A Physiological Perspective on the Response of Body Size and Development Time to Simultaneous Directional Selection†

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SYNOPSIS. Natural selection typically acts on multiple traits simultaneously. Quantitative genetics provides the theory for predicting the response to selection of multiple traits and predicts symmetrical responses to selection (the response to upward selection on both traits is equal to their response to downward selection). In reality, however, the response to simultaneous selection on two traits is often asymmetrical. We provide a physiology-based framework to explain the asymmetrical response to simultaneous selection on two important life history traits: body size and development time. The tobacco hornworm, Manduca sexta, is particularly well suited for such a study, as the physiological control of body size and development time is well known in this species. Three physiological factors control both life history traits in M. sexta: growth rate, the critical weight that measures the timing of the onset of the cessation of juvenile hormone secretion (which initiates the processes leading to pupation) and the time interval between the critical weight and secretion of the molting hormone 20-hydroxyecdysteroid (the interval to cessation of growth, ICG). Asymmetry in the response to simultaneous selection on the two life history traits is due to the different types of selection acting on the three physiological factors. The critical weight and ICG are always under synergistic selection when both focal traits are selected in the same direction and under antagonistic selection when the focal traits are selected in opposite directions. Growth rate follows the opposite pattern. We propose a general model to explain the asymmetric response to simultaneous selection. This model emphasizes the importance of physiological processes in understanding evolutionary responses to selection and the control of complex traits.

INTRODUCTION

Selection experiments are an important part of the toolbox in evolutionary biology and their use is becoming more prevalent in other fields as well. Ecologists have recently begun to use selection experiments to evaluate the long term effects of ecological processes (see the recent Special Feature: Selection Studies in Ecology: Concepts, Methods and Directions [Ecology 84 (7): 1649–1712]). Although there is an increasing awareness of the usefulness of selection experiments to explore physiological traits (Gibbs, 1999), selection experiments are still an under-utilized tool in many sub fields in physiology (Garland, 2003). Here we demonstrate how an understanding of the underlying physiological mechanisms of life history traits, can provide important insight into their response to simultaneous selection.

A fundamental assumption of life history evolution is the tradeoff (Roff, 1992). Given a finite amount of resources, an organism allocates those resources so as to maximize fitness. Within an individual, the allocation of resources to one trait necessarily comes at the expense of allocation to another trait, hence the tradeoff. Many such life history decisions involve the growth and development of the whole organism, in contrast to that of a single trait within the organism. Coordination of growth and development throughout the whole organism is in the purview of the hormone system (Nijhout, 1994, 1999). It is not surprising then, that the regulation of body size and development time, two important life history traits that affect the whole organism, are coordinated through the hormone system (Davidowitz et al., 2003, 2004; Davidowitz and Nijhout, 2004).

The tobacco hornworm (Manduca sexta: Sphingidae) is an excellent model organism to study the hormonal regulation of life history traits in insects. Probably more is known of the physiology and endocrinology of growth and development in M. sexta than any other insect (Nijhout, 1994; Gilbert et al., 1996). The short generation time of five weeks, egg to egg, and the ease of rearing it on an artificial diet (see diet recipe in Davidowitz et al., 2003) make it particularly appealing to study in laboratory conditions and particularly well suited for selection experiments. The hormonal regulation of pupation and metamorphosis has been particularly well studied in M. sexta (Gilbert and Frieden, 1981; Riddiford and Truman, 1993; Gilbert et al., 1996; Riddiford et al., 1999). These studies have shown that the initiation of pupation is regulated by the timing of two hormonal events: the timing of the initiation of juvenile hormone (JH) decay in the middle of the last larval stadium, and the timing of surges in the secretion of the molting hormone ecdysone in the latter period of the last instar larva (see Nijhout [1994], Riddiford [1994] and Gilbert et al. [1996] for reviews).
Body size is a function of the duration of the growth period and mass accumulated during the growth period. Recent work (D’Amico et al., 2001; Davidowitz et al., 2003, 2004; Davidowitz and Nijhout, 2004) has shown that peak larval body size in *M. sexta* is determined by three physiological factors: the timing of the cessation of *JH* secretion, the timing of surges in ecdysone secretion, and the growth rate. Together, the first two endocrine-based physiological traits determine the duration of the growth period of the larval stages of development. The growth rate determines how much mass is gained during the growth period (Davidowitz and Nijhout, 2004).

**The physiological background**

Davidowitz and Nijhout (2004) propose a physiological mechanism demonstrating how these three physiological factors control both body size and development time. We briefly describe their mechanism and provide a simplified schematic of this mechanism in Figure 1. As with other insects, adult *M. sexta* do not grow. The size that a larva attains at the time of metamorphosis can largely define the body size of the adult insect (Davidowitz et al., 2004). Insect growth is exponential, such that in *M. sexta*, 90% of the increase in mass occurs during the last larval instar (Davidowitz et al., 2003). A last instar larva feeds and grows until it attains a critical weight. At the critical weight the corpora allata, the glands that synthesize and secrete *JH*, switch off (Nijhout and Williams, 1974). In the presence of *JH*, secretion of the prothoracicotrophic hormone (PTTH) and ecdysteroids are inhibited (Nijhout and Williams, 1974; Rountree and Bollenbacher, 1986). PTTH induces secretion of ecdysone, which directly regulates pupation and metamorphosis. In *M. sexta* PTTH and ecdysone secretion cannot occur until all *JH* has been cleared from the hemolymph. A small peak in ecdysteroid titer effectively terminates the feeding and growth period of the larva. The time interval between when the larva attains the critical weight and when PTTH and ecdysone are secreted is called the Interval to Cessation of Growth (ICG, Fig. 1, Davidowitz et al., 2004; Davidowitz and Nijhout, 2004). PTTH can only be secreted during a fixed window during the daily light-dark photoperiodic cycle. This time window is termed the photoperiodic gate for PTTH secretion and recurs each day at the same time (Fig. 1). Thus, after *JH* is cleared from the hemolymph, and the larva is competent to secrete PTTH, it must wait for the next photoperiodic gate to secrete PTTH and ecdysone (Truman, 1972; Truman and Riddiford, 1974). Larval growth stops when the sequence of events initiated by the critical weight culminates in secretion of ecdysone. Thus, development time is determined by the timing of ecdysone secretion, and peak larval size is determined by how much additional growth occurs during the ICG (larvae nearly double their weight during this period, Davidowitz et al., 2004).

That the critical weight, ICG and growth rate affect the response to selection and hence, the evolution of body size was demonstrated by D’Amico et al. (2001). They showed that over a period of 220 generations (≈ 30 years), body size in a laboratory colony of *M. sexta* evolved, increasing 50%. Concomitant with this increase in size, the critical weight, ICG and growth rate also increased. Together they explained over 95% of this increase in peak larval size in *M. sexta*.

In following up on these findings, we have found that knowledge of the physiological control of body size and development time offers a unique opportunity to address a vexing problem in evolutionary biology: why are the responses to selection often asymmetric? Asymmetric responses to selection occur when the response to upward selection differs from the response to downward selection. Theory typically predicts symmetric responses, although asymmetric responses to selection are generally seen empirically (Roff, 2002).

**The simultaneous response to selection**

Natural selection generally tends to minimize development time: faster growing individuals reduce the risk of predation resulting in a higher probability of survivorship (Roff, 1992). Selection would also favor larger sized individuals, as larger females tend to be
more fecund (Roff, 1992). Natural selection, however, does not typically act on a single trait; rather, it acts on multiple traits simultaneously.

Simultaneously selected traits may be constrained from evolving in a preferred direction, due to tradeoffs between them. Tradeoffs are typically revealed by negative genetic correlations between traits. These correlations, however, only identify the existence of tradeoffs; they cannot explain the underlying cause of the tradeoff (Zera and Harshman, 2001).

The response to selection and the evolution of traits depends on the underlying genetic architecture, particularly the amounts of additive genetic variances and covariances. For single traits, the evolutionary trajectory can be described by the breeders’ equation \( R = h^2 S \), where \( R \) is the response to selection, \( h^2 \) is the heritability of the trait and \( S \) is the selection differential. For multiple traits, both the direct effects of selection as well as responses due to the genetic correlation between traits need to be taken into account. This can be accomplished using the multivariate extension of the breeders equation \( \Delta z = GP^{-1}S \), where \( \Delta z \) is the vector of changes in trait means, \( G \) is the matrix of additive genetic variances and covariances, \( P \) is the phenotypic variance-covariance matrix and \( S \) is a vector of selection differentials (Lande and Arnold, 1983).

Both the single and multivariate breeders’ equations predict symmetric responses to selection. In other words, the response to upward selection (selection to increase the value of a trait) will be equal to the response to downward selection (selection to decrease a trait’s value). Although symmetric responses are frequently found when selection is on morphological traits, asymmetric responses are more typical for life-history traits such as development time and fecundity (Frankham, 1990; see also Fig 2.21 in Roff, 2002). A few artificial selection studies have examined simultaneous selection on multiple traits (Bell and Burris, 1973; Rutledge et al., 1973; Nordskog, 1977; Beldade et al., 2002). These studies suggest that the response to selection is often far from symmetric and that selection that is contrary to the sign of the genetic correlation typically produces results that cannot be predicted from the simple multivariate form of the breeders’ equation (\( \Delta z = GP^{-1}S \)).

The observation of asymmetric responses to selection highlights the insufficiency of quantitative genetic analyses based solely on estimates of genetic variances and covariances (Nordskog, 1977). One way to resolve the problem of asymmetric responses to selection is to dissect in greater detail the underlying physiological and developmental mechanisms that generate phenotypic and genetic correlations (Riska, 1986). Here we show how an understanding of the physiological control of two complex traits, development time and body size, may provide a better understanding of how these traits respond to selection and why their response to simultaneous selection is asymmetrical.

A PHYSIOLOGICAL FRAMEWORK FOR THE CONTROL OF THE RESPONSE TO SIMULTANEOUS SELECTION

Using physiology to predict the response to selection

Changes in the critical weight, ICG and growth rate affect peak larval size and development time (Fig. 2). Increasing growth rate results in a larger body size, but shortens development time (Fig. 2A). Increasing ICG also leads to larger body size but a longer development time, because the secretion of PTTH and ecdysone occurs later and at a larger size (Fig. 2B) allowing more time for the larva to accumulate mass. Increasing the critical weight increases both body size and development time, because the cascade of events leading to pupation starts later and at a larger size (Fig. 2C). In real life, all three factors are likely to change...
simultaneously under selection and the final development time and body size will be a function of the interaction of all three factors (Davidowitz et al., 2004).

Understanding the underlying physiology allows us to make predictions about the physiological response to simultaneous selection of the two traits as shown in Figure 3. On the abscissa is development time, which increases to the right. On the ordinate is body size, which increases upwards. The filled circle at the center of the diagram indicates the population before selection. The response to selection would move the population from the central circle to one of the four quadrants as indicated by the arrows (Fig. 3). For example, simultaneously increasing both traits would move the population from the central circle to the upper right quadrant. Simultaneous selection acting to decrease development time but increase body size would move...
the population from the center towards the upper left quadrant.

The **bold** type within each quadrant show how each of the three physiological factors can control the response to selection on a single focal trait (based on the principles of Fig. 2). For instance, body size can increase (two upper quadrants on body size axis, Fig. 3) via an increase in the growth rate (GR), in the interval to cessation of growth (ICG) or in the critical weight (CW), or some combination of the three. A decrease in development time (left two quadrants on development time axis) can occur either by increasing GR or decreasing ICG and CW, or by a combination of these.

To increase two traits simultaneously, however, the control of the individual traits need also be examined simultaneously. This reveals a physiological conflict between the control mechanisms of both traits. For example, an increase in both development time and body size (upper right quadrant in Fig. 3) can occur by an increase in both CW and ICG (Fig. 2B, C). Growth rate (GR), however, should increase to increase body size, but at the same time, decrease to increase development time (Fig. 2A). Thus, under simultaneous selection for increased development time and body size, CW and ICG are under synergistic selection while GR is under antagonistic selection (Fig. 3).

The upper half of the inner boxes in Figure 3 show the type of selection (antagonistic or synergistic) acting on each of the three physiological factors (CW, ICG, GR) during simultaneous selection of the two focal traits, development time and body size. Antagonistic selection on a physiological factor occurs when selection on one focal trait causes this factor to increase while selection on the other focal trait causes it to decrease. Synergistic selection occurs when the regulatory factor increases (or decreases) in the same direction in response to selection on both focal traits. We predict the type of selection (antagonistic or synergistic) based on the response of the physiological factors to selection on the focal traits as shown in **bold** (which were determined from Fig. 2, see above). For example, we predict that simultaneously selecting for increased development time and body size (upper right quadrant) should generate antagonistic selection on GR (it should increase because of selection upwards on body size, and decrease because of selection upwards on development time). Similarly, we hypothesize that ICG and CW are under synergistic selection, as both would increase in simultaneous upward selection on the two focal traits.

As another example, imagine selecting for increased body size and decreased development time (upper left quadrant in Fig. 3). In this case ICG and CW are under antagonistic selection (as they would tend to increase with increased body size and decrease with shorter development time (Fig. 2B, C), and GR is under synergistic selection, as it would increase with an increase of both focal traits.

With this framework, we predict that the simultaneous response to selection for increased body size and decreased development time will be constrained by the ICG and CW, and determined primarily by GR, as indicated in the lower portion of the inner box in the upper left quadrant of Figure 3. In the first example, where simultaneous selection acts to increase both development time and body size, the response to selection will be constrained by GR and determined primarily by CW and ICG. In general, we propose that simultaneous selection will be constrained by whichever underlying physiological factors are under antagonistic selection.

In Fig. 3 we assume a positive genetic correlation between development time and body size (a test of this assumption will be reported elsewhere). A positive correlation between development time and body size indicates that both traits will, for example, increase under simultaneous upward selection. When both focal traits are under upwards selection, growth rate is under antagonistic selection. It will increase as body size increases but decrease as development time increases (Fig. 3). Therefore the response to selection for an increase in both traits will be constrained by growth rate and determined primarily by the critical weight and the ICG, which are both under synergistic selection and will increase as the focal traits increase.

In our other example, body size increases while development time decreases (upper left quadrant in Fig. 3). Here we predict the response to selection will be constrained by the critical weight and ICG and determined primarily by the growth rate, as the former two are under antagonistic selection and growth rate is under synergistic selection.

We note, however, that the predictions for the constraints on selection would be reversed, if the genetic correlation were negative. In the examples above, simultaneous selection to increase both traits would be constrained by the ICG and the CW and determined primarily by GR while selection for decreased development time and increased body size would be constrained by GR and determined by ICG and CW.

It is important to note that the predictions on the constraints to selection are the same along each diagonal (upper-right/lower-left and upper-left/lower-right) but differ between diagonals (upper-right/lower-right, upper-left/lower-left, upper-right/upper-left, and lower-right/lower-left, in Fig. 3): the response to selection is constrained by growth rate when both development time and body size either decrease or increase (upper right and lower left quadrants in Fig. 3) and is constrained by the ICG and the critical weight when one focal trait is selected upwards and the other downwards (upper left and lower right quadrants in Fig. 3). This may explain why the patterns of the responses to simultaneous selection often differ between diagonals. For example, Beldade et al. (2002) produced symmetric responses to selection on butterfly eyespots along the diagonals, but asymmetric responses across diagonals. Bell and Burris (1973) selected simultaneously for larval weight (at day 13) and pupal weight.
in the flour beetle (*Tribolium castaneum*). The genetic correlation between the two traits was positive (0.54 ± 0.13). Their results demonstrated a symmetric response when selection increased or decreased both traits simultaneously and an asymmetric response when one trait increased and the other decreased. Nordskog (1977) demonstrated the difficulty of selection increasing one trait and decreasing the other, when the two traits, body weight and egg weight (in poultry) were positively correlated.

We propose a simple, general, physiology-based model that explains an asymmetric response to simultaneous selection (Fig. 4). A and B represent two focal traits such as body size or development time. The arrows indicate selection. Two physiological factors are represented here for simplicity, but there can be any number of factors (greater than one), provided they have large effect on the focal traits. The model predicts that when the underlying physiological factors are under synergistic selection, the response to selection of the focal traits will be symmetric (Fig. 4; upper panels). When the underlying physiological factors are under synergistic selection, the response to selection of the focal traits will be asymmetric. The degree of the asymmetrical response depends on the strength of the genetic and phenotypic correlations represented in the physiological factors and focal traits and among the physiological factors themselves.

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REFERENCES


