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Critical weight in the development of insect body size

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SUMMARY Body size is one of the most important life history characters of organisms, yet little is known of the physiological mechanisms that regulate either body size or variation in body size. Here, we examined one of these mechanisms, the critical weight, which is defined as the minimal mass at which further growth is not necessary for a normal time course to pupation. The critical weight occurred at 55% of peak larval mass in laboratory-reared larvae of the tobacco hornworm *Manduca sexta.* We examined the effects of genetic and environmental variation in the critical weight on body size. As in many other insects, *Manduca* larvae reared on poor diets were smaller and those reared at lower temperatures were larger than control animals. We demonstrated that the critical weight was lower on low quality diets but did not change with temperature. There was significant genetic variation for body size, for plasticity of body size, and for critical weight, but not for plasticity of critical weight. Variation in the critical weight accounted for 73% of between-family variance in peak larval size, whereas plasticity of critical weight was not significantly correlated with plasticity of body size. Our results suggest that although critical weight is an important factor in determining body size and enabling the evolution of body size, it may, at the same time, act as a constraint on the evolution of plasticity of body size. Thus, the determinants of body size and the determinants of plasticity of body size do not need to be identical.

INTRODUCTION

Body size is one of the most important life history characters of an organism. Its effects on fitness are well documented and have been extensively studied both theoretically and empirically (Calder 1984; Schmidt-Nielsen 1984; Roff 1992; Stearns 1992). Body size evolution is one of the dominant features of evolution in many lineages of animals (McShea 1998). Body size is also plastic: It can change in response to different environmental conditions. For example, insects that develop at higher temperatures are generally smaller than those that develop at lower temperatures (Atkinson 1994), and well-fed organisms are typically larger than those fed a poor-quality diet (Chapman 1998).

Despite a long-standing interest in the ecology and evolution of body size, remarkably little is known about the developmental and physiological mechanisms that determine body size or about the mechanisms by which an organism translates different environmental signals that result in plasticity of body size (Stern 2001). In essence, little is known about *how* an animal comes to be the size that it is.

Recently, D'Amico et al. (2001) analyzed the physiological basis of body size evolution in the tobacco hornworm (*Manduca sexta*: Sphingidae). In this species the final body size of the adult is determined by five variable factors: (a) the initial size of the last larval instar, (b) the growth rate during that instar, (c) the critical weight, (d) the time delay between achieving the critical weight and the initiation of prothoracicotropic hormone (PTTH) secretion (the PTTH delay time), and (e) the timing of the photoperiodic gate for PTTH secretion. They demonstrated that in a continuous laboratory culture over a 30-year period (approximately 220 generations) body size of this insect increased by 50%. This evolutionary increase in body size could be accounted for by changes in three of the five factors: growth rate, critical weight, and PTTH delay time (the remaining two factors remained unchanged during this period). The quantitative change in these three factors was shown to account for over 95% of the evolutionary change in body size (D'Amico et al. 2001). The relationship between critical weight, PTTH delay time, and growth rate is shown in Figure 1.

The five factors that regulate body size were first elucidated in the 1970s because of their importance in controlling the timing of metamorphosis. In final (fifth) instar larvae of *M. sexta*, somatic growth is causally associated with the timing of a number of endocrine events that induce the onset of pupation and metamorphosis (Nijhout and Williams 1974a,b; Nijhout 1981). Growth in the final instar stops, and metamorphosis begins, with the secretion of PTTH and ecdysteroids. PTTH and ecdysteroid secretion are inhibited by the presence of juvenile hormone (JH) (Nijhout and Williams 1974b; Rountree and Bollenbacher 1986). The level of JH circulating in the hemolymph is high during the first few days of the instar but drops dramatically when the larva attains a specific critical

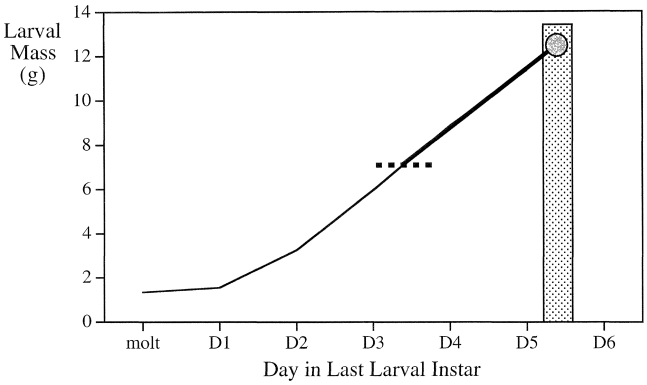


Fig. 1. The critical weight in relation to growth in the last larval instar of *Manduca sexta.* The thin line is the average growth curve of larvae reared on the standard rearing diet at 25°C. The critical weight is depicted as the horizontal dashed line and here is 7.0 g. The thick diagonal line represents the growth that occurs during the prothoracicotropic hormone (PTTH) delay time: the time from the critical weight until the secretion of PTTH (see text). The vertical stippled bar represents the photoperiodic gate for PTTH release and the circle within the gate represents the time and the weight at which PTTH and ecdysteroids are secreted (see text).

weight (Nijhout and Williams 1974b). This disappearance of JH sets into motion the cascade of endocrine events that leads to metamorphosis. The critical weight, the focus of this study, is operationally defined as the minimal weight in which further feeding and growth are not required for a normal time course to metamorphosis and pupation (Nijhout and Williams 1974a; and see below). Once the larva attains the critical weight, the corpora allata, the glands that synthesize and secrete JH, turn off, resulting in the drop in JH titer. Shortly after JH is cleared from the hemolymph, the activity of the enzyme JH esterase increases dramatically and is believed to be required for scavenging the last remaining traces of JH (Hammock 1985; Baker et al. 1987; Browder et al. 2001). At this point the larva becomes competent to secrete PTTH. The actual secretion of PTTH occurs during the first photoperiodic gate that follows after the clearance of JH (Truman 1972; Truman and Riddiford 1974). The PTTH stimulates the secretion of ecdysteroids that cause the larva to stop feeding and induce the switchover to pupal commitment (Riddiford 1985; Nijhout 1994). Thus, larval growth stops when the sequence of endocrine and physiological events initiated by the critical weight culminate in the secretion of ecdysteroids (Fig. 1). As in other insects, adults do not grow. As a result, the size that a larva attains at the time of metamorphosis completely defines the body size of the adult. Therefore, understanding how genetic and environmental factors determine and alter the value of the critical weight is of critical importance for understanding how body size and plasticity of body size are regulated and how they may evolve.

Here we examine the effects of genetic and environmental variation on the critical weight and demonstrate the key role that the critical weight plays in determining adult body size in response to diet and temperature variation. We examine environmental effects by comparing critical weight of last instar *M. sexta* reared on diets of different nutritive values and at different temperatures. We then examine genetic effects on critical weight by means of a quantitative genetic study and estimate the additive genetic variance of critical weight and body size and the genetic correlation between them. Finally, we examine phenotypic plasticity of critical weight in response to two diet treatments.

MATERIALS AND METHODS

All experiments were done using a hybrid colony of *M. sexta* outcrossed from colonies maintained at the University of Washington, North Carolina State University, and the University of Arizona. This colony was specifically created to increase genetic variation of body size. To minimize maternal effects, all experiments were conducted a minimum of eight generations after the hybrid colony was established.

Diet and temperature effects on critical weight

Larvae were maintained at a 16:8-h light:dark photoperiod and a constant temperature of 25°C and fed on the standard diet (100% diet, see Appendix) except where noted. 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. To estimate temperature effects on critical weight, we measured critical weight as described below at three temperatures: 20, 25, and 30°C. To estimate the effect of diet on critical weight, fifth instar larvae were fed 100% (standard rearing diet), 60%, and 40% diets. The nutritional components of these diets were reduced by the appropriate percentages, with the remaining volume made up by adding 50% cellulose (Alphacel, ICN, Aurora, OH, USA) as non-nutritive bulk. The vitamin, antibacterial, and antifungal components were identical in all three diets. Diet formulae are given in the Appendix. 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.

Larvae that are starved at or above the critical weight metamorphose at the same time as larvae that are allowed to continue to feed normally. By contrast, if larvae are starved before they have achieved the critical weight, metamorphosis is delayed compared with larvae that continue to feed. We measured critical weight as in Nijhout and Williams (1974a) and D'Amico et al. (2001). Briefly, we assigned final (fifth) instar larvae into weight classes at 0.5-g intervals. Within each weight class, 15–20 larvae were allowed to feed normally and an additional 15–20 were starved (they were provided with a 2% agar block to prevent desiccation). Larvae were chosen at random from the colony and were assigned at random to experimental treatment and to weight class within each treatment. The time to PTTH and to ecdysteroid secretion of each individual were then recorded. PTTH and ecdysteroid secretion can be unambiguously identified, because they initiate two readily observable physiologi-

cal changes in the larva: the deposition of pink ommochrome pigment along the dorsum and the exposure of the dorsal vessel due to a clearing of the tissues around the heart (Nijhout and Williams 1974a). Significance of differences between treatments were tested using *t*-tests ($\alpha = 0.05$). The *t*-tests were conducted independently for each weight class, because the larvae used, as well as each weight class, were independent of all others (Sokal and Rohlf 1995; and see above). We note that the critical weights as measured above may differ somewhat using different sample sizes or weight classes (e.g., weight classes of whole grams or 1/4 grams); thus, our definition of the critical weight is specific to the methods used here.

Two statistical issues needed to be addressed in this study: the treatment of outliers and the use of nominal weight classes. The differences between the fed and starved treatments were tested using a *t*-test, which can be sensitive to outliers. We therefore conducted each *t*-test twice, once including all individuals and once excluding extreme outliers (4.2%) that were greater than 1.5 times the interquartile range (JMP 4.04, SAS Institute, Cary, NC). All but one yielded identical estimates of the critical weights, independent of whether or not we excluded the extreme outliers. These results indicate that our estimates of the critical weight are robust. In the single experiment on 40% diet, the estimate of the critical weight changed from no critical weight to a critical weight of 6.5 g. We note that this is the most stressful of the treatments based on mortality and growth rate data (data not shown). Results presented below include outliers.

The weight classes used to measure critical weight in the different temperature and diet treatments were determined to the nearest 0.2 g (i.e., the 7-g weight class, for example, included individuals ranging from 6.8 to 7.2 g). Therefore, there is a potential error in estimating larval weights from the nominal weight class if by chance more individuals at one extreme of the range were chosen for a particular nominal weight class. However, of the 35 weight classes used, in all but 3 (91%) the mean weight of larvae used within each weight class did not deviate significantly from their respective nominal weight class values (*t*-test, $\alpha = 0.05$). In those three cases the deviations were 0.06 g or less.

Genetic analysis of critical weight

To estimate genetic variation, it would be preferable to compare critical weights across families (Roff 1997). This was not feasible in the present experiments because the large number of animals required would have been prohibitively expensive. Instead, we estimated individual critical weights indirectly as follows. We took advantage of the fact that the timing of the photoperiodic gate for PTTH secretion and the time required for larvae to become competent to secrete PTTH after reaching the critical weight (the PTTH delay time) are both known quantities. Knowing the growth rate of a larva and the time at which it reaches its peak weight then allows back-calculation of its critical weight. In a separate study (Davidowitz and Nijhout, unpublished data), we determined that the middle of the photoperiodic gate for PTTH secretion occurred at 2.5 h after onset of the photophase. The time needed for JH to be cleared from the hemolymph was calculated as the number of days to PTTH release for larvae *at* the critical weight (the mean experiment PTTH delay time). We used the value of the colony mean PTTH delay time as a constant to determine the individual critical weight in a separate quantitative genetic experiment.

To obtain the quantitative genetic parameters for the critical weight, we mated 35 single pairs of *M. sexta* in individual mating cages and allowed the females to oviposit. Their F_1 larvae were kept at 25°C on a 16:8-h light:dark photoperiod and fed the standard rearing diet (Appendix). Individual critical weight was measured as $icw = ipm - (mdt \cdot igr)$, where *icw* is the individual critical weight, *ipm* is the individual peak mass as measured below, *mdt* is the mean experiment PTTH delay time (a single value for each experiment, see above), and *igr* is the individual growth rate. The use of a single value of *mdt* to calculate *icw* does not bias the genetic correlation between size and critical weight (unpublished data). Growth rate in the fifth instar was recorded for each of these offspring by weighing them daily until exposure of the dorsal vessel occurred. Individual growth rate (grams per day) was determined from the last 3 days before exposure of the dorsal vessel, during which time growth rate is linear.

To determine the significance of critical weight in the determination of adult body size, we regressed individual body size, measured as peak larval mass, on individual critical weight using least-squares linear regression. Larval mass measurements were taken daily at the end of the photoperiodic gate for PTTH release. Therefore, the larva grew an additional 19 h, on average, before PTTH release at the next photoperiodic gate. We calculated individual peak mass as $ipm = llm + (igr \cdot agt)$, where *ipm* is individual peak mass, *llm* is the last larval mass measured before PTTH release, *igr* is the individual growth rate measured as grams per day (as above), and *agt* is the additional growth time from *llm* to the middle of the next photoperiodic gate (see above).

The genetic correlation between critical weight and body size was calculated using parent–offspring covariances (Roff 1997), and its standard error was estimated using the jackknife method of Roff and Preziosi (1994). To avoid spurious correlations due to the interdependency of the methods of calculating the individual critical weight and individual peak mass, we estimated the genetic correlations using pupal mass as our measure of body size. Genetic variation for body size and critical weight were calculated using mid-parent mean-offspring regression.

Phenotypic plasticity of critical weight

To examine phenotypic plasticity of critical weight in response to diet, we analyzed the same 35 single-pair families described above in a full-sib split-brood design. All F_1 larvae were fed 100% diet until they reached the fifth instar. At the molt to the fifth instar, an average of 12 offspring from each family were allowed to continue feeding on 100% diet and an average of 14 additional offspring from the same families were fed on 60% diet. Individual critical weights for the larvae reared on 60% diet were calculated as described above for the larvae on 100% diet. The middle of the photoperiodic gate for larvae on the 60% diet was determined to be 5 h after onset of the photophase (Davidowitz and Nijhout, unpublished data), and the PTTH delay time was determined from the plot of critical weight on the 60% diet, as outlined above. Growth rates on 60% diet were recorded in the same manner as those for larvae on the 100% diet.

Phenotypic plasticity was measured as the family mean difference between treatments (Scheiner 1993a). We regressed plasticity of body size (dependent variable) on plasticity of critical weight (independent variable) to examine the importance of phenotypic

plasticity of critical weight in determining phenotypic plasticity of body size. We regressed the dependent variable of family mean body size on the independent variable of family mean critical weight to determine the importance of critical weight in determining body size. First, we calculated the family mean for each *diet* treatment and then calculated the family mean of both diets (a mean of means). The use of family means in both regressions allow direct comparisons between them. A mixed-model analysis of variance (ANOVA) with *family* and *diet by family* as random effects and diet as a fixed effect was used to determine the significance of effects for body size and critical weight. Between-diet genetic correlations of body size and critical weight and their associated F tests were estimated as in Fry (1992). Variance components were calculated using restricted maximum likelihood (REML, PROC MIXED, SAS Institute 1997)

As demonstrated by Nijhout (1981), we tested whether larvae that were larger at the molt from the fourth to the fifth instar had higher critical weights. We did this using linear regression of individual critical weight of all offspring from both diets ($n = 882$) on both the mass at the beginning of the last larval instar and on head capsule width (Nijhout 1981).

RESULTS

Diet and temperature effects on critical weight

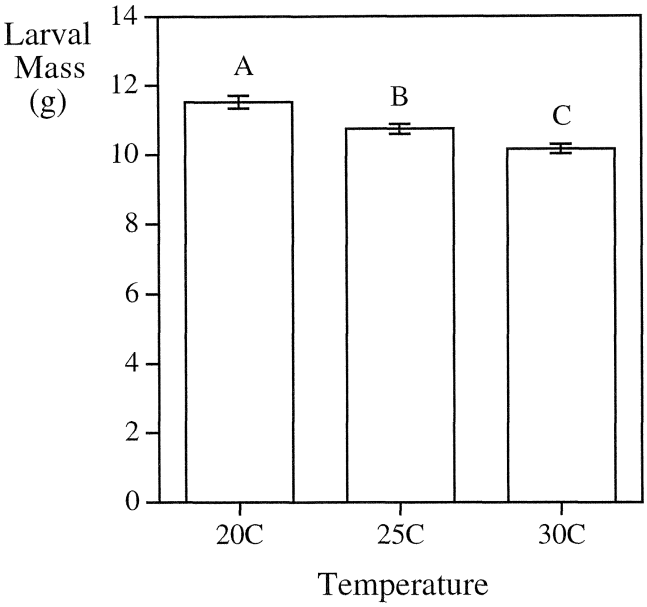
Body size increased with decreasing temperature and increasing diet quality (Fig. 2). The critical weight did not appear to be sensitive to temperature and remained at 7 g across all temperatures (Fig. 3). By contrast, the critical weight decreased with decreasing food quality. On 100% diet the critical weight was 7 g, on 60% diet it was 6.5 g, and on 40% diet there was no critical weight because the time to PTTH secretion was the same at all weights, whether the larva was starved or allowed to feed (Fig. 4).

Genetic analysis of critical weight

The genetic correlation between critical weight and pupal mass was 0.43 ± 0.23. The phenotypic correlation between critical weight and peak mass was 0.68 ($P < 0.0001$) and that between critical weight and pupal mass was 0.51 ($P < 0.0001$). The between-diet genetic correlation for critical weight was 1.03, which was significantly different from zero ($F = 7.78$, $P << 0.001$). A genetic correlation greater than 1.0 can occur when there is a large among-family component of variation. The between-diet genetic correlation for body size measured as peak mass was 0.66 ($F = 3.53$, $P < 0.001$). The parent–offspring regressions revealed additive genetic variation for both measures of body size (peak mass: $h^2 = 0.22 \pm 0.09$; pupal mass: 0.44 ± 0.11) and critical weight ($h^2 = 0.35 \pm 0.10$).

Phenotypic plasticity of critical weight

Seventy-three percent of the among-family variation in peak larval mass could be accounted for by variation in the mean critical weights of those families (Fig. 5). In contrast, there was no significant relationship between phenotypic plasticity of critical weight and plasticity of peak mass among fam-



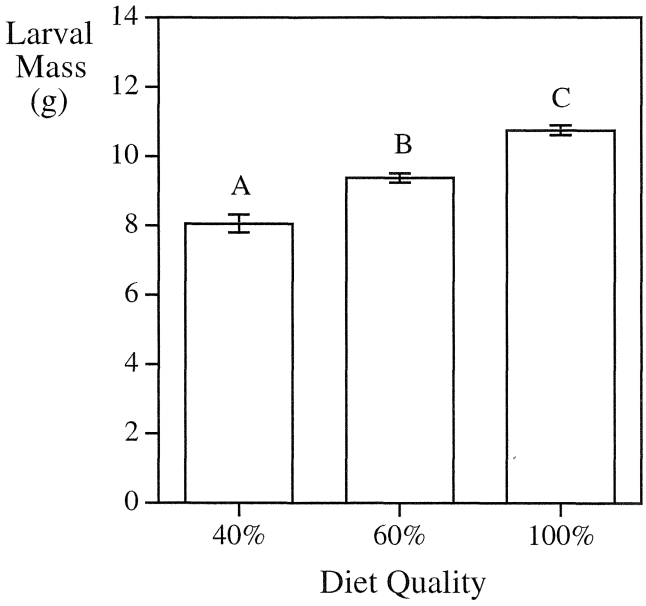


Fig. 2. Maximal mass of last instar *Manduca sexta* larvae reared under different temperatures and diets. All larvae were reared on standard rearing diet (100%) at 25°C from hatching until the molt to the last instar. Larval weights were as measured on the day before prothoracicotropic hormone secretion and are thus smaller than the individual peak mass (see text). Error bars are one standard error of the mean. Different letters indicate treatments that differ significantly at $\alpha = 0.05$ (Tukey-Kramer pairwise comparison).

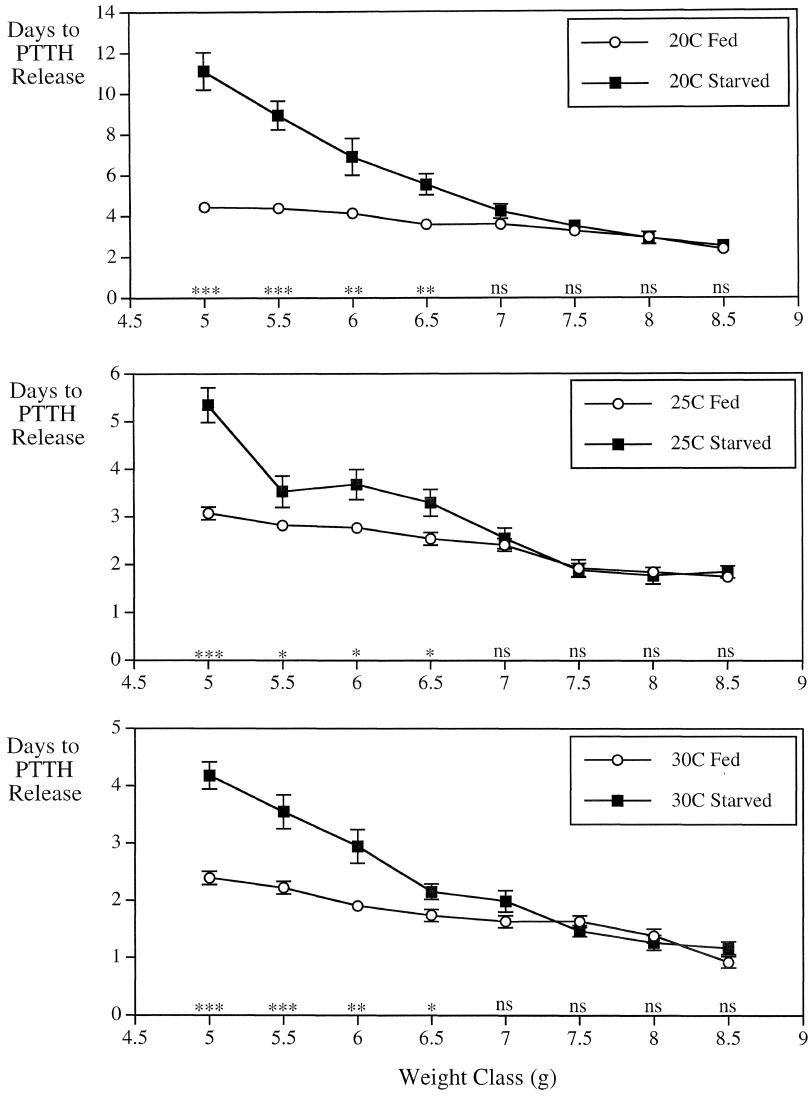


Fig. 3. The effect of temperature on the critical weight. Each data point represents 15–20 larvae. Error bars are one standard error of the mean and in some cases are smaller than the symbol. ns, nonsignificant at $\alpha = 0.05$; $0.05 > * > 0.01$; $0.01 > ** > 0.001$; $*** < 0.001$ (*t*-test). PTTH, prothoracicotropic hormone.

ilies (Fig. 6). ANOVA revealed significant genetic and diet effects for both body size and critical weight (Tables 1 and 2). The G × E component of variation was significant for body size (Table 1) but not for critical weight (Table 2).

Variation in individual critical weight accounted for only 20% of the variation of individual peak mass on both 100% and 60% diets (Fig. 7). The relationship was stronger within diets ($r^2 = 0.46$ and $r^2 = 0.57$ for 100% and 60% diets, respectively). These regressions also revealed a pronounced diet effect of critical weight on peak mass: For any given critical weight, larvae reared on 60% diet had smaller peak mass than those reared on 100% diet (Fig. 7).

Regression of the individual critical weight on head capsule width revealed a significant positive relationship (*icw* = 1.91 + 3.61 × *capsule width*; F = 279.9, $P < 0.0001$, $r^2 = 0.24$), as did the *icw* with weight at the beginning of the last larval instar (*icw* = −6.2 + 2.2 × *initial weight*; F = 118.9, $P < 0.0001$, $r^2 = 0.13$).

DISCUSSION

The evolution and plasticity of body size have been extensively studied at the phenomenological level (Calder 1984; Schmidt-Nielsen 1984; Roff 1992; Stearns 1992; Schlichting and Pigliucci 1998). However, we still have only a rudimentary knowledge of the underlying mechanisms that cause variation in body size (Stern 2001). Body size variation is typically described by fitting an arbitrary function, such as a linear, polynomial, Gompertz, or Bertalanffy equation (Cock 1966; Forbes and Lopez 1989; Jolicoeur 1989; Nijhout and Wheeler 1996), whose parameters have no par-

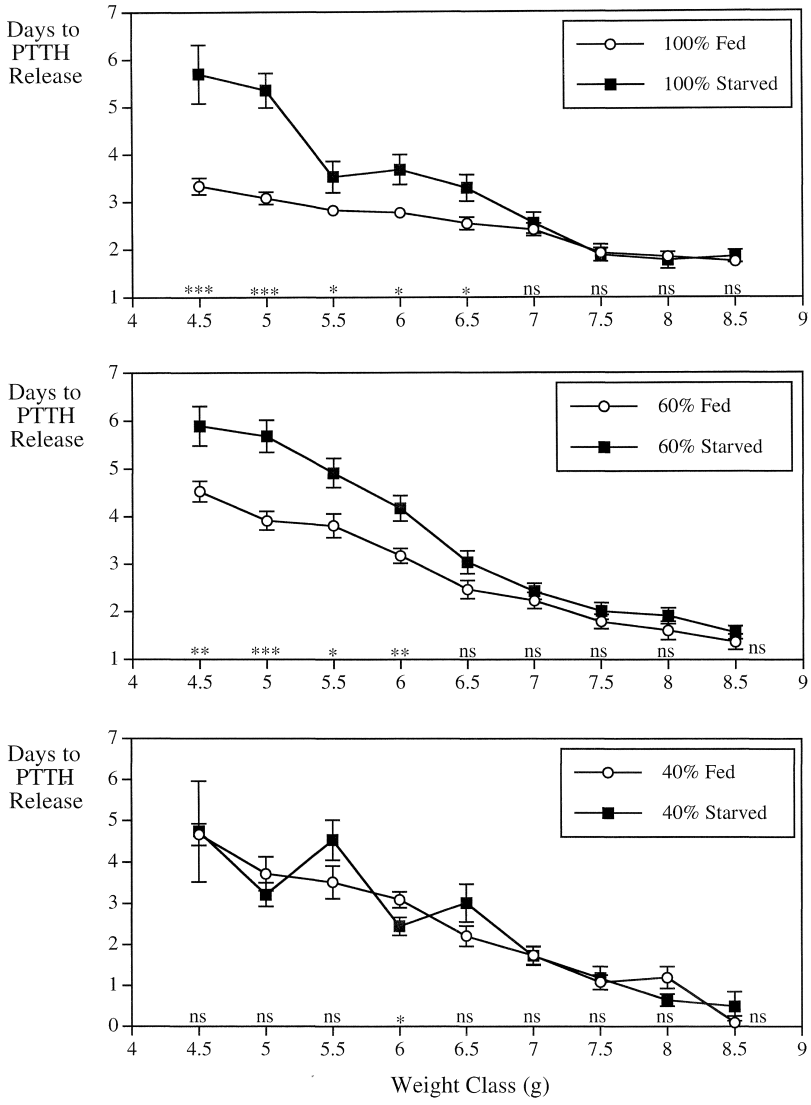


Fig. 4. The effect of diet quality on the critical weight. Each data point represents 15–20 larvae. Error bars are one standard error of the mean and in some cases are smaller than the symbol. ns, nonsignificant at $\alpha = 0.05$; $0.05 > * > 0.01$; $0.01 > ** > 0.001$; $*** < 0.001$ (*t*-test). PTTH, prothoracicotropic hormone.

ticular relationship to the causal mechanisms generating body size. Indeed, in most organisms the mechanisms underlying body size regulation are entirely unknown. Accordingly, little is known about how the developmental and physiological determinants of body size change as body size itself evolves. Likewise, the developmental and physiological mechanisms by which environmental variation affect growth, and that result in plasticity of body size, are also poorly understood (Stern 2001).

However, our current understanding of the timing of metamorphosis in *M. sexta* provides insight into the regulation of insect body size. The inhibition of PTTH and ecdysteroid secretion by JH in the last larval instar is believed to be an evolved mechanism that prevents the initiation of metamorphosis until all traces of JH have disappeared (Nijhout 1994). This inhibitory effect of JH is unique to the last larval instar: In earlier instars, PTTH and ecdysteroid secretion occur in the presence of JH, leading to a larval–larval molt. The critical weight in the last larval instar is, in effect, the trigger that sets in motion the suppression of JH secretion and the expression of the JH-scavenging enzyme, JH esterase (Browder et al. 2001). Once these processes begin, the larva is set on an inexorable course toward metamorphosis. Body size of the adult is therefore determined by the value of the critical weight and the amount of growth the larva can achieve during the interval between the critical weight and the secretion of PTTH. This knowledge, together with information on the dynamics of growth, can be used to study how body size is determined and to elucidate the mechanisms by which genetically or environmentally induced

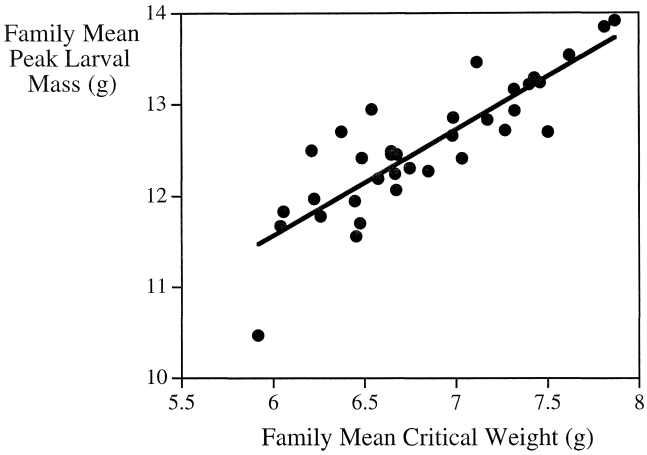


Fig. 5. Regression of peak larval mass on critical weight. Data points are family means (see text). $y = 4.6 + 1.16x$, $n = 35$, F = 91.07, $P < 0.0001$, $r^2 = 0.73$.

variation in the determinants of size translates into variation in body size.

Genetic and environmental variation

As stated above, in *M. sexta* body size is determined by a sequence of events that is initiated when the last instar larvae attains its critical weight. The critical weight occurs at about 55% of peak larval mass. In normally growing animals, body size is determined by both the critical weight and by the amount of growth that occurs between the attainment of the critical weight and the secretion of PTTH and ecdysteroids

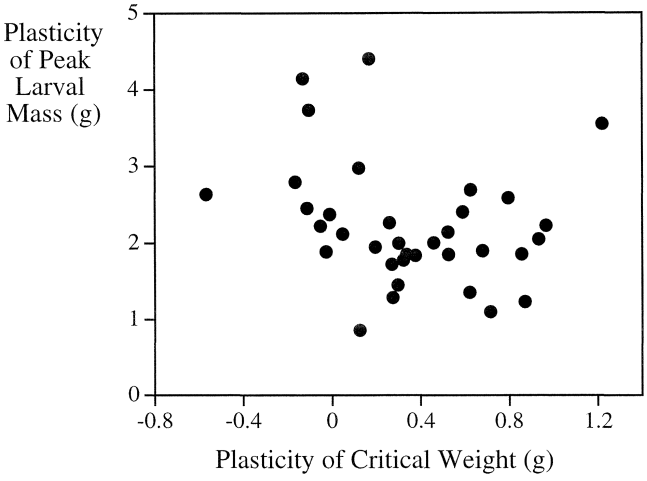


Fig. 6. Regression of plasticity of peak larval mass on plasticity of critical weight. Plasticity was measured as the mean family difference between the two (100%, 60%) diet treatments (see text). $y = 2.4 - 0.48x$, $n = 35$, F = 1.9, $P = 0.1737$, $r^2 = 0.06$.

Table 1. Mixed-model analysis of variance of body size

Source	*df*	SS	MS	F	*P*
Model	69	1672.2	24.2	18.5	<0.0001
Family	34	478.3	14.1	10.7	<0.0001
Diet	1	1002.0	1002.0	764.3	<0.0001
Family × diet	34	136.2	4.01	3.06	<0.0001
Error	810	1061.9	1.3		

that initiate the metamorphic process. The high coefficient of determination of critical weight and body size ($r^2 = 0.73$) and the high genetic correlation between critical weight and body size (0.43 ± 0.23) further demonstrate that critical weight is an important determinant of body size in this insect.

Plasticity of body size in response to environmental variables, such as temperature and nutrition, must be mediated through the physiological mechanisms, such as the critical weight, that determine the size at the onset of metamorphosis. We found that variation in temperature had no effect on the critical weight, although food quality did. The critical weight decreased with decreasing diet quality and was apparently no longer functional in larvae reared on a very poor (40%) diet. On 40% diet larvae grew very slowly, and there was no difference in the timing of metamorphosis of feeding and starved larvae at almost all weights we tested. 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. A test of the repeatability of estimating critical weight (data not shown) indicated that in all cases critical weight decreased with decreasing diet quality, whereas the variation in critical weight due to temperature was less than the variation due to environmental chambers, further suggesting that critical weight does not change with temperature.

The critical weight accounted for a large proportion of the phenotypic variation in body size. This is not surprising, because the two are not completely independent (see above). At the same time, however, there was no relationship

Table 2. Mixed-model analysis of variance of critical weight

Source	*df*	SS	MS	F	*P*
Model	69	305.7	4.43	4.37	<0.0001
Family	34	235.6	6.93	6.84	<0.0001
Diet	1	25.1	25.1	24.8	<0.0001
Family × diet	34	30.2	0.89	0.88	0.6712
Error	810	820.7	1.01		

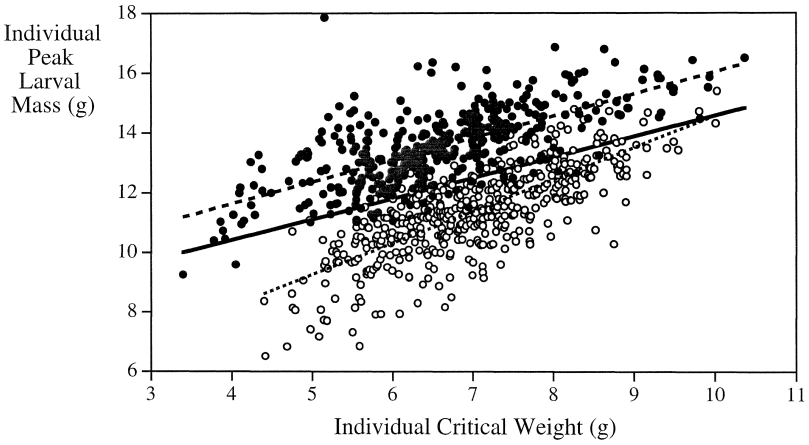


Fig. 7. Regression of individual peak larval mass on individual critical weight. The open circles are larvae fed 60% diet (n = 471), and filled circles are larvae fed 100% diet (n = 409). The solid-lined regression is for all data combined, $y = 7.63 + 0.69x$, n = 880, F = 218.8, $P < 0.0001$, $r^2 = 0.2$. The regression of larvae fed 60% diet only (dashed line), $y = 3.91 + 1.07x$, n = 471, F = 631.3, $P < 0.0001$, $r^2 = 0.57$. The regression of larvae fed 100% diet only (dotted line), $y = 8.7 + 0.73x$, n = 409, F = 351.4, $P < 0.0001$, $r^2 = 0.46$.

between plasticity of the critical weight and plasticity of body size. This suggests that body size and plasticity of body size are not regulated via the same mechanism. An exciting prospect for future work is to determine how the signaling and regulatory mechanisms of the critical weight change in response to environmental variation.

Our results revealed significant additive genetic variance for both body size and critical weight, so both of these factors should be able to respond to selection on body size. Phenotypic plasticity of body size should also be able to respond to selection because our results indicate significant genetic variation for plasticity of body size as well. The high between-diet genetic correlation of body size (0.66) and critical weight (1.0) suggests that both are regulated by the same genetic mechanisms in both high and low quality diets. A between-environment genetic correlation of $+1$ or -1 indicates a lack of genetic variation for phenotypic plasticity (Via and Lande 1985, 1987) that has often been cited as one of the primary constraints on the evolution of phenotypic plasticity (Via and Lande 1985, 1987; Scheiner 1993a; Via et al. 1995; DeWitt et al. 1998). The lack of genetic variation for plasticity of critical weight as indicated by the between-diet genetic correlation was substantiated by the lack of significant variation of the interaction term in the mixed-model ANOVA. Our results, therefore, suggest that although the critical weight may be an important factor enabling the evolution of body size, it may simultaneously constrain the evolution of plasticity of body size.

Support for the hypothesis that change in critical weight is one of the mechanisms by which holometabolous insects change body size comes from the work of D'Amico et al. (2001) on *M. sexta.* They showed that an evolutionary increase in body size (from 7.8 to 11.1 g) over approximately 220 generations was associated with an increase in the critical weight (from 5 to 6 g).

Larvae that are larger at the onset of the last larval instar have higher critical weights (Nijhout 1981). There is a strong positive nonlinear relationship between the head capsule width of the last larval instar and the critical weight as well as a positive linear relationship between the initial mass of the last larval instar and the critical weight (Nijhout 1981). Our results show significant positive correlations, although the coefficients of determination reveal much weaker relationships (r^2 = 0.24 and 0.13, respectively) than have been previously reported. The reason we detected a weaker relationship was probably due to the fact that Nijhout (1981) used various combinations of temperature, diet quality, and starvation to enhance the range of the head capsule sizes and initial larval masses over which the relationship to critical weight could be measured. The laboratory conditions used in the present study generated only about 15% of the range produced by Nijhout (1981), which is the normal range encountered by larvae in the laboratory, and this reduced our ability to detect a stronger correlation.

Is there additional evidence for the role of the critical weight in regulating body size from other systems? A confusion has arisen over the years about the exact definition of the critical weight, and a number of studies that have purported to study critical weight have studied other phenomena instead. For example, Jones et al. (1981) examined the critical weight in the caterpillar *Trichoplusia ni.* However, their weight classes were defined at the time of the molt to the last instar (and not during the last instar), and because they did not directly compare the timing of PTTH release between starved and fed larvae, it is unclear how their measure of the critical weight relates to that defined by Nijhout and Williams (1974a). Woodring (1983), Ochieng Odero (1990), and De Moed et al. (1999) all defined the critical weight as the weight at which 50% of the larvae were able to pupate. This is not a measure of critical weight as originally defined (Nijhout and Williams 1974a) but is more akin to a "minimal viable size," which is the minimal mass necessary for a larva to successfully pupate. The critical weight has a special significance because it initiates a known developmental cas-

cade of events that determines the timing of metamorphosis and thus adult body size. The minimal viable size, by contrast, describes the minimal amount of resources necessary for a developing larva to successfully pupate. The minimal viable size essentially defines the lower limit of body size, which may be substantially smaller than the critical weight, and is not associated with any known developmental or physiological events. Because they did not measure the actual critical weight, the results of these various studies are not directly comparable with those presented here.

A number of other mechanisms for body size regulation have been proposed. For example, Partridge et al. (1994), Van Voorhies (1996), De Moed et al. (1997), Sibly and Atkinson (1994), Oldham et al. (2000), and Brogiolo et al. (2001) have proposed that body size is regulated by the size and the number of cells in the organism. The role of the critical weight in regulating body size is consistent with this, because the mechanism regulating cell size and cell number operates at the cellular and organ levels, whereas the critical weight operates on the level of the whole organism. The critical weight mechanism determines the time and size at which growth will stop and appears to be independent of the mechanisms that regulate growth at the cellular level.

Variation in body size can have large consequences to an individuals fitness (Calder 1984; Schmidt-Nielsen 1984; Roff 1992; Stearns 1992; Schlichting and Pigliucci 1998) or to species evolution (McShea 1998). Part of this variation is due to phenotypic plasticity. It is well established that phenotypic plasticity is environment specific in that different environmental stimuli are known to produce different degrees of plastic response in a given trait (Bradshaw 1965; Schlichting 1986; Scheiner 1993a). Our results suggest that different environmental stimuli may have their primary effect on different portions of the mechanism that determines the value (e.g., size) of a trait.

Recently, whether phenotypic plasticity is regulated by specific genes or is a by-product of selection on the mean values of a trait in different environments has been discussed (Scheiner 1993b; Schlichting and Pigliucci 1993, 1998; Via 1993a,b; Via et al. 1995; Pigliucci 2001). The positive correlation between body size and critical weight and the lack of a correlation between plasticity of size and plasticity of critical weight indicate that in this species, plasticity of size is not a by-product of the size of larvae reared in different environments. This suggests that the mechanism regulating body size differs from the mechanism regulating plasticity of body size. Thus, the determinants of the value of a trait and the determinants of the plasticity of that trait do not need to be identical.

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Appendix. Diet quality formulae

Ingredient	100%	60%	40%
Wheat germ (g)	109	65.4	43.6
Casein (g)	49	29.4	19.6
Torula yeast (g)	21.8	13.08	8.72
Wesson salts (g)	16.4	9.84	6.56
Cholesterol (g)	4.8	2.88	1.92
Sucrose (g)	43	25.8	17.2
Cellulose (g)	0	48.8	73.2
Methylbaraben (g)	1.4	1.4	1.4
Sorbic acid (g)	2.7	2.7	2.7
Ascorbic acid (g)	6.8	6.8	6.8
Streptomycin (g)	0.27	0.27	0.27
Penicillin (g)	1	1	1
Vanderzandt vitamin mix (g)	0.67	0.67	0.67
Water (l)	1	1	1
Linseed oil (ml)	5	5	5
Formalin (ml)	5	5	5

The 100% diet is the standard rearing diet.