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Conflicting processes in the evolution of body size and development time

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Body size and development time of *Manduca sexta* are both determined by the same set of three developmental–physiological factors. These define a parameter space within which it is possible to analyse and explain how phenotypic change is associated with changes in the underlying factors. Body size and development time are determined by the identical set of underlying factors, so they are not independent, but because the mechanisms by which these factors produce each phenotype are different, the two phenotypes are only weakly correlated, and the correlation is context dependent. We use a mathematical model of this mechanism to explore the association between body size and development time and show that the correlation between these two life-history traits can be positive, zero or negative, depending entirely on where in parameter space a population is located, and on which of the underlying factors has a greater variation. The gradient within this parameter space predicts the unconstrained evolutionary trajectory under directional selection on each trait. Calculations of the gradients for body size and development time revealed that these are nearly orthogonal through much of the parameter space. Therefore, simultaneous directional selection on body size and development time can be neither synergistic nor antagonistic but leads to conflicting selection on the underlying developmental parameters.

Keywords: body size; mathematical model; morphospace; selection; *Manduca sexta*; growth

1. INTRODUCTION

The evolution of body size is one of the most common and widespread trends we see in all of evolutionary biology. This is because body size is typically positively correlated with fitness (Calder 1984; Schmidt-Nielsen 1984; Roff 1992; Stearns 1992). Development time is also likely to be under selection. Insect larvae such as those of our experimental species, the tobacco hornworm *Manduca sexta*, are exposed to predators and parasitoids, and mortality due to these causes can exceed 95 per cent (Bernays 1997; Mira & Bernays 2002). It is therefore reasonable to expect that short development time would increase fitness by reducing the time the animal is exposed to parasitoids. Insect larvae, like those of *M. sexta*, typically grow at an exponentially increasing rate (Nijhout *et al.* 2006), so even relatively small changes in the timing at which growth stops can have a profound effect on the final body size. Body size and development time are therefore likely to be correlated traits (reviewed in Roff 2000).

It seems reasonable to assume that there should be a positive relation between body size and development time for the simple reason that, other things being equal, animals that grow for a longer time should grow bigger. However, animals have size regulating mechanisms that result in a species-characteristic size that is fairly independent of growth rate and development time. Yet, at the same time, most species exhibit a range of adult body sizes owing to environmental and genetic variation. In insects, for instance, increased nutrition increases growth rate and adult body size; by contrast, an increase in temperature increases growth rate but results in smaller adult body size (Davidowitz & Nijhout 2004; Davidowitz *et al.* 2004).

Here, we report on a study of the relationship between body size and development time, based on a detailed quantitative and experimental understanding of the developmental and physiological mechanisms that control these two traits. We have developed a mathematical description of the processes that control body size in *M. sexta* (Nijhout & Williams 1974*a*,*b*; Davidowitz *et al.* 2003, 2005; Davidowitz & Nijhout 2004; Nijhout *et al.* 2006), which allows us to examine the functional relationships between body size and development time.

Manduca sexta has long been the principal model system for the physiology, endocrinology and post-embryonic development of insects. We initially developed our mathematical description of the mechanisms that regulate body size and development time as a means of testing the quality of our understanding of the processes that regulate these important life-history traits. Mathematical modelling is an excellent way of testing one's understanding of how a system operates because it forces one to be completely explicit about all hypotheses and assumptions that go into that understanding. Our mathematical model is not an abstract theoretical model but a quantitative

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description of the underlying processes by which body size and development time come about during the life of an individual. Our mathematical model predicts body size and development time with 95 per cent accuracy, and predicts how these two traits will vary owing to variation in the underlying developmental, physiological and environmental factors. We can use the model to explore the causal relationships between body size and development time, and how these relationships are affected by quantitative changes in the underlying factors.

2. MATERIAL AND METHODS

Throughout this paper we refer to *body size* as the maximum size the larva reaches before beginning the wandering stage and entering metamorphosis. As in other insects, there is no growth after metamorphosis, so the size the larva has attained at the time it starts wandering defines the size of the pupa and that of the adult. Because larval growth is exponential, about 90 per cent of the increase in mass occurs during the last larval instar. We refer to *development time* as the duration of the last larval instar, from the moult to the beginning of the wandering stage.

(a) *The mathematical model for the control of size and development time*

The derivation of the mathematical model we use here is described in detail in Nijhout *et al.* (2006). This model describes growth during the last (fifth) larval instar. *Manduca* fifth instar larvae grow from a mass of about 1.2–12 g, so they gain 90 per cent of their total body mass during this instar. Thus, the events that control growth during this instar largely determine the final body size.

The logical structure of the model is illustrated in figure 1. This figure shows that continued growth depends on a series of checkpoints (depicted by diamond-shaped boxes) at which certain physiological events occur. The first checkpoint is the critical weight. The critical weight is defined operationally as the mass at which no further nutrition or growth is required for a normal time course to metamorphosis. What happens when a larva reaches the critical weight is that the secretion of juvenile hormone (JH) stops and the expression of its catabolizing enzyme (JH-esterase) increases dramatically. The second checkpoint monitors whether JH has been completely cleared. During the last larval instar, JH actively inhibits secretion of the prothoracicotropic hormone (PTTH) and ecdysone. When JH degradation has gone to completion, the secretion of these hormones is disinhibited. PTTH stimulates ecdysone secretion, and ecdysone causes the larva to stop feeding, initiates a wandering phase and initiates metamorphosis. Thus, ecdysone secretion effectively ends the growth phase, and since adult insects do not grow, the size the larva has attained at the time of ecdysone secretion determines the adult body size. Once these endocrine events are set in motion (upon achieving the critical weight) they go to completion in a stereotyped pattern and are unaffected by further nutrition. These endocrine events take some time (1–3 days) to go to

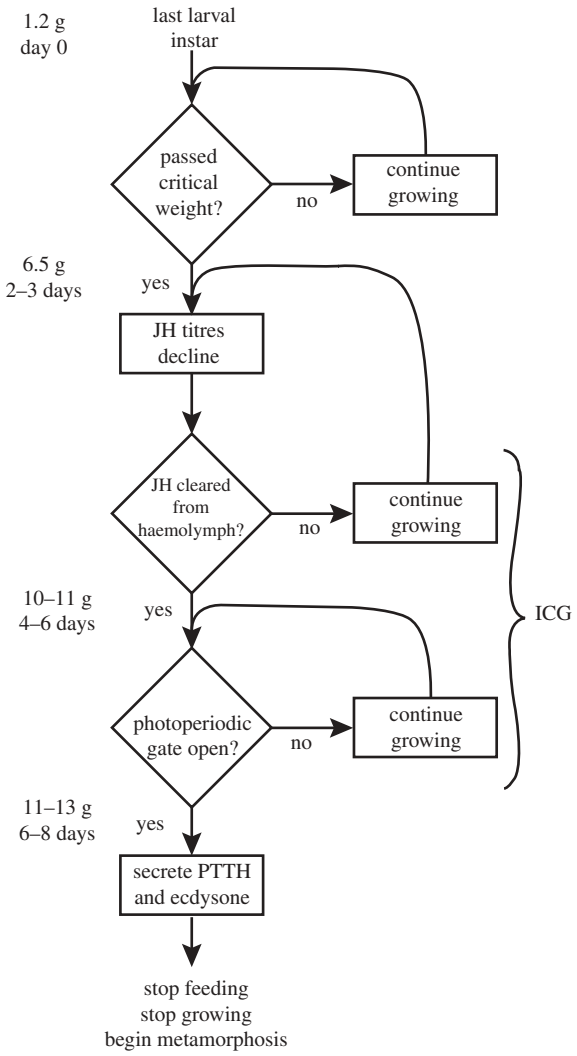

Figure 1. Logic diagram of the mathematical model for body size and development time in the final (fifth) larval instar of *M. sexta*. Diamonds represent checkpoints. Approximate masses and times for the wild type at each stage are indicated. ICG, interval to cessation of growth; JH, juvenile hormone; PTTH, prothoracicotropic hormone.

completion, however, and during this time the larva continues to grow. The third checkpoint comes when the secretion of PTTH and ecdysone are disinhibited because the actual timing of secretion of these hormones is controlled by a circadian clock that restricts their secretion to an 8 h long gate on a given day. If these hormones are disinhibited while the gate is open they are secreted immediately, but if the gate is closed their secretion is delayed until the next gate opens on the following day. During this delay, the larva continues to feed and grow normally. The period between the achievement of the critical weight and the actual secretion of PTTH and ecdysone is called the interval to cessation of growth (ICG). The critical weight is achieved half-way through the fifth instar, so the mass accumulated during the ICG can make up as much as half the final mass of the animal (figure 1).

The mathematical model is a quantitative description of this series of physiological events. We have continued to refine and update the equations that describe these relationships since we first published

our model (Nijhout *et al.* 2006), and we can now summarize the model as the following set of equations:

$$\text{Final size} = w5 * \exp\left(\left(k * \text{ICG} + \ln 5.33 - \frac{0.8}{W_0}\right) * \left(1 - 0.073 * \text{ICG}\right)\right), \quad (2.1)$$

where W_0 is the initial weight of the instar and k is the growth exponent, which is calculated from the growth rate (GR) during the third day of the instar as follows:

$$k = 0.15 * \exp(-0.65 * W_0) * \text{GR} + 0.27. \quad (2.2)$$

The duration of the instar (development time) is given by

$$\text{Duration of instar} = \frac{\ln(5.33 - 0.8/W_0)}{k} + \text{ICG}. \quad (2.3)$$

We note that these equations do not include the photoperiodic gating (i.e. as written, they stop after the second checkpoint). We calculate the gating numerically by knowing when the larva starts growing to the nearest hour and calculate whether equation (2.3) predicts a time inside a gate; if it does not then we add the appropriate time interval to the ICG term in equations (2.1) and (2.2).

The model thus requires only three easily measurable inputs, which we call the *underlying factors*. These are (i) the growth rate, (ii) the initial weight and (iii) the ICG. The critical weight (CW) is related to the initial weight of the instar by the linear function $\text{CW} = 5.33 * W_0 - 0.8$ (Nijhout *et al.* 2006). In the figures used in this paper, we show body size and development time as functions of the CW. The model accurately predicts individual final weights and development times for the entire physiologically relevant range of the three underlying factors, which are growth rate = $1\text{–}4\ \text{g}\,\text{d}^{-1}$, ICG = 16–96 h and CW = 3–9 g. The mathematical model is implemented in MatLab (The Math Works, Natick, MA, USA), and the visualizations of the multivariate data as well as the calculations of the gradients were done in Amira (Mercury Computer Systems, Chelmsford, MA, USA).

3. RESULTS

(a) *Overall relation between body size and development time*

Natural variation in body size and development time comes about through variation in the underlying processes, which may be due to genetic and environmental causes. Simulations with the model can be used to determine the relative importance of each of the underlying developmental–physiological parameters in determining body size, development time and the relation between the two.

We allowed the three underlying factors to vary randomly, with a uniform distribution, over their entire physiological range of values and calculated body size and development time for 64 000 'individuals'. We found an overall positive relationship between body size and development time (figure 2), but the relationship is somewhat complex, in that development time is not continuously represented. This is because the termination of growth, which is due to the secretion of ecdysone, is gated by the photoperiod and cannot occur during a portion of the day (Nijhout *et al.* 2006). A similar pattern was observed when the underlying factors were allowed to vary over a small range that could represent normal variation in a population. This is shown in figure 3 where the three factors were allowed to vary with a normal distribution around a mean and with a 10 per cent standard deviation. Small subsamples of a population show a variable and generally weak relationship between body size and development time.

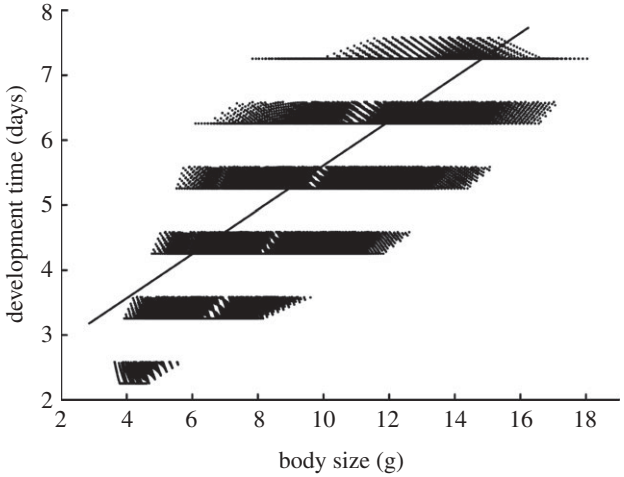

Figure 2. Relationship between body size and development time simulated with the model. The three underlying factors were sampled independently from a uniform distribution, with a range for each factor spanning the known physiological range. The linear regression is $y = 0.35x + 2.2$, $r^2 = 0.63$ and $n = 64\,000$.

(b) *Correlations between body size and development time when variation is due to different causes*

From a developmental viewpoint it is of interest to know the relative effect of each of the underlying factors on body size and development time. The independent effects of each of the three parameters, when the others are held constant, are shown in figure 4. Growth rate is generally positively correlated with body size and negatively correlated with development time, as one would expect (figure 4*a*), but the relation is not simple. There is a sawtooth-like pattern, which is due to the fact that the secretion of ecdysone (which terminates the growth phase) is gated by the photoperiod and can only occur during an 8 h period each day (Nijhout *et al.* 2006). Thus, if a larva becomes competent to secrete ecdysone after a photoperiodic gate has closed, it continues to feed and grow until the next gate opens. Because of photoperiodic gating, the timing of cessation of growth is not continuous, and the final body size is continuously distributed.

The data in figure 4*a* were calculated over a range of growth rates that corresponds to the entire range we have been able to document for diverse genetic strains. For any given strain or population, the range is rather small and is thus represented by only a segment of the

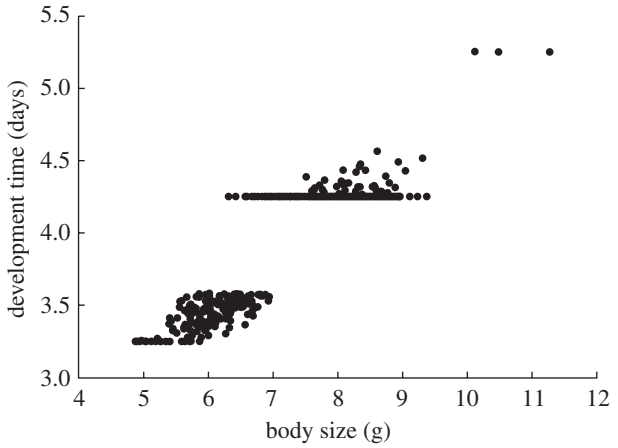

Figure 3. Relationship between body size and development time calculated with the model. The three underlying factors were sampled from normal distributions with the means: growth rate, 2 g d^{-1}; critical weight, 5 g; ICG, 24 h; and with standard deviations 10% of the mean, $n = 1000$.

curves shown. Over a small range of growth rates, however, there is no clear relationship between growth rate and body size or development time; it can be positive, near zero or negative, and a genetic or environmental change in growth rate can alter this relationship. The overall relationship between body size and development time is negative (i.e. development time goes down as body size goes up).

The relationship between the critical weight, body size and development time is equally complex (figure 4*b*), as is the relationship between body size, development time and the JH-dependent ICG (figure 4*c*). In these cases, the relationship with development time is positive, although there are regions where the relationship is effectively zero. The relationship with body size ranges from negative (in the region around 1.5 g d^{-1}; figure 4*c*) to strongly positive (figure 4*b*). The relationship between body size and development time is positive when variation is due to either the critical weight or the ICG.

Several of the curves in figure 4 have large horizontal flat regions (see also figure 6), which implies that in that range, variation of the underlying factor has no effect on the phenotype. In those regions of parameter space, the phenotype is robust to variation in that factor. The causes and mechanisms of robustness in development are of considerable interest because they help explain the remarkable stability of phenotypes in the face of genetic and environmental variation (Gilchrist & Nijhout 2001; Nijhout 2002; Nijhout *et al.* 2003; Ciliberti *et al.* 2007; Munteanu & Solé 2008). In the present case, robustness is an emergent property of the temporal gating of hormone secretion by a photoperiodic clock.

(c) *The phenotypic landscapes for body size and development time*

Rice (1998, 2002, 2004, 2008) has developed a complete theory of microevolution that is a theoretical elaboration of Price's (1970) theorem. Rice's theory is general and subsumes quantitative genetics and population genetics. Rice (2004, 2008) has shown

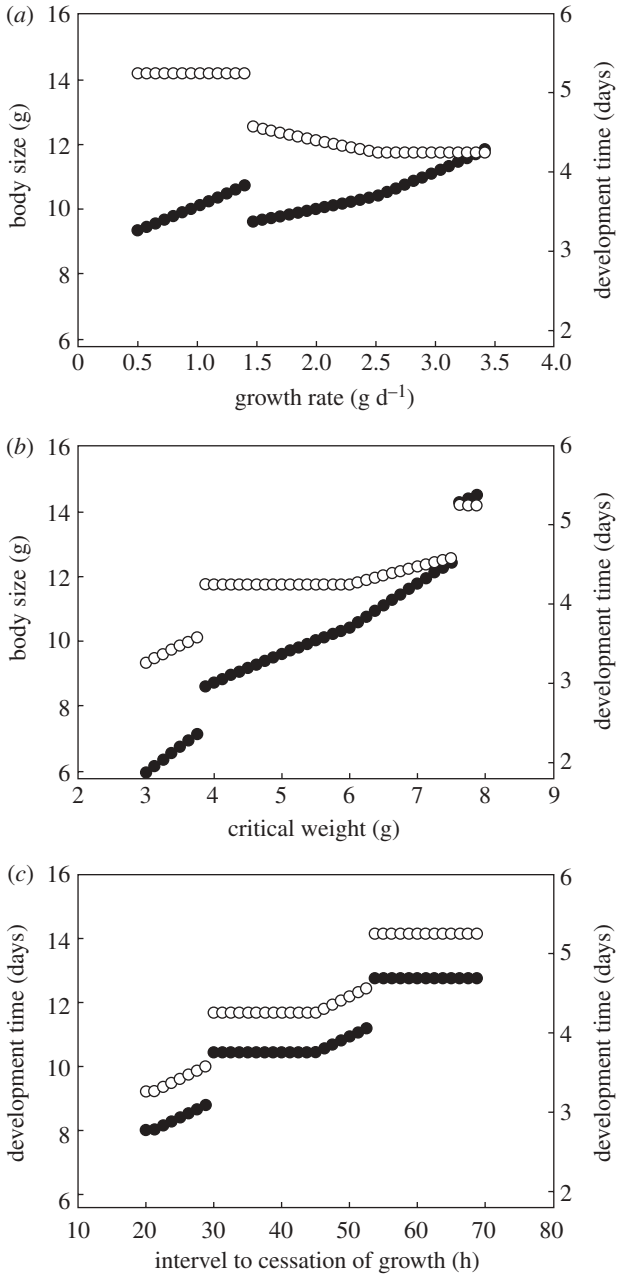

Figure 4. Body size and development time as functions of either the growth rate (*a*), critical weight (*b*), or ICG (*c*), when the other factors are held constant. The constant values were growth rate, 2.5 g d^{-1}; critical weight, 6 g; and ICG, 48 h. Filled circles, body size; open circles, development time.

how this theory links developmental mechanism with phenotypic evolution via the concept of phenotypic landscapes. Phenotypic landscapes are plots of the value of a phenotypic trait as a function of the underlying causal factors (see also Alberch 1991). These factors can be genes, physiological factors, developmental modules and environmental factors that affect the phenotype. A phenotypic landscape is a multidimensional surface whose dimensions are usually the same as the number of underlying causal factors. An individual is a point on that surface, and a population is a distribution of points, and Rice provides an explicit theory for evolution *on* phenotypic landscapes, independent of the shape of the landscape and the distribution of the population on the landscape.

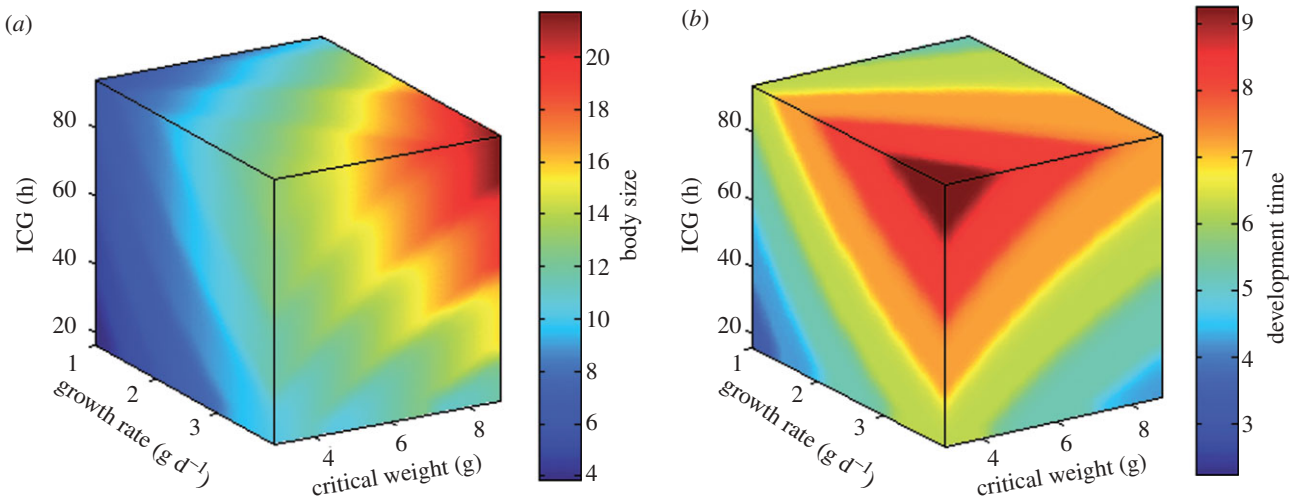

Figure 5. Phenotypic landscapes of body size (*a*) and development time (*b*) as functions of the growth rate, critical weight and ICG. Orientation of axes is the same for the two landscapes. Body size (g) and development time (days) are indicated by colour scales.

Our model provides a mechanism for generating phenotypic landscapes. When all three factors are allowed to vary over their entire range of observed values we can compute the full three-dimensional phenotypic landscapes for both body size and development time. Here, we keep the range of variation in the underlying factors within the experimentally observed range, so the phenotypic landscape is not projected beyond real data (Nijhout *et al.* 2006).

Because both body size and development time are determined by the exact same set of underlying factors, it is possible to plot the phenotypic landscape for both traits on the same axes. There are only three underlying factors, so the phenotypic landscapes can be depicted as a three-dimensional volume where the three axes are the independent variables (the underlying factors) and using a colour scale to represent phenotypic values within the volume (figure 5). The landscape for body size shows the sawtooth pattern imposed by the circadian clock for ecdysone secretion. The landscape for development time is much smoother than that for body size. The colour gradients show that the two landscapes are not congruent, in that the combination of parameter values that give the largest body size is not the same as the combination of those that give the longest development time. Thus, selection that favours large body size would favour a high growth rate and a high critical weight, whereas selection that favours long development time would favour a high growth rate but a low critical weight.

Sections through the three-dimensional landscape allow one to examine the effects of simultaneous variation in two of the parameters, while the third is held constant. An example of such a phenotypic sub-landscape for two parameters, growth rate and ICG (with the critical weight held constant), is shown in figure 6. This particular landscape can be used to examine the effects of temperature on body size. Davidowitz *et al.* (2004) have shown that the critical weight is not affected by temperature, but the growth rate and ICG are. The landscape in figure 6 is for a

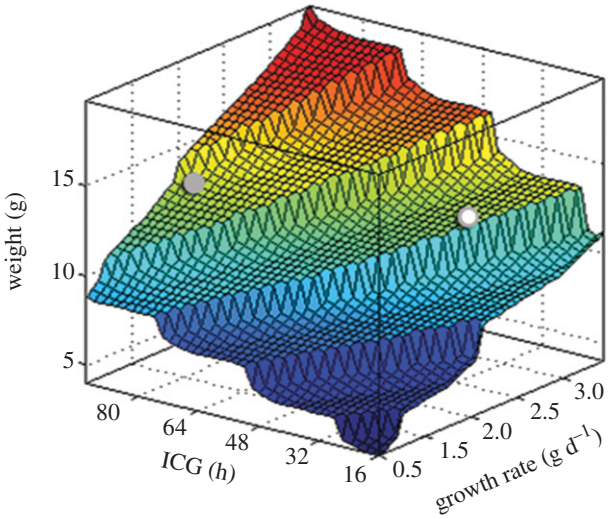

Figure 6. Phenotypic landscape for body size when critical weight is held constant at 7 g. The two circles indicate phenotypes at two different temperatures: 30°C (on the right) and 20°C (left).

critical weight of 7.0 g. When the temperature is changed from 20 to 30°C, the growth rate goes from 1.7 to 2.9 g d^{-1} and the ICG goes from 86 to 40 h. This shift in parameter values is plotted in figure 6 and predicts that the mean body size will change from 11.6 to 10.4 g as the temperature goes from 20 to 30°C, which is in accord with the data of Davidowitz *et al.* (2004), and the general finding that in insects body size decreases with increasing temperature.

Virtually all the causal factors have highly nonlinear effects on the phenotype, as the figures presented above illustrate. These nonlinearities make the relationship between any given underlying factor and the trait context dependent. The value of the trait associated with a given value of one underlying variable depends entirely on the values of the other underlying variables. Likewise, the effect of a given amount of variation in one underlying variable on variation in a trait can be profound or negligible, depending again on the exact values of the other

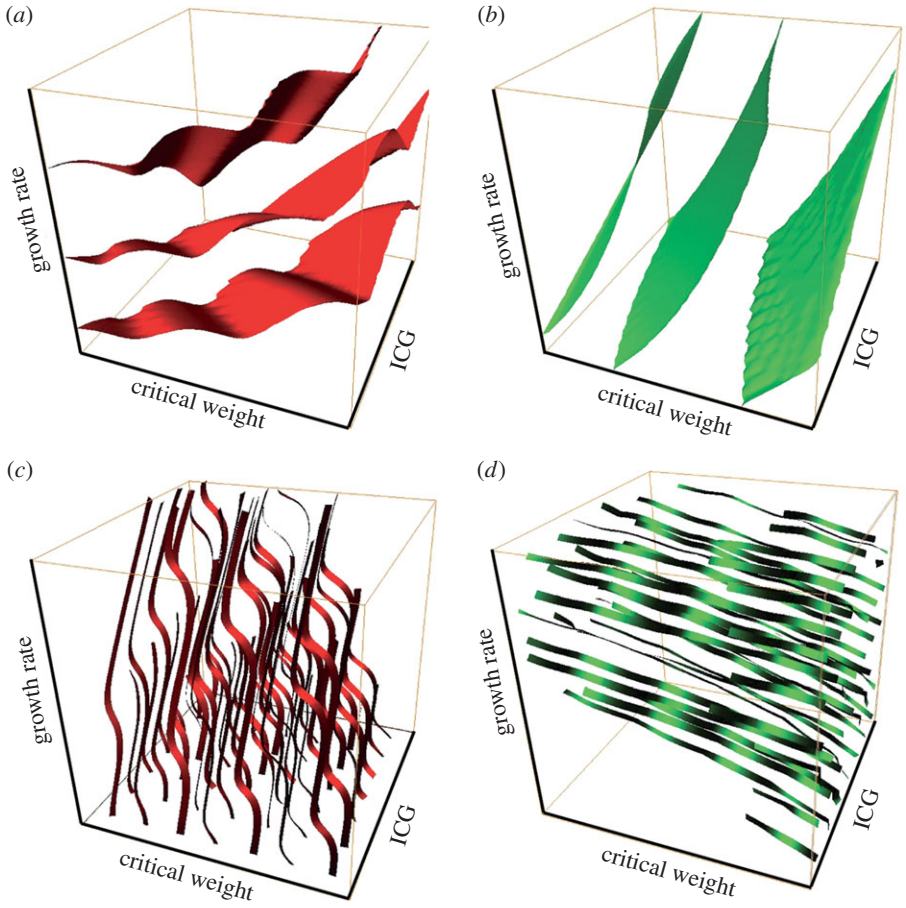

Figure 7. Selected isosurfaces for (*a*) body size (red) and (*b*) development time (green). Orientation of the phenotypic landscape is the same in all panels. Isosurfaces are combinations of parameter values that produce the same phenotype. The gradients of the phenotypic landscapes for (*c*) body size and (*d*) development time. The gradients are shown as ribbons that follow the steepest internal slopes of the phenotypic landscapes. The gradients are orthogonal to the isosurfaces.

underlying variables. This means that the correlation between a given factor and a trait will depend on both the exact location and the dispersal of the population on the phenotypic landscape. This in turn implies that the evolution of the phenotype and its causal underlying factors will be affected by exactly where in parameter space the population starts from.

(d) *Evolution on a phenotypic landscape*

It is difficult to depict the inside of the phenotypic landscapes in detail, but one can get a sense of the internal structure by plotting some of the isosurfaces. These are surfaces of identical phenotype and correspond to the contours on a two-dimensional landscape. The isosurface shows the various combinations of values of the underlying factors that give rise to an identical phenotype. Three isosurfaces for three different phenotypic values in each phenotypic landscape are shown in figure 7*a*,*b*. It is obvious that there are an infinite number of combinations of values of the three parameters that can produce the identical phenotypic value. Thus, a population could drift widely through parameter space even if one of the phenotypes is under strong stabilizing selection. But such drift would be accompanied by significant changes in the other phenotype. If both phenotypes are under stabilizing selection the parameter values would be constrained to a line formed by the intersection of their isosurfaces.

Figure 7*a*,*b* shows that the isosurfaces of the two landscapes are almost orthogonal to each other in many regions of parameter space. This turns out to be an important observation for understanding the interaction of body size and development time. To see why this is the case, consider the idealized evolution on a phenotypic landscape. Lande & Arnold (1983) and Rice (2004) have shown that in the absence of genetic and demographic constraints, under directional selection a population will follow a trajectory that takes it up the steepest local slope of the phenotypic landscape (see also Gilchrist & Kingsolver 2001). In three-dimensional space, this would be the same as taking the shortest distance from one isosurface to another. Thus, the evolutionary trajectory under directional selection on a phenotype will be normal to the local isosurface of the phenotype.

We can compute such idealized trajectories by calculating the three-dimensional gradients within the phenotypic landscape volumes. These gradients are depicted by ribbons in figure 7*c*,*d*. Each ribbon represents a trajectory along which the underlying parameters would change in response to directional selection on the phenotype, from a given starting point within parameter space. Thus, directional selection on body size will change the values of the

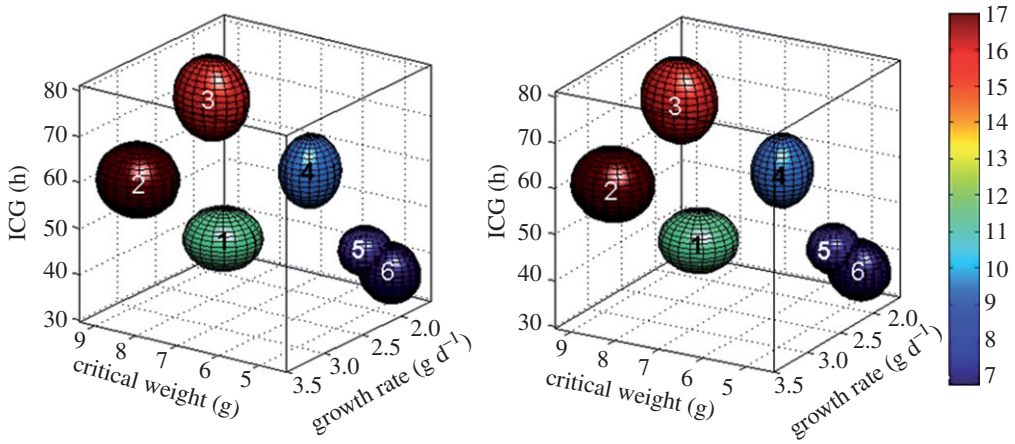

Figure 8. Stereo-pair showing distributions of six strains of *M. sexta* within the phenotypic landscape of body size. Strain 1 is the ancestor of strains 2–5, which were derived by selection for increased or decreased body size and development time. Strain 6 is the black larval mutant. The dimensions of the axes of the spheroids correspond to standard deviations around the mean for each of the underlying factors. Colour of the spheroids indicates body size.

underlying parameters (again, assuming there are no constraints on their variation), and this will cause a correlated change in development time.

The univariate data shown in figure 2 indicate a positive correlation between body size and development time, so one might expect that simultaneous selection for increasing body size and increased development time (and vice versa) would be synergistic because they would both favour changes of the underlying factors in the same direction. By contrast, selection for increased body size and decreased development time would be antagonistic because they would favour changes of the underlying factors in opposite directions. The three-dimensional analysis with the model shows that the gradients for body size and development time are nearly orthogonal almost everywhere in parameter space, which implies that selection cannot act strictly synergistically on these two traits. Nor does simultaneous selection act strictly antagonistically. An inevitable conflict arises when the two traits are under simultaneous selection.

(e) *The distribution of populations on the phenotypic landscape*

The gradients depicted in figure 7*c*,*d* are idealizations of potential evolutionary trajectories that assume that continuous variation over the entire range of underlying factors is available. In real populations, this variation will not be homogeneous over the entire parameter space. We have calculated the means and standard deviations of the underlying factors for several genetic strains of *Manduca* that have different body sizes and development times, and these are illustrated in figure 8. The population labelled 1 is our wild-type control population that was the source from which populations 2–5 were derived by selection (Davidowitz *et al.* 2005). Population 2 was selected for large body size and short development time, population 3 for large body and long development time, population 4 for small body and short development time and population 5 for small body and long development time. Population 6 is the black larval strain, which is due to a single recessive mutation that alters JH production (Safranek & Riddiford 1975). Evolutionary increases in body size were accomplished in large measure by increases in the ICG and the growth rate, whereas decreases in body size involved mostly a decrease in the critical weight and the growth rate. Selection for short development time was associated with both an increase and a decrease in growth rate, depending on the direction of simultaneous selection on body size, whereas selection for long development time was associated with a decreased growth rate independent of whether simultaneous selection favoured large or small size. Population 6 illustrates the effect of a single mutation that reduces the level of JH. This mutation reduces body size to about half of wild type and, evidently, does so through a decrease of all three of the underlying factors. A decreased JH level shortens the ICG, but is not known to have an effect on the growth rate in the fifth instar. The effect on growth rate and the critical weight must be due to the fact that reduced JH causes earlier larval instars to moult at smaller sizes, so that the initial size of the fifth instar is smaller than normal. This smaller initial size would account for both the decreased critical weight as well as the decreased growth rate of the fifth instar.

4. DISCUSSION

The developmental causes of both body size and development time in *M. sexta* can be reduced to three fundamental developmental–physiological factors: the growth rate (GR), the critical weight (CW) and the juvenile-hormone-dependent interval between the attainment of the critical weight and the cessation of growth (ICG). A mathematical model that simulates the normal physiology of growth of *Manduca* can, using these three parameters, accurately predict body size and development time for diverse genetic strains, and under a variety of environmental conditions (D'Amico *et al.* 2001; Nijhout *et al.* 2006).

(a) *Genes and environment*

The three factors that determine body size and development time are themselves complex traits that are the

result of interactions among several genetic and environmental variables. For instance: *the growth rate* depends on environmental variables like nutrition and temperature, but also on genetic or heritable variables like nutrient assimilation, metabolism, insulin and ecdysone synthesis, endocrine signal transduction, protein synthesis and cell division; the value of *the ICG* depends on temperature and on genetic variables like the circadian clock and the regulation of JH synthesis and catabolism; and *the critical weight* depends on nutrition of the earlier instars, and on the genetic mechanism by which the animal monitors its size. If these determinants vary independently of each other they can be depicted as additional mutually orthogonal axes of variation that replace each of the three axes in figure 8. Therefore, we can think of each of the axes as projections of high-dimensional hyperspaces. The important point is that the three axes of variation represent empirically measurable underlying factors, and these factors alone are sufficient to predict body size and development time with 95 per cent accuracy (Nijhout *et al.* 2006). So even though each axis can be decomposed into a complex hyperspace, all the relevant information of that space seems to project neatly onto the three axes.

Because the position of an individual on each axis is determined by both genetic and environmental factors, this view of a phenotypic landscape naturally puts genes and environment on a common and equal footing. Imagine, for instance, a genetically homogeneous population subject to variation in temperature; such a population would have a distribution along the growth rate axis that would be indistinguishable from that of a population in a temperature-invariant environment that has genetic variation in nutrient assimilation (and thus in growth rate). Thus, the phenotypic effect of variation along the growth rate axis can represent genetic variation, or a reaction norm, depending entirely on the amount of genetic and environmental variation of a population.

Nutrition and temperature are the environmental factors whose effects on body size and development time are best understood. But their effect is highly indirect and complex. The complexity of the interaction can be understood by considering the effect of nutrition. Nutrition affects the growth rate and thus the time required to get to the critical weight, but it does not affect the value of the critical weight. And while nutrition does not affect the duration of the ICG it does affect the mass that can be accumulated during that time interval. Thus, nutrition affects both development time and body size, but it does so through very different mechanisms that operate at different times in the last larval instar.

Nutrition also has an indirect effect on body size by affecting growth of the early instars. One could imagine that low nutrition throughout early larval life would have a knock-on effect, by causing each larval stage to moult to the next at a progressively worsening subnormal size, resulting in a final instar larva of a severely subnormal initial size, and thus having a corresponding subnormal critical weight. In general, this effect is likely to be small for the following reason. We have recently found that each preceding larval instar also has a critical weight (operationally measured as described in Davidowitz *et al.* 2003), but in these early stages this critical weight is close to the normal final weight of each instar (V. Callier & H. F. Nijhout 2009, unpublished data), and thus constrains mass at each larval moult to a relatively narrow range. This in effect acts as a robustness mechanism for body size because it prevents the exponentially cumulative effect of low nutrition in which each successive instar moults at a smaller size increment.

Genetic effects on the phenotype are just as indirect and complex as those of environment. As noted above, genetic variation in nutrient assimilation (for instance, owing to polymorphisms at various metabolic enzymes) would be expected to result in variation in growth rate (*ceteris paribus*), with the exact same consequences for body size and development time as those produced by environmental variation in nutrition. The same argument can be made for genetic effects of variation in the insulin and ecdysone signalling pathways, both of which also affect the growth rate in insects (Nijhout 2003*a*,*b*).

This brief consideration of the effects of genetic and environmental variation on size and development time, via their effect on the growth rate, illustrates how difficult it is to disentangle the effects of genes and environment on a complex trait. In the laboratory, one can establish inbred strains that can be maintained under stable environmental conditions, but in nature both environmental and genetic variation are complex and their effects on the phenotype are largely unknown. This explains why heritabilities and genetic correlations among complex traits are low, and why they vary in often apparently irrational ways with variation in environment and genetic background.

Knowledge of the developmental and physiological mechanisms that produce complex traits makes it possible to ascribe phenotypic variation to specific underlying factors that are causal. This, in turn, may provide a way to a deeper understanding of the patterns of statistical associations. Using a mathematical description like the one we have explored here provides a model that is realistic and explicit, which can be used to provide a more deeply informed analysis of inheritance and evolution of complex traits than is possible with the standard linear-additive model. Studies to this end are underway in our laboratories.

(b) *Conflicting forces*

Because the determinants of body size and development time can be reduced to only three independent variables, it is possible to depict the entire parameter space for body size of the two phenotypic traits as a pair of volume graphs, which constitute the phenotypic landscapes of body size and development time (figure 5). Evolution within these phenotypic landscapes can be predicted by Rice's (2004, 2008) theory, if the distribution of a population on the landscape and the pattern of selection are known.

The model shows that the phenotypic landscapes for body size and for development time are almost orthogonal to each other in large regions of parameter space (figure 7). This means that body size and development

time will not be strongly correlated with each other and that processes that increase body size will not necessarily also increase development time. This goes against one's intuition, which is that longer development time should be associated with a larger body size (and vice versa). But it can be seen from figure 6, for instance, that if an increase in body size is due to an increase in the critical weight, this would necessarily be associated with a decrease in development time.

Because both traits are produced by the same underlying factors, selection on one trait will produce a correlated response in the other. The joint response to selection will depend on where the population is located and how widely it is dispersed on the landscape. There are pockets within parameter space where the gradients of the two landscapes are not orthogonal, and these presumably give rise to the variable correlation between body size and development time seen in figure 3. The gradients within these volumes predict the unconstrained evolutionary trajectories and show how each of the underlying parameters would change under directional selection on the phenotype (figure 7*c*,*d*). In a real system, these trajectories will of course be constrained by the uneven distribution of genetic variation along each of the three axes and by sampling error. In *Manduca*, there appears to be a lot of genetic variation for the three underlying factors, as indicated by the fact that artificial selection was able to take the distributions of populations throughout most of the depicted parameter space (figure 8).

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