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The (ongoing) problem of relative growth

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Differential growth, the phenomenon where parts of the body grow at different rates, is necessary to generate the complex morphologies of most multicellular organisms. Despite this central importance, how differential growth is regulated remains largely unknown. Recent discoveries, particularly in insects, have started to uncover the molecular-genetic and physiological mechanisms that coordinate growth among different tissues throughout the body and regulate relative growth. These discoveries suggest that growth is coordinated by a network of signals that emanate from growing tissues and central endocrine organs. Here we review these findings and discuss their implications for understanding the regulation of relative growth and the evolution of morphology.

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Introduction

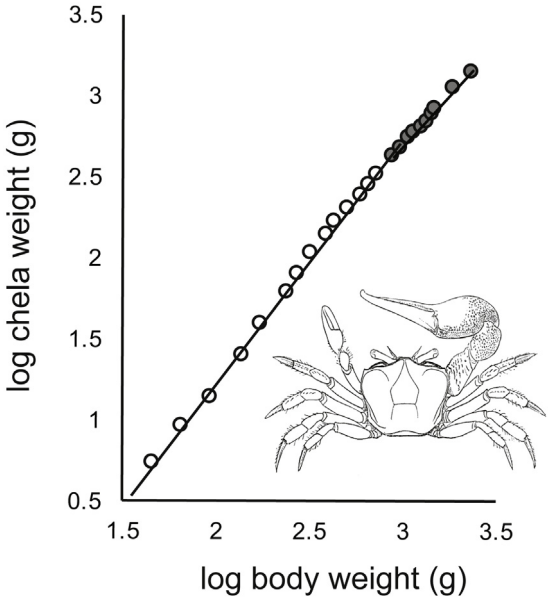
"*The problem of differential growth is a fundamental one for biology, since, all organic forms, save the simplest . . . are the result of differential growth, whether general growth which is quantitatively different in the three planes of space, or growth localized at certain circumscribed spots*". So wrote Julian Huxley at the beginning of his book, *Problems of Relative Growth* [1]. Huxley, along with D'Arcy Thompson a generation before him [2], recognized that morphological diversity is dominated by variation in body proportion, and that body proportion is in turn produced through differential growth of the body's constituent parts. Consequently, if we are to understand the evolution of morphology, we need to identify the developmental mechanisms that regulate differential growth. Nevertheless, Huxley notes "*But the subject has received little consideration*"; unfortunately, this statement has remained true for much of the eighty-five years since it was written. With the rise of developmental physiology, we are starting to discover how the growth of structures is coordinated throughout the body. In this review, we outline recent work that has begun to elucidate the developmental regulation of relative growth, and propose foci of research that will better help us solve the vexing problem of how growth is integrated across traits to produce a properly proportioned adult, even in the face of environmental variation. As much of this new research uses insects as models, we draw on these organisms heavily to illustrate our points. However, the problem of relative growth is of the widest biological relevance, and so we also employ non-insect examples where possible.

Allometry and differential growth

As Huxley observed in *Problems of Relative Growth*, apart from simple spherical or amoeboid organisms, the form of all multicellular organisms is generated through differential growth among body parts. This is most apparent in the exaggerated morphological traits males use to compete for females, such as those observed in stalk-eyed flies and horned beetles. Indeed, Huxley used the exaggerated claw (chela) of the male fiddler crab, *Uca pugnax*, to illustrate his most important contribution to the field of morphometrics: the allometric equation $y = bx^k$ (Figure 1). This equation describes the scaling relationship between covariation in the size of trait y (typically a focal trait of interest) and trait x (typically the whole body) through ontogeny. While Huxley dismissed b (the intercept) as being biologically unimportant (although see [3]), he recognized that k, the allometric coefficient, captures the growth rate of y relative to x. He further argued that the allometric coefficient is constant, at least within each growth cycle (e.g. larval instar), such that a plot of log y against log x generates a straight line of slope k, the *constant differential growth-rate*, and intercept log b; that is $\log y = \log b + k \log x$. Thus a plot of log(*chela mass*) against log(*body mass*) for male fiddler crabs at different points in ontogeny generates a straight line with a slope 1.62 for the first phase of growth and 1.26 for the second phase of growth (Figure 1, [1]). When the allometric coefficient is <1 or >1, trait y grows disproportionally slower or faster relative to trait x, a condition called *heterogony*. In contrast, when the allometric coefficient is 1, the two traits grow at the same rate, or proportionally, a condition called *isogony*.

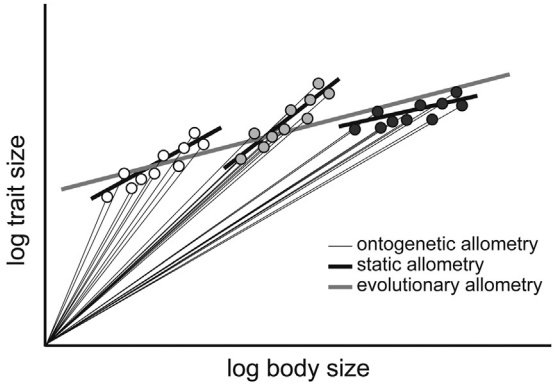
In addition to variation in relative size that occurs through ontogeny, size also varies among adult individuals within a population or species, and among species themselves. Here, the scaling relationship between y and x can also be captured by the allometric equation, but the relationships

Figure 1



Ontogenetic allometry of chela size against body size in the fiddler crab *Uca pugnax*. Open points are the first phase of growth (slope = 1.62). Closed points are the second phase of growth (slope = 1.26). Data from [1]. Image from [87].

Figure 2



The relationship between ontogenetic, static and evolutionary allometry. The thin black lines are the *ontogenetic allometry* between body and trait size. These end at the final adult body and trait size (circles). The thick black line shows the *static allometry* between body and traits size among adults in a population or species. Each species is represented by a different colored circle (white, gray or black). The thick gray line shows the *evolutionary allometry* between body and trait size among species. Note that each static allometry represents a different category of morphological scaling; the population of open circles exhibit a hyperallometric relationship, gray circles illustrate isometry, and black circles reflect hypoallometric scaling.

are called *static allometry* and *evolutionary allometry*, respectively. Nevertheless, for all allometries, the two traits have the same relative size when $k = 1$, regardless of overall size (i.e. the traits change size in constant proportion to one another). When $k > 1$, trait y becomes disproportionally larger relative to trait x as size increases, whereas when $k < 1$, trait y becomes disproportionally smaller as the size of x increases. Because of the generality of these effects on overall shape, the term heterogony has been replaced by *hyperallometry* ($k > 1$) and *hypoallometry* ($k < 1$), and isogony has been replaced by *isometry*. Technically *allometry* refers to any condition that is not isometric, but recently it has come to refer to scaling relationships in general [4], and will be used in this way here. Further, the concept of allometry has been expanded beyond the morphological to include the relationship between body size and biochemical, physiological and ecological processes, such as metabolic rate, the cost of locomotion, or population density. There is a rich literature exploring these scaling relationships, many of which can also be modeled using the allometric equation [5–7].

Ontogenetic, static and evolutionary allometries describe different, but related, relationships (Figure 2). An ontogenetic allometry describes the relative size of traits throughout the growth of an individual. In as much as the purpose of ontogeny is to generate a correctly proportioned adult, the ontogenetic allometry describes how this is achieved. A static allometry describes the relative size of traits, among individuals at the same developmental stage (typically adult), within populations, species or other biological groups. The static allometry is generally thought of as the 'proper' scaling relationship for the group. Evolutionary allometries describe the pattern of evolutionary divergence in relative trait size among these same biological groups. Static and evolutionary allometries have been the subject of extensive recent research, and this work has been widely reviewed [4,8–11,12••,13], so will only be touched on here. In contrast, while the pattern of ontogenetic allometries has been well studied (e.g. [14–20]), the developmental mechanisms that regulate differential growth remain poorly understood. It is variation in these mechanisms among individuals, revealed as variation in the length and slope of ontogenetic allometries, that generates static allometries, and it is evolved changes in these mechanisms that produce the patterns revealed through evolutionary allometries.

The utility of the allometric equation to describe differential growth lies in the observation that differential growth-rates remain constant for prolonged periods of development. Huxley provided many examples of linear ontogenetic allometries between different trait pairs, including the chela and body size of male fiddler crabs, tail and body length in mice, face and cranium length in dogs, and stem and root weight in various plants [1]. Huxley initially considered the allometric equation to be a consequence of the multiplicative nature of growth, that is, the notion that traits grow exponentially, and he argued that the equation approximated a general law of

differential growth [1]. However, Haldane observed that if two individual traits, for example the length of the femur and the length of the tibia, scale with a second trait, for example body size, according to $y = bx^k$, then the total size of these individual traits, in this case leg length, cannot also scale with body size according to $y = bx^k$ (p. 81 in [1]). Consequently, the allometric equation is an empirical description of growth that fits the data adequately, rather than a theoretical descriptor. Indeed, many log–log ontogenetic allometries are better captured by a smooth curve using a quadratic function ($\log y = \log b + k_1 \log x + k_2(\log x)^2$) or higher order polynomial [21], or by using functions that assume individual traits grow following a logistic or Gompertz function [22], than they are by the linear log–log allometric equation.

Regardless of the shape of the ontogenetic allometry, the gradient near any point on the allometry captures the growth rate of trait y relative to trait x [23]. Properly proportioned adult morphology requires that the ontogenetic allometries that lead to final trait and body size be tightly controlled, and that different ontogenetic allometries be integrated across traits within individuals. Differences in body proportion among individuals, between sexes, among populations, or among species must result from variation in the ontogenetic allometries of traits. Thus, to understand how body proportion is maintained within groups and how it evolves to generate morphological diversity requires solving the problem of relative growth. The solution lies in determining the developmental mechanisms that regulate and integrate ontogenetic allometries.

Mechanisms regulating differential growth

There are at least three developmental pathways through which the phenomenon of relative growth can be achieved. First, differential growth may be a consequence of trait-autonomous growth, where each trait grows independently to a target size. Second, differential growth may result from different trait sensitivities to circulating growth factors. Finally, differential growth may be regulated through a network of signals acting directly among traits and/or indirectly from traits to central systemic growth regulators and back out to other traits. These mechanisms are not mutually exclusive and there is evidence that all three are involved in the generation and regulation of differential growth. Below we discuss each in turn.

Mechanism 1: trait-autonomous differential growth

Under this model, each trait grows independently, and ceases growth once its individual target size is achieved. The rate and duration of trait growth is regulated in an organ-autonomous manner, and the ontogenetic allometry is therefore an epiphenomenon of the independent growth of traits. Evolutionary shifts in the duration or rate of relative growth are consequently a result of changes in the mechanisms that regulate the target size of an individual trait, leading to changes in its ontogenetic allometric relationship with other traits.

Evidence that organs regulate their final size intrinsically comes from transplant experiments where traits do not grow beyond their 'normal' size despite being maintained in a growth-permissive environment. For example, *Drosophila* imaginal discs do not grow beyond the size they would achieve *in situ* when transplanted into the growth-permissive abdomens of adults [24]. In cockroaches, limbs regenerate to their proper size through consecutive molts following removal of a proximodistal segment and reattachment of the distal portion to the stump [25]. In humans, a similar phenomenon is seen where one lobe of the liver is transplanted to a recipient, and the remnants in both donor and recipient regrow to near their original size [26]. Collectively, these data suggest that organs have an intrinsic sense of how big they need to be and grow to achieve and maintain that size. Recent studies using the wing imaginal disc of *Drosophila* as a model have begun to elucidate the mechanisms that might control target size. Growth in wing imaginal discs appears to be regulated both by disc-autonomous patterning signals (e.g. Wg and Dpp morphogen gradients), and by mechanical feedback from forces generated by the growing tissues (see [27] for review). More recently, there is evidence that both types of disc-autonomous growth regulation converge on the Wart/Hippo signaling pathway [28–30], a key regulator of cell proliferation and apoptosis in animals.

If a target size does exist, then the mechanisms that regulate it must respond to the environmental and genetic factors that generate variation in body size to ensure a correctly proportioned adult. A recent study has begun to reveal how proper target size may be achieved autonomously, showing that nutritional signals impinge on Wart/Hippo signaling via the TOR signaling pathway to affect trait growth [31••]. The TOR pathway regulates growth rate in response to nutritional conditions both directly, via cellular levels of amino acids, and indirectly, via the insulin/insulin-like growth factor signaling (IIS) pathway [32]. The convergence of TOR with Wart/Hippo signaling therefore allows organ-autonomous growth-control to respond to environmental conditions via circulating hormones and amino acids. However, if this were the case, it suggests that differential growth rates among traits are not solely a consequence of traits growing independently to individual target sizes: there appears to be a role for systemic regulation of differential growth.

Mechanism 2: systemically regulated differential growth

Under this model, growth is regulated by circulating growth factors, be they hormones or other signaling molecules. Differential growth results from variation among traits in their response to these factors; traits that are more sensitive grow relatively faster than less sensitive traits. Evolutionary shifts in differential growth, and

thus in ontogenetic allometry, result from changes in trait sensitivity to these circulating growth factors.

The existence of circulating hormones has been known since the mid-19th century, when both Arnold Berthold and Claude Bernard recognized that organs can communicate chemically with each other. The term 'hormone' was not coined, however, until Enest Starling used it in 1905 [33], and it was not until 50 years later that growth factors were actually characterized, the first being nerve growth factor by Rita Levi-Montalcini and Stanley Cohen in 1956 [34].

Huxley proposed circulating hormones as regulators of differential growth in *Problems of Relative Growth*. A key example he drew on was Hammett's work on the growth-retarding effect of thyroidectomy on male rats [35]. Loss of the thyroid gland inhibits the growth of some organs much more than others; thyroidectomized males are small but possess proportionally large eyes and nervous systems and disproportionally small viscera and glands. Although Huxley did not discuss the mechanism by which the thyroidectomy influenced relative growth, he noted that various tissues in anurans differed in their sensitivity to thyroid-hormone. Thus, Huxley appears to have argued implicitly that the effects of hormones on differential growth are due to relative variation in sensitivity to hormones among traits.

Additional evidence that differential growth rate is regulated by circulating growth factors comes from experiments where growing organs are ablated. In a now classic series of experiments, Nijhout and colleagues demonstrated that removing the hind-wing imaginal disc of the butterfly *Precis coenia* caused a corresponding increase in the size of the forewing, the thorax and the forelegs [36]. They further demonstrated that decreasing the horn size in male *Onthophagus* beetles, either through hormonal manipulation or through artificial selection, increased the size of the eyes. They interpreted these results as evidence for allocation trade-offs between traits competing for common resources, although they were agnostic as to what these resources might be. Nevertheless, these findings suggest that differential growth of insect organs appears to be mediated through competition for circulating growth factors, be they hormones or nutrients.

While there has been extensive research to elucidate the endocrinology of growth hormones, growth factors, and the proximate mechanisms of their action, very little work has been directed toward determining their role in differential growth (although see [14]). However, recent work on insects has started to reveal the developmental mechanisms by which hormones — particularly insulin-like peptides, ecdysone and juvenile hormone (JH) — regulate relative growth and body proportion. Below, we discuss how each of these regulators is believed to affect relative growth.

Insulin-like peptides and relative growth

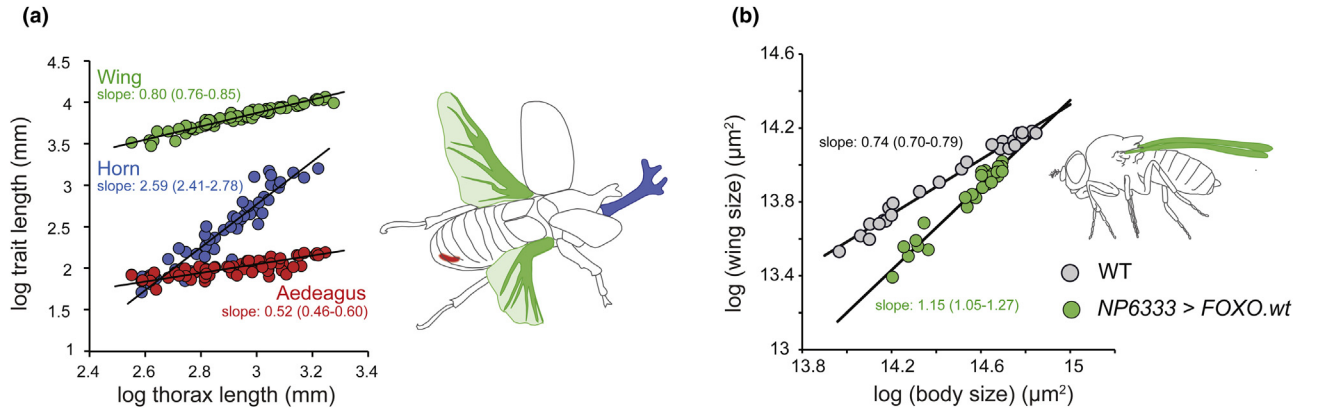
In most animals, organs vary in how sensitive their growth, and ultimately their size, is to changes in nutrition. There has been considerable work on the role that insulin-like-peptides (ILPs) play in regulating body proportion and static allometry. Insulin regulates growth with respect to nutrition in almost all animals by activating the insulin-like/IGF-signaling pathway in proliferating cells [37]. Most traits have insulin-sensitivity similar to that of the body, and so changes in nutrition affect growth of these traits and the body similarly. Traits that are insulin-insensitive, however, grow in a largely insulin-independent manner, and so maintain growth when nutrition and insulin-levels are low [38–40]. A consequence of differential insulin-sensitivity is that traits that are insulin-hyposensitive, for example the male genitalia in *Drosophila*, are disproportionally large in small individuals [38]. Conversely, traits that are insulin-hypersensitive, for example the horns of male rhinoceros beetles, are disproportionally large in large individuals [40]. Such variation in sensitivity is reflected in differences among traits in their static allometric relationships with body size (Figure 3a). Importantly, changing the insulin-sensitivity of a trait in *Drosophila* is sufficient to alter the slope of that trait's static allometry (Figure 3b) [41].

While the effects of insulin-sensitivity on static allometries have been well documented, how these effects are manifested through changes in ontogenetic allometries is less clear (Figure 2). Only one study has measured ontogenetic allometry among traits where insulin-sensitivity was perturbed [42]. Here, constitutive activation or suppression of insulin-signaling in the anterior compartment of wing imaginal disc in *Drosophila* changed the relative size of the anterior compartment during the final larval instar, as expected; however, it did not affect the slope of the allometry between the anterior and posterior compartments [42]. This suggests that insulin-signaling does not affect the differential growth rate of these compartments. One possible explanation for this unexpected outcome is that insulin-signaling affects differential growth before the final instar, which could help explain why the anterior compartment is disproportionately large or small even at the beginning of the stage. However, this would mean that additional mechanisms prevent the IIS from affecting differential growth in the anterior and posterior compartments during the third larval instar.

Ecdysone and relative growth

Although ecdysone is canonically known as the hormone responsible for insect molting and developmental transitions, an increasing amount of research indicates that it is an important growth regulator in its own right. This was

Figure 3



The influence of insulin-sensitivity on static and ontogenetic allometry. **(a)** The slope of the static allometry reflects each trait's insulin sensitivity in male rhinoceros beetles (*Trypoxylus dichotomus*). Traits that are highly insulin-sensitive (horn) exhibit steeper static allometries with body size than do traits that are moderately insulin-sensitive (wing) or insulin-insensitive (aedegus). Data and figure adapted from [40]. **(b)** Increasing the insulin-sensitivity of the wing by upregulating expression of *FOXO* (*UAS-FOXO.wt*) using a wing-specific GAL4 driver (*np6333-GAL4*) increases the slope of the wing-body static allometry. Data from [41]. Slopes are given with 95% confidence interval. Lines are fitted using major axis regression.

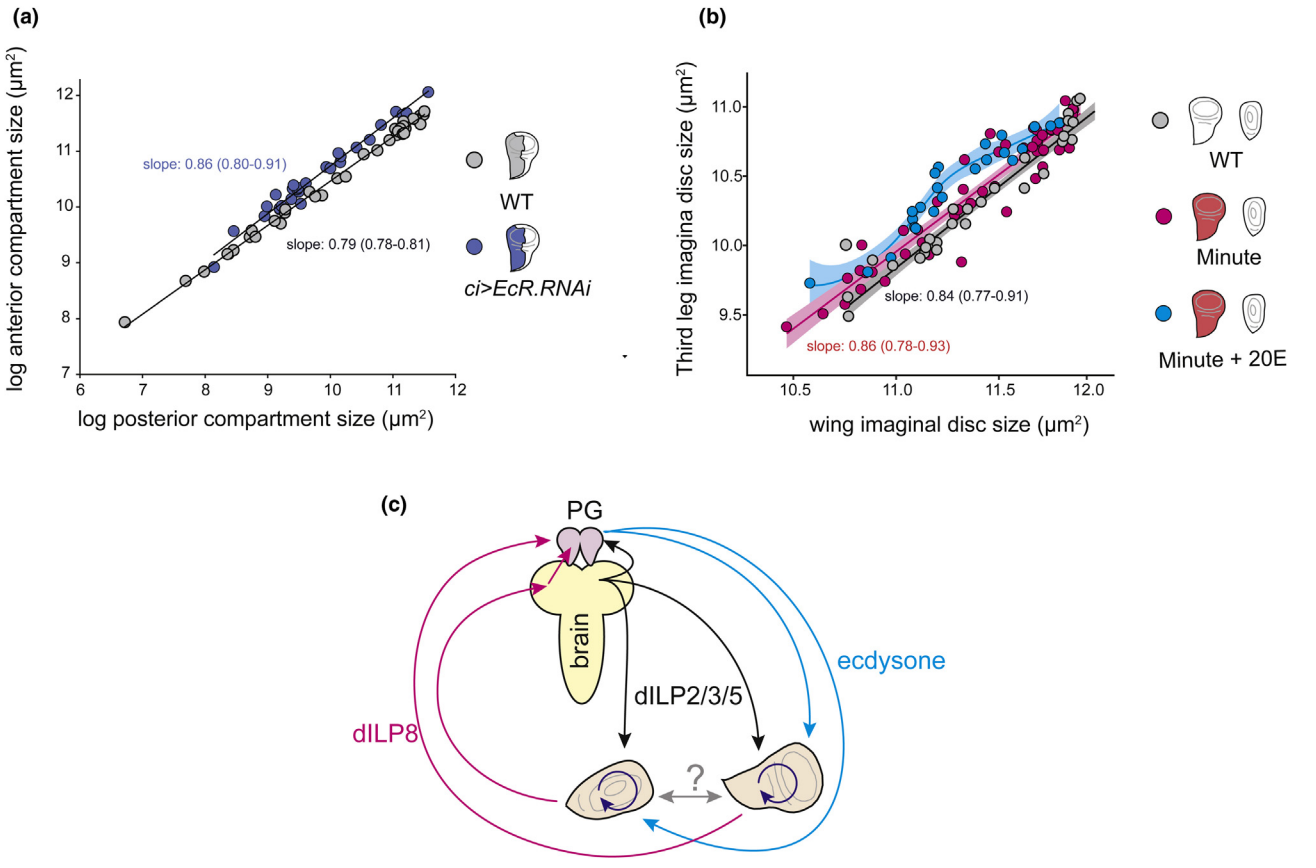
elegantly revealed in a series of studies in *Drosophila* showing that up-regulating ecdysone synthesis slows larval growth, while down-regulating synthesis had the opposite effect [43–45]. These effects on larval growth are mediated by the insulin-signaling pathway: upregulation of ecdysone activates ecdysone-signaling in the fat-body, which in turn suppresses the release of ILPs from the brain and causes a systemic reduction in IIS and in whole-body growth [44–46]. Thus, ecdysone can, in principle, influence differential growth through the same mechanisms as do the ILPs. However, in contrast to its negative effects on whole-body growth, ecdysone also acts directly on imaginal discs to promote growth [47•]. Consequently, an up-regulation of ecdysone synthesis generates an adult fly with a small body and disproportionally large wings [43]. More direct evidence of ecdysone being a regulator of differential growth comes from *Drosophila* experiments where ecdysone signaling is upregulated in the anterior compartment of the wing imaginal disc alone [48•]. The result is an increase in the slope of the anterior–posterior ontogenetic allometry during the third larval instar, consistent with a change in differential growth (Figure 4a). These data may explain why changing insulin-sensitivity does not appear to affect differential growth during the third larval instar in *Drosophila*: during this period of development differential growth may be primarily regulated by ecdysone.

Juvenile hormone and relative growth

One of the major factors that have led to the ecological success of insects is the prevalence of polyphenisms: the ability to generate distinct phenotypes under different environmental conditions. In very many cases, these polyphenisms are marked by distinct changes in body proportion that reflect different seasonal morphs (for example the seasonal wing polyphenisms in soapberry bugs, *Jadera haematoloma*) [49], sexual morphs (for example horned and hornless males in the beetle *Onthophagus taurus*) [50], and castes (for example the castes of *Pheidole* ants) [51]. A change in body proportion necessarily requires shifts in differential growth rates, unless mediated solely through a change in trait-specific growth period. Consequently, it is highly likely that the generation of differently proportioned morphs involves some change in differential growth. In many cases, juvenile hormone (JH) has been implicated in the regulation of polyphenism expression. For example, ectopic application of JH analogs induces horn development in otherwise hornless male *Onthophagus taurus* [50], increases the proportion of short-winged soapberry bugs [49], and changes queen/soldier/worker caste identity in *Pheidole morrisi* [51].

While JH has been implicated in the phenomenon of differential growth, how it mediates its effects at a developmental level is unknown. However, there is increasing evidence of cross-talk among JH signaling, insulin/insulin-like growth factor signaling and TOR signaling. For example, studies in *Manduca sexta* indicate that JH increases the sensitivity of growing imaginal discs to nutrition [52]. Starvation normally suppresses disc growth in *M. sexta* larvae, but growth is rescued when the corpora allatum (which synthesizes JH) is also ablated. Similarly, growth of the prothoracic gland (PG) becomes independent of TOR-signaling in JH-deficient *black* mutant larvae [53]. In wild-type *M. sexta* larvae, suppression of TOR differentially reduces growth of the PG relative to the body as a whole, lowering the slope of the PG-body ontogenetic allometry. This does not occur in *black* mutant larvae, suggesting that JH also regulates the

Figure 4



The influences of ecdysone-signaling on ontogenetic allometry in *Drosophila*. **(a)** Up-regulating ecdysone signaling in the anterior compartment of the third-instar wing imaginal disc, by driving expression of RNAi against the ecdysone receptor (UAS-*EcR.RNAi*) using the *cubitus interruptus* driver (*ci-GAL4*), causes an increase in the slope of the anterior-posterior ontogenetic allometry. Data from [42]. **(b)** The slope of the ontogenetic allometry between the wing and third-leg imaginal disc during the third instar is the same even when the wing imaginal disc is growth-perturbed, by driving expression of RNAi against the ribosomal protein *RpS3* (UAS-*RpS3.RNAi*) using the *Bx-GAL4* driver. Application of 20-hydroxyecdysone (20E) increases the relative growth of the third-leg disc, indicated by an increase in slope of the ontogenetic allometry in smaller discs. Data from [63]. Slopes are given with 95% confidence interval. Straight lines are fitted using major axis regression. Curved line is fitted using a cubic spline. Shading indicates 95% confidence interval. **(c)** Summary of the mechanisms that coordinate relative growth among imaginal discs in *Drosophila*. As yet, it is unclear whether discs can regulate each other's growth directly, independent of ecdysone.

nutritional sensitivity of the PG. In *Drosophila*, in contrast, JH does not appear to play a role in sensitizing tissues to nutritional signals, but rather regulates IIS directly. Specifically, *Drosophila* larvae that lack a corpora allatum grow more slowly and have suppressed IIS [54]. Interestingly, this appears to be a consequence of increased ecdysone signaling, a hypothesis supported by the observation that the effects of allatectomy can be reproduced by knocking down the JH receptor *Met* in the prothoracic gland alone [54].

Mechanism 3: network-regulated differential growth

Trait-autonomously and systemically regulated differential growth mechanisms can function redundantly to ensure that tissues grow at the appropriate rate to generate a correctly proportioned adult. However, both mechanisms suffer from a lack of robustness: any perturbation in the growth of an individual tissue will result in improper differential growth and inappropriate body proportions in the adult. Indeed, if traits compete for nutritional resources or growth factors, as suggested by Nijhout and colleagues' work [36,55,56], then any small perturbation in the growth of one organ may be exaggerated through development as it grows disproportionately and increasingly impacts the growth of other traits. Thus, under these models, changes in the pattern of growth of one organ are expected to affect the size of others growing at the same time, potentially producing dramatically misproportioned adults. However, research in *Drosophila* and other holometabolous insects indicates that the patterns of differential growth are far more robust than this, and that they may derive this stability in part from regulatory communication among growing traits.

In *Drosophila*, damage to growing imaginal discs retards ontogeny, ostensibly to allow the damaged disc time to regenerate before metamorphosis [57–59]. A similar phenomenon is observed in cockroaches if one or more of their legs are removed [60], in the wax moth *Galleria mellonella* if there is nerve damage [61], and in *Ephestia kuhniella* when immature imaginal discs are implanted in a more mature individual [62]. Despite this prolongation of ontogeny, however, while the focal organ catches up with the rest of the body, the remaining discs and tissues do not overgrow. In *Drosophila*, such overgrowth is prevented by a slowing of growth in the undamaged discs [63]. This signal involves a suppression of ecdysone release, since feeding ecdysone to larvae with growth-perturbed wing discs causes un-manipulated discs to accelerate growth, altering their ontogenetic allometry with wing disc size (Figure 4b). dILP8 is the signal from the slow-growing disc that regulates ecdysone synthesis [64,65]. dILP8 binds to the receptor Lgr3 in the brain to regulate the timing of metamorphosis via PTTH [66,67,68••], and binds to Lgr3 in the PG to regulate the growth of the undamaged imaginal discs via NOS [69•,70••]. Importantly, dILP8 is not just produced by discs when they are damaged. *dILP8* expression is high at the beginning of the third larval instar, and declines as the instar progresses [65], suggesting that dILP8 plays an important role in coordinating growth in normally growing larvae. Indeed, loss of dILP8 leads to an elevated level of fluctuating asymmetry, consistent with a key role in regulating differential growth [64]. As an added nuance, similar signals appear to coordinate growth among different parts of the same organ in *Drosophila* [48•]. Further, there is evidence that dILP8 expression is regulated by Warts/Hippo signaling in imaginal discs [71••] and in the PG [72•], connecting organ-autonomous growth-regulation with growth coordination across the whole body.

The problem of relative growth: a new role for imaginal discs

The three mechanisms of differential growth regulation discussed above are not mutually exclusive and may act redundantly to ensure that body proportion is maintained in the face of genetic variation, environmental effects, and stochastic developmental perturbations. Nevertheless, there is increasing evidence that differential growth is regulated democratically via a network of signals from growing tissues rather than through the dictatorship of central endocrine organs. These new data also force us to reconsider allocation tradeoff models of differential growth, where organs compete amongst themselves for limited resources or growth factors. Such trade-off models have been used to explain patterns of evolutionary and static allometry, where the size of one trait, for example the horn in *Onthophagus taurus* males, correlates negatively with that of other traits, for example the copulatory organs [36,73,74]. As described above, these allocation trade-off models are supported by data that show reciprocal changes in size in one trait when another trait is either ablated, changed through (artificial) selection, or subject to endocrine manipulation [36,55,75]. However, these trade-offs do not apply to all organs. For example, ablation of the hindwing of *Precis coenia* does not affect the size of the head or abdomen. Similarly, a reduction in horn size in *Onthophagus taurus* through artificial selection does not affect the size of any structure apart from the eyes. One explanation for this is that only traits that are physically close to each other compete for resources. However, this does not explain the apparent trade-off between horn size and genital size in *Onthophagus taurus*, which are located at opposite ends of the body. It is also possible that only fast growing organs or organs that grow at the same time compete for resources. However, in *Drosophila*, the wing imaginal disc grows at the same time as the leg- and eye-discs and the rate of cell proliferation is approximately the same across all discs. Nevertheless, other discs exhibit no evidence of taking competitive advantage of growth-perturbed discs.

An alternative explanation for the apparent phenomenon of resource allocation trade-offs during development is that ablation of discs disrupts the signaling among them that coordinates their growth. In *Drosophila*, *dILP8* expression is high at the beginning of the third larval instar and becomes lower as the instar progresses [65], corresponding to an increase in the ecdysone titer and, presumably, an increase in ecdysone-regulated growth. Complete ablation of individual discs early in the third instar may therefore reduce levels of circulating dILP8, which increases ecdysone levels and promotes growth of the remaining discs. Further, because discs can have different threshold responses to ecdysone, changes in ecdysone may affect the growth of one disc type more than another.

It is important to note that, even though differential growth may not be developmentally regulated by direct competition among traits for limited resources, there may be selective pressures to trade-off the size of one trait against another [75]. For example, in horned beetles, males can pursue alternate mating strategies: large males compete for and guard females while small males sneak copulations [76,77]. Consequently, selection for large horns and increased fighting ability may relax selection for large copulatory apparatus and testis that are favored in relatively smaller, 'sneaky' males, and vice versa. Because male body size is dependent on larval nutrition, genes in the male do not 'know' whether they will be in a large or a small male. Consequently, developmental mechanisms will be favored that alter differential growth to produce a negative correlation between horns versus genitalia and testis when body size varies with nutrition, or any other environmental factor.

The observation that growth is coordinated via signals to and from growing tissues echoes recent findings from

studies of gene regulation and the rise of systems biology, where gene expression is no longer thought to be controlled exclusively through linear signaling pathways, but rather through regulatory networks [77]. In particular, it suggests a new role for imaginal discs, not as sole generators of adult structures but also as nodes in the network of signals that coordinate growth and development. This network approach to developmental physiology leads to a number of exciting research questions regarding the problem of relative growth, of which we highlight just three:

(1) *What are the signals that imaginal discs produce and receive to regulate differential growth?* While dILP8 and its receptor Lgr3 have been identified as necessary for the developmental delay induced by perturbing the growth of individual imaginal discs, questions remain regarding how these regulate differential growth, and whether there are additional players involved. For example, does dILP8 also act on imaginal discs to regulate their growth directly, or is growth entirely coordinated by ecdysone? If growth is coordinated by ecdysone, how is this achieved? Is it the level of circulating ecdysone that regulates growth rate, or the temporal dynamics of changes in the ecdysone titer that coordinates growth? Under the circulating ecdysone model, ecdysone level sets the growth rate of all discs, and growth-perturbed discs lower the level of ecdysone, slowing the growth of other discs. Under the temporal dynamics model, discs grow incrementally, with each growth increment being permitted only when the ecdysone titer exceeds a particular threshold. Here, growth-perturbed discs therefore reduce growth by slowing the rate at which each threshold is passed.

(2) *How can we apply mathematical models to developmental physiology?* A key component of the emerging field of systems biology is the modeling of gene-regulatory networks, metabolic networks, and signaling transduction cascades *in silico* using dynamic mathematical modeling. Such models have proved invaluable as a method to generate novel hypotheses and make predictions about the emergent properties of biological networks at a cellular and molecular level; these are then tested using data from genomics, transcriptomics and proteomics. Physiological networks can, in principle, be modeled similarly, although the interacting components are more complex than enzyme-substrate or receptor-ligand binding systems, or even the myriad molecular interactions that regulate transcription and translation. Nevertheless, with an increased understanding of the nodes and links of the physiological network that regulates growth throughout the body, we should be able to build rudimentary models from first physiological principals and test their predictions regarding how differential growth is controlled.

(3) *To what extent are signals from imaginal discs involved in the evolution of morphology?* Perhaps the most exciting aspect of the discovery that imaginal discs are involved in the regulation of relative growth is that it gives imaginal discs a new role as participants in the evolution of relative growth and morphological diversification. In many flightless holometabolous insects, the wing imaginal discs or wing buds are retained throughout a period of development, in some cases only degenerating at the very end of development [51,78–83]. One explanation for these observations is that they are examples of developmental drift: there may be negligible selection on when wings degenerate, save that they are absent before adult eclosion. Under the allocation tradeoff model of differential growth-regulation, however, these discs should be eliminated as early development as possible to maximize the growth of other organs. An alternative explanation for the developmental persistence of wing imaginal discs is that, if they play a role in regulating differential growth, then selection may retain them in this role, even as they lose their adult function. The hypothesis that imaginal discs have a function during development distinct from an adult function may also explain why, in many insects, imaginal discs overgrow during development and are subsequently 'trimmed' through apoptosis during metamorphosis [84–86].

Conclusion

As this review reveals, we are making significant progress in finally solving Huxley's problem of relative growth. Historically, the research focus on organismal form has taken a 'top-down' approach; in part this focus has been born of necessity, because measuring morphology is straightforward. Only recently have the tools become available to reveal and dissect the mechanisms that coordinate growth across the body and that regulate the development of form. While the focus of this review has been on the developmental regulation of differential growth, the underlying motivation is to understand how ontogenetic allometries give rise to static allometries, and how changes in ontogenetic allometries produce patterns of morphological change revealed through evolutionary allometries. It is this latter point, how ontogenetic allometries underlie the evolution and diversification of organismal shape, that may prove the most interesting new area for research. The relative role of external natural selection and internal developmental processes in shaping static and evolutionary allometries is an open, and critical, subject in the evolution of organismal form. Huxley clearly recognized the importance of understanding the developmental regulation of differential growth when explaining morphological change, and even hypothesized the types of genes that underlie evolutionary changes in relative size. Nevertheless, his hypotheses were constrained by his (unavoidably) limited understanding

of how differential growth is regulated. With the rise of developmental physiology and molecular genetics, we can finally start to address the problems he first posed eighty years ago.

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