

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 developmental 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), α 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

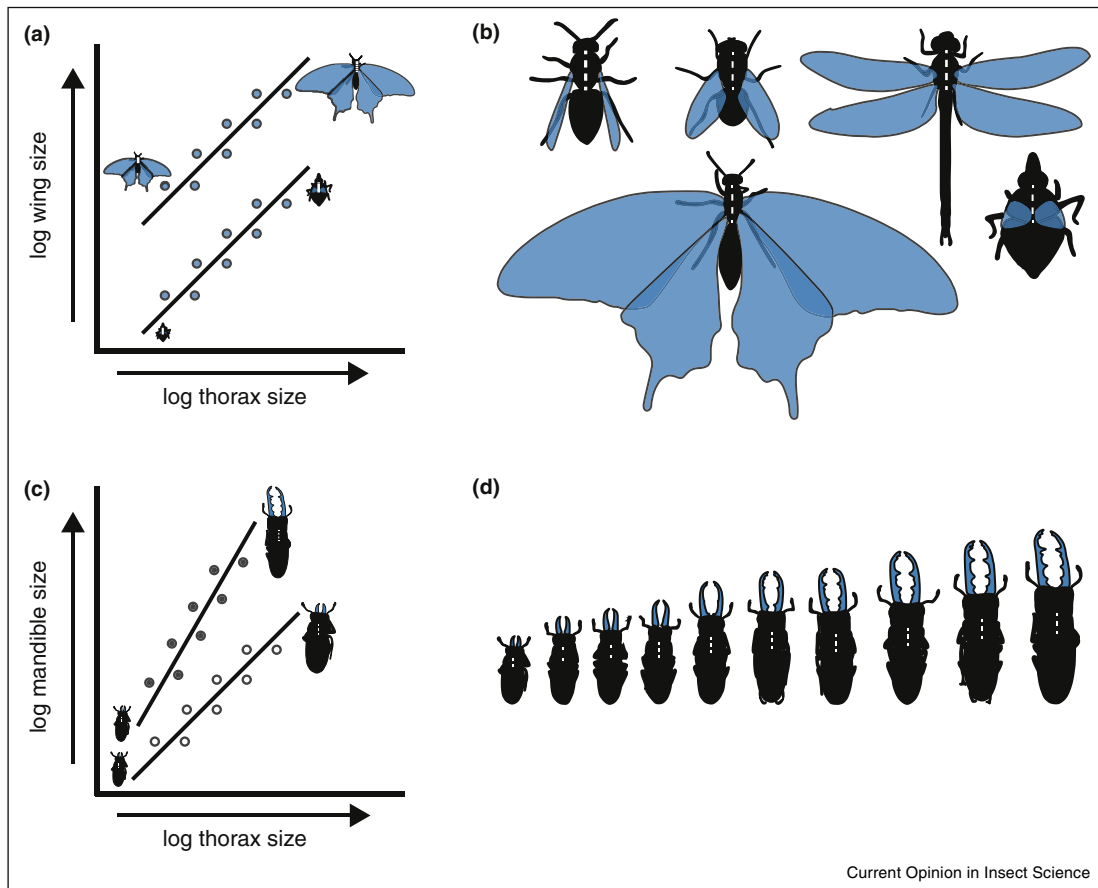
beetles (Figure 1c and d) [7], or the antlers of red deer [8]. When $\alpha < 1$, a condition called *hypoallometry*, y increases in size at a rate below that of x , becoming disproportionately small as x increases. Hypoallometric traits include brain size in mammals [9] and male genital size in arthropods [10]. Variation among groups in b reflect differences in organ size relative to the body (e.g. Figure 1a and b), but such relative differences will remain constant across body sizes if the groups share the same α (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 α) 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].

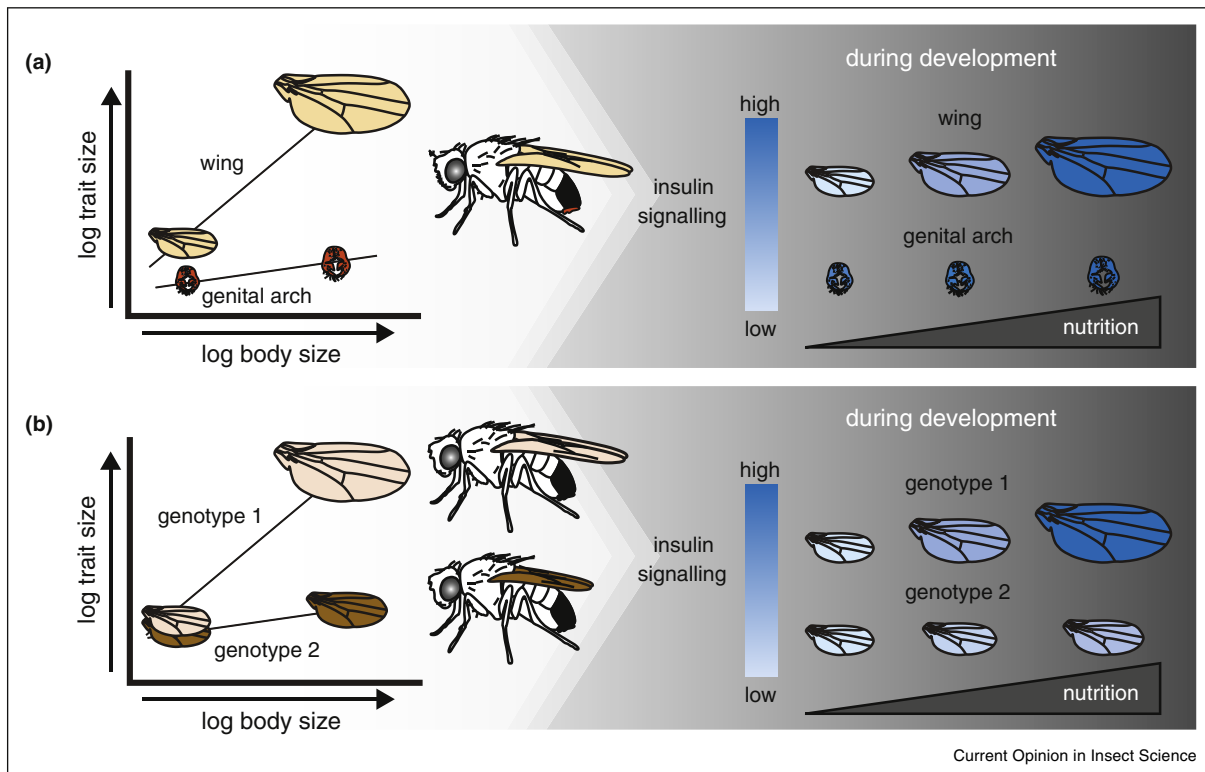
Two controversies have emerged regarding the evolution of allometry. The first concerns the evolvability of the parameters of allometric relationships. Some hold that phylogenetic conservatism and long periods of evolutionary stasis in the slope of scaling relationships may indicate that this parameter 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^{*}].

Figure 1



The diversity of insect shape results from changes in the relative size of body parts. **(Panel a)** Scaling relationships fit to hypothetical populations of butterflies and planthoppers illustrate differences in the size of the forewing relative to the thorax, but a constant ratio of wing/thorax size across all body sizes. **(Panel b)** Morphological diversity among insect orders is produced by changing the size of the wings relative to the body, as in Panel a. Images are scaled to the same thorax length (dashed white line), and the wings of wasps, flies, dragonflies, planthoppers, and butterflies (clockwise from top left) are highlighted in blue. **(Panel c)** Scaling relationships fit to hypothetical populations of male (filled circles) and female (open circles) stag beetles. Mandible size increases disproportionately with thorax size in males, producing changes in overall shape as size increases. Conversely, mandible size increases proportionally with increasing thorax size in females and thus shape does not change with size. **(Panel d)** Mandible size increases disproportionately with increasing thorax length (dashed white line) in male stag beetles, as shown in the scaling relationship in Panel c.

Figure 2



Differences in scaling relationships between organs in the same individual and within organs across genotypes. **(Panel a)** illustrates wing and genital allometries with respect to body size and shows how these relate to differences in insulin signalling between these organs in larvae exposed to different nutritional environments during development. In **(b)**, we hypothesize that genetic differences in wing allometry result from genotype-specific differences in insulin signalling during wing development in larvae exposed to different nutritional environments.

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 ovariole number, results from changes in ovary growth rate and the rate of ovariole 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 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, *Trypoxylus 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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