Allometry and size control: what can studies of body size regulation teach us about the evolution of morphological scaling relationships?

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The relationship between organ and body size, known as morphological allometry, has fascinated biologists for over a century because changes in allometry generate the vast diversity of organism shapes. Nevertheless, progress has been limited in understanding the genetic mechanisms that regulate allometries and how these mechanisms evolve. This is perhaps because allometry is measured at the population level, however adult organ and body size depends on genetic background and the developmental environment of individuals. Recent findings have enhanced our understanding of how insects regulate their organ and body sizes in response to environmental conditions, particularly nutritional availability. We argue that merging these developmental insights with a population genetics approach will provide a powerful system for understanding the evolution of allometry.

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Introduction
Individuals regulate the growth of their organs and body to ensure that the size of morphological traits match final body size, regardless of the environmentally-induced variation encountered during ontogeny [1]. The maintenance of relative organ size across body sizes can be visualized as a morphological scaling relationship for a population, species or other biological group (Figure 1a and b). Over a century of effort describing such scaling relationships has revealed that the same organs can vary considerably in relative size, producing, for example, gross differences in body shape among insect orders (Figure 1c), or variation in how shape changes with size within species (Figure 1d). In fact, morphologies diversify by changing organ scaling [2,3]. Since scaling is generally tightly regulated within species, this leads to the question: how does genetic variation among taxa change scaling relationships?

Progress in understanding how scaling relationships evolve has been limited by a lack of information regarding how animals developmentally control organ/body size scaling developmentally. However, recent elucidation of the developmental processes that regulate organ and body growth, particularly from studies dissecting the molecular mechanisms that regulate growth with respect to nutrition, promises to fill this gap [4]. In this review, we discuss current information regarding the developmental processes controlling organ and body size in the context of morphological scaling. From this perspective, we propose a strategy to uncover how scaling relationships evolve.

Evolution and allometry
Morphological allometry, henceforth termed allometry, describes how organ size scales with the size of the body or its constituent parts. The relative size of organs can vary within an individual over development time, known as ontogenetic allometry, across conspecifics of the same developmental stage, called static allometry, or across species of the same stage, termed evolutionary allometry. Traditionally, allometries are modelled using the allometric equation: \( \log(y) = \log(b) + \alpha \log(x) \), where \( y \) is the size of a trait (e.g. leg size), \( x \) is the size of another trait (typically body size), \( \alpha \) is the allometric coefficient (the slope of the relationship), and \( b \) is a constant (the intercept of the relationship) [5]. A proportional increase in organ size with trait size, a condition called isometry, occurs when \( \alpha = 1 \); in this case large individuals are uniformly magnified versions of smaller ones. Shape changes with size when \( \alpha \neq 1 \). When \( \alpha > 1 \), a condition called hyperallometry, \( y \) becomes disproportionately large as \( x \) increases. Examples of hyperallometric traits include the exaggerated secondary sexual characteristics that males use to compete for females, such as the horns of male rhinoceros beetles [6], the mandibles of male stag...
beetles (Figure 1c and d) [7], or the antlers of red deer [8]. When $\alpha < 1$, a condition called hypoallometry, $y$ increases in size at a rate below that of $x$, becoming disproportionately small as $x$ increases. Hypoallometric traits include brain size in mammals [9] and male genital size in arthropods [10]. Variation among groups in $b$ reflect differences in organ size relative to the body (e.g. Figures 1a and b), but such relative differences will remain constant across body sizes if the groups share the same $\alpha$ (i.e. when the scaling relationships are parallel) [11].

Because static allometries describe how organs and the body scale, they capture the relationship between size and shape. Comparative work has revealed that the parameters of morphological scaling relationships (i.e. $b$ or $\alpha$) can vary considerably for different organs within the same species (Figures 1 and 2), or for the same organ among species (Figure 1). Evolutionary biologists have therefore come to recognize that changes in the parameters of scaling relationships are a central component of the evolution of morphology [1,3,12].

Two controversies have emerged regarding the evolution of allometry. The first concerns the evolvability of the parameters of allometric relationships. Some hold that phylogenetic conservatism and long periods of evolutionary stasis in the slope of scaling relationships may indicate that this parameter is developmentally and evolutionarily constrained [13*]. In contrast, others argue that genetic manipulation and artificial selection experiments on organ size that change the allometric slope suggests they are evolutionary labile [11,14*].
The second controversy centres on the nature of the selective forces that change the slope and intercept of allometric relationships. Models addressing allometry evolution [10,13*,15–18] are sensitive to the biological assumptions they employ, and thus can generate very different predictions regarding how scaling relationships will respond to the same patterns of selection. This lack of consensus is heightened by artificial selection experiments that change the slope of a trait’s allometric relationship with body size when there is direct selection on organ size [19,20], but not when there is direct selection on the slope of the allometry [21].

These controversies may result from researcher’s focus on the allometries themselves, which are group-level phenomena, rather than the actual targets of selection — the variation among individuals in the developmental-genetic mechanisms that control trait size, body size, and the relationship between the two. Work over the last decade has made great progress in identifying how organ [18,22–25] and body size are regulated [4,26,27**]. An explicit integration of these findings into theories of allometry evolution is essential if we are to resolve these controversies and deepen our understanding of morphological evolution.

The developmental physiology of size regulation

In most insects, adults do not moult and their sclerotized bodies impede further growth. Thus, organ and body size are determined by growth during the larval and pupal stages. In holometabolous insects, most adult organs arise from populations of cells that are sequestered during embryogenesis, the imaginal tissues or discs, which grow, undergo patterning, and differentiate during the late larval instars and pupal stage. Final organ and body size are thus a function of their initial size at the beginning of growth, growth rate, and the duration of growth [28,29]. Variation in organ and body size arises from genetic differences among individuals and from developmental plasticity, that is, developmental modifications induced by the environment. Much is known about how organ development responds to nutritional variation. For example, plasticity in ovary size in the fruit fly (*Drosophila melanogaster*), as determined by ovarioc number, results from changes in ovary growth rate and the rate of ovarioc addition in response to change in access to nutrition in larvae [30]. In the tobacco hornworm, *Manduca sexta*, nutrition-induced differences in wing size result from
changes in the rate and duration of wing disc growth during the larval and pupal periods [31].

The processes that generate variation in organ or body size within species via developmental plasticity overlap at least partially with those that generate fixed, genetic differences in organ and body size among and within species. For example, differences in ovary size between D. simulans and D. melanogaster arise from differences in ovary growth rate after the third instar [30]. In contrast, however, populations of D. melanogaster vary in ovary size due to differential allocation of ovarian somatic cells to alternate cell fates [32], a process not involved in regulating ovary size in response to nutrition in this species. Such findings have lead investigators to ask to what extent do the mechanisms that produce fixed, genetic differences in organ and body size share common pathways with the mechanisms that underlie developmental plasticity in size?

Three hormones, the insulin-like peptides, the steroid moulting hormone ecdysone, and the sesquiterpenoid hormone juvenile hormone (JH), have overlapping roles in regulating the rate and duration of growth, and hence are central to size regulation in insects [4]. The insulin-like peptides regulate growth rate and growth duration in response to nutritional availability, and potentially to other environmental signals, in most animals [33,34]. Consequently, levels of insulin signalling transduce nutritional variation into variation in body size and organ growth rates in D. melanogaster, M. sexta, and the buckeye butterfly Precis coenia. Both ecdysone and JH are typically considered morphogenetic hormones, inducing key developmental transitions that influence organ and body size such as moulting and metamorphosis in insects, and their synthesis can be influenced by environmental conditions including access to nutrition [35*36,37].

Both ecdysone and JH have opposing effects on organ and body growth. In D. melanogaster, M. sexta and P. coenia, ecdysone suppresses the growth rate of the body yet promotes growth of the wing imaginal discs [31,38,39,40*]. In M. sexta and P. coenia, ecdysone and insulin-like peptides interact synergistically to induce growth in the wing discs [38,41]. In addition, while JH induces growth of the body in M. sexta and D. melanogaster [42*,43**], it represses the growth of imaginal tissues when insulin signalling is low due to starvation [44,45]. Such differential effects of these regulators on organ and body growth could have important impacts on the regulation, expression, and evolution of morphological allometries.

In addition, the same hormonal pathway can affect the growth of various organs to differing degrees. Although D. melanogaster wings scale isometrically with the body in response to nutrition, the genital arch is hypoallometric [22,46] (Figure 2a). Thus, small males have proportionally larger genital structures than large males. This is because the genital disc responds less to starvation than the wing disc, as the genitals express less Forkhead Box O, a negative regulator of insulin signalling [23]. This reduces the insulin-sensitivity of the genitals, such that insulin signalling remains high in the genital disc irrespective of nutritional conditions, whereas insulin signalling tracks nutrition in the wing disc (Figure 2a). In the rhinoceros beetle, Trypoxylon dichotomus, increased insulin-sensitivity appears to induce hyperallometric growth of the male horn, since reduced insulin-signalling affects horn length more than it does wing or genital size [18]. Thus, the levels of insulin signalling within an organ and how that tracks whole-body nutritional status appear to be crucial determinants of its scaling relationship with the body.

In extreme cases, scaling relationships become non-linear with some insects switching between alternate scaling relationships according to environmental cues, a phenomenon known as polyphenism. For example, the migratory brown planthopper, Nilaparvata lugens, typically develops short forewings and stub-like hindwings. However, under high densities differential expression of alternate forms of the Insulin Receptor in the wing induce the production of long fore-wings and hind-wings for dispersal, changing the intercept of the wing-body size scaling relationship [47**]. Thus, the same pathway responsible for generating differences in scaling relationships between organs, such as the wing and genitals of D. melanogaster and the horns and wings of rhinoceros beetles, can also modify allometry within an organ, namely the wing, in planthoppers.

There is some evidence that these mechanisms that regulate the effect of nutrition on organ size within species account for differences in organ size between species [30]. As discussed above, ovary size in D. melanogaster is regulated by developmental nutrition and this regulation occurs via the insulin signalling pathway [48**]. Intriguingly, insulin signalling is suppressed in the ovaries of D. sechellia and this correlates with a corresponding decrease in ovary size compared to D. melanogaster [48**]. Thus the insulin signalling pathway may control both the plastic response of ovary size to changes in developmental nutrition, as well as differences in ovary size between species. Whether interspecific differences in insulin signalling account for differences in morphological scaling, that is relative as well as absolute organ size, is unclear.

A developmental perspective on the evolution of allometry
Static allometries are a population-level phenomenon; they are estimated empirically by fitting a function to groups of individuals spanning the full range of body and organ size. While utilitarian, this population-level approach potentially confounds genetic and environmental sources of variation in organ and body size among individuals. This matters, as it is the genetic variation among individuals in the mechanisms that regulate growth in response to the
environment — for example, allelic variation among individuals in the insulin signalling pathway genes — that will respond to selection on the scaling relationship itself. To understand how allometries evolve, studies of scaling need to move below the population level and focus on the individual variation in relative organ size across body sizes. This, however, presents a challenge for empiricists: individuals express only one adult size, and so fitting a function to describe the morphological phenotypes produced by an individual across the full range of body sizes — that is, the scaling relationship for an individual genotype — would seem at the surface to be impossible.

However, the recent development of genome reference panels in *D. melanogaster* [49,50] provides new tools to study the evolution of developmental plasticity and how it relates to the evolution of allometry. These panels consist of hundreds to thousands of fully-sequenced, isogenic lineages. Because individuals within each lineage share a single genotype, they can be reared at different nutrition levels to produce the full range of size phenotypes for each genotype, to which a scaling relationship can be fit. Characterizing the morphological scaling relationships of individual genotypes in this manner offers a means to quantify genetic variation in morphological scaling relationships, and can be used to conduct association mapping of the genetic underpinnings of phenotypic variation in scaling. A similar approach using clonal insects, like aphids, would provide a tractable alternative, although the genomic resources have yet to be developed.

Coupled with an in-depth understanding of the developmental mechanisms underlying organ and body size plasticity, this provides a powerful entryway into understanding how allometries evolve. As a hypothesis, variation among individuals in organ allometry could result from variation in the same pathways that generate differences between organ allometries within individuals (Figure 2b). If this were true, genotypes that proved to have the least plasticity in, for instance, wing/body size scaling might also show reduced variation in insulin signalling in their wings across nutritional conditions. The approach outlined above would provide a means of addressing the degree to which the signalling pathways that regulate plasticity in organ and body size contribute to the evolution of allometry. Furthermore, they might provide an inroad into predicting how allometries evolve.

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**References**


This review addresses the concept that allometric relationships show low evolvability, thus constraining phenotypic diversification. The authors review the evidence for the evolution of allometry, suggesting that the slope of allometric relationships does appear constrained. They conclude that the understanding of how selection acts on allometry remains insufficient to resolve whether it truly acts as a constraint.


In this article, Stillwell and co-authors use artificial selection to explore whether scaling relationships between the wing and body can evolve independently of trait size. They find after 17 generations of selection, the slope of the morphological scaling relationship does change independently of the bivariate mean, although the degree of change is small. They conclude that understanding how scaling relationships evolve requires a more in depth understanding of how individual genotypic and phenotypic variation relates to population-level patterns of allometry.


In this comprehensive review outlining the mechanisms of extreme trait growth, the authors identify four key pathways that lead to exaggerated trait growth: the sex determination pathway, the appendage patterning pathway, the ecdysone/JH pathways, and the insulin signalling/target of rapamycin pathway. Further, the authors postulate that the developmental mechanisms underlying extreme trait growth reflect the type of selection acting on a given trait.


This article identifies a molecular mechanism through which nutrition modifies the timing of the critical weight ecdysone peak in D. melanogaster. In pre-critical weight larvae, a negative regulator of insulin signalling, FoxO, binds to a component of the ecdysone receptor, Ultraspire, in the prothoracic gland and represses ecdysone synthesis. With continued feeding, insulin signalling rises in the prothoracic gland causing FoxO to become displaced from the nucleus, thereby permitting ecdysone synthesis.


Here, Herbsos et al., 2015 show that while increasing ecdysone reduces the growth of the body, it promotes growth in the wing imaginal discs and other imaginal tissues. In the wing, ecdysone exerts its action by modulating the activity of Thor/4E-BP, a negative regulator of insulin signalling.


This article demonstrates that JH regulates body size by modifying growth rates but not developmental timing in D. melanogaster. Further, it shows that JH regulates growth rates by modifying the activity of the insulin-signalling pathway and by altering ecdysone synthesis.


This study highlights the role of JH in regulating the balance between nutrient-dependent signalling and body-size monitoring to determine final body size in Manduca sexta. Using black mutant larvae, which bear a mutation known to reduce JH synthesis, the authors find that if they reduce both JH synthesis and Target of Rapamycin signalling, they increase developmental time without affecting growth rate relative to control.


In this work, the authors provide an in-depth study of the mechanism that results in alternative wing morphs in the planthoppers. They find that the difference in growth between long-winged and short-winged planthoppers results from the activity of either homodimeric insulin receptors, in the case of the long winged morph, or of a heterodimer of two insulin receptors in the short wing. Insulin receptor 2 acts to suppress insulin signalling, and hence wing growth, in short-winged animals.


Here, Green and Extaavour show that interspecific difference in ovariole number between D. sechellia and D. melanogaster arise from differences in the levels of insulin signalling. Further, they show that allistic variation in the insulin receptor accounts for some of this variation. Finally, they show that the evolution of substrate specialization in D. sechellia and D. erecta correlates with reduced plasticity in ovariole number.
