Evolution and the regulation of growth and body size

Alexander W. Shingleton

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Introduction

The relationship between body size and life-history parameters has been long recognized. Correlates between body size and clutch size, life span, gestation time and age at maturation have been well studied in myriad taxa. Similarly, changes in relative organ size are associated with major life-history transitions; the decision to grow horns in beetle, become a worker or queen ant, or develop a defensive helmet in Daphnia all involve changes in organ growth, and have important life-history implications. Developmentally, final body and organ size is regulated by mechanisms that control the rate and duration of growth, two primary life-history parameters. At many levels, therefore, the genetic, developmental and physiological mechanisms that regulate body and organ size are intimately involved with those that regulate life-history.

Changes in body and organ size will consequently impact other life history traits, and vice-versa, and bias their collective evolution (Roff 2002; Stearns 1992). Work over the last thirty years has seen a dramatic deepening of our understanding of the mechanisms that regulate growth and body size, and how these mechanisms influence other aspects of life history. The challenge for evolutionary biologists is to integrate this new mechanistic information with an evolutionary perspective to understand how growth and body size evolves. In other words, we need to link the proximate with the ultimate causes of body size evolution.

This chapter is divided into two parts. In the first part I will concentrate on the regulation of body size. I will describe the molecular and physiological mechanisms that regulate the duration and rate of growth, explore how these mechanisms work together to create variation in body size, and review how they influence other aspects of life history. This first part will concentrate on body size regulation from an environmental rather than genetic perspective. This is because most of our mechanistic understanding of body size regulation comes primarily from studies of how body size changes in response to environmental factors. In the second part I will focus on body size evolution. I will consider observed patterns of body size evolution in response to natural and artificial selection, and ask whether these patterns can be explained in terms of the genetic, developmental and physiological mechanisms that regulate body size. I will then explore the relationship between environmental and evolutionary changes in body size, before asking...
whether we can predict which size-regulatory mechanisms are the target for selection on body size.

I will largely, but not exclusively, concentrate on growth regulation in holometabolous insects. This is not solely a personal bias. First, we perhaps know more about the physiological and molecular regulation of growth in insects than in any other animal. Second, as will become clear, these mechanisms are highly conserved evolutionarily and aspects of them are likely shared among all animals.

**The regulation of body size in insects**
Holometabolous insects, as their name suggests, undergo complete metamorphosis, molting through a series of grub-like larval stages before pupating and metamorphosing into their final adult form. Adult insects, like all arthropods, have a stiff growth-restricting exoskeleton, so adult size is entirely regulated by growth during the premetamorphic larval stages. This is true both for the body as a whole and for the external organs (wings, legs, eyes, etc), which grow as imaginal discs within the developing larva. Maximal body and organ size is therefore fixed at the point of metamorphosis.

The physiological processes that control the initiation of metamorphosis, and hence define the duration of growth, have been best described in large lepidoptera such as the tobacco hornworm *Manduca sexta* and the silkworm, *Bombyx mori* (Ishizaki & Suzuki 1994; Nijhout & Williams 1974a; Nijhout & Williams 1974b; Truman & Riddiford 1974). These physiological processes take the form of a hormone cascade, illustrated in Figure 1. At some point in the final larval instar of a lepidopteran, attainment of a particular body size, referred to as critical size or critical weight, is associated with a drop in the level of circulating juvenile hormone (JH). This is a consequence of a reduction in the production of JH by the corpora allata (Nijhout & Williams 1974a) and an increase in the production of JH esterase (Browder et al. 2001; Roe et al. 1993; Sparks et al. 1983). Once JH levels fall below a certain threshold, this de-inhibits the release of prothoracicotropic hormone (PTTH) (Nijhout & Williams 1974a), which in turn stimulates the synthesis of ecdysone by the prothoracic gland (PG). The subsequent peaks in ecdysteroid levels (the metabolic derivatives of ecdysone) ultimately cause the cessation of feeding, then, after a
period of larval wandering, initiate pupation itself. Growth of the body stops at the cessation of larval feeding. However, the imaginal discs continue to grow until the beginning of the pupal period, when even higher levels of circulating ecdysteroids causes them to stop growing and differentiate into their respective adult structures (Champlin & Truman 1998a; Champlin & Truman 1998b; Chihara et al. 1972). While the hormonal regulation of growth cessation has been less well elucidated in other holometabolous insects, the processes are likely similar to those of the lepidopterans, albeit with some key differences, as I will describe below.

(Figure 1 here)

This cascade of hormonal events means that there is a delay between the attainment of critical size and the termination of body and organ growth, called the terminal growth period (TGP) (Shingleton et al. 2007). Final body size in holometabolous insects is thus regulated by three basic mechanisms: (1) critical size; (2) the duration of the TGP; and (3) growth rate during the TGP (Davidowitz & Nijhout 2004; Nijhout et al. 2006; Shingleton et al. 2007; Shingleton et al. 2008). The same is true for the organs, although because they continue to grow after the cessation of body growth, their TGPs are longer than the TGP of the body. The regulation of body size in holometabolous insects can therefore be approached by considering these three developmental processes separately.

**The regulation of critical size**

While the hormonal link between attainment of critical size and the initiation of pupariation was elucidated in *M. sexta* (Nijhout & Williams 1974a; Nijhout & Williams 1974b), critical size was first described in *Drosophila* (Beadle et al. 1938). It was recognized as the minimal size at which transient starvation no longer delays metamorphosis: withdrawing food and then re-feeding larvae smaller than critical size delayed metamorphosis but did not affect final adult size, while the same manipulation in larvae larger than critical size did not delay metamorphosis but reduced final adult size. Thus critical size represents a size checkpoint, attainment of which is dependent on nutritionally regulated growth. How does an insect know when it has achieved critical size? Three mechanisms that regulate critical size have been identified (Figure 2): insulin-signaling in
the PG, the production of PTTH by neurosecretory cells in the brain, and growth of the imaginal discs.

(Figure 2 here)

Work in *Drosophila* indicate that nutritional regulation of critical size is via insulin/insulin-like growth factor signaling (IIS) (Shingleton et al. 2005). The IIS signaling pathway is the major molecular mechanisms regulating growth with respect to nutrition, in all animals (see below). It is activated by the nutritional release of insulin-like peptides (ILPs) from insulin-producing cells in the brain (IPCs) and from endocrine tissue around the body (Figure 3). As with transient starvation, suppression of the IIS pathway early in development delays attainment of critical size. Conversely activation of the IIS pathway by increasing the expression of ILPs causes precocious metamorphosis (Walkiewicz & Stern 2009). These effects appear to be controlled by IIS signaling in the PG. An increase in IIS signaling in the PG alone is sufficient to accelerate the attainment of critical size, resulting in a premature release of ecdysone and precocious metamorphosis in larvae of a smaller size (Caldwell et al. 2005; Mirth 2005). Suppressing the IIS in the PG has the opposite effect.

More recently, ablation of PTTH-producing neurosecretory cells in the *Drosophila* brain has been shown to severely delay the release of ecdysteroids, retarding metamorphosis and increasing in adult size (McBrayer et al. 2007). PTTH promotes ecdysteroidogenesis in the PG by acting as a ligand for the receptor *torso* and activating the Ras-Raf-MAPK pathway. Changes in *torso*, *Ras* and *Raf* expression in the PG consequently alter critical size and final body size (Caldwell et al. 2005; Rewitz et al. 2009). In lepidopterans, PTTH also appears to activate the synthesis of ecdysone via the Ras-Raf-MAPK pathway (Rybczynski et al. 2001; Smith et al. 2003). Interestingly, PTTH transcription in wild-type flies shows a cyclic profile during the third-and-final larva instar (Mcbrayer et al. 2007). PTTH synthesis may therefore impose circadian rhythm on the attainment of critical size, the release of ecdysone and the timing of the resulting developmental events (Walkiewicz & Stern 2009).
Finally, there is increasing evidence that the imaginal discs also regulate critical size. Slowing the growth of the imaginal discs alone by inducing DNA damage using X-irradiation, or by repressing the expression of ribosomal proteins using RNAi, delays attainment of critical size and retards metamorphosis in Drosophila (Poodry & Woods 1990; Stieper et al. 2008). This seems to be a repair mechanism that ensures damaged or slow–growing imaginal discs are given additional developmental time to recover to their normal size before differentiating into their adult structures (Simpson et al. 1980). Damage to the imaginal disc delays critical size in part by inhibiting the transcription of the gene encoding PTTH, via retinoid signaling: inhibiting retinoid signaling prevents larvae with damaged discs from delaying metamorphosis (Halme et al. 2010). The same may be true for larvae with slow-growing discs. Intriguingly, however, larvae with slow-growing or damaged discs pupariate at their normal size, unlike larvae in which PTTH production is directly inhibited. Thus, either the PTTH-inhibiting signaling or some other signal produced by slow-growing/damaged imaginal discs must also slow growth, or prevent overgrowth, of the rest of the body.

The regulation of TGP

In lepidopterans, the duration of the TGP is regulated by the timing of the hormonal cascade that is initiated at attainment of critical size (Figure 1) (Browder et al. 2001). Two factors affect the timing of this cascade: (1) the speed at which JH is cleared from the haemolymph and (2) the time between the clearing of JH to the synthesis of PTTH (Davidowitz et al. 2002). The rate at which JH declines is in part regulated by the activity of JH esterase (Roe et al. 1993). Once JH has been cleared from the haemolymph, the precise timing of PTTH release is regulated by photoperiod: PTTH is only released during the first night after JH has been cleared from the haemolymph (Truman 1972).

The hormonal dynamics that follow attainment of critical size in Drosophila are less well elucidated and appear to be somewhat different than in M. sexta. The ecdysone titre show multiple peaks throughout the third larval instar in Drosophila, each of which initiates a particular developmental process. The first peak just precedes attainment of critical size, the second peak occurs just before the synthesis of glue protein by the salivary glands, the third precedes larval wandering and the cessation of body growth, while the fourth initiates
pupariation itself (Berreur et al. 1979; Warren et al. 2006). All of these peaks are preceded by a spike in PTTH synthesis (Mcbrayer et al. 2007), suggesting that the control of the body and organs’ TGPs is also regulated by PTTH action in Drosophila. Further, there is some evidence that the timing of development is influenced by photoperiod in Drosophila (Mirth et al. 2005), suggesting that the cycles of PTTH transcription may also be photoperiodically regulated. However, loss of PTTH (Mcbrayer et al. 2007) or application of JH mimics (Riddiford & Ashburner 1991) delays but does not prevent pupariation in Drosophila, so there must be additional mechanisms that regulate the TGP apart from JH and PTTH.

It is not only the temporal dynamics of JH, PTTH and ecdysone that defines the body’s and organs’ TGPs, however, but also the sensitivity of growing tissues to these hormones. For example, in M. sexta body growth stops with the cessation of feeding, when ecdysteroids rise above a minimum threshold in the absence of JH and acting on the nervous system to initiate larval wandering (Dominick & Truman 1986a; Dominick & Truman 1986b). In contrast, the eye-antennal imaginal disc continues to grow until ecdysteroid levels rises above a higher threshold during pupal development (Champlin & Truman 1998a; Champlin & Truman 1998b). The cessation of growth and the initiation of differentiation also seems to be dependent on levels of ecdysteroids in developing Drosophila (Chihara et al. 1972; Cullen & Milner 1991; Currie et al. 1988; Mirth 2005; Peel & Milner 1992). Consequently, different organs may have different sensitivities to the circulating hormone that regulate developmental timing, and this may account for observe differences in their periods of growth and the timing of growth cessation (Emlen & Allen 2003).

The regulation of growth rate

The second determinant of body size in animals is growth rate. A key regulator of growth rate is metabolic rate, and much has been written about broad interspecific relationships between growth rate and metabolic rate, and metabolic rate and body size (Calder 1984; Schmidt-Nielsen 1984; West et al. 1997; West et al. 2001; West et al. 2003). Nevertheless, among individuals within a population there may be considerable variation in growth rate, much (if not most) of which is due to environmental factors such as nutrition, temperature, and oxygen level. Research
over the last decade has made substantial progress in elucidating how these factors influence growth rate at a molecular level. In doing so they provide a deeper understanding of how growth rate is controlled and how this control may change through evolutionary time.

In almost all animals, reduced developmental nutrition lowers growth rate and reduces final adult size. This relationship is intuitive: growth is through the conversion of nutrition to tissue, and the more limited the nutrition, the more limited the growth. Remarkably, in all animals this relationship appears to be regulated by the same signaling pathways: the insulin/insulin-like growth factor signaling (IIS) pathway, the Target of Rapamycin (TOR) signaling pathway, and the AMP-dependent kinase (AMPK) pathway, collectively called the IIS/TOR system (Figure 3) (Conlon & Raff 1999; Edgar 2006; Edgar 1999; Oldham et al. 2000; Shingleton 2005). The IIS/TOR system ultimately regulates growth rate by regulating transcription, translation, endocytosis, autophagy, and mitosis and is essentially identical in insects and vertebrates.

(Figure 3 here)

The IIS/TOR system responds to nutrition in two ways (Figure 2). The first is indirect, through the nutritionally-dependent release of insulin-like peptides (ILPs) and their subsequent binding to the insulin receptor (Inr). The second is more direct, through the cell autonomous response of TOR to cellular levels of amino acids and glucose, the latter via the AMPK pathway. Suppression of the IIS system, for example by mutation of Inr or TOR, genocopies starvation and result in a reduction in growth rate and final adult size, both in insects (Chen et al. 1996), nematodes (Kimura et al. 1997) and vertebrates (Baker et al. 1993).

For ectothermic animals, growth rate is also regulated by temperature: an increase in temperature results in an increase in growth rate. Canonically, this is thought to be a consequence of the effect of temperature on biochemical kinetics (Gillooly et al. 2002). However, as I discuss below, while biokinetics undoubtedly has a substantial influence on growth rate and hence body size, there is compelling evidence that animals can regulate the effect of temperature on the rate of growth, even at the level of individual tissues. The molecular mechanisms by which this is
achieved remains unknown, leaving a conspicuous hole in our understanding of the molecular regulation of body size.

Finally, a reduction in oxygen reduces growth rate and decreases final adult size in many animals. For example, Drosophila reared under hypoxic conditions are smaller than when reared under normoxic conditions (Peck & Maddrell 2005). The trend for amphipods to be larger at increasing latitude, once seen to be a result of decreasing temperature, is now thought to be dependent on oxygen availability in the ambient water (Chapelle & Peck 1999). Similar trends are seen in deep-sea turrid gastropods (McClain & Rex 2001) and oceanic nematodes (Soetaert et al. 2002). Much research has been directed to the molecular and physiological response to hypoxia in mammals, although this has only recently been extended to Drosophila. In mammals, the transcriptional response to hypoxia is mediated by the hypoxia-inducible factors α and β (HIF1-α and HIF1-β) (Déry et al. 2005). In Drosophila, over-expression of Sima, the homolog of HIF1-α, protein genocopies hypoxia and causes an autonomous reduction in cell size (Centanin et al. 2005), in part by suppressing TOR signaling (Reiling & Hafen 2004). In mammals, HIF1 activates FOXO3a (Bakker et al. 2007), and the same may be true for FOXO in Drosophila. Thus the mechanisms that regulate body and organ size in response to oxygen concentration appear to converge on the mechanisms that regulate size in response to nutrition.

Environmental variation in body size: The functional interaction between critical size, TGP and growth rate in insect size regulation

Understanding the individual mechanisms that control growth rate and growth duration is necessary but not sufficient to explain the regulation of body and organ size. For example, changes in growth rate need not result in a change in final body size if there is a compensatory increase in developmental time. Therefore, we must consider the functional interaction between the mechanisms that regulate growth rate and duration if we are to understand the regulation and evolution of body and organ size. This interaction can be best appreciated by examining how size variation is achieved in response to environmental regulators of size, that is the phenotypic plasticity of body size.
As discussed above, a reduction in developmental nutrition reduces adult body size in almost all animals. In *Drosophila* this is primarily achieved through the negative effects of reduced IIS/TOR signaling on growth rate. Malnourished flies do not adjust either their critical size or lengthen their TGP to compensate for their reduced growth rate (Shingleton et al. 2005), although see (Layalle et al. 2008). Consequently, such flies grow more slowly during their TGP resulting in a reduction in final body and organ size (Figure 4) (Shingleton et al. 2005; Shingleton et al. 2008). In *M. sexta*, the size response to developmental nutrition is mechanistically similar, but not identical, to *Drosophila*. As for *Drosophila*, a reduction in size through nutritional deprivation is largely through a reduction in growth rate during a nutritionally-insensitive TGP (Davidowitz et al. 2004). However, dietary restriction also reduces critical size in *M. sexta*, providing a second mechanism by which nutrition affects final body size (Davidowitz et al. 2004).

(Figure 4 here)

The thermal response of the mechanisms that regulate growth rate and duration also explain why *M. sexta* larvae reared at higher temperatures produce smaller adults. Critical size is unaffected by temperature in *M. sexta*, but both the duration of the TGP and growth rate during the TGP are temperature-sensitive (Davidowitz et al. 2004). However, because an increase in temperature shortens the duration of the TGP more than it increases growth rate, the result is an overall decrease in body size. It is unclear whether the same is true for *Drosophila*.

In the majority of ectotherms hitherto studied, an increase in rearing temperature leads to a decrease in body size, dubbed the temperature-size rule (Atkinson 1994). One explanation for this rule is that growth duration/developmental rate is more sensitive to changes in temperature than growth rate, as observed in *M. sexta* (van der Have & de Jong 1996). Further, these same principles can explain why, in many animals, individuals reared at higher temperatures also have smaller cells: temperature may have a greater effect on the rate of cell division that on the rate of cell growth (Angilletta et al. 2004). The result is a decrease in cell size as temperature increases to maintain energy budget.
It is tempting to view the thermal response of growth and differentiation, and hence the effect of temperature on size, as simply being a result of the underlying biochemical kinetics, and consequently non-adaptive. However, there is extensive evidence for population and individual variation in thermal sensitivity for body size (Angilletta et al. 2004). Thus, animals appear to be able to modulate how temperature effects their growth and development rate. Intriguingly, this control of thermal sensitivity can be seen at the level of different organs within an individual. For example, in *Drosophila* the size of the wing is much more thermally sensitive than the size of other organs and size of the body as a whole and means that individuals reared at lower temperatures have proportionally larger wings, relative to body size (Shingleton et al. 2009). A recent study has shown that flies with lower wing loadings (wing area divided by body area) perform better at lower temperatures, so the high thermal sensitivity of the wings appears to be an adaptation to flight at different temperatures (Frazier et al. 2008). Because the TGP of the wing is regulated by the same hormonal cascade that regulates the TGP of the body and all imaginal discs, this difference in thermal sensitivity likely reflects a difference in the way temperature effects growth rate rather than growth duration.

In general, which of the three major regulators of body size in holometabolous insects (critical size, TGP or growth rate) underlies size plasticity varies between environmental factors and between species. Consequently, we might expect the evolutionary response of insects to selection on body size to be similarly variable.

**Trade-offs between body size and other traits**

The mechanisms used to regulate body size in insects also regulate other life-history traits. These interactions have the potential to create trade-offs, such that an increase in one life-history trait negatively impacts another life-history trait. In some cases, these trade-offs are an inevitable consequence of the way that body size is physiologically regulated. In other cases, trade-offs exist because the same genes and signaling pathways are co-opted to regulate multiple developmental and physiological processes. Consequently, these trade-off may be apparent when body size changes in response to one factor, for example developmental temperature, but not another, for example direct selection on body size.
An example of a life-history trait that is inevitably affected by the physiological mechanisms that regulate body size in holometabolous insects is developmental time. All other things being equal, natural selection favors an increase in body size (larger females tend to be more fecund) and a decrease in developmental time (a shorter generate time increases reproductive rate) (Roff 2002). Increasing body size by increasing critical size or the duration of the TGP alone will coincidentally increase developmental time, potentially ameliorating any fitness benefits gained from a larger adult size (Davidowitz et al. 2003). In contrast, increasing body size by increasing growth rate will shorten developmental time by accelerating attainment of critical size. Which size-regulatory mechanism is targeted by natural selection to increase body size presumably accommodates these potential trade-offs in developmental time and reflects the ecological context in which selection is acting.

An example of a signaling pathway that is utilized in the regulation of multiple life-history traits is the IIS/TOR system. IIS signaling not only affects growth rate (and hence developmental time), but also aging and egg-production. A reduction in IIS causes an increase in longevity in *Drosophila* (Tatar et al. 2001b), nematodes *Caenorhabditis elegans* (Dillin et al. 2002; Lee et al. 2003) and mouse *Mus musculus* (Holzenberger et al. 2003). In *Drosophila* this is mediated through the action of FOXO in the adult fat body, the insect equivalent of the mammalian liver and the major nutritional storage organ. Constitutive activation of FOXO in the fat body leads to an increase in median lifespan by as much as 35% (Hwangbo et al. 2004). Further, in the adult ovary, suppression of IIS in follicle cells reduces their proliferation and inhibits the ability of ovarian cells to enter vitellogenesis (Drummond-Barbosa & Spradling 2001; LaFever & Drummond-Barbosa 2005). This, combined with a decrease in proliferation of both germ-line and somatic stem cells in the ovary, results in a 60-fold reduction in the rate of egg-production in protein-starved adult female flies (Drummond-Barbosa & Spradling 2001). These pleiotropic effects of IIS means that any changes in systemic IIS activity will not only influence body size and developmental time, but may also coincidentally affect fecundity and longevity.

Changes in the expression and activity of the hormones that regulate critical size and the timing of metamorphosis may also impact adult phenotype. Both JH and ecdysteroids are implicated in the regulation of longevity and in egg production. In monarch butterflies and several grasshopper
species (Herman & Tatar 2001; Pener 1972), removal of adult JH synthetic tissue arrests reproduction and increases longevity. In *Drosophila* these same phenotypic effects are seen in adults undergoing reproductive diapause, which is marked by a decline in JH (Tatar et al. 2001a). Flies that are heterozygous for a mutation of the ecdysone receptor (EcR) are also long lived but do not show a reduction in fecundity (Simon et al. 2003), although flies with a heteroallelic combination of two EcR mutations do show reduced oogenesis (Carney & Bender). There is also evidence that ecdysone and IIS interact antagonistically during development (Colombani et al. 2005). Up-regulating ecdysteroidgenesis during the final larval-instar not only accelerates metamorphosis but also reduces growth rate, by reducing IIS in the fat body. Systemic changes in the synthesis, release and response to both JH and ecdysone may therefore not only impact the timing of larval developmental events, but also influence growth rate, longevity and fecundity.

Further details of the molecular and physiological regulation of reproduction (Chapman, this book), metamorphosis (Erezyilmis, this book), and aging (Bauer & Helfand, this book) are described elsewhere in this book. These chapters, along with the chapters on diapause (Schmidt, this book), social interactions (Sinervo & Lancaster, this book), and immunity (McKean & Lazzaro, this book), serve to emphasize that the mechanisms that regulate growth and body size also regulate multiple life-history traits. This creates potential trade-offs among life-history traits that, as I discuss below, have important implications for the evolution of individual traits and for the integrated phenotype as a whole. An open question is why developmental and physiological systems have evolved so that these trade-off exist in the first place. In some cases, the trade-offs may be an inevitable consequence of processes that depend upon each other (West-Eberhard 2003), for example the interaction between developmental time and growth in holometabolous insects. However, why the same signaling systems, for example the ecdysone-signaling pathway, should be used to regulate as disparate processes as the timing of critical size and egg production is less clear. Such pleiotropy may be a consequence of natural selection linking developmental and physiological processes that maximize fitness in a particular environmental context. Alternatively, it may reflect the general pattern of evolution co-opting pre-existing signaling pathways for novel developmental functions (True & Carroll 2002).
The evolution of body size

From the above discussion it is evident that we have a good (and improving) understanding of the developmental and physiological mechanisms that regulate body size in insects, particularly in response to environmental variation. It is also clear that there are multiple mechanisms by which body size can change, each of which may trade-off with other aspects of life-history. What, if anything, does this knowledge add to our understanding of how body size evolves? To answer this question I will briefly review some of the evolutionary trends in body size variation, and the selective forces that are thought to underlie them, as well as studies that have used artificial selection and experimental evolution to alter body size in a population. I will then examine some of the few example where we know the developmental mechanisms that underlie evolved changes in body size, and explore whether these are the same as the mechanisms that control environmental variation in body size. Finally I will pose the question “Can we predict which size-regulatory mechanisms are the target for selection on body size?” The goal of this section is to begin to clarify how the proximate (mechanistic) causes of body size evolution interact with the ultimate (selective) causes of body size evolution.

Evolutionary trends

There is a tendency for body size within a lineage to increase over evolutionary time. This macroevolutionary trend, referred to as Cope’s Rule, has been documented in a wide variety of animal and plant taxa and appears to be driven by individual selection on size within populations (Kingsolver & Pfennig 2004). There are a number of hypotheses as to the selective pressures that drive this trend. Larger individuals may utilize a larger share of resources within local ecosystems, can more easily avoid predation whilst being more effective predators, are more successful in mating and intraspecific competition, and are more resistant to environmental perturbation (Hone & Benton 2005). Although Cope’s law does not apply to insects throughout their evolutionary history (gigantism was widespread amongst the insects during the Palaeozoic, see below), it does appear apply to insects after the Permian mass extinction, at least in the Coleoptera, Hymenoptera, Diptera and Lepidoptera (Chown & Gaston 2010).

A second pattern is for species to become larger with latitude and altitude, known as Bergmann’s rule. Although the rule was first applied to interspecific patterns in endotherms, it also applies to
intraspecific patterns (where it is also referred to as the James’ rule) and to ectotherms (Chown & Gaston 2010). The trend has been particularly well studied in *Drosophila*. For example a genetic increase in body size with latitude is observed in *D. melanogaster* from western Europe and Africa (Capy et al. 1993), North America (Coyne & Beecham 1987), South America (Van't Land et al. 1995) and Australia (James et al. 1997). Similar clines are seen in other *Drosophila* species (James et al. 1997). These clines have apparently evolved quickly: *D. subobscura* populations introduced into North and South America in the 1970’s have evolved genetic wing size clines that are similar to those observed in endemic European populations (Calboli et al. 2003). The nature of the selective pressures that underlie Bergman’s rule remains controversial, but include changes in temperature, oxygen level, starvation resistance and duration of growing season with latitude (Chown & Gaston 2010). It should be noted, however, that that Bergman’s rule is not immutable. Many species show a negative correlation between body size and latitude (Mousseau 1997).

Finally, for insects at least, there is also a pattern of increased body size in species living in the late Palaeozoic. Gigantism was common among insect taxa during this period, with dragonflies growing wing spans as wide as 710 mm (Wootton & Kukalova-Peck 2000). A common explanation for this phenomenon is increased oxygen levels and atmospheric pressure during the Palaeozoic (Dudley 1998). Support for this hypothesis is the loss of gigantism with decreased hyperoxia during the late Permian and a second spike in gigantism coinciding with an oxygen peak during the Cretaceous. However, increased body size in Palaeozoic may also have been in response to an increase in predation by vertebrates (Chown & Gaston 2010). The phenomenon of gigantism may therefore be another example of Cope’s rule.

**Artificial Selection**

Artificial selection has been most commonly used to alter body size in domesticated animals and plants. Examples include toy and giant breeds of dogs, and for increased size in cattle (Yerex et al. 1988) and swine (Partridge et al. 1999). There have also been a numerous of studies in which insect body size has been changed through artificial selection on body size itself (Partridge et al. 1999; Teuschl et al. 2007) or as a correlated response to selection on another trait, for example
developmental time (Pijpe et al. 2006) or stress resistance (Bubliy & Loeschcke 2005). Finally, body size has evolved through experimental evolution, where laboratory populations are reared in environmental conditions thought to select for a changes in body size. Examples include rearing populations of *Drosophila* in low temperature or nutrition environments (Bochdanovits & de Jong 2003; Partridge et al. 1995), and bacteria in low-glucose environment, which resulted in an increase in cell size (Mongold & Lenski 1996).

**The developmental mechanisms underlying the evolution of body size.**

Despite the amount of experimental and observational evidence that body size can and does evolve, the developmental mechanisms underlying evolved changes in body size have generally been poorly elucidated. Nevertheless, there are a few examples where we have some idea of the proximate mechanisms that underlie evolved changes in body size.

**Evolution of body size in Manduca sexta**

*Manduca sexta* have been used as a model for insect physiology for over 30 years. Consequently, colonies have been reared under laboratory conditions for extended periods of time. One such colony, maintained for over 30 years, has shown an increase in average body size of 50%.

Subsequent investigation revealed that this increase in body size was due to an increase in critical size, an increase in the delay between attainment of critical size and the release of PTTH (that is the TGP) and an increase in growth rate (D'Amico et al. 2001); that is, all three regulators of body size in holometabolous insects.

The precise genetic basis for these physiological changes are unknown. However, the experimental manipulations of the mechanisms that regulate critical size, TGP and growth rate described above, hint at what some of these changes might be. For example, in *Manduca sexta* (unlike *Drosophila*) critical size is increased in well-fed animals. The evolved changes in critical size might be a consequence of modifications in the production of ILPs and/or the autonomous response of the PG to these peptides. Alternatively, changes in critical size may be a result of changes in the synthesis of, or response to, PTTH. Changes in TGP could be due to alterations in the expression and/or efficacy of JH esterase; in the suppressive effect of JH on PTTH synthesis;
in the effect of PTTH on ecdysteroidgenesis; or the sensitivity of growth tissue to changes in the ecdysteroid titre. Finally, whole body growth rates may evolve through changes in the production of, and/or response to, ILPs.

**Evolution of body size in Drosophila.**

There have been several studies where *Drosophila* body size has been subjected to artificial selection. In one such study (Partridge et al. 1999), direct selection for increased body size (thorax length) was associated with an increase in larval development time, and an increase in the Mean Viable Weight for pupariation (MVW), but no change in growth rate. In contrast, selection for a decrease in body size was associated with a reduced growth rate and a decrease in MVW but no change in developmental time. The MVW is the point in development at which starvation no longer prevents a larva from pupariation, and occurs at approximately the same time as critical size in *Drosophila*. Consequently, it is seems likely that the observed changes in body size were due to changes in critical size. The developmental response of *Drosophila* to selection on body size therefore uses all of the mechanisms that account for changes in body size in *M. sexta*, but in a different pattern depending on the direction of selection. Further, because there are many processes that regulate growth rate, critical size and the duration of growth in insects, the molecular-genetic response to selection on any one aspect of size regulation may be very different in the two species.

**Evolution of body size in domestic dogs.**

Domestic animals are commonly subjected to artificial selection on size. In the case of dogs, this selection appears to have targeted the IIS/TOR system (Sutter et al. 2007). Within a breed (Portuguese Water Dogs) variation in body size is associated with variation at the Insulin Growth Factor 1 (IGF1) allele, the mammalian homolog of insulin-like peptides in insects (Sutter et al. 2007). Like dILPs, IGF1 promotes growth and its serum levels are regulated by nutritional status (Donahue & Phillips 1989). Variation in IGF1 serum levels correlate with variation in body size both within and between breeds (Eigenmann et al. 1984a; Eigenmann et al. 1984b). Among breeds, there is evidence of a selective sweep at the IGF1 allele, with single IGF1 SNP haplotype common to nearly all small breeds but generally absent in large breeds (Sutter et al. 2007).
The relationship between evolutionary and environmental variation in body size.

The above examples suggest that the mechanisms that account for evolved genetic variation in body size converge on the mechanisms that control environmental variation in body size. To a certain extent this may be because body size is primarily regulated by critical size, TGP and growth rate in insects, and so both natural selection and phenotypic plasticity are constrained to target these mechanisms. Consequently, there will inevitably be overlap between the developmental and physiological mechanisms responsible for evolutionary and environmental variation in body size. However, critical size, TGP and growth rate are regulated through multiple processes, so evolution need not affect these in the same way as environmental factors. Nevertheless, because the processes involved in the environmental regulation of size by definition affect body size, they may provide additional targets for selection on body size and facilitate its evolution.

Alternatively, phenotypic plasticity may play a more central role in evolutionary change, through the process of genetic assimilation (West-Eberhard 2003). Genetic assimilation occurs where a phenotype formally produced only in response to a particular environment becomes stably expressed independent of the environmental effect (Flatt 2005). During genetic assimilation, the phenotype therefore loses its plasticity and becomes canalized. For this to occur there must be genetic variation in phenotypic plasticity, that is there is a genotype by environment (GxE) interaction for a trait. There is abundant evidence for GxE interactions for traits in general (Schlichting & Pigliucci 1998) and body size in particular (Bergland et al. 2008). Consequently, genetic assimilation is an appealing mechanism for body size evolution.

Genetic assimilation should be evident from examining the evolved developmental response of flies to a particular environmental pressure. If body size has evolved through genetic assimilation then, where evolution is a response to a particular environmental pressure, for example low nutrition, temperature or oxygen levels, the mechanisms targeted by natural selection should be the same as those utilized in the plastic response to that environmental pressure. More specifically, evolution should target those components of the plasticity mechanism that regulate the extent of the phenotypic plasticity.
Experimental evolution of *Drosophila* reared at different temperatures provides only partial support for body-size evolution by genetic assimilation. Both the evolved and plastic response to higher temperatures is a reduction in body size mediated primarily through changes in cell size, at least in the wing (Partridge et al. 1994). This is unlike the plastic response to nutrition which involves changes in both cell size and cell number. However, the effect of thermal selection on other regulators of body size show a different pattern. The plastic response to increased temperature results from an increase in growth rate but a decrease in developmental time, while the evolved response is exactly the opposite (when comparing high- and low-temperature lines reared at the same temperature) (Partridge et al. 1994). Thus thermal selection appears to target some but not all aspects of the mechanisms that underlie the thermal plasticity of body size.

The developmental response of body size to temperature has been hypothesized to underlie Bergman’s rule in *Drosophila*, whereby individuals get larger with increasing latitude, and presumably decreasing temperature (James et al. 1997; Zwaan et al. 2000). Again, this trend may arise through genetic assimilation of an initially plastic responses to temperature. However, in *D. melanogaster* the plastic response of the wing size to temperature is mediated primarily through changes in cell size while the latitudinal cline in wing size is a result of changes in both cell size and cell number (James et al. 1997). Further, the relative importance of cell size versus cell number in explaining the cline differs between different continents (Zwaan et al. 2000). The same is true for *D. obscura* (Gilchrist et al. 2004). Thus the same selective pressure can result in the same gross morphological response but a for different developmental reasons. The incongruence between the developmental mechanisms that underlie the latitudinal cline and thermal plasticity of body size suggest that either the latitudinal cline is not driven solely by thermal selection, or that thermal selection does not affect body size solely through genetic assimilation (or both).

Despite the lack of support for the role of genetic assimilation in the evolution of body size in *Drosophila*, there is evidence that it has occurred in snakes. Head size is smaller but more plastic in tiger snake populations isolated on islands for the last 30 years than in populations isolated for 6,000 – 9,000 years (Aubret & Shine 2009). Nevertheless, the details of the size regulatory
mechanisms that account for this pattern are unknown. In general, additional studies elucidating the molecular genetic basis for body size plasticity and evolution are necessary before firm conclusions on the relationship between the two can be drawn. Experimental evolution and artificial selection provides a particularly powerful method for testing theories of genetic assimilation (Frankino et al. 2009), and for understanding body size evolution in general.

Can we predict which size-regulatory mechanisms are the target for selection on body size?

The extensive pleiotropic effects that changes in different developmental regulators of body size have on other life-history traits will determine how body size evolves through space and time. They also make it very difficult to predict a priori which mechanisms will be targeted by which selective pressures. In general, we might expect that changes in those size regulatory mechanisms that have the fewest pleiotropic effects will occur first, since pleiotropy is thought to constrain evolutionary change (Hansen & Houle 2004). For example, changes in cell size should have fewer pleiotropic effects than changes in cell number, because the former can, in principle, occur at the very end of development, while the latter requires alterations in the rate and duration of cell proliferation during development. However, there are also likely limits on the extent to which body size can be affected solely through changes in cell size, due to functional constraints on cell size itself. Consequently, short-term thermal selection may initially affect body size through changes in cell size, while longer-term thermal selection along a latitudinal cline may subsequently break life-history trade-offs and affect body size further through changes in cell number also.

Trade-offs between different size regulatory mechanisms and other life-history traits need not always constrain evolution. Moderate levels of pleiotropy may enhance the evolvibility of a trait through an increase in mutational target, as long as directional selection on the target trait, in this case body size, is sufficiently large relative to stabilizing selection on the pleiotropic traits, for example developmental time (Hansen 2003). Further, variation among pleiotropic traits need not always be antagonistic. For example, certain environmental conditions may select for both a
reduction in body size and a reduction in developmental time, in which case selection may target
growth period rather than growth rate as the mechanism for reduced body size.

The picture that emerges of the developmental mechanisms that underlie body size evolution is a
complex one. In general, it would be surprising if the evolution of body size did not reflect the
many different physiological and molecular mechanisms by which body size is regulated. Just as
the mechanisms insects use to adjust body size in response to the environment varies with
environmental factors, so too might we expect the mechanistic targets for natural selection on
body size to vary with selective pressure. Life-history trade-offs, general pleiotropy, and possibly
 genetic assimilation, will play important roles in determining precisely what these targets are.
Consequently, we should expect considerable variation among insects specifically, and animals
in general, in the mechanisms used by natural selection to alter body size. Only with a more
profound understanding of the mechanisms that regulate body size will we be able to better
explain why, and predict which, mechanisms are targets for selection on body size.

Summary

• Body size is regulated by mechanisms that control the duration and rate of growth. In
holometabolous insects these mechanisms regulate body size through their influence on
critical size, TGP and growth rate.
• We have a good, and improving, understanding of the molecular and physiological
regulation of critical size, TGP and growth rate, in particular how this regulation
generates variation in body size in response to environmental factors, i.e. phenotypic
plasticity. Different environmental factors influence body size through different
mechanisms in different species.
• The molecular and physiological regulators of body size are also intimately involved in
regulating other life-history traits, for example life-span and fecundity. This creates life
history trade-offs that will influence how an animal responds to an environmental factor
that favors change in body size, on both developmental and evolutionary time-scales.
• Body size is evolutionarily labile and responds rapidly to natural and artificial selection.
Which size-regulatory mechanisms are the proximate target for selection varies between
species and with the nature of the selective agent. Nevertheless, selection appears to
frequently target mechanisms involved in the environmental regulation of size. This may be because these plasticity mechanisms provide additional targets for selection on size. Alternatively, body size may evolve through the process of genetic assimilation.

• A deeper understanding of the mechanisms that regulate body size and how these mechanisms regulate other life-history traits is essential if we are to understand intra- and interspecific patterns of body size variation through space and time.

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**Figure Captions**

Figure 1: The physiological regulation of metamorphosis in *M. sexta*. Attainment of critical size (*a*) is associated with decline in the JH titre. The elimination of JH from the haemolymph de-inhibits PTTH secretion (*b*), which stimulates ecdysteroidgenesis by the prothoracic gland. The subsequent rise in the ecdysteroid titre causes the end of feeding (*c*), delimiting the TGP of the body (also called the interval to cessation of growth, ICG). A second peak in PTTH (*d*) causes another rise in the ecdysteroid titre. This, combined with a rise in JH, initiates pupation (*e*). Subsequent peaks in ecdysone during pupation (not shown) initiate imaginal disc differentiation, ending growth and delimiting the TGP of the imaginal discs. (Redrawn after Nijhout 1994)

Figure 2: A model of critical size regulation in *Drosophila*. Critical size is regulated by larval and imaginal signals. The larvae signals comprise (*a*) a nutritional/size signal from the IPCs, and (*b*) a temporal signal from the PTTH-producing neurons. The imaginal signal (*c*) is inhibitory and affects the synthesis of PTTH, in part in a retinoid-dependent manner. It is the balance of these signals that ultimately regulates the release of ecdysteroids (*d*).

Figure 3: The insulin/IGF-signaling system in *Drosophila*. Variation in nutrition influences the release of ILPs, possibly by the action of sNPFs and AKH. ILPs bind to Inr which initiates a
signal transduction cascade involving the phosphorylation of multiple intermediate proteins. Downstream growth regulators include FOXO, which is deactivated by IIS via phosphorylation by AKT, and S6K, which is activated by IIS via PDK1. S6K is also a target of TOR, which additionally restricts the effects of FOXO by inhibiting one of FOXO’s transcriptional targets, 4EBP. TOR is regulated indirectly by IIS via the action of AKT on TSC1/2. TOR also responds to amino acids, by an unknown mechanism, and glucose, via the AMPK pathway. Both FOXO and TOR regulate the activity of multiple growth inhibitors and promoters, respectively. Data from (Edgar 2006; Harrington et al. 2004; Miron et al. 2003; Oldham & Hafen 2003; Radimerski et al. 2002; Rintelen et al. 2001). Dotted lines are un-elucidate/putative relationships. Reproduced from (Shingleton 2010).

Figure 4: The physiological regulation of body and organ size in Drosophila. (A) Larvae grow until they reach a critical size at the beginning of the third larval instar. Attainment of critical size begins a terminal growth period for the body (TGP_{body}) and the imaginal discs (TGP_{disc}). Final body and organ size is controlled by the size of the body and discs at critical size, plus the amount of growth achieved during the subsequent TGP_{body} and TGP_{disc}. (B) Reduced nutrition slows growth rate but does not lower critical size nor extend the TGP_{body} and TGP_{disc}. Dietary restriction before critical size delays attainment of critical size and extends total developmental time, but does not reduce final body size. Dietary restriction after critical size slows growth during the TGP_{body} and TGP_{disc} and reduces final body and organ size, but does not increase total developmental time (Redrawn after (Shingleton et al. 2008)).
damaged/immature imaginal Disc

imaginal signal

larval signal

PTTH

PG

ILPs

IPCs

larval brain

ecdysteroid

PG neurons
(A) During development, there is a critical size that determines the final body size. The imaginal disc size also increases with time, passing through a TGP disc stage. (B) Similar process for final organ size, with the same stages and timing.