

PREDICTING THE RESPONSE TO SIMULTANEOUS SELECTION: GENETIC ARCHITECTURE AND PHYSIOLOGICAL CONSTRAINTS

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A great deal is known about the evolutionary significance of body size and development time. They are determined by the nonlinear interaction of three physiological traits: two hormonal events and growth rate (GR). In this study we investigate how the genetic architecture of the underlying three physiological traits affects the simultaneous response to selection on the two life-history traits in the hawkmoth *Manduca sexta*. The genetic architecture suggests that when the two life-history traits are both selected in the same direction (to increase or decrease) the response to selection is primarily determined by the hormonal mechanism. When the life-history traits are selected in opposite directions (one to increase and one to decrease) the response to selection is primarily determined by factors that affect the GR. To determine how the physiological traits affect the response to selection of the life-history traits, we simulated the predicted response to 10 generations of selection. A total of 83% of our predictions were supported by the simulation. The main components of this physiological framework also exist in unicellular organisms, vertebrates, and plants and can thus provide a robust framework for understanding how underlying physiology can determine the simultaneous evolution of life-history traits.

KEY WORDS: Critical weight, growth rate, interval to cessation of growth, *Manduca sexta*, physiological antagonism, physiological synergism.

Body size and development time, arguably the two most important life-history traits, have been the focus of a vast literature over many decades of research (see almost any issue of this and similar journals). We know a great deal about how these traits are correlated to fitness in a wide variety of systems, as well as how these traits may respond to selection at the phenotypic level. Only recently have we begun to understand how these traits are regulated at the physiological and developmental levels.

The most frequently used method for studying the response to selection of life-history traits, such as body size and develop-

ment time, is to select on one trait and measure the correlated response to selection of another trait (Lande and Arnold 1983). Natural (and artificial) selection, however, acts on multiple traits simultaneously. It is surprising then that simultaneous selection on multiple traits has rarely been studied relative to selection on single traits (but see, Robertson and Reeve 1952; Bell and Burris 1973; Rutledge et al. 1973; Nordskog 1977; Beldade et al. 2002; Zijlstra et al. 2003; Frankino et al. 2005).

In the present article we investigate how the underlying physiology can affect the response to simultaneous selection on both

body size and development time. Our interpretation of these experiments is informed by recent work describing the physiological mechanism that determines most of the variation in body size and development time. This allows us to make explicit a priori predictions as to how the underlying physiology enables or constrains the response to simultaneous selection on these two life-history traits.

Davidowitz and Nijhout (2004) have identified the physiological mechanism that accounts for over 95% of the variation in both body size and development time in the insect *Manduca sexta* (tobacco hornworm: Sphingidae). This mechanism explains the evolution of body size (D'Amico et al. 2001) and plasticity of body size (Davidowitz et al. 2004). For brevity, only the relevant salient components of this mechanism are presented here. Further details of this mechanism can be found in D'Amico et al. (2001); Davidowitz et al. (2003, 2004, 2005); Davidowitz and Nijhout (2004); Mirth et al. (2005); Shingleton et al. (2007); Nijhout et al. (2006, 2010).

Three physiological traits determine body size and development time: (1) The critical weight (CW) is a threshold trait that initiates the cascade of events that lead to the cessation of growth and pupation. At the CW the corpora allata, the glands that synthesize and secrete juvenile hormone (JH) switch off and JH titers start to decay. The CW is typically about 54% of peak larval mass (Davidowitz et al. 2004), it is sensitive to diet quality but not to temperature (Davidowitz et al. 2003, 2004), and is not a fixed trait but can evolve (D'Amico et al. 2001). Larger CWs result in larger peak larval sizes and longer development times (Davidowitz et al. 2005; Nijhout et al. 2006) as the cascade of events that leads to the cessation of growth starts later and at a larger size.

(2) The time interval between attainment of the CW and the secretion of the ecdysteroids, the molting hormones that directly regulate molting and pupation in insects, is called the Interval to the Cessation of Growth (ICG) (Davidowitz and Nijhout 2004). During the ICG, the larva continues to feed and grow normally, and can nearly double its body mass (Davidowitz et al. 2004). In contrast to the CW, the duration of the ICG is sensitive to temperature but not to diet quality (Davidowitz et al. 2004). A longer 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 CW and the ICG are bioindicators for hormonal traits: the CW is an indicator for the cessation of JH secretion and the ICG is an indicator for the secretion of ecdysteroids. Together, these two factors determine the duration of the growth period (Davidowitz and Nijhout 2004; Nijhout et al. 2006).

(3) The third physiological factor that determines body size and development time is growth rate (GR) and is typically measured as mass accumulation per unit time. GR interacts differently with the CW than with the ICG to determine final larval size and

total development time of the last larval instar. The GR determines how fast a larva will attain the CW, but not the value of the CW. The GR also controls the amount of mass accumulated during the ICG but not the duration of the ICG. Thus, the GR comes into play in determining size only during the ICG, but it affects development time only in the time required to reach the CW. These three physiological factors, the CW, ICG, and GR interact in a nonlinear fashion to determine body size and development time (Nijhout et al. 2006, 2010).

The sequence of events leading to pupation and metamorphosis that are initiated at the CW are a fixed sequence of events (Nijhout 1994). However, the values of the components of this sequence as measured here by CW, ICG, and GR can vary and evolve (D'Amico et al. 2001). To understand how the physiology underlying life-history traits enable, or constrain, the response to simultaneous selection on the life-history traits, Davidowitz et al. (2005) proposed a physiological framework to explain the response to simultaneous selection on body size and development time. This framework starts with physiological first principles and shows that when GR increases, body size increases and development time decreases. When the CW and ICG increase, both body size and development time increase (see above). The converse is true when the values of the physiological factors decrease. As a consequence, depending on the direction of selection acting on the two life-history traits, the three factors that regulate the life-history traits, GR, ICG, and CW, will be under either synergistic or antagonistic selection (Davidowitz et al. 2005). The framework assumes that the response to simultaneous selection will be enabled by those physiological traits that are under synergistic selection, and constrained by those under antagonistic selection (Davidowitz et al. 2005, Fig. 1, below). How the physiological factors that are under antagonistic selection affect the response to simultaneous selection is determined by their genetic correlations with the life-history traits and with each other (see below).

The concept of constraint has historically been confusing in the evolutionary literature largely due to a lack of explicit definitions of what is considered a constraint (Antonovics and van Tienderen 1991; Roff and Fairbairn 2007, and references therein). Some constraints can impede evolution in particular directions but not stop it. In this sense, all character states are possible. Other constraints may not allow a response to selection in certain evolutionary trajectories (Roff and Fairbairn 2007), "evolutionary forbidden trajectories" sensu Kirkpatrick and Lofsvold (1992). Using a mathematical simulation of the mechanism described in this study, Nijhout et al. (2010) showed there to be almost infinite combinations of the three physiological factors (GR, CW, ICG) that can produce particular values of body size and development time. Furthermore, there does not seem to be any area of morphospace that is "forbidden" from evolving. We, therefore, in the context of this study, define "constraint" as the

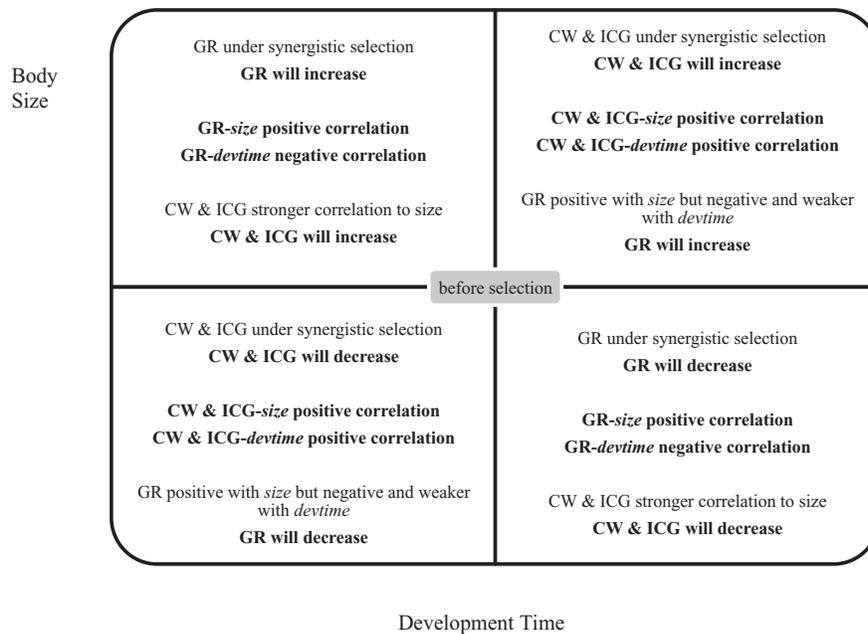


Figure 1. Predictions of the physiological framework and predictions due to genetic correlations of the response to simultaneous selection on body size and development time. Quadrants represent direction of simultaneous selection on body size and development time (e.g., in the lower right quadrant body size is selected to decrease while development time is selected to increase). The top couplet in each quadrant gives the predictions of the physiological framework in Davidowitz et al. (2005) for the traits under synergistic selection. The middle couplet represents predictions of the genetic correlations between the physiological traits under synergistic selection and the life-history traits. The bottom couplet represents predictions based on the genetic correlations for the physiological factors under antagonistic selection. Abbreviations: GR, growth rate; CW = critical weight; ICG, interval to cessation of growth; *size*, body size, *devtime*, development time. The couplets in bold are the a priori predictions of: top—the framework, middle—genetic correlations of the physiological factors under synergistic selection, and bottom—changes in the physiological factors under antagonistic selection based on the genetic correlations. The conditions leading to these predictions are in regular font (not bold).

impediment to evolution of body size and/or development time, imposed by antagonistic selection on one or more of the underlying three physiological factors. The strength of this constraint will be determined by the relative strengths of the genetic correlations among those factors and the two life-history traits (see Fig. 1).

As the framework is based on the underlying physiology, it allows explicit a priori predictions on how the physiological factors will change to enable the response to simultaneous selection on the two life-history traits. These a priori predictions are given in the top couplets of Figure 1. When body size and development time are both selected to increase (upper right quadrant of Fig. 1), the response to selection will be determined primarily by an increase in CW and ICG and constrained by GR. When body size is selected to increase and development time is selected to decrease (upper left quadrant in Fig. 1), the response to simultaneous selection is determined primarily by an increase in GR and constrained by CW and ICG. When the two life-history traits are selected to decrease (lower left quadrant in Fig. 1), the response to simultaneous selection on the two life-history traits will be determined largely by a decrease in CW and ICG and constrained by GR. Finally, when body size is selected to decrease and development time is selected to increase (lower right quadrant in Fig. 1), the

response to simultaneous selection is determined primarily by a decrease in GR and constrained by CW and ICG.

For these a priori predictions to hold, the framework requires specific signs (positive or negative) of the genetic correlations between the physiological traits under synergistic selection and the two life-history traits (middle couplets in Fig. 1): (1) when both life-history traits are simultaneously selected to either increase or decrease, CW and ICG should have a positive genetic correlation with both body size and development time and (2) when body size and development time are selected in opposite directions, one to increase and the other to decrease, for the framework to function GR should have a positive genetic correlation with body size and a negative genetic correlation with development time.

We note that the physiological framework makes predictions regarding the sign (positive or negative) of the genetic correlations. It makes no predictions regarding the strength of these correlations. The strength of the genetic correlations will affect how fast the life-history traits respond to selection.

The physiological framework of Davidowitz et al. (2005) makes no a priori predictions regarding the physiological factors that are under antagonistic selection. The effects of these traits on the response to selection are determined by the strength and

direction of their genetic correlations (see below) and can only be made a posteriori.

Here we test whether the genetic architecture of the physiological factors and life-history traits support the a priori and a posteriori predictions of the physiological framework in how the underlying physiology enables or constrains the response to simultaneous selection on body size and development time. We examine the heritabilities and genetic and phenotypic correlations of a population of tobacco hornworm before selection (Fig. 1; Davidowitz et al. 2005). We present a simulation of how the genetic and phenotypic architecture of the physiological traits can affect the response to selection of the two life-history traits. We simulated the predicted response to 10 generations of 25% simultaneous directional selection. A test of this framework following selection will be presented elsewhere.

Materials and Methods

The colony of *M. sexta* (Sphingidae) used in this study was out-crossed from colonies from Duke University, The University of Arizona, and the University of Washington. To minimize maternal effects, the data were collected eight generations after the colonies were out-crossed.

Single male and female pupae that were ready to eclose were chosen at random and placed together in a brown paper bag (11.3 L). Upon eclosion, within each bag, moths were given ad libitum 25% (v/v) sucrose solution as a nectar source in white cone drinking cups to simulate a natural hawkmoth flower (Raguso and Willis 2005), a genpak waterpik (133 mL) with a dentist cotton roll wick for humidity, and a styrofoam platform (approximately 50 cm²) with an ethanol leaf extract (5:1 v/v) of the native host plant (*Datura wrightii*: Solanaceae) to stimulate female oviposition. The nectar, waterpik, and oviposition platform were replenished daily within each bag and eggs were collected daily from all bags.

All larvae, parents, and offspring were reared on a 16:8 (L:D) photoperiod at 25°C on the standard rearing diet ad libitum (100% diet in Davidowitz et al. 2003). Larvae in instars one to four were reared individually in 29.6 mL (Solo) cups with a perforated lid for gas exchange. Last (fifth) instar larvae were transferred individually to 266 mL (Solo) cups with straw slits in the lid for gas exchange. Upon the initiation of pupation (Davidowitz et al. 2004), wandering larvae were placed in 266 mL cups half filled with potting soil. As all the individuals were reared under identical conditions, we assume all variation among families and individuals to be genetic.

MEASUREMENTS AND STATISTICS

This study included 1195 offspring from 165 families and their parents, with six to eight (mode = 8) offspring per family.

Larval peak mass (*peak*, g) was measured 3 h before the scotophase prior to wandering at which time body mass reaches its maximum (G. Davidowitz, unpubl. pilot data). Pupal mass (*pupa*, g) was measured on pupae seven days following the onset of wandering. This is the first day that pupae can be handled without damage. Pupal mass was used as the measure of body size under selection. GR (*gr*, g/d) was measured for the 24-h period between the third and second to last days of larval growth. This ensured all individuals were measured during the linear phase of growth (Nijhout et al. 2006). In this species, GR is linear within an instar with the exception of short durations immediately prior to, and following, a molt (Nijhout et al. 2006). Three measures of development time were recorded. Total development time (*dt*) was measured as the number of days between hatching and the onset of wandering when the larvae cease feeding and prepare for pupation. This measure includes the entire larval period and was the target of selection on development time. Development time of the first four instars (*dt14*) was measured as the number of days from hatching to the molt to the fifth instar. Development time of the fifth (last) instar larvae (*dt5*) was measured as the number of days from the molt to the fifth instar and wandering. About of 90% of larval growth occurs during this last instar (Davidowitz et al. 2004).

Direct measures of hormonal titers in insects require sacrificing the organism or, in the case of large insects such as *M. sexta*, extracting a sufficiently large amount of hemolymph that drastically alters growth. It is therefore not possible to measure the cessation of JH secretion or the first peak of ecdysone associated with the termination of growth and measure body size and development time on the same individual. We therefore, needed to develop an indirect proxy for these hormonal events. To do this we made use of the functional assay of estimating CW (Nijhout and Williams 1974; Davidowitz et al. 2003). This method, however, is a population-level assay that requires over 400 individuals for accurate estimation (Nijhout and Williams 1974; D'Amico et al. 2001; Davidowitz et al. 2003, 2004), and is not, by itself, appropriate for individual-level estimates of CW (cw_i) and ICG (icg_i). For this study we developed an indirect method for estimating cw_i and icg_i that combines population level measures of CW (cw_p) and ICG (icg_p) with individual GR (gr_i) and individual peak mass ($peak_i$) that provides individual-level estimates that can then be used to calculate genetic correlations and heritabilities. In this colony (population), the CW (cw_p) was 7.0 g and the population ICG (icg_p) was 1.92 days estimated in a separate preliminary experiment as in Davidowitz et al. (2003). Using this, we then indirectly estimated the individual-level CW as $cw_i = peak_i - (icg_p \times gr_i)$, where cw_i is the individual CW, $peak_i$ is the individual peak mass, icg_p is the population level estimate of ICG (1.92 days), and gr_i is the individual GR. In a similar fashion, the individual ICG was calculated as $icg_i = (peak_i - cw_p)/gr_i$,

where icg_i is the individual ICG, $peak_i$ is the individual peak mass as above, cw_p is the population CW (7.0 grams), and gr_i is the individual GR as above.

To validate our method for estimating cw_i and icg_i , we performed two tests. First, we tested whether our indirect estimates of the two proxies for hormonal traits (cw_i and icg_i) were biased from the population-level assay of CW (cw_p) and ICG (icg_p). The mean individual CW of all offspring ($n = 1195$) calculated with the above equation was not significantly different from the population CW ($cw_p = 7.0$ grams), mean offspring $cw_i = 7.02$ g, $t = 0.5663$, $df = 1194$, $P = 0.5713$. The mean offspring ICG was 1.95 days, and was not significantly different from icg_p of 1.92 days ($t = 1.8721$, $df = 1194$, $P = 0.0614$). Second, we determined how the variances of these indirectly calculated estimates were related to the variances of the three measures of mass ($peak_i$ and the two masses used to calculate gr_i) that were directly collected from each individual (see Appendix). Examination of the variances of the indirectly estimated measures of cw_i and icg_i with those of the directly measured masses of each individual, showed that while there can be significant variability in the weights measured, there will be little variance in GR. The variance of cw_i is proportional to the variance of $peak_i$ and the variance of icg_i is proportional to the variance of the measured weights (see Appendix). These two analyses lend confidence that our method of estimating individual-level measures of CW and ICG (cw_i and icg_i , respectively) is unbiased and provides robust proxies for the hormonal traits at the population level (cw_p and icg_p , respectively). It further demonstrates that the variances of the measured weights ($peak_i$ and the two weights used to measure gr_i) do not bias the variances of the three physiological factors.

The traits cw_i and icg_i (above) as estimated are compound traits with gr_i and $peak_i$ in common (see equations above). We therefore did not calculate genetic correlations for the combinations of cw_i and icg_i with gr_i and $peak_i$ and between themselves (Table 1), because shared traits would artificially inflate these estimates. These correlations are left blank in Table 1.

Due to space and personnel limitations, data for this study were collected in three cohorts ($n = 27, 75, 63$ families). Data collection for each cohort lasted three to four weeks with a four-to-five-week interlude between cohorts. An ANOVA showed significant differences in the mean of all eight traits between cohorts (although the differences were not large as seen in r^2 values ranging from 0.02 to 0.08). A Levene's test for homogeneity of variances showed that half the variables (gr , cw , $dt5$, $dt14$) had unequal variances among cohorts. We, therefore, first z -transformed the data within each cohort to have a mean = 0 and a standard deviation = 1. All correlations and heritabilities were estimated from the transformed data.

Heritabilities, genetic, and phenotypic correlations were estimated using both parent-offspring and full siblings. Full-sibling genetic correlations and heritabilities were estimated as in Roff (1997). Standard errors were estimated using the jackknife procedure in Roff and Preziosi (1994). We considered a full-sibling genetic correlation and heritability to be different from zero if the genetic correlation $\pm 2SE$ (where SE is standard error) did not include zero (Table 1). Pearson product-moment phenotypic correlations and their significance tests were calculated in the standard way. Parent-offspring heritabilities were calculated using the standard regression method with the associated standard error of the mean (SEM) and significance test. Parent-offspring genetic correlations were calculated as in equations (3.12) and (3.13) of Roff 1997. All analyses were done in either SAS (9.2) or JMP (8.0.1).

Recent work (Stillwell and Davidowitz 2010) showed that the sexual size dimorphism (10%) in this species is due to a larger cw , icg , and $dt5$ in the females (the GR did not differ between the sexes). We tested for and found only minor sex effects on the correlations. We therefore present the correlations uncorrected for sex (Table 1).

SIMULATION

To determine the predicted response to selection on the two traits, we used estimates of the genetic and phenotypic covariance matrices from the full-sibling analysis (the two estimates of genetic correlations gave similar results, see below. We used the full-sibling covariance matrices for the simulation because of their smaller standard errors). The model requires phenotypic and genetic covariances among all traits. Even although we did not calculate these for cw_i and icg_i with gr_i and $peak_i$ because of potential bias (see above and Table 1), we needed these correlations for the simulation. These were estimated as follows (phenotypic correlation ± 1 SEM, followed by full-sibling genetic correlation ± 1 SEM): $peak-cw$ 0.72 ± 0.03 , 0.81 ± 0.07 ; $peak-icg$ 0.72 ± 0.03 , 0.87 ± 0.09 ; $gr-cw$ 0.28 ± 0.03 , 0.34 ± 0.21 ; $gr-icg$ 0.22 ± 0.03 , 0.46 ± 0.26 ; $cw-icg$ 0.94 ± 0.03 , 0.97 ± 0.01 . We note that inclusion of these biased correlations does not affect the value of the equilibrium reached in the simulation only the rate at which the equilibrium is reached. Because of the complexity of the model, we used numerical simulation of an individual variance components model, adapting coding given in scenario 6 of chapter 5 of Roff (2010). To create a deterministic solution population size was set at 1000 individuals. Bivariate truncation selection of the required 25% was accomplished by first selecting the appropriate 50% of one trait and then 50% of the other trait within this selected group. We ran the simulation both by alternating between generations the trait selected first and also without alternation: the results were not noticeably different. Trait means start at 0 with variance of 1

Table 1. Heritabilities, genetic, and phenotypic correlations between body size (*pupa*, *peak*), development time (*dt*, *dt14*, *dt5*), critical weight (*cw*) interval to cessation of growth (*icg*), and growth rate (*gr*). All estimates are from z-transformed data with a mean = 0 and SD = 1. Pearson phenotypic correlations are above the diagonal with those significantly different from zero in bold. Genetic correlations are below the diagonal. Parent offspring genetic correlations are above the full-sibling genetic correlations. All estimates include ± 1 SEM. Genetic correlations in which ± 2 SEM do not include zero are in bold. On the diagonal (in gray) are parent offspring heritabilities above the full-sibling heritabilities, both with ± 1 SEM. Significance of parent offspring heritabilities are calculated in the usual way. Full-sibling heritabilities in which ± 2 SEM do not include zero are in bold. For all estimations, $n = 1195$ individuals from 165 families. Estimates with an asterisk (*) denotes marginal significance ($0.06 < P < 0.08$).

	<i>pupa</i>	<i>peak</i>	<i>gr</i>	<i>cw</i>	<i>icg</i>	<i>dt</i>	<i>dt14</i>	<i>dt5</i>
<i>pupa</i>	0.25±0.04	0.89±0.01	0.42±0.03	0.63±0.02	0.63±0.02	-0.08±0.03	0.00±0.03	-0.12±0.03
	0.52±0.06							
<i>peak</i>	0.98±0.02	0.23±0.04	0.45±0.03			-0.12±0.03	-0.07±0.03	-0.11±0.03
	0.91±0.03	0.41±0.06						
<i>gr</i>	0.90±0.16	0.99±0.16	0.09±0.04			-0.43±0.03	-0.17±0.03	-0.47±0.03
	0.71±0.1	0.83±0.08	0.32±0.05					
<i>cw</i>	1.06±0.13			0.08±0.04		-0.2±0.03	0.05±0.03*	0.25±0.03
	0.78±0.07			0.17±0.05				
<i>icg</i>	1.03±0.17				0.07±0.03	0.09±0.03	0.01±0.03	0.11±0.03
	0.85±0.08				0.14±0.04			
<i>dt</i>	-0.62±0.41	-0.67±0.41	-0.65±0.42	-0.63±0.67	-0.56±0.71	0.05±0.05	0.76±0.02	0.68±0.02
	-0.22±0.18	-0.34±0.17	-0.51±0.17	-0.04±0.19	-0.19±0.25	0.55±0.06		
<i>dt14</i>	-0.09±0.3	-0.21±0.3	-0.01±0.41	-0.3±0.41	-0.28±0.44	0.68±0.21	0.01±0.05*	0.05±0.03
	0.05±0.14	-0.12±0.13	-0.11±0.14	-0.08±0.16	-0.20±0.20	0.82±0.05	0.79±0.07	
<i>dt5</i>	-0.68±0.25	-0.56±0.25	-0.78±0.21	-0.43±0.44	-0.37±0.47	0.28±0.49	-0.54±0.42	0.08±0.05*
	-0.47±0.13	-0.40±0.16	-0.7±0.12	0.05±0.15	-0.04±0.22	0.46±0.17	-0.12±0.16	0.48±0.06

and so the responses to selection are in standard deviation units (Table 2).

Results

The mean and SEM for all eight trait values were ($n = 1195$) as follows: *peak* (12.02 ± 0.04 g), *pupa* (6.60 ± 0.02 g), *dt* (18.3 ± 0.03 d), *dt14* (12.1 ± 0.02 d), *dt5* (6.24 ± 0.02 d), *gr* (2.61 ± 0.01 g/d), *cw* (7.02 ± 0.03 g), *icg* (1.95 ± 0.01 d).

The heritabilities and correlations are given in (Table 1). All eight traits (*pupa*, *peak*, *gr*, *cw*, *icg*, *dt*, *dt14*, *dt5*) had significant full-sibling heritabilities. The narrow sense heritabilities estimated by parent offspring regression were also significant with the exception of *dt* which was not significantly different from zero and those of *dt14* and *dt5* were marginally significant ($0.06 < P < 0.08$). In all cases, the full-sibling heritabilities were larger than the parent-offspring heritabilities suggesting the presence of nonadditive genetic variation. Combined, the heritability estimates indicate that both the life-history traits (development time and body size) and the underlying physiological factors (CW, ICG and GR) can respond to selection.

The two estimates of genetic correlations (full sibling and parent-offspring) gave largely the same results as 91% of the estimates (21/23) were within 1 SEM of each other and 100% within 2 SEM. The two measures of body size (*peak*, *pupa*) had

weak, negative ($r_p \geq -0.12$) or insignificant phenotypic correlations with the three measures of development time. *Pupa* had strong negative genetic correlations with *dt* and *dt5* (*pupa* and *dt* were the targets of selection) although the large SEM could not exclude zero. *Peak* also had a significant negative genetic correlation with *dt5* and *dt*. All three physiological factors had high genetic ($r_g \geq 0.71$) and phenotypic ($r_p \geq 0.42$) correlations with body size (*pupa*). The genetic correlations of the two hormonal traits, *cw* and *icg*, were strongly negatively correlated with all three measures of development time, although the large SEM could not exclude zero. The trait *gr* was strongly negatively correlated with *dt5* and *dt*.

All genetic correlations with any of the three measures of development time have very large standard errors suggesting they are influenced by an additional, unknown factor that we have not accounted for. It is unlikely that this unknown factor is microhabitat variation within the environmental chambers or day-to-day variation in diet. If this were the case, the standard deviations of the phenotypic correlations would also be large which was not the case in our study (Table 1).

SIMULATION

Results of the simulation are given in Table 2. The response to simultaneous selection in all four combinations of body size and development time was consistent with the appropriate direction

Table 2. Simulation of predicted response to 10 generations of simultaneous 25% truncation selection (see text). Trait values are in standard deviation units. In the first column: Big—refers to selection to increase size, Small—selection to decrease size, Slow—selection to increase development time, and Fast—selection to decrease development time. Predictions are those given in Figure 1. Support for the predictions for the increase or decrease of a trait are from this Table. Support for the predictions of the sign of a genetic correlation between traits are from Table 1. Symbols: the diagonal arrows (↖ ↗ ↘ ↙) in the first column refer to the appropriate quadrant in Figure 1. The up (↑), down (↓) arrows refer to an increase or decrease of a trait following 10 generations of simulated selection, respectively. Genetic correlations are positive (+ +) or negative (– –). Abbreviations: cw, critical weight; dt, development time; gen, generation; gr, growth rate; icg, interval to cessation of growth; size, body size.

Line	gen	pupa	peak	gr	cw	icg	dt	Predictions	Support
Big	1	-0.02	-0.03	-0.01	-0.03	-0.02	0.01	cw ↑	Yes
Slow	2	0.37	0.22	0.11	0.15	0.13	0.34	icg ↑	Yes
	3	0.68	0.44	0.16	0.35	0.30	0.76		
↗	4	1.04	0.67	0.19	0.58	0.44	1.07	cw-size + +	Yes
	5	1.26	0.78	0.27	0.63	0.51	1.41	icg-size + +	Yes
	6	1.62	1.06	0.36	0.86	0.69	1.68		
	7	1.96	1.30	0.49	1.02	0.82	2.04	cw-dt + +	No
	8	2.34	1.56	0.57	1.24	0.99	2.35	icg-dt + +	No
	9	2.67	1.76	0.70	1.36	1.11	2.64		
	10	2.99	1.96	0.78	1.51	1.21	3.06	gr ↑	Yes
Big	1	-0.02	-0.03	-0.01	-0.03	-0.02	0.01	gr ↑	Yes
Fast	2	0.44	0.38	0.39	0.11	0.16	-0.50		
	3	0.90	0.81	0.70	0.34	0.39	-0.86	gr-size + +	Yes
↖	4	1.36	1.21	1.04	0.51	0.56	-1.38	gr-dt - -	Yes
	5	1.74	1.54	1.39	0.59	0.71	-1.89		
	6	2.27	2.05	1.75	0.86	1.01	-2.45	cw ↑	Yes
	7	2.75	2.47	2.18	1.00	1.18	-3.02	icg ↑	Yes
	8	3.32	3.01	2.57	1.27	1.46	-3.55		
	9	3.78	3.43	2.95	1.43	1.68	-4.18		
	10	4.22	3.82	3.33	1.56	1.84	-4.66		
Small	1	-0.02	-0.03	-0.01	-0.03	-0.02	0.01	cw ↓	Yes
Fast	2	-0.34	-0.21	-0.04	-0.20	-0.15	-0.41	icg ↓	Yes
	3	-0.69	-0.41	-0.14	-0.33	-0.25	-0.72		
↙	4	-1.00	-0.63	-0.24	-0.49	-0.43	-1.04	cw-size + +	Yes
	5	-1.43	-0.93	-0.32	-0.76	-0.62	-1.45	icg-size + +	Yes
	6	-1.75	-1.10	-0.43	-0.86	-0.70	-1.77		
	7	-2.13	-1.39	-0.52	-1.10	-0.90	-2.16	cw-dt + +	No
	8	-2.40	-1.49	-0.54	-1.19	-0.98	-2.54	icg-dt + +	No
	9	-2.78	-1.76	-0.64	-1.40	-1.14	-2.88		
	10	-3.10	-1.96	-0.64	-1.62	-1.32	-3.21	gr ↓	Yes
Small	1	-0.02	-0.03	-0.01	-0.03	-0.02	0.01	gr ↓	Yes
Slow	2	-0.46	-0.44	-0.37	-0.19	-0.22	0.54		
	3	-0.91	-0.87	-0.81	-0.31	-0.37	1.16	gr-size + +	Yes
↘	4	-1.35	-1.30	-1.20	-0.49	-0.61	1.72	gr-dt + +	Yes
	5	-1.92	-1.87	-1.62	-0.78	-0.89	2.27		
	6	-2.37	-2.29	-2.05	-0.90	-1.06	2.86	cw ↓	Yes
	7	-2.82	-2.73	-2.37	-1.12	-1.31	3.39	icg ↓	Yes
	8	-3.27	-3.12	-2.73	-1.27	-1.50	3.89		
	9	-3.80	-3.58	-3.12	-1.46	-1.71	4.44		
	10	-4.31	-4.05	-3.50	-1.69	-1.97	5.05		

of selection: the values of the traits decreased when selected to decrease and increased when selected to increase. In all, of the 24 predictions made, 20 (83%) were upheld (Table 2). All six a priori predictions for the change in the physiological factors under synergistic selection were supported. All six a posteriori predictions for the change in the physiological factors under antagonistic selection were also supported. The four predictions for the sign of the genetic correlation of CW and ICG were supported, as were the four predictions of GR with size and development time. The only predictions not supported were the predictions for a positive correlation of CW and ICG with development time.

Discussion

How body size and development time, two life-history traits that are highly correlated with fitness, respond to selection has been the focus of research for many years (Kingsolver and Pfennig 2004; Kingsolver and Huey 2008). This study increases our understanding of how the underlying physiology can constrain or enable their response to simultaneous selection.

Previous studies (Davidowitz and Nijhout 2004; Nijhout et al. 2006) have shown that both body size and development time are regulated by the same three physiological factors, CW, interval to cessation of growth, and GR. This provides us with a unique opportunity to examine how these two fundamental life-history traits may respond when both are selected upon simultaneously, as is often the case in natural conditions.

Simultaneously selected traits may be constrained from evolving in a preferred direction, due to constraints between them. Constraints are typically revealed by negative genetic correlations among traits. These correlations, however, only suggest the existence of constraints; they cannot explain the underlying cause of these constraints (Zera and Harshman 2001). How simultaneously selected traits respond to selection will depend on the genetic architecture of the underlying mechanism that determines these focal traits, particularly the additive genetic variances and covariances.

We found that in *M. sexta*, body size (*peak, pupa*), and development time (*dt*) have strong negative phenotypic and genetic correlations (although, in some instances the large SEM may not preclude zero) (Table 1). These two life-history traits are, therefore, likely to be constrained from evolving independently when both are simultaneously selected to evolve (see Figure 2 and below).

Davidowitz et al. (2005) provided a framework to explain how the three underlying physiological factors constrain or enable the response to simultaneous selection on body size and development time. In this framework, the underlying physiological factors (*cw, icg, gr*) are either under synergistic or antagonistic selection, depending on the direction of simultaneous selection on

the two focal life-history traits (body size and development time). Regardless of the direction of selection, when selection on the life-history traits pulls the underlying physiological traits in opposite directions, the physiological traits are under antagonistic selection. When selection on the life-history traits pulls the underlying physiological traits in the same direction, the physiological traits are under synergistic selection. This framework assumes that the response to selection is constrained by the underlying factors that are under antagonistic selection and predominantly determined by those factors that are under synergistic selection (top couplets in Fig. 1).

When body size and development time are both selected in the same direction—both are selected to increase (upper right quadrant Fig. 1) or both are selected to decrease (lower left quadrant in Fig. 1)—the response to simultaneous selection on body size and development time will be determined primarily by the two bioindicators of hormonal events, CW and ICG (as these factors are under synergistic selection). When both size and development time are selected to increase, CW and ICG are predicted to increase. When both are selected to decrease, however, CW and ICG are predicted to decrease (Fig. 1). These two predictions were supported in our simulation of selection (Table 2). Consequently, the physiological framework predicts that CW and ICG should have a positive genetic correlation with both life-history traits (middle couplet in Fig. 1). This prediction is supported for body size where the genetic correlations are positive and high ($> +1.0$). The genetic correlations between CW and ICG and development time, however, are negative (Table 1) contradicting the prediction of the framework. The analysis of Nijhout et al. (2010) used a phenotypic model of the physiological underpinnings of growth and development in *M. sexta*. This model showed that at the phenotypic level, any particular combination of body size and development time could be produced by a large number of combinations of the three physiological traits (GR, CW, ICG). Whether such combinations are attainable via selection will depend upon the genetic variance–covariance matrix of these three traits: provided that none of the eigenvalues of this matrix is zero then evolution will not be prevented from occurring in any direction dictated by selection (Dickerson 1955). Demonstrating that a given eigenvalue is zero with any degree of statistical confidence is generally not possible (Roff et al. 2012) and given the sample size, not feasible in the present analysis. However, a minimal condition for the direction of the evolutionary response to be the same as that predicted by the physiological model is that the signs of the genetic correlations match those of the phenotypic correlations. Examination of Table 1 shows that this is not the case for the correlations between ICG, CW, and the three measures of development time (DT, DT14, DT5). This contradiction, although puzzling, can be explained through the negative correlation of body size and development time. All three physiological factors

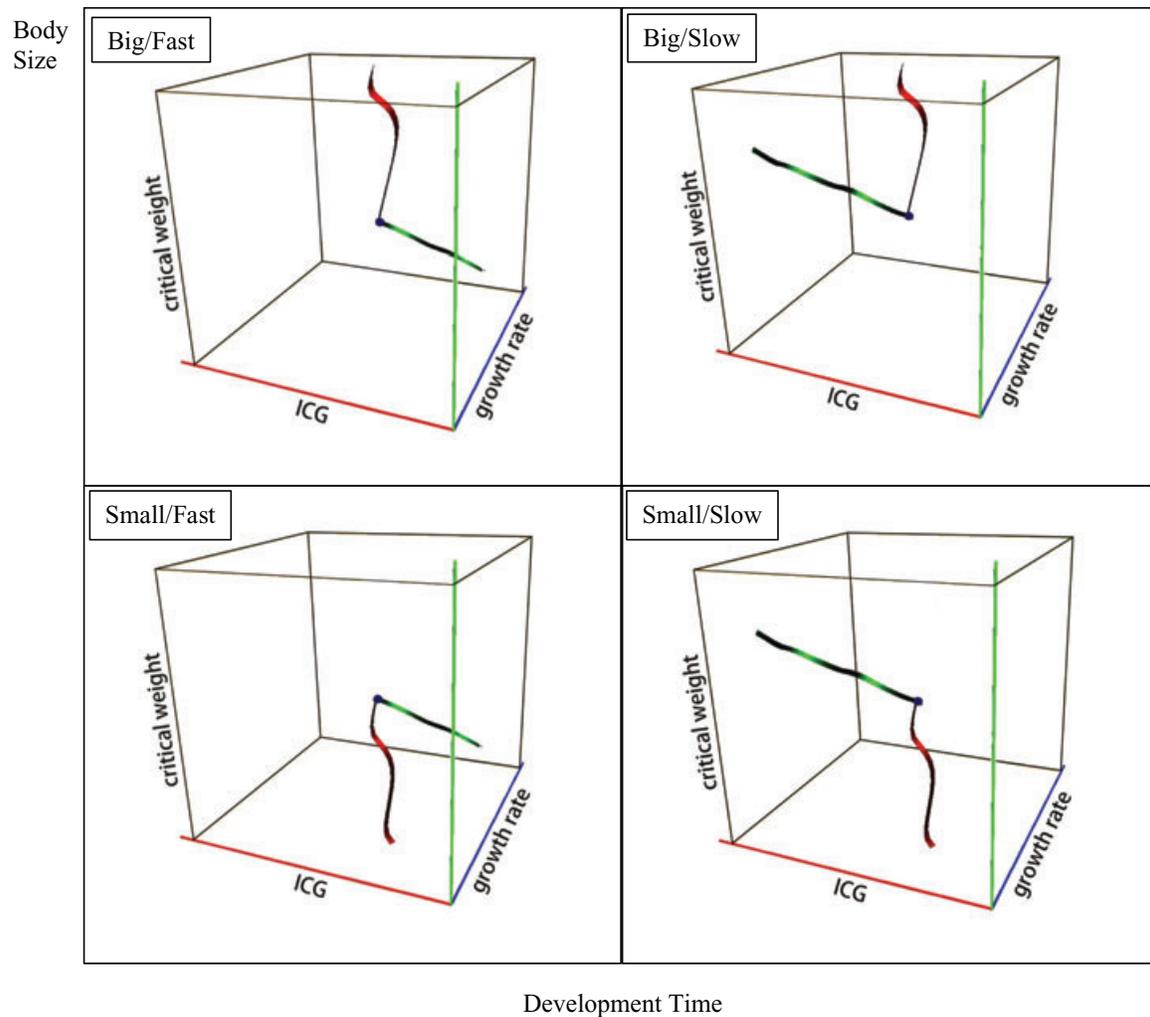


Figure 2. Response to simultaneous selection on all four combinations of body size and development time. The filled circle represents a population before selection. The ribbons represent the response to selection for body size (red) and development time (green) following the mathematical model in Nijhout et al. (2010) using the method of Lande and Arnold (1983). This figure illustrates how the underlying physiological factors are pulled in different directions when the life-history traits are selected upon, imposing a constraint on the response to selection.

are predicted to increase (upper right quadrant), or decrease (lower left quadrant), in the same direction with both development time and body size. But because body size and development time are strongly negatively correlated, and the CW and ICG are positively correlated with body size, they are constrained to have a negative correlation with development time under synergistic selection on body size and development time.

When body size and development time are selected in opposite directions (upper left-lower right quadrants in Fig. 1), GR is under synergistic selection and is predicted to be the primary physiological determinate of the response to selection. The predictions for the changes in GR following selection were supported by our simulation (Table 2). GR is predicted to have a positive correlation with body size and a negative correlation with development time (middle couplets in Fig. 1). The estimated genetic correla-

tions between GR and body size (parent-offspring = 0.90, full sibling = 0.71), and between GR and development time (parent-offspring = -0.65, full sibling = -0.51) support these predictions (Table 1).

The original framework of Davidowitz et al. (2005) did not make predictions regarding the physiological factors under antagonistic selection, as their effects on the response to selection can only be known once the genetic correlations are estimated. Having these correlations (Table 1), we are now able to make predictions regarding these, which are given in the bottom couplets of Figure 1. In the instances where body size and development time are selected in the same direction, GR is under antagonistic selection and has a slightly stronger genetic correlation with body size (parent-offspring = 0.90 ± 0.16 , full sibling = 0.71 ± 0.1) than it does with development time (parent-offspring = $-0.65 \pm$

0.42, full sibling = -0.51 ± 0.17), we can predict, therefore, that GR will increase, or decrease, in the same direction as body size, which our simulation shows it does (Table 2). We note, however, that in both these instances, the change in GR is an order of magnitude smaller than the change in any of the other traits. This is consistent with our analysis based on the variances of the traits (Appendix) where there is predicted to be little or no change in gr_i following selection on the two life-history traits. These results also reinforce the overall prediction of the framework that when body size and development time are both selected in the same direction, the response to selection will be predominately determined by the two hormonal traits CW and ICG.

When body size and development time are selected in opposite directions, CW and ICG are under antagonistic selection. These hormonal traits are more positively correlated with body size ($1.06 > r_g \geq 0.78$) then they are negatively correlated with development time ($-0.04 \leq r_g \leq -0.63$) (Table 1). We predict, therefore, that CW and ICG will increase following selection. This prediction is consistent with that made using the variances of the traits (Appendix), which showed that the change in cw_i and icg_i should be proportional to body size ($peak_i$). Our simulation following selection supports these predictions (Table 2).

Although this is not an explicit prediction of the framework, the simulation also shows that the response to selection of those traits under synergistic selection were much larger than those traits under antagonistic selection, as we would expect (Table 2).

Overall, the physiological framework proposed by Davidowitz et al. (2005) provides a robust predictive framework for understanding how underlying physiology can constrain or enable the response to selection of two important life-history traits: body size and development time (D'Amico et al. 2001; Davidowitz and Nijhout 2004; Nijhout et al. 2006, 2010). The genetic architecture of the five traits largely supports this framework.

Recently, the authors (Nijhout et al. 2010) modeled the mechanism described here to determine how body size and development time can evolve over a broad range of physiological parameter space. Body size and development time in their study is equivalent to $peak$ and $dt5$, respectively, in this study. Their results show that the phenotypic landscapes of the underlying physiological factors are orthogonal to each other over much of the joint parameter space of body size and development time. In other words, the physiological parameters are being pulled in different directions by selection on the two life-history traits. This implies that selection cannot act in a fully synergistic direction on both these life-history traits, as the physiological traits are antagonistic over much of the parameter space.

This can be seen clearly in Figure 2. We extended the model of Nijhout et al. (2010) to show how CW, ICG, and GR change when body size and development time are selected upon in all four combinations of selection (Fig. 2) as in this study. The three phys-

iological factors that determine body size and development time evolve along the trajectories shown by the ribbons. From Figure 2 it can be seen that the physiological factors impose a constraint on the simultaneous selection on body size and development time because they are pulled in different directions regardless of the direction in which the two life-history traits are selected. This figure illustrates the constraint imposed by the negative genetic correlation between development time and the two hormonal traits as explained above. Kingsolver and Pfennig (2004) showed that the majority (79%) of studies show directional selection acts to increase body size: larger individuals tend to have greater survival (76%), greater fecundity (85%), and greater mating success (74%). Kingsolver and Huey (2008) showed that directional selection tends to decrease development time in nature (84%). From these, in the context of this study, natural selection would tend to select in the direction of the upper left quadrant (Big/Fast) of our Figure 1.

It is important to emphasize that although the physiological framework discussed here is built on the physiological mechanism for the regulation of body size and development time in *M. sexta* (Davidowitz and Nijhout 2004; Nijhout et al. 2006), it is not restricted only to this species. Increasing evidence demonstrates its relevancy for the regulation of these traits in other insects such as *Drosophila* (Mirth and Riddiford 2007; Shingleton et al. 2007) and the butterfly *Bicyclus anynana* (Davidowitz and Brakefield, unpubl. ms.).

This mechanism has three main components: a size threshold that initiates the termination of growth (in insects this is the CW), a time interval between when this threshold is passed and when growth ceases (in *M. sexta* this is the ICG which is a special case of the "terminal growth period," Shingleton et al. 2007), and the GR.

A mechanism for the cessation of growth is a feature common to all organisms with determinate growth. For example, thyroid hormone secretion triggers cessation of the tadpole life stage and initiates metamorphosis in anurans (Allen 1918; Denver 1996). In mammals, growth of the long bones are regulated by a complex network of endocrine signals (Nilsson and Baron 2005), but it is the exhaustion of chondrocyte proliferation inherent within the growth plate followed by epiphyseal fusion of the long bones which lead to the termination of growth (Nilsson and Baron 2004, 2005). In *Chlamydomonas* there is both a "sizer" and a "timer" that terminate growth and lead to cell division (Donnan and John 1983) and in *Arabidopsis thaliana*, there are either five or two QTL on either three or two chromosomes (for two RI lines, respectively) that are involved in determining bolting time (Ungerer et al. 2002). The interval to cessation of growth (or terminal growth period) may occur a significant period of time after the threshold to terminate growth is reached, as in *M. sexta* (Davidowitz et al. 2004), or it may coincide with the threshold as in

Drosophila (Mirth et al. 2005). All growing organisms have, by definition, a GR.

It is likely then that the main features of the physiological framework for body size and development time, involving a size threshold plus a developmental delay while the endocrine machinery that controls growth is reset, will apply to many organisms. The details of the mechanism of the threshold and delay period will of course differ among taxa. Understanding the genetic architecture of such frameworks can provide insight as to how evolution of the underlying developmental physiological mechanism affects the evolution of size and development time. A comparison of the genetic architecture of similar physiological frameworks in other taxa may allow us to elucidate the commonalities and divergences of the developmental regulation and evolution of body size and development time among diverse taxa.

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Appendix

The three physiological traits, individual growth rate (GR) (gr_i), individual critical weight (CW) (cw_i), and the individual interval to cessation of growth (ICG) (icg_i), are themselves compound traits that operationally are estimated using individual weights at age (w_1 , w_2 , $peak_i$: these are in order of the last three mass measurements of an individual larva). Using these “lower level” traits, we can determine how the measures at weight affect the mean and variance of the physiological traits, and make predictions as to how the physiological traits will respond to selection given that they were calculated from the weights at age.

OPERATIONAL TRAIT DEFINITIONS

Individual GR (gr_i)

This is operationally defined as the change in weight on the two days prior to exposure of the dorsal vessel,

$$gr_i = w_2 - w_1,$$

where w_i is the weight on day i . In this study they were the third (w_1) and second (w_2) to last days of larval growth, with $peak_i$ being the last day.

Individual CW (cw_i)

$$\begin{aligned} cw_i &= peak_i - (icg_p \times gr_i) = peak_i - (1.92 \times gr_i) \\ &= peak_i - 1.92(w_2 - w_1), \end{aligned}$$

where cw_i is the individual CW, $peak_i$ is the individual peak larval mass, icg_p is the population mean icg ($= 1.92$ days) and gr_i , w_2 , and w_1 are as above.

Individual interval to cessation of growth (icg_i)

$$icg_i = (peak_i - cw_p)/gr_i = (peak_i - 7.0)/(w_2 - w_1),$$

where icg_i is the individual interval to cessation of growth cw_p is the population (colony) mean cw ($= 7.0$ g) and $peak_i$, gr_i , w_2 , and w_1 are as above.

WHAT IS THE VARIANCE IN THE THREE PHYSIOLOGICAL FACTORS GIVEN THE MEASURES AT WEIGHT?

Variance in individual GR (gr_i)

From standard statistics the variance in individual GR is

$$\sigma_{gr_i}^2 = \sigma_{w_1}^2 + \sigma_{w_2}^2 - 2\sigma_{w_1w_2},$$

where σ_x^2 designates variance of x and σ_{xy} designates covariance between x and y .

Dividing both sides by $\frac{1}{\sigma_{w_1}^2 \sigma_{w_2}^2}$

$$\frac{\sigma_{gr_i}^2}{\sigma_{w_1}^2 \sigma_{w_2}^2} = \frac{1}{\sigma_{w_1}^2} + \frac{1}{\sigma_{w_2}^2} - \frac{2\sigma_{w_1w_2}}{\sigma_{w_1}^2 \sigma_{w_2}^2} = \frac{1}{\sigma_{w_1}^2} + \frac{1}{\sigma_{w_2}^2} - \frac{2}{\sigma_{w_1}^2 \sigma_{w_2}^2} r,$$

where r is the correlation between the two weights $\sigma_{w_1w_2}/\sigma_{w_1}\sigma_{w_2}$. w_2 and w_1 are very highly correlated ($r_p = 0.93$, $n = 1195$, $P < 0.0001$), and the variances are the same (say σ_w^2 : two-sided F -test, $F = 1.0975$, $df = 1194$, $P = 0.1080$). Hence we have

$$\frac{\sigma_{gr_i}^2}{\sigma_w^2} \approx \frac{1}{\sigma_w^2} - \frac{1}{\sigma_w^2} \times 1 = 0.$$

Whereas there can be significant variability in the weights at age ($\sigma_{w_1}^2 = 1.44$, $\sigma_{w_2}^2 = 1.58$, $n = 1195$), the above derivation indicates that there should be little variance in GR, which is what was observed ($h^2 = 0.09$, $\sigma_{gr_i}^2 = 0.2$).

Variance in CW (cw_i)

$$\begin{aligned} \sigma_{cw_i}^2 &= \sigma_{peak_i}^2 + 1.92^2 \sigma_{w_2-w_1}^2 - 2\sigma_{peak_i(w_2-w_1)} \\ &= \sigma_{peak_i}^2 + 3.69 \sigma_{w_2-w_1}^2 - \sigma_{peak_i(w_2-w_1)} \approx \sigma_{peak_i}^2 \end{aligned}$$

as the variance in $w_2 - w_1$ is very small ($\sigma_{w_2-w_1}^2 = 0.22$) and the covariance between peak mass ($peak_i$) and $w_2 - w_1$ is also very small ($= 0.26$). The variance of cw_i should roughly equal that of $peak_i$, as was the case ($\sigma_{cw_i}^2 = 1.39$, $\sigma_{peak_i}^2 = 1.56$, Levene's test: $F = 1.0785$, $df_{1,2388}$, $P = 0.2991$).

Variance in interval to cessation of growth (icg_i)

To find the variance of icg_i we need the variance of a ratio (see Lynch and Walsh 1998, p. 818)

$$\begin{aligned} \sigma_{icg_i}^2 &= \left(\frac{\mu_3}{\mu_{w_2-w_1}} \right)^2 \left(\frac{\sigma_{peak_i}^2}{\mu_3^2} - \frac{2\sigma_{(peak_i-7.0),(w_2-w_1)}}{\mu_3 \mu_{w_2-w_1}} \right) \\ &= (\mu_{w_2-w_1})^{-2} \left(\sigma_{peak_i}^2 - \frac{2\mu_3 \sigma_{(peak_i-7.0),(w_2-w_1)}}{\mu_{w_2-w_1}} \right) = \frac{\sigma_{peak_i}^2}{\mu_{w_2-w_1}^2}, \end{aligned}$$

where μ_3 is the mean value of $peak_i - 7.0$ and $\mu_{w_2-w_1}$ is the mean GR. In this study, these values are very similar ($\sigma_{icg_i}^2 = 0.27$ and $\frac{\sigma_{peak_i}^2}{\mu_{w_2-w_1}^2} = 0.23$), confirming the relationship between icg_i and the weights at age.

Predicting the response to selection given that the physiological factors were determined from weights at age.

(1) Changes in gr_i

Given the short space of time between weighings (one day), pupal weight (w_p), w_1 and w_2 are highly correlated ($r_{(w_p, w_1)} = 0.67$, $P < 0.0001$; $r_{(w_p, w_2)} = 0.79$, $P < 0.0001$). Consequently, selection on w_p will lead to a corresponding increase in w_2 and w_1 . Because of the high correlation between w_2 and w_1 ($r_{(w_1, w_2)} = 0.93$) the difference $w_2 - w_1$ will stay approximately the same (i.e., both increase by approximately the same fraction). Consequently we can predict that following selection, there will be little or no change in gr_i .

(2) Changes in cw_i

Following selection, the change in cw_i will be the same as for $peak_i$.

(3) Changes in icg_i

Following selection, icg_i will increase, but the increase will be equal to $(\frac{peak_i - 7.0}{gr_i})$. As we predict little change in gr_i following selection (above), the change in icg_i will be proportional to $peak_i$.

The predictions for cw_i and icg_i (2 and 3, above) are consistent with the high genetic correlation (≈ 1.0) between these traits and $peak_i$ (Table 1). All the above predictions were supported by the simulation in Table 2.