. 1).

Insect growth from instar to instar is typically exponential so that most of growth occurs in the last

larval instar. In *M. sexta*, 90% of mass accumulation occurs in the 5th (final) instar (Davidowitz *et al.*, 2004). Within the final instar, growth is approximately linear after the first day. In the strain used for the present studies, larvae reach a peak mass at about 12 grams, after which they stop feeding, purge their gut contents, enter a wandering stage, and form a pupa about 6 days later. The pupal mass is, on average, 54%, and the moth 25%, of the peak larval mass. As with other insects, adults do not grow and therefore peak larval mass largely determines the size of the adult moth (Davidowitz *et al.*, 2004). Because growth ends with pupation in the final instar and most of the growth occurs in the last larval instar, our model concentrates on the processes that terminate the growth period in the final instar larvae.

A final instar larva feeds and grows until it reaches a threshold mass termed the critical weight (Nijhout and Williams, 1974). At the critical weight the corpora allata (CA) stop secreting JH (Nijhout and Williams, 1974, loop I in Fig. 1). The critical weight is typically about 54% of peak larval mass (Davidowitz *et al.*, 2004). The critical weight is not a static trait of a species but can evolve (D'Amico *et al.*, 2001; Davidowitz *et al.*, 2003), and is sensitive to diet quality but not to temperature (Davidowitz *et al.*, 2003, 2004). Larger critical weights result in larger peak larval sizes and longer development times (Davidowitz *et al.*, 2005).

The circulating level of JH is high during the first few days of the instar, but drops precipitously after the larva reaches the critical weight (Baker *et al.*, 1987; Riddiford, 1994, 1995). This rapid decline of JH is due to a sharp rise in the activity of the enzyme JH esterase, which accelerates the rate of degradation of JH (Vince and Gilbert, 1977; Sparks *et al.*, 1983; Hammock, 1985; deKort and Granger, 1996; Browder *et al.*, 2001).

In the last larval instar JH inhibits PTTH and ecdysteroid secretion (loop II in Fig. 1). (Bollenbacher *et al.*, 1979; Rountree and Bollenbacher, 1986). Once JH is cleared from the hemolymph some time after the larva passes the critical weight, secretion of PTTH and ecdysteroids is disinhibited.

The actual 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 (loop III in Fig. 1). Secretion of PTTH occurs during the first photoperiodic gate that follows after complete clearance of JH (Truman, 1972; Truman and Riddiford, 1974). This latter point is important because if JH is cleared from the hemolymph shortly after the closing of a gate, for example, secretion of PTTH cannot occur until the next gate opens and so the larva has an additional 16 hours (on average) to continue to feed and grow. The duration and timing of the photoperiodic gate is sensitive to the nutritional quality of the diet consumed by the larva (G.D., unpublished data) but not to temperature (Truman, 1972).

We call the time interval between attainment of the critical weight and the secretion of PTTH and ecdysteroids the Interval to the Cessation of Growth (ICG). During the ICG, the larva continues to feed and grow normally (D'Amico *et al.*, 2001; Davidowitz *et al.*, 2003). The length of the ICG is determined by two factors (Fig. 1): the time required to eliminate JH, and the time subsequent to the clearing of JH, to the opening of the next photoperiodic gate. The time required to eliminate traces of JH is determined in part by the activity of JH esterase (Browder *et al.*, 2001).

The ICG is sensitive to variation in temperature but not to diet quality (Davidowitz *et al.*, 2004). Increasing the ICG allows a larva more time to feed which results in a larger body size and a longer development time (Davidowitz *et al.*, 2005). The duration of the ICG is an important factor in the control of body size, because during this period the larva can more than double its mass.

When a larva reaches the critical weight and the corpora allata switch off, the physiological processes that culminate in pupation and metamorphosis cannot be stopped or reversed, so the larva is set on an irrevocable course to pupation at this time (Nijhout and Williams, 1974; D'Amico *et al.*, 2001). The growth period until the critical weight is, therefore, a pre-commitment phase of growth and the growth period after reaching the critical weight is the post-commitment phase of growth (Fig. 1, Davidowitz *et al.*, 2004). As will be shown below, this distinction is important in understanding the control of development time along a thermal gradient.

The timing of the critical weight and the timing of the interval to cessation of growth together determine the duration of the growth period of the last instar larva. Growth rate interacts differently with these two factors to determine final larval size and total development time of the last larval instar (Fig. 1). The growth rate determines how fast a larva will attain the critical weight, but not the value of the critical weight. The growth rate also controls the amount of mass accumulated during the ICG but not the duration of the ICG. Thus, the growth rate comes into play in determining size only during the ICG, but it affects development time only in the time required to reach the critical weight (Fig. 1).

In summary, the endocrine-physiological mechanism (Fig. 1) includes one threshold trait (the critical weight), three regulatory loops (critical weight, time for JH decay, photoperiodic gate for PTTH) and a pre-commitment and post-commitment phase of growth. The mechanism explicitly incorporates the regulation of the growth period (Fig. 1), the growth rate, as well as the interaction between the two (arrows connecting the left and right hand sides).

As is the case in other insects, *Manduca sexta* grows more slowly and has a prolonged development time at lower temperatures, but it grows to a significantly larger size at lower temperatures (Fig. 2). Below we show that this physiological mechanism can explain how
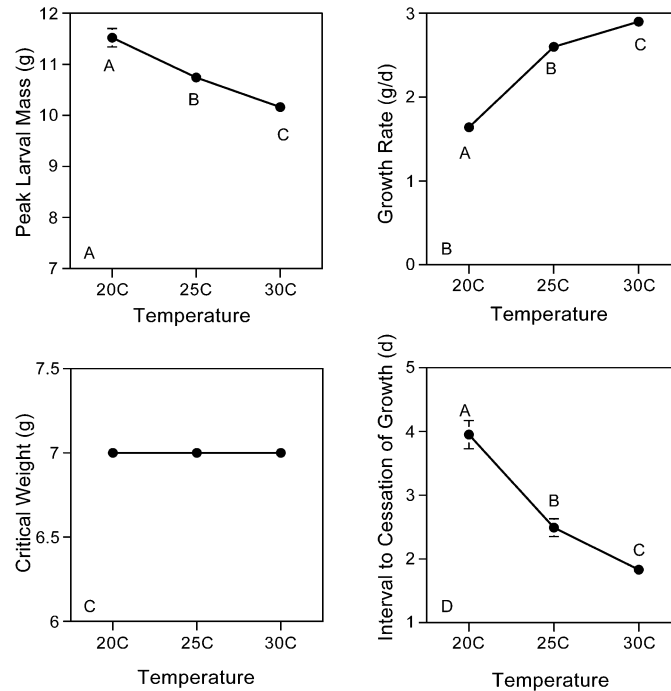
FIG. 2. Response of (A) larval body size, (B) growth rate, (C) critical weight and (D) interval to cessation of growth, to variation in temperature. In A, B and D, each data point represents about 55 individuals. The error bars represent 1 SEM and in some cases are smaller than the symbol. Different letters indicate treatments that differ significantly at α = 0.05 (Tukey-Kramer pair-wise comparison). Redrawn after Davidowitz *et al.* (2004).
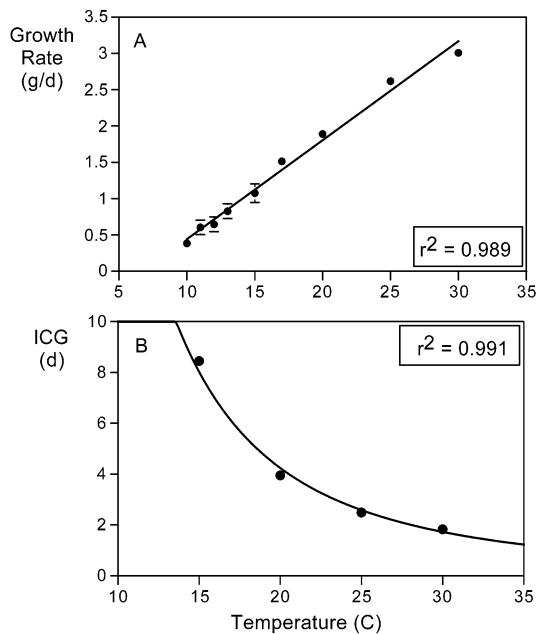

FIG. 3. Empirical relationship between (A) growth rate and (B) interval to cessation of growth (ICG) and temperature in the last larval instar of *Manduca sexta*. Growth rate, y = 0.136x − 0.922, n = 9, r^2 = 0.989. For 10°C–17°C each data point represents about 12 individuals and for 20°C–30°C each data point represents about 55 individuals. The error bars represent 1 SEM and in some cases are smaller than the symbol. ICG, y = 3238.610x$^{-2.216}$, n = 4, r^2 = 0.991. Each data point is a single value determined from an experiment involving about 300 individuals (for details see Davidowitz *et al.*, 2003).

body size and development time are controlled in response to variation in temperature.

CONTROL OF BODY SIZE ALONG A THERMAL GRADIENT

As noted above, body size is the product of growth rate and the duration of the growth period. The duration of the growth period is determined by both the ICG and the critical weight. Since the critical weight does not change with temperature (Fig. 2C), we examined peak larval size along a thermal continuum as a function of growth rate and the ICG. We measured growth rate at nine temperatures ranging from 10°C–30°C and determined that there was a linear positive relationship between growth rate and temperature (Fig. 3A). In a similar fashion, we found that the relationship between the ICG and temperature (Fig. 3B) is best described by a power function. In both instances, temperature accounted for over 98% of the variation in growth rate and ICG (Fig. 3).

Using the equations obtained from these relationships (Fig. 3), we calculated body size as the product of ICG and growth rate along a thermal continuum at 1°C intervals (Fig. 4A). We chose the lower bound of this continuum as 8°C because Davidowitz *et al.* (2004*a*) showed that the minimum temperature required for a non-zero positive growth rate was 7.8°C. The upper bound was chosen as 45°C, the lethal temperature for *M. sexta* (Casey, 1976). We note that body size in Figure 4A is not the peak larval size. To obtain the values for peak larval size in Figure 4A it is nec-
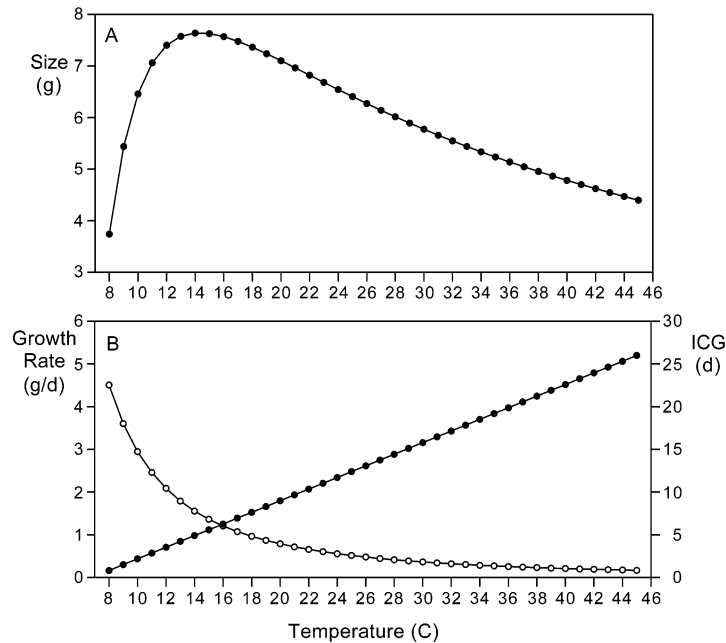
FIG. 4. Relationship of (A) body size, (B) growth rate (closed circles) and ICG (open circles) to temperature. Growth rate and the ICG were calculated from equations in Figure 3. Body size was calculated as the product of growth rate and the ICG. To calculate peak body size in (A), add 7 g to each value (see text).

essary to add the critical weight of 7 g (Fig. 2C) to each of the plotted values.

The results of this analysis show that body size increases with temperature, peaks at 14°C, then declines with increasing temperature (Fig. 4A). This indicates that the general trend of larger insect sizes at lower temperatures (Atkinson, 1994) is correct only for the warmer part of the thermal gradient (in this case, temperatures above 14°C).

The relative contribution of growth rate and ICG to body size along the thermal gradient can be deduced from the plots in Figure 4B. At lower temperatures, the duration of the growth period contributes most to body size, whereas growth rate contributes more to body size at higher temperatures (Fig. 4B). This may have important implications for the evolution of life histories. For body size to evolve, populations from colder environments may require a change in the duration of the growth period, but selection on growth rate will be more effective in populations from warmer environments. The genetic correlation between the ICG and growth rate is -0.06 ± 0.4 (G.D., unpublished data) indicating each should be able to evolve independently of the other, and that evolution of body size can thus proceed via different mechanisms in different regions of a thermal gradient.

CONTROL OF DEVELOPMENT TIME ALONG A THERMAL GRADIENT

Development time includes the period of larval growth from hatching to pupation. Here, we are concerned more with the mechanism that terminates the growth period so we focus on development time in the last larval instar when most of the mass of an individual insect accumulates.

In the last larval instar, development time is the sum of the time required to attain the critical weight and the time spent in the ICG (the time from the critical weight to the secretion of ecdysteroids). To examine how these two components of development time change along a thermal gradient, we calculated the total growth period and the time required to attain the critical weight, at each of six temperatures (Fig. 5). The r^2 values were >0.90%. The ICG was calculated as in Figure 3B. Using the equations derived by non-linear regression from these data plots, we calculated the values for each of these three traits along a thermal gradient as we did for body size (above). These results (Fig. 6) show that total development time, the ICG and the time to critical weight all decrease non-linearly with temperature (Fig. 6), The range of temperatures shown in Figure 6 is probably broader than those typically encountered in nature, so it is likely that a real population of animals will fall somewhere within the range shown.

Figure 6 also shows the relative contributions of the two components of development time to total development time. Total development time is predominantly due to the time to reach the critical weight particularly at lower temperatures. This is the pre-commitment phase of growth (Fig. 1). Davidowitz *et al.* (2004) showed how the control of plasticity of body size can be better understood when examined in the context of plasticity in the pre-commitment and post-commitment phases of growth (Fig. 1). Our results suggest this may be true for development time as well.
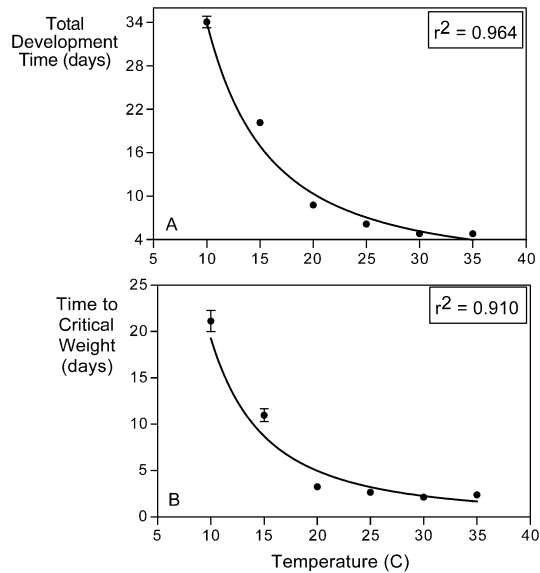
FIG. 5. Empirical relationship between temperature and (A) total development time in the last larval instar of *Manduca sexta* and (B) time to attain the critical weight (CW) in last instar *M. sexta*. Total development time, $y = 1777.669x^{-1.718}$, n = 6, $r^2 = 0.964$. Time to reach the CW, $y = -1746.514x^{-1.958}$, n = 6, $r^2 = 0.910$. Each data point represents about 50 individuals. The error bars represent 1 SEM and in most cases are smaller than the symbol.
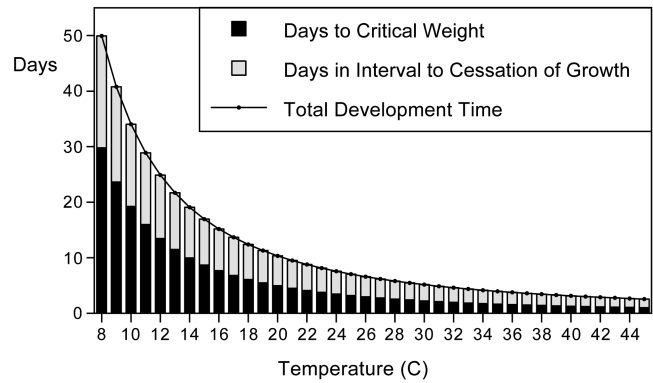

FIG. 6. Relationship between the total number of days in last larval instar (line) and the relative contributions of the time to reach the critical weight (solid bars) and time in the interval to cessation of growth (stippled bars) and temperature. The values of total development time and time to reach the critical weight were calculated from the equations in Figure 5. The values of the ICG were calculated from the equation in Figure 3B.

As we have seen, the relative roles of the different components of the physiological mechanism that controls body size and development time change along the thermal gradient. At lower temperatures, the duration of the growth period is more important in determining body size, while growth rate is more important in determining size at higher temperatures. Development time at lower temperatures is determined more by the time required to reach the critical weight while at higher temperatures the ICG and time to the critical weight have a more equal contribution. These changes in the relative importance of different aspects of the physiological mechanism with temperature may have important consequences for the evolution of life histories.

There are numerous examples of population differentiation in body size and development time along latitudinal and elevational clines (Endler, 1977; Huey *et al.*, 2000; Ashton, 2004; Blankenhorn and Demont, 2004). Our results suggest that the targets of selection leading to these differences in adaptive life histories may shift along these gradients: for body size, selection may act on the duration of the growth period in the colder, high altitude and high latitude populations, while selection may target the growth rate at the warmer lower elevations and latitudes. A similar shift in the targets of selection may occur between early spring and summer cohorts in individual populations. Similarly, for a change in development time to occur, selection may target the pre-commitment phase of growth at higher latitudes and altitudes or in the early spring cohorts. On a practical level, the evolution of body size along thermal gradients may be better addressed if studied at the level of its two constitutive components, growth rate and the duration of the growth period, and development time at the levels of the time to reach the critical weight and the interval to the cessation of growth.

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