Size and shape: the developmental regulation of static allometry in insects

Alexander W. Shingleton,[1]* W. Anthony Frankino,[2] Thomas Flatt,[3] H. Frederik Nijhout,[4] and Douglas. J. Emlen[5]

Summary

Among all organisms, the size of each body part or organ scales with overall body size, a phenomenon called allometry. The study of shape and form has attracted enormous interest from biologists, but the genetic, developmental and physiological mechanisms that control allometry and the proportional growth of parts have remained elusive. Recent progress in our understanding of body-size regulation provides a new synthetic framework for thinking about the mechanisms and the evolution of allometric scaling. In particular, insulin/IGF signaling, which plays major roles in longevity, diabetes and the regulation of cell, organ and body size, might also be centrally involved in regulating organismal shape. Here we review recent advances in the fields of growth regulation and endocrinology and use them to construct a developmental model of static allometry expression in insects. This model serves as the foundation for a research program that will result in a deeper understanding of the relationship between growth and form, a question that has fascinated biologists for centuries. ***BioEssays*** **29:536–548, 2007.**

[1]Department of Zoology, Michigan State University.
[2]Department of Ecology and Evolutionary Biology, Princeton University.
[3]Department of Ecology and Evolutionary Biology, Brown University.
[4]Department of Biology, Duke University.
[5]Division of Biological Sciences, University of Montana.
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*Correspondence to: Alexander W. Shingleton, Department of Zoology, Michigan State University, 203 Natural Science, East Lansing, Michigan 48910. E-mail: shingle9@msu.edu
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Abbreviations: ICG, Interval to the cessation of growth (*M. sexta*); JH, Juvenile hormone; PTTH, Prothoracicotropic hormone; TGP, Terminal growth period.

Introduction

It seems intuitive that within a species, individuals with larger bodies also have larger constituent parts. Larger humans tend to have longer legs, arms and torsos, bigger livers and larger hearts. This scaling relationship between the sizes of individual traits and the size of the whole body is called allometry.(1) Allometry describes how the characteristics of an organism scale with each other and with body size (Box 1). For morphological characteristics, allometries can be best visualized as plots of the size of a trait against the size of the body. When these plots are made from measurements of conspecific individuals at the same life stage, the relationship is called a static allometry (Fig. 1). Even a cursory survey of static allometries reveals considerable variation in their slope. Slopes vary between species for the same trait (Fig. 1a), and between traits for the same species (Fig. 1b). Often, morphological traits scale proportionally with the body, a condition called isometry, so that the relative size of the trait is independent of body size (e.g. maxillary palps in *Drosophila melanogaster*, Fig. 1b). However slopes can be very steep, such that traits become relatively larger with increasing body size, or very shallow or flat, such that traits become relatively smaller with increasing body size (e.g. male genitals in

Box 1: Glossary

Allometry: The proportional change in the dimensions of one trait relative to another trait or to overall body size; the scaling relationship between traits.

Critical size: The point in development when further growth is no longer necessary for a normal time course to pupation.

Imaginal discs: Developing adult organs within larval holometabolous insects.

Reaction norm: The pattern of phenotypes generated by a single genotype under varying environmental conditions.

Terminal growth period: The remaining period of growth available to the body and organs once critical size is attained.

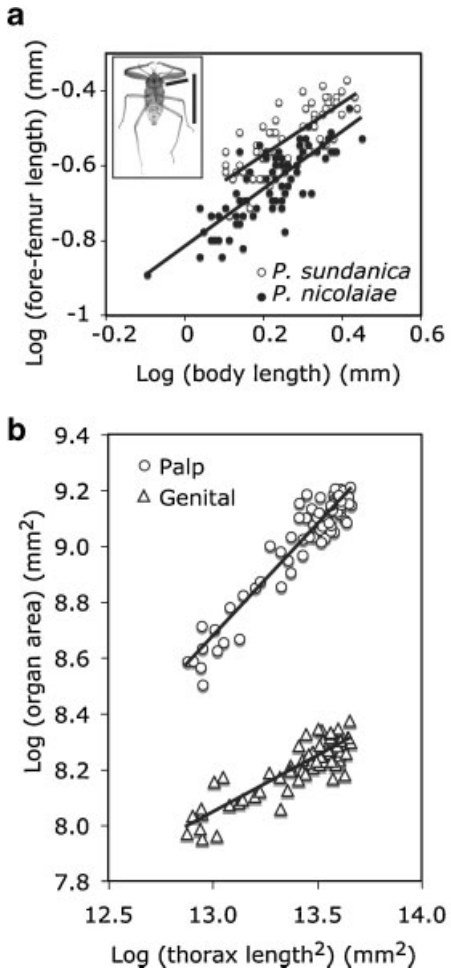

Figure 1. Static allometries can vary for the same organ between species and between different organs within a species. **a:** The static allometry of fore-femur length against body length for two soldier-aphid species, *Pseudoregma sundanica* (open circles) and *P. nicolaiae* (closed circles). Inset shows measurements on *P. sundanica*.[84] **b:** The static allometry of maxillary palp area and genital arch area for male OreR *D. melanogaster*. The palps are almost isometric to body size, while the genitals have a flatter allometry, such that larger flies have proportionally smaller genitals.

D. melanogaster, Fig. 1b). Slope can even be negative, such that traits become absolutely smaller with increasing body size. The shape of allometries are often modelled using the allometric equation (Box 2), which can be applied to traits that scale linearly on a Log–Log scale. However, static allometries need not be linear on a Log–Log scale, or even linear on any scale. They can be sigmoidal or discontinuous, depending on the trait, the species and the unit of measurement.[2]

Irrespective of their shape, static allometries reveal how the relative sizes of a trait scale with each other and with overall body size, and so capture the relationship between size and form in complex organisms. Variation in the shape of static allometries is therefore an important component of phenotypic diversity, and there has been extensive work on the evolutionary relevance of allometry (for review see Ref. 3). Despite this, there is very little understanding of the developmental processes that create allometries, and how these processes can be modified to produce the variety of scaling relationships that we see both within and between species. Recent advances concerning body and organ size control, however, provide the first clues as to how static allometries may be developmentally regulated. These discoveries, which have been made principally in holometabolous insects, concern the genetic and physiological mechanisms that regulate body and organ size in response to variation in nutrition.

Here we review these findings and hypothesize the developmental mechanisms that regulate static allometry in holometabolous insects. We begin by discussing the different types of allometry and the developmental phenomena that produce them. We then use a 'reaction norm' approach to argue that the developmental processes that regulate static allometries may be based on those that regulate phenotypic plasticity. We next review the genetic and physiological mechanisms that regulate nutritional reaction norms for body and organ size. We synthesize these mechanisms to produce a developmental model to explain how static allometry is regulated in holometabolous insects. Finally we show how changing certain parameters in this developmental model can alter the shape of a scaling relationship, providing candidate mechanisms for the evolution of static allometries and thus of animal form.

Box 2

Allometries are traditionally modelled using the allometric equation $y = ax^b$, where x and y are two given traits.[82,83] A Log-transformation of this equation produces the linear equation Log $(y) = \text{Log}(a) + b\text{Log}(x)$, and log–log plots of the size of different traits within a species often reveal linear allometries with a slope of b (Fig. 1). Such allometries are classified according to their slope and the value of b. Morphological traits scale isometrically when $b = 1$ (e.g. palp size against body size in *D. melanogaster* Fig. 1b), hypometrically when $b < 1$ (e.g. genital size against body size in *D. melanogaster* Fig. 1b), and hypermetrically when $b > 1$. This classification has utility in summarising the myriad slopes of different allometries, although it cannot be applied to those allometries that are not linear on a log–log scale.

Types of allometry—partitioning sources of variation

Traditionally, allometries are classified into three types: ontogenetic, evolutionary and static allometries.[3,4] Ontogenetic allometries are growth trajectories and describe the growth of an organ relative to the growth of another organ or growth of the body, in a single individual. Evolutionary allometries describe the relative size of different organs among individuals at the same developmental stage across species. Static allometries are similar but describe the relative size of different organs among individuals at the same developmental stage within a species. Both evolutionary and static allometries arise because there is covariation in the size of body parts among individuals at a particular developmental stage. Since variation in organ and body size at any particular developmental stage is a consequence of variation in growth up to that stage, it follows that both evolutionary and static allometries are a consequence of changes in ontogenetic allometries. This relationship between ontogenetic, and evolutionary and static allometries is illustrated in Fig. 2.

To elucidate the developmental mechanisms that regulate static allometries, it is necessary, therefore, to understand the developmental mechanisms that create variation in the relative growth of individual organs and the body. For evolutionary allometry, this variation in growth is presumed to be caused by evolved genetic differences between individuals of different species. For static allometry, however, variation in growth may be due to genetic differences between individuals, to differences in the environment in which they developed, or due to the interaction between the two. Static allometries may therefore arise as a consequence of genetic and/or environmental influences on growth. This is a problem if we are to identify the developmental mechanisms that create static allometries: environmental factors may influence growth through completely different mechanisms than genetic factors.

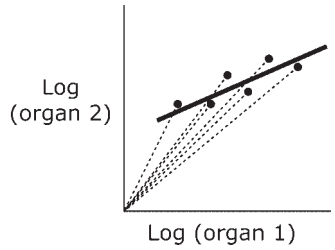

Figure 2. The relationship between evolutionary, static and ontogenetic allometries. If each point represents the organ sizes of a different species at the same developmental stage, then the solid line is an evolutionary allometry. If each point represents the organ sizes of a different individual within a species at the same developmental stage, then the solid line is a static allometry. In both cases, the allometry arises due to variation in the relative growth trajectories, or ontogenetic allometry, of the two organs (broken lines).

We suggest that static allometries can therefore be subdivided into (1) environmental static allometries, where each point on an allometric chart represents the same genotype in different environments, and (2) genetic static allometries, where each point on an allometric chart represents a different genotype within a single environment. Different sources of environmental variation, for example temperature, nutrition, sunlight, etc, each could generate a particular allometric relationship. Similarly, there may be different sources of genetic variation, such that allelic variation at one locus may produce a different allometry than variation at another. Further, there are almost certainly gene–environment interactions, with genetic variation in the mechanisms that control environmental static allometries.

Such distinction between genetic and static allometries is not a new concept.[4] A static allometry describes covariation in the size of two traits across individuals within a species, and quantitative geneticists have long partitioned the genetic and environmental components of variation in correlated traits.[5] However, in quantitative genetics, the environmental component of phenotypic covariation is typically that which is not explained by the genetic component.[6] This quantitative genetic approach acknowledges the importance of environmental sources of covariation, but does not address the nature of this source; environmental covariation is more defined by what it is not (i.e. not genetic) rather than what it is.

In this paper, we focus on the developmental mechanisms that create environmental static allometries, specifically those that result from variation in the nutritional environment. While our classification of static allometries into genetic and environmental static allometries is an idealization, the conceptual distinction between the two is important. It allows clear identification of the mechanisms that create static allometries. Further, investigating the developmental basis of environmental static allometries has wider implications. Evidence increasingly suggests that both genetic and environmental variation converge on the same regulatory pathways to control the expression and evolutionary diversification of phenotypic variation.[7] Understanding how the environment modifies development to generate static allometries may therefore help elucidate how evolution has modified development to generate evolutionary allometries.

The mechanism of static allometry expression—a reaction norm approach

Environmental static allometries arise because both the body and the organs within it respond in similar ways, either directly or indirectly, to environmental factors that regulate the rate and duration of cell growth and division. This developmental response to the environment is a form of phenotypic plasticity,

the phenomenon whereby a particular genotype produces different phenotypes in different environments. Plotting trait size against the value of a particular environmental variable for a single genotype reveals the environment-specific phenotype, or reaction norm. A reaction norm therefore describes the pattern of phenotypic variation produced by a single genotype reared under a range of environmental conditions. In *D. melanogaster* for example, as with most metazoans, malnutrition during development reduces final adult size. Figs. 3a and 3b show the reaction norms for wing area thorax area of *D. melanogaster* as a function of larval nutrition. Combining and re-plotting two reaction norms generated by the same environmental variable reveals an environmental static allometry. From such plots, it is clear that two organs will have an isometric relationship to each other if they share the same reaction norm to an environmental variable. They will have a negative, positive or non-linear allometric relationship if they have different reaction norms to the same environmental variable.

To understand what determines the nature of a given environmental static allometry, it is therefore necessary to (i) elucidate the developmental mechanisms that create a particular set of reaction norms, and (ii) understand how those reaction norms interact. This coordination of reaction norms is an example of phenotypic integration; that is, how traits are genetically or functionally interrelated.[3,8] For example, the allometric relationship between wing size and thorax size in an insect may be a consequence of both the wing and thorax independently responding to variation in nutrition. Alternatively, only thorax size may respond directly to variation in nutrition, with variation in wing size being, mechanistically, a secondary consequence of variation in thorax size. Both these alternative mechanisms would produce a correlation between the reaction norms for thorax and wing size. Although several studies have explored correlations among reaction norms,[3,8,9] the genetic and physiological mechanisms that underlie such 'plasticity correlations' remain unknown.

Nutritional static allometries in holometabolous insects

Nutrition is the best-studied factor that affects body and/or organ size and can generate an environmental static allometry. Almost ubiquitously among the metazoans, nutritional restriction during development produces adults with reduced body and organ size. In recent years, the genetic and physiological processes that regulate this response have been uncovered. Thus it is now possible to explore how nutrition controls the absolute and relative sizes of the body and organs. In the next section, we consider the mechanisms that regulate the nutritional reaction norms of insect body and organ size. We then explore how the nutritional reaction norms of different traits interact to regulate each other and nutritional static allometry. Finally, we synthesize the findings into a developmental model of nutritional static allometry expression in holometabolous insects.

Mechanisms that generate nutritional reaction norms for body size

Comprehensive reviews of the developmental mechanisms that control adult body size in insects have recently been published in this journal[85] and elsewhere.[10] Consequently, we will only briefly cover these mechanisms here, and encourage the interested reader to refer to these other papers for further details.

In holometabolous insects, growth is restricted to the embryonic and larval stages (Fig. 4a,b). The developing insect moults through a series of larval instars before it stops feeding, pupates, undergoes metamorphosis, and finally ecloses from the pupal case as a fully formed adult. Adult size is limited by the size of the larvae when it stops feeding, and hence stops growing. The physiological process that controls this growth cessation is best understood in the tobacco hornworm *Manduca sexta*. At some point in the final larval instar, attainment of a particular body size is associated with a reduction in the levels of circulating juvenile hormone (JH).[11–14] This size is called 'critical size' or 'critical weight'. Falling levels of JH allow the release of prothoracicotropic hormone (PTTH) which, in turn, stimulates the prothoracic gland to release ecdysteroids and terminate feeding.[15] There is temporal separation between attainment of critical size and the termination of growth, which in *M. sexta* is called the interval to cessation of growth (ICG). Once critical size is attained, a larva irreversibly initiates the hormonal cascade that ends in metamorphosis, and the remaining period of growth available to the larva is fixed. The final size of *M. sexta* is thus determined by the critical size, the duration of the ICG and the rate of growth during the ICG.[16]

The physiological mechanisms of size regulation in other holometabolous insects have been less well elucidated, but are thought to be similar. Like *M. sexta, D. melanogaster* has a critical size, and there is also a delay between the attainment of critical size and the termination of body growth. The termination of growth is also caused by a rise in ecdysone levels.[17,18] However, the physiological mechanisms that link the attainment of critical size with this rise in ecdysone have not been elucidated in any insect other than *M. sexta*. Consequently, it is not clear that *Drosophila* has a delay that is mechanistically identical to the ICG of *M. sexta*. We will therefore refer collectively to the delay between the attainment of critical size and the termination of growth in holometabolous insects as the body's 'terminal growth period' (TGP), of which a special case is the ICG of *M. sexta*.

We propose the following general model for the control of body size in holometabolous insects. Final body size is the

critical size plus the amount of subsequent growth achieved during the body's TGP, which is in turn determined by the growth rate and the duration of the body's TGP (Fig. 4b). More formally,

$$B_F \approx B_{CS} + S_B \cdot \Delta t_B \quad (1)$$

where B_F is the final body size, B_{CS} is critical size, s_B is the rate of growth and Δt_B is the duration of the body's TGP. There are therefore three ways by which nutrition can potentially generate a reaction norm for body size in holometabolous insects: (i) by influencing growth rate during the body's TGP (s_B), (ii) by influencing the critical size (B_{CS}), and (iii) by influencing the duration of the body's TGP (Δt_B).

Nutrition and growth rate during the TGP (s_B)

Nutrition influences growth rate substantially during the body's TGP in *Drosophila* and *M. sexta*. The insulin-signaling pathway coordinates growth rate with nutritional conditions in most metazoans (for review see Refs. 19–23). In *Drosophila*, insulin-like peptides (dILPs) are produced by neurosecretory cells in the brain in response to nutrition. dILPS are also produced by the gut, the ovaries, the imaginal discs and various other tissues.[24–27] These dILPs bind to the *trans*-membrane insulin-like receptor (dInr) of dividing cells. This initiates a signal-transduction cascade that ultimately stimulates cell proliferation in developing tissues. Starvation down-regulates the insulin-signaling pathway in growing organs in a number of ways. It is sensed directly by dividing cells and suppresses the insulin-signaling pathway through an unknown mechanism via dTOR (target of rapamycin).[28,29] Nutrition also regulates the insulin-signaling pathway indirectly, through a reduction in the release of dILPs from neurosecretory cells in the brain,[24] and through an unknown humeral mechanism via the fat body (the insect equivalent of the liver).[30] Finally, the insulin signaling pathway is also regulated by other hormones, for example ecdysone,[31–33] JH[34] and Imaginal Disk Growth Factors,[35] the release of which may also be nutritionally regulated. All growing organs are likely exposed to the same concentration of dILPs and nutrition in the circulating haemolymph,[36] and so the insulin-signaling pathway can coordinate growth throughout the body in response to nutritional conditions.

In *M. sexta*, the action of insulin signaling on growth rate during the body's TGP/ICG is also likely to be an important component of the nutritional regulation of size.[13,16] However, in *M. sexta*, growth rate declines after critical size is attained and *M. sexta* larvae barely double in mass during their body's TGP/ICG.[37] In contrast, while growth rate also declines somewhat after critical size in *Drosophila*, fruit fly larvae more than triple in mass in their TGP. Consequently, the nutritional regulation of growth rate during the body's TGP may have less influence on variation of final body size in *M. sexta* than in *Drosophila*. This suggests that different insects emphasize different mechanisms to regulate the body's nutritional reaction norm.

Nutrition and critical size (B_{CS})

Neither nutrition[14] nor insulin signaling[38] appear to influence critical size in *Drosophila*. Larvae that are malnourished or insulin-suppressed early in development initiate metamorphosis at the same size as well-fed larvae:[14,39] food deprivation simply slows their growth and delays when they reach critical size. In *M. sexta*, however, critical size is a function of larval size at the transition to the final larval instar,[12,38] and is influenced by nutrition.[13] This indicates again that different insects utilize different mechanisms to regulate the body's nutritional reaction norm.

Nutrition and the body's TGP (Δt_B)

Nutrition has no influence on the duration of the body's TGP/ICG in *M. sexta*.[13] Nutrition has only a small effect on the duration of the body's TGP in *Drosophila*, with starvation slightly accelerating metamorphosis.[32] Such acceleration will cause a reduction in final body size. However, the effect is likely small relative to the effect nutrition has on growth rate, and may only occur when the larva is completely starved rather than reared on a low quality diet.

In summary, the nutritional reaction norm of the body is largely regulated through the effect of insulin signaling on growth rate during a fixed TGP in *Drosophila*. This mechanism also seems to be important in *M. sexta,* although body size is further regulated through nutritional influences on critical size.

Mechanisms that generate nutritional reaction norms for organ size

Surprisingly little is known of the developmental mechanisms that generate nutritional reaction norms for final organ size. However, the data that are available suggest that the mechanisms are similar to those that generate the nutritional reaction norm for final body size, but with a few important differences.

In holometabolous insects, adult organs develop as imaginal discs within the growing larvae. However, the timing of disc growth is not the same as for the body. The point in development when discs initiate growth varies intraspecifically among discs, and varies interspecifically among homologous discs. For example, in *Drosophila*, the wing and the eye discs begin proliferation in the first larval instar, while the leg and genital discs begin at the start of the second instar.[40] In *M. sexta,* the wing discs grow throughout larval development, while the eye and the leg discs initiate growth only in the last larval instar, after attainment of critical size.[41] Similarly, different discs stop growing and differentiate into adult structures at different points in development. For example, in *Drosophila* the eye disc completes growth by pupariation while the wing continues to grow for an additional 24 hours. Despite

this variation in when different discs start and stop growing, the cessation of disc growth is always after the attainment of critical size. Like the body, therefore, all organs have a 'terminal growth period' (Fig. 4d). For many organs this TGP is longer than the TGP of the body.[42,43] Further, the TGP for discs that begin growth after attainment of critical size (late-growing discs) is effectively their entire growth period.

The TGP for a particular disc, like the TGP of the body, appears to be controlled by fluctuating hormones. For example, cell proliferation in the eye in *M. sexta* is stimulated by an increase in ecdysteroid levels above a minimum threshold, just before the cessation of feeding. Proliferation continues until ecdysteroid levels rise above a maximum threshold in the middle of pupal development,[44,45] where upon the cells differentiate into their final adult states. Growth and differentiation of the imaginal discs of *Drosophila* also appear to be contingent upon certain ecdysteroid levels.[46–50] These data suggest that the duration of growth of imaginal discs may be regulated by response thresholds to fluctuating levels of hormones.[51] Variation among discs in response thresholds may be the proximate mechanism for among-disc variation in TGP.[51]

The physiological mechanisms that regulate the rate and duration of organ growth appear very similar to those that regulate the rate and duration of body growth. We can extend the model of body size regulation in holometabolous insects (Fig. 4b), and apply it to organ size regulation (Fig. 4d). More formally:

$$D_F \approx D_{CS} + S_D \cdot \Delta t_D \qquad (2)$$

where D_F is the final organ size, D_{CS} is organ size at critical size, s_D is the rate of organ growth and Δt_D is the duration of the organ's TGP. As with body size, nutrition might therefore influence the final size of individual organs by influencing (1) imaginal disc size at critical size (for discs that begin proliferation early in development) (2) the duration of a disc's TGP, and (3) the rate of growth during a particular disc's TGP.

Nutrition and disc size at critical size (D_{CS})

Data from the buckeye butterfly *Precis coenia* suggest that the growth of the discs and the growth of the body are closely matched, so that the size of the wing disc relative to the size of the body is constant, independent of growth rate.[52] These data are appealing since all growing tissues are likely exposed to the same concentration of nutrition and insulin-like peptides in the circulating haemolymph.[36] Consequently, if critical body size were affected by nutrition, as it is in *M. sexta*, the size of the discs at critical size would also be affected. Conversely, if critical size is unaffected by nutrition, then disc size at critical size would also be unaffected. There is evidence that this latter situation applies to *Drosophila*—suppression of the insulin-signaling pathway before critical size has no substantial effect on critical size of final organ size.[38]

Nutrition and disc-specific TGPs (Δt_D).

Data are scant concerning nutritional effects on the cessation of disc growth and the initiation of differentiation. In *Drosophila*, suppression of insulin signaling autonomously within the eye slightly retards differentiation, potentially lengthening the eye's TGP.[53] Insulin signaling also appears to influence the timing of differentiation in other imaginal discs.[53]

Nutrition and disc growth rate (s_D)

Nutrition affects substantially the rate of disc growth in *P. coenia*,[52] acting through the insulin-signaling pathway.[54] The same appears to be true for the imaginal discs of *Drosophila*. Insulin influences the rate of cell proliferation of imaginal disc cell lines in vitro.[35] Various components of the insulin-signaling pathway have been shown to affect cell cycle progression.[55,56] However, direct measurements of the effect of insulin signaling on the rate of *Drosophila* disc growth in vivo have, surprisingly, not been made.

As is the case for the body, it seems likely that nutrition and insulin signaling regulate final organ size primarily through influencing the rate of disc growth during an organ's TGP. This assessment is not based upon direct evidence, however. The effect of insulin signaling on disc size at critical size (D_{CS}), the duration of a disc's TGP (Δt_D) and the rate of disc growth (s_D) have not been well elucidated.

Additional mechanisms that influence organ size

When imaginal discs are excised from young *Drosophila* larvae and cultured in the abdomens of adults, they grow slowly to a size approximately equal to the size of non-excised discs at pupariation. This suggests that discs have an organ autonomous target size. Nutrition may conceivably influence final organ size by regulating the target size of the organ, rather than via the factors discussed above. Organ size is also regulated by short-range paracrine signals (morphogens) that pattern the developing discs. These morphogens define compartments within developing organs, and cell proliferation within a compartment is controlled by the levels of morphogens that specify it.[57,58] Manipulations of morphogen expression levels change the size and shape of the resulting organ,[59–61] suggesting that target size represents an organ-specific final distribution of these morphogens.

One mechanism by which nutrition and insulin signaling could alter final organ size is therefore by altering target size. Target size may reflect the nutritional condition of a larva at a particular point in development, for example, at the cessation of feeding. However, changes in insulin signaling in *Drosophila* affect final wing size throughout the wing's TGP even after feeding has ceased. Consequently, if insulin-signaling affects target wing size, this size must be modified continually throughout these periods of development. This suggests two hypotheses. First, target size may represent a mean (or minimum or maximum) organ size, with nutrition and

insulin-signaling regulating variation around the target size. Second, the insulin-signaling pathway may influence the expression and distribution of morphogens within an organ and how cells respond to them. Like the insulin-signaling pathway, many of these morphogens also regulate the rate of cell growth and division.[62] Morphogens may therefore interact with the insulin-signaling pathway during each organ's TGP to control the rate of organ growth while maintaining correct organ patterning. In support of this hypothesis is the fact that both the insulin receptor[63] and the morphogens Decapentaplegic (Dpp) and Wingless (Wg)[64] are known to regulate the Ras/Ras pathway, which in turn regulates the rate of cell division. Nevertheless, in both hypotheses, nutrition and insulin-signaling influences final organ size by influencing the rate of cell growth and division during an organ's TGP.

The interaction of nutritional reaction norms

Understanding how nutritional reaction norms are generated in developing insects only partially explains how nutritional allometries are expressed. A complete developmental model of nutritional static allometry must include information on how these reaction norms interact to produce a fully integrated phenotype (Fig. 3).

As discussed above, two traits will exhibit nutritional isometry with each other if they share the same nutritional reaction norm. This occurs if both traits autonomously respond identically to the same level of nutrition. Alternatively, the response of one trait could mechanistically control the response of the other. More generally, the nutritional reaction norm of a particular trait will depend on a combination of two factors: (1) The trait's autonomous, or direct, response to changes in nutrition and insulin-signaling, and (2) the trait's non-autonomous, or indirect, response that is mediated through the nutritional responses of other traits.

We have hypothesized that there are three primary factors through which nutrition can influence final body and organ size and generate nutritional reaction norms—critical size (B_{CS} and D_{cs}), duration of TGP (Δt_B and Δt_D) and growth rate during the TGP (s_B and s_D). We can now ask, to what extent does nutritionally induced variation in one of these factors in one trait, for example the TGP of the body (Δt_B), affect the same factor in another trait, for example the TGP of an organ (Δt_D), and by what mechanism?

Interaction of body and organ size at critical size (B_{CS} and D_{CS})

Critical size is, by definition, a characteristic of the body as a whole, rather than of individual organs within it. As discussed above, growth of the imaginal discs in *P. coenia*, closely matches that of the body as whole. The same is likely true for other holometabolous insects, including *Drosophila* and *M. sexta*. It therefore appears that the size of individual organs at critical size is controlled entirely by the size of the body at critical size. Nutritional variation of the body's critical size in *M. sexta* will consequently create nutritional variation in the size of the organs at critical size. However, there is some evidence that the developing organs may also regulate critical size. In *Drosophila*, underdeveloped or continuously growing wing discs can inhibit ecdysteroid release and delay metamorphosis.[65-69] Whether continuously growing discs postpone metamorphosis by delaying attainment of critical size or by extending the body's TGP is unknown. However, if discs do need to be of a certain size for the attainment of critical size, then nutritional variation in disc size could generate nutritional variation in the body's critical size.

Interaction of body and organ TGPs (Δt_B and Δt_D)

The TGPs of the body and of the organs all appear to be controlled by fluctuating levels of ecdysteroids and JH. Changes in the body's or an organ's TGP may result from changes in the dynamics of these hormone fluctuations, in which case all structures will be affected similarly. Alternatively, it may be due to organ-specific changes in response to the hormones. In both cases, the TGP of one structure will not directly influence that of another.

Interaction of body and organ growth rates (s_B and s_D)

In *Drosophila,* reduced nutrition, specifically reduced amino acid levels, can be sensed directly by dividing cells, which leads to a reduction of the insulin-signaling pathway within those cells and a reduction in growth rate.[28,29] This is theoretically sufficient to control the allometric relationship between organ size and body size.[70] However, the nutritional allometries of organ size against body size cannot simply be a consequence of both independently responding to what the developing insect is eating. This is because many internal structures continue growth after feeding and overall somatic growth have stopped. After the cessation of feeding, the imaginal discs must rely on stored nutrients in the fat body for further growth. The fat body constitutes the majority of a larva's body mass at the cessation of feeding. Consequently, the body's growth rate during its TGP determines the maximum size of the body and the amount of nutrients stored within. This in turn influences the imaginal discs' growth rates by regulating the level of nutrients available to them after the cessation of feeding.

Recent evidence from *Drosophila* indicates that the fat body also regulates organ growth before the cessation of feeding, by modifying the effects of insulin signaling on organ growth. Autonomously depriving the fat body of amino acids reduces fat body growth, but also reduces insulin-signaling and growth in peripheral tissues, even when the larva is still feeding.[30] Further, the fat body is known to regulate the production JH esterase, an enzyme that degrades JH.[71] JH has been shown to affect the influence of nutrition on imaginal disc growth in *M. sexta*—in the absence of JH, starvation

slows, but does not inhibit, disc growth.[34] The fat body could thus regulate disc growth by regulating levels of circulating JH. Collectively, these data suggest that the nutritional reaction norm of the body influences the nutritional reaction norms of the organs, and hence partly regulates the allometric relationship between body and organ size.

There is also evidence of interactions among growing imaginal discs. In *Drosophila*, imaginal discs produce dILPs and other growth factors, and so growth of one disc may regulate the growth of another. Ablation experiments provide evidence of negative interactions between growing imaginal discs. In *Precis coenia*, larger wings result when one wing disc is destroyed,[72] and the effects of such organ ablation are both local and additive.[73] Similar experiments in horned beetles reveal that the effects of such disc–disc interactions are affected strongly by the relative timing of the primary growth periods of the organs; larger structures result from experimental removal of organs growing during the same point in ontogeny.[74] In each case, these experiments reveal competitive interactions among growing structures that may affect their allometry with each other and with body size. However, the effects of these disc–disc interactions on nutritional static allometries may be relatively small. In *Drosophila,* eye overgrowth, caused by driving the insulin-signaling pathway in the developing eye alone, does not affect the size of any other organs in otherwise wild-type flies.[70] In contrast, in flies mutant for the insulin-receptor substrate *chico*, a mutation that genocopies starvation, eye overgrowth does cause a small (5%) reduction in wing size. These data suggest that disc–disc competitive interactions may only be important when nutrition is limiting and/or insulin signaling is impaired.

A developmental model of nutritional static allometries in insects

The evidence reviewed above suggests a specific developmental model of how nutritional static allometries are generated in holometabolous insects (Fig. 5). Nutrition, acting through its influence on levels of amino acids in the hemolymph or through its influence on circulating insulin-like peptides, can regulate the size of the body and organs at critical size (B_{CS} and D_{CS}), the duration of their TGPs (Δt_B and Δt_D) and their growth rate during their TGPs (s_B and s_D). Further, there are interactions between developing traits within the insect, so that the nutritional effects on the final size of one trait influences the final size of another trait. These interactions may also regulate the size of the body and organs at critical size, the duration of their TGPs and their growth rate during their TGPs. This combination of direct and indirect nutritional effects on growing structures links reaction norms, underlies phenotypic integration and ultimately regulates static allometry. Although the mechanistic aspects of this model need further elucidation, the model serves as a blueprint for future research and provides a conceptual framework for understanding the genetic and physiological processes that influence allometric expression. The model can also be refined into a mathematical description of allometry expression, which would allow further exploration of the factors that regulate static allometry in silico.

The evolution of static allometries

Identifying the proximate basis of reaction norm expression, and elucidating how reaction norms interact to produce allometries, is important if we are to understand how allometries evolve. Firstly, identifying the proximate basis of allometry expression allows us to understand better how development can change to produce observed differences in allometries among lineages, sexes or other groups. Secondly, dissection of the proximate basis of allometry expression permits the identification of candidate genes responsible for allometry evolution. Finally, understanding the proximate basis of allometry expression is necessary to resolve controversies regarding adaptation versus developmental constraint in the evolution of scaling relationships.[75]

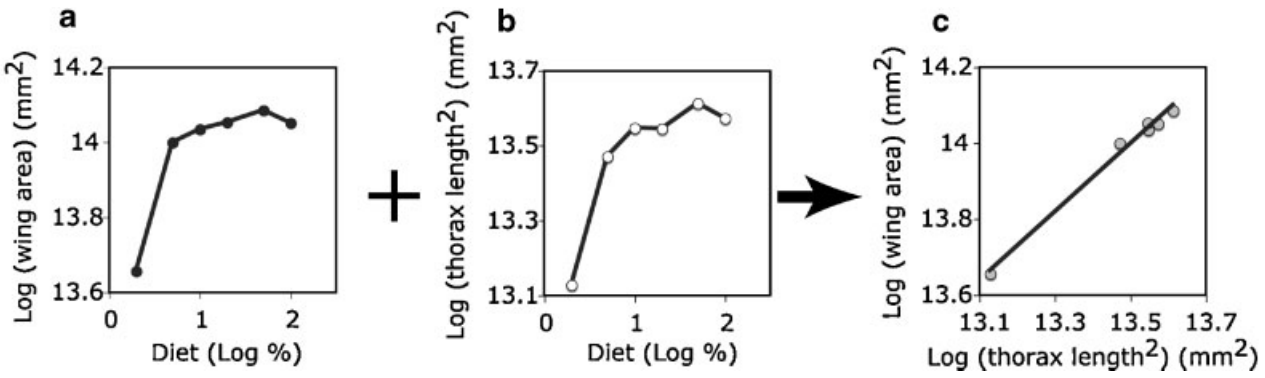

Figure 3. The relationship between reaction norms and allometries. The relationship between larval nutrition and **a** wing area and **b** thorax length in *Drosophila.* **c:** The two reaction norms can be re-plotted to reveal the nutritional static allometry between wing area and thorax length. All the flies were wild-type reared on a corn flour and molasses medium, diluted by 2% agar in water.

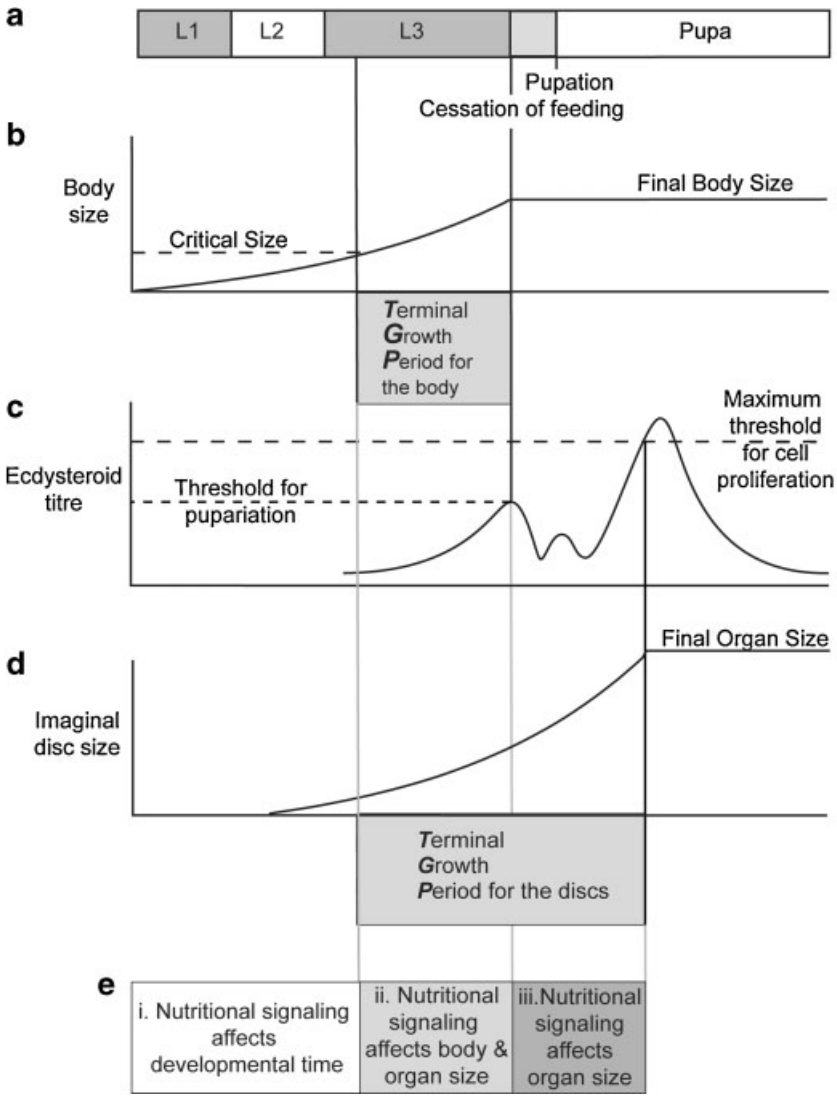

Figure 4. A model of the nutritional regulation of body and organ size in a holometabolous insect. **a:** Holometabolous insects moult through a series of larval instars before they stop feeding, pupate and metamorphose into an adult. **b:** Body size increases through the larval period until attainment of a critical size, which is associated with the initiation of pupation. There is a delay between attainment of critical size and the beginning of pupation called the body's terminal growth period (TGP). **c:** The beginning of pupation and the duration of the body's TGP are regulated by fluctuating hormones, in particular ecdysteroids. When ecdysteroids rise above a certain threshold, the larvae stops feeding and its final body size is fixed. **d:** The imaginal discs stop growing sometime after pupation, probably in response to ecdysone levels rising above a maximum threshold for cell proliferation. Like the body as a whole, the discs also have a terminal growth period, although this interval can be longer than for the body. **e:** Nutritional signaling regulates the growth rate of the body and the developing imaginal discs. **e,i:** Prior to critical size, nutritional signaling regulates the rate of growth to the critical size and principally influences developmental time. **e,ii:** After critical size, the remaining periods of growth for the body and organs are fixed. Nutritional signaling again regulates growth rate, but now influences the final body and organ size. **e,iii:** After the cessation of feeding final body size is fixed, but nutritional signaling continues to influence final organ size.

Linear environmental static allometries, and the reaction norms that produce them (Fig. 3), can evolve through changes in intercept or slope. Fig. 6 shows how changing either of these in one organ's reaction norm can influence the organ's allometric relationship with another organ. Allometries can also be modified through changes in the mechanisms by which reaction norms interact. Using nutritional static allometries as an example, we suggest a few mechanisms by which changes in the slope and intercept of an organ's allometric relationship with body size may evolve.

Evolutionary changes in the slope of a static allometry

The slope of the nutritional allometry for traits will depend on the similarity of their nutritional reaction norms. If their reaction norms are identical then their allometry will be isometric. If one trait's reaction norm becomes flatter than the other, then the allometry of the first trait against the second trait will also become flatter. If the first trait's reaction norm becomes steeper than the second, then the allometry will become steeper (Fig. 6). (If both slopes change equally, the derived allometry will be an extension or retraction of the ancestral allometry, with the same slope and intercept). Finally, if the reaction norm of one trait becomes non-linear, then the allometry will become non-linear.

In *Drosophila*, the slope of an organ's nutritional reaction norm will be influenced by its response to changes in insulin signaling. If an organ's growth were unaffected by insulin signaling, then that organ might have a size independent of food level, which would produce a flat nutritional reaction norm. The organ would then have a hypometric relationship with body size. This seems to account for the allometry between genital size and body size. Unlike the wing or mouthparts, *Drosophila* genitals do not show a substantial reduction in size when the insulin-signaling pathway is suppressed.[38] This lack of response may underlie the shallow allometric relationship between genital and body size seen in many insects and other arthropods.[76] The precise developmental basis for this phenomenon is unclear. One hypothesis is that the genitals express particularly high levels of insulin receptor, and so almost always have at least some receptor-bound insulin to activate growth-promoting pathways. Alternatively, downstream components of the pathway may be constitutively active (or inactive, in the case of the growth inhibitor dFOXO) so growth occurs even when insulin levels are low. Fine-scale adjustments to the response to insulin signaling in different organs may similarly allow evolutionary changes in their allometric relationships.

Evolutionary changes in the intercept of a static allometry

The intercept of a nutritional static allometry will reflect the intercepts of the reaction norms that produce it. The allometry's intercept will change if the intercept of one of the reaction norms increases or decreases relative to the other (Fig. 6). (If both intercepts change equally, the derived allometry will be an extension of the ancestral allometry, with the same slope and intercept).

One mechanism by which this can occur is by changing the TGP of either the body or the organs. As discussed above, juvenile hormone and ecdysteroids appear to define the onset and duration of cell proliferation in the imaginal discs. Divergent periods of cell proliferation among discs suggest that they may differ in their sensitivities to these fluctuating hormones. Evolutionary changes in the sensitivities of specific discs to such regulatory hormones will influence disc size at the critical size, and the duration of disc TGPs, by influencing the initiation and cessation of cell proliferation, respectively. Interestingly, altering the duration of a disc's TGP may also affect the slope of a nutritionally generated allometry. The longer an organ's TGP, the greater the influence nutrition has on its final size. As an extreme example, if a *Drosophila* disc were to stop growing prior to critical size then it would have a zero TGP. Since nutrition appears to affect final organ size in *Drosophila* primarily by affecting growth rate during an organ's TGP, a disc without a TGP would grow to the same size irrespective of nutritional changes in body size.

It is not known which, if any, of these changes in growth regulation occur more frequently during allometry evolution. Comparative data from natural populations are lacking, although some information may be gained from examining patterns in the response to artificial selection on body or organ size. This has been done for body size in *M. sexta*,[77] and implicates changes in critical size, duration of the body's TGP and rate of growth during the body's TGP. Selection to change the intercept of the forewing–body size allometry and

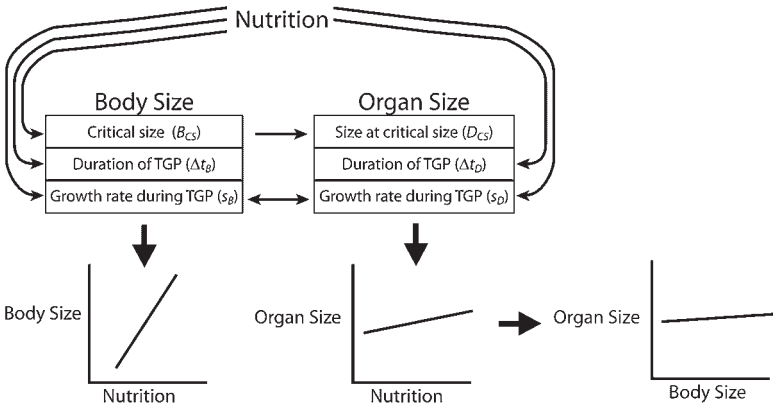

Figure 5. A model of how nutritional reaction norms interact to produce nutritional static allometries in holometabolous insects. Arrows indicate mechanisms through which nutrition influences the reaction norms of the body and organs, and how those mechanisms interact between the body and the organs. Not all interactions may be important in all species of holometabolous insects. See text for more details.

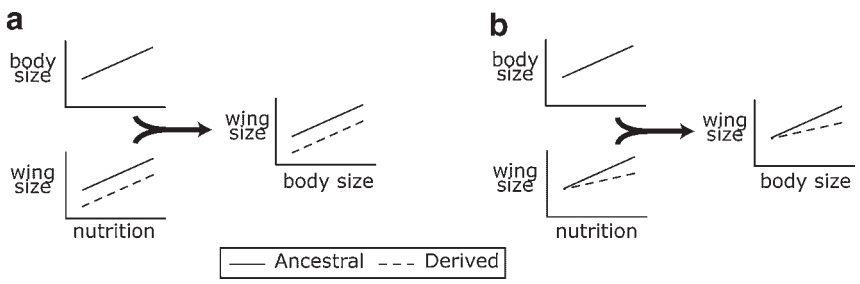

Figure 6. Evolutionary change in either the intercept (**a**) or slope (**b**) of a trait's reaction norm can influence allometry.

forewing–hindwing size allometry in lineages of the butterfly *Bicyclus anynana* produced new scaling relationships in just 13 generations.[78,79] Surprisingly, different lineages responded to artificial selection for allometric changes in different ways, which reflects variation in the proximate physiological mechanisms that can contribute to allometry evolution. Artificial selection experiments that target allometries directly (e.g. Refs 80,81) provide a rich approach for exploring variability in the proximate basis of allometry evolution.

Conclusions

It has been long recognized that changes in the scaling relationships among traits accounts for much of the morphological diversity present among metazoans. Although we are still a long way from understanding the mechanisms that underlie nutritional static allometries, let alone those that underlie other types of allometries, recent work has taken the first steps towards elucidating the proximate basis of allometry expression, integration and evolution. This research highlights two important factors that need to be considered by those researching the developmental basis of allometry. First, it is important that the type of allometry under study is identified. The source of variation that creates an allometry, be it environmental or genetic, defines the developmental mechanisms that produce it. Second, an integrative approach towards studying allometry expression and evolution is necessary. It is clear that the regulation of organ size involves both non-autonomous and autonomous effects, which implicates hormones and organ-specific responses to those hormones, respectively. Future research must therefore combine molecular biology, physiology, and—because static allometries are only observed at the level of the population—population biology. Comparative data from a variety of sources are needed about the proximate basis of allometry diversification. Different species, populations, sexes and even seasonal or other kinds of alternative morphs all can exhibit different allometries. Moreover, artificial selection can be used to create novel, derived allometries. Such naturally occurring and artificially produced variants are a rich resource for studying how allometries are produced and how they diversify.

It is an exciting time for those studying the regulation and evolution of body and organ size. Recent studies have begun to reveal how allometries are controlled and hint at how they might evolve. At the moment, much of what we know is speculative, as we pull together strands of evidence from different insect species, and from different fields of study. Nevertheless, over the next few years and with increasing understanding of how different components of the mechanisms controlling body and organ size fit together, we will finally have a developmental understanding of a phenomenon that has, for so long, fascinated biologists.

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