REVIEW ARTICLE

The developmental basis for allometry in insects

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SUMMARY

Within all species of animals, the size of each organ bears a specific relationship to overall body size. These patterns of organ size relative to total body size are called static allometry and have enchanted biologists for centuries, yet the mechanisms generating these patterns have attracted little experimental study. We review recent and older work on holometabolous insect development that sheds light on these mechanisms. In insects, static allometry can be divided into at least two processes: (1) the autonomous specification of organ identity, perhaps including the approximate size of the organ, and (2) the determination of the final size of organs based on total body size. We present three models to explain the second process: (1) all organs autonomously absorb nutrients and grow at organ-specific rates, (2) a centralized system measures a close correlate of total body size and distributes this information to all organs, and (3) autonomous organ growth is combined with feedback between growing organs to modulate final sizes. We provide evidence supporting models 2 and 3 and also suggest that hormones are the messengers of size information. Advances in our understanding of the mechanisms of allometry will come through the integrated study of whole tissues using techniques from development, genetics, endocrinology and population biology.

Key words: Static allometry, Hormones, Insects, Evolution, Allometry

INTRODUCTION

In 1917 D'Arcy Wentworth Thompson published a treatise, *On Growth and Form*, in which he argued that mathematical and physical laws not only underlie biological form, they actually generate biological form (Gould, 1992). Although such a heterodox view has not overtaken biology, Thompson's visual approach proved aesthetically compelling. In particular, his use of Cartesian coordinates to 'analyze' changes in animal form influenced generations of biologists; it is hard to imagine any biologist of the past eighty years who has not seen a reproduction of one of Thompson's 'transformed' fish (Fig. 1). Despite its intuitive appeal, which was highlighted in both Thompson's work and in Julian Huxley's *Problems of Relative Growth* (1932), the problem of how body parts scale with total body size has attracted little attention from mechanistic biologists.

It is worth reflecting on what Thompson and Huxley were trying to do. Both distilled the obvious complexity of variations in organismic form into simple patterns, in an attempt to identify what they considered the essence of the problem. Huxley's approach, of measuring the relative sizes of organs, typically along a single dimension such as length, was arguably more analytical than Thompson's approach. Huxley found that simple models of growth provided good fits to bivariate plots of these linear data and thus believed that he had identified laws of growth. The field has, however, failed to progress past these types of observations. It seems likely that we cannot simply infer the mechanisms that control relative growth from the patterns of differential growth, and that we require a mechanistic understanding of growth that is only now becoming accessible.

The mechanistic study of relative growth is essentially a new field with little data. Many exciting discoveries lie in the future. We do believe, however, that there is sufficient evidence to begin forming a framework for study and to provide some simple hypotheses that can be tested in the near future. Most importantly, we wish to turn scientists minds back towards this classical problem by marshaling the relevant data for one group of animals, the insects.

DEFINITIONS OF ALLOMETRY

The term allometry refers to three alternative phenomena (Cock, 1966; Cheverud, 1982; Klingenberg, 1996; Schlichting and Piggliucci, 1998): ontogenetic, static and evolutionary allometry. Ontogenetic allometry is the growth trajectory of an
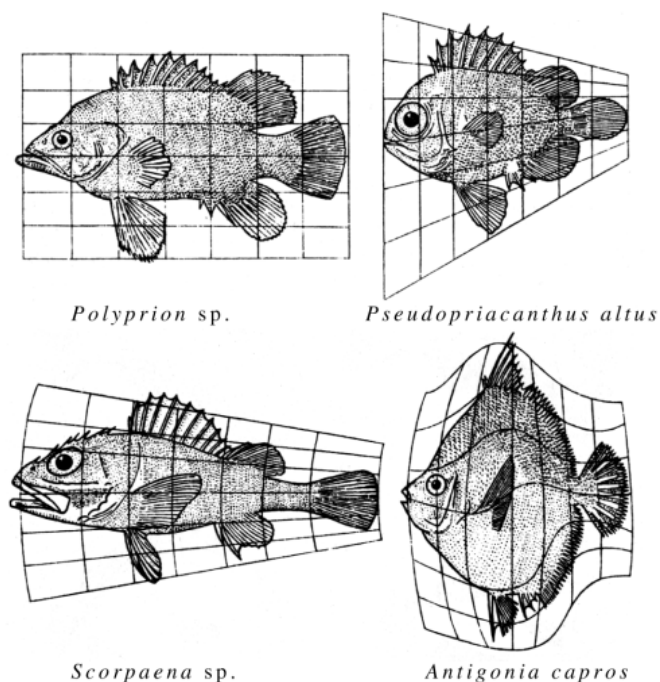
Fig. 1. Acanthopterygian fish with superimposed transformed Cartesian coordinates, reproduced from Thompson (1917) figures 150-153. The regular grid was placed over *Polyprion* sp. and then transformed with angular or radial transformations to 'best fit' the other three fish.

organ relative to body size during the growth of a single individual. Static allometry is the scaling relationship *among individuals* between one organ and total body size, or between two organs, after growth has ceased or at a single developmental stage (Fig. 2). Evolutionary (or phylogenetic) allometry is the size relationship between organs *across species*. The slopes of such scaling relationships often vary for different organs and are often not equal to 1; that is, large insects are not uniformly scaled-up versions of small insects. Although the study of allometry originated as a problem in morphology, almost any feature of an organism (e.g. metabolic rate) can be compared to size to reveal possible functional relationships (Schmidt-Nielsen, 1984; Reiss, 1989).

Huxley (1932) switched easily between ontogenetic and static allometry, seeing them as two parts of a single problem. We feel that it is more useful to keep these two types of allometry separate, and here we focus on the problem of static allometry, or how different-sized individuals produce adult organs that scale appropriately to body size.

Our starting point is the fact that a genetically homogeneous population produces individuals of different sizes depending on the environmental conditions. By focusing on variability generated by the environment, we are not ignoring the genetic control of development. Instead, we are asking how environmental differences are translated, via interactions with the genotype, into phenotypic differences.

LARVAL AND IMAGINAL TISSUES

We will focus our discussion on the growth of the imaginal cells, which form the adult (imago) tissues of holometabolous

(completely metamorphic) insects. The imaginal cells are set aside during embryogenesis and, for some tissues in some species, they invaginate into the body cavity as small pockets of cells, in which case they are called imaginal discs.

The distinction between imaginal and larval tissues highlights one of the crucial points in understanding allometry, that different body parts grow in different ways. In holometabolous insects, imaginal tissues grow primarily after larvae have ceased feeding (Fig. 3) and different imaginal tissues grow at different times and rates (Williams, 1980). Therefore, attainment of total body size is temporally dissociated from growth of individual adult organs (pp. 55-61 of Huxley, 1932; Wilson, 1953; Nijhout and Wheeler, 1996). This fact is often overshadowed by the abundant work on the imaginal discs of *Drosophila*. In this atypical insect, the imaginal discs of the head and thorax proliferate during the larval instars. However, even *Drosophila* imaginal tissues grow after feeding has ceased; after pupariation, the wings (Milán et al., 1996) and the abdominal imaginal tissues (Fristrom and Fristrom, 1993) proliferate extensively. [Also in vertebrates,
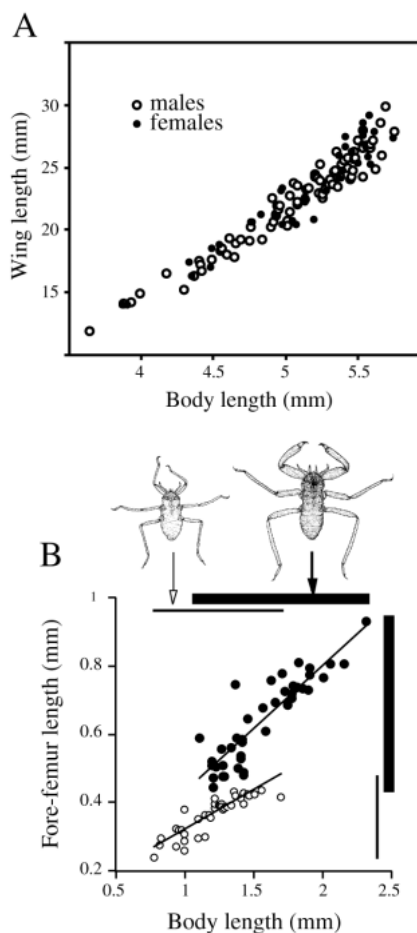

Fig. 2. Examples of static allometry. (A) The size of beetle wings, *Onthophagus acuminatus* (Scarabaeidae), covaries with body size. Individuals were sampled from a population on Barro Colorado Island, Panama (D. J. Emlen, unpublished observations). (B) Fore-femur length covaries with body length in the soldier-producing aphid, *Pseudoregma alexanderi*, and soldiers (bold symbols) follow a different allometry than non-soldiers (open symbols). Lines top and right indicate data ranges for each morph. From Stern et al. (1996).
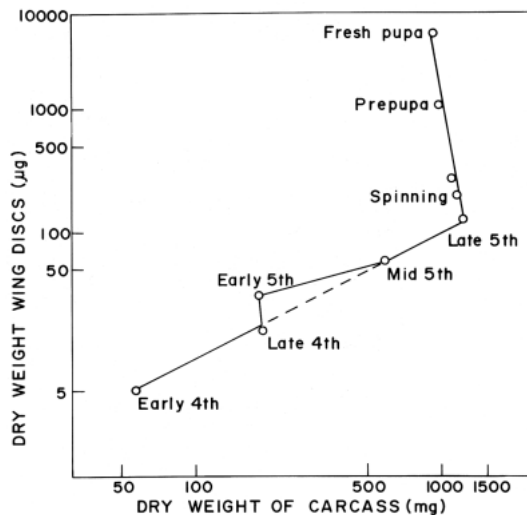
Fig. 3. The wing imaginal discs of the silkworm *Cecropia* grow primarily after larvae have ceased feeding in the late 5th instar. Dry weights of an average wing disc (*n*=16 discs) are plotted on double log scale as a function of carcass dry weight. Stage of development is indicated adjacent to data points. From Williams (1980).

organs do not grow at constant rates, nor do they necessarily grow at the same time (Boag, 1984; Cane, 1993).] Static allometry can therefore be defined for our purposes as the coordination of final adult organ sizes with the total body size attained at the end of larval feeding.

Below, we suggest that the mechanisms of allometry consist of at least two distinct developmental processes: (1) the specification of organ identity, including the basic properties of growth of that organ, and (2) modulation of organ growth in response to the conditions encountered by animals as they develop and, in particular, in response to their actual body size. We then present three models to explain the second process and highlight the potential role of hormones in communicating size information.

PROCESS 1. SPECIFICATION OF ORGAN IDENTITY

Kopec (1922) first demonstrated the autonomous identity of imaginal discs, by transplanting discs between individuals (see also Wigglesworth, 1953; Williams, 1961; Pohley, 1965; Garcia-Bellido, 1965). For example, if a wing disc is surgically removed from one individual, and placed within the abdomen of another, that disc still produces a wing, despite the fact that it is physically separated from its typical neighboring tissues. Several recent reviews have discussed the development of imaginal discs (e.g. Cohen, 1993; Williams and Carroll, 1993; Serrano and O'Farrell, 1997). We broadly review three of the main points from this work and, for each, we discuss observations relevant to growth control that are often overlooked.

Point 1. The establishment of the identity of an imaginal disc involves the generation of a spatial map within the growing disc
The establishment of imaginal disc identity can be viewed,

broadly, in the following terms (French et al., 1976; Bryant and Simpson, 1984; Bryant, 1987, 1993; Couso et al., 1993). . Early in development, the imaginal disc is divided into territories defined by the expression of selector genes in subsets of cells (e.g. *engrailed* is expressed only in the posterior region of the disc). These initial cellular territories are inherited from the positions of these cells within the embryo. The boundaries of these territories serve a particularly important role, since the boundaries provide unique spatial information. That is, a field of contiguous cells all expressing the same complement of regulatory genes have, to a first approximation, the same 'identity' and thus the same positional value, but boundaries between these fields provide information that uniquely defines position (Meinhardt, 1983). For example, the position defined by two abutting fields is a line and a unique point is defined where two such lines cross. In this way, a 'grid' consisting of boundaries and points is established within the developing disc from the partially overlapping territories of selector gene expression.

The boundaries and points of intersection sometimes serve as signaling centers. Cells at these positions release molecules that spread through the field of contiguous cells. These signals can induce neighboring cells to take on new identities, often depending on the concentration of the signal that they receive. This defines new fields within the older, broader fields. The intersections of these new fields then generate another set of unique positions that could be further used as new signaling centers. In this way, the complex patterning of adult organs is built up hierarchically by the iterative definition of new territories within old ones as the organs grow.

Point 2. The approximate sizes of organs appear to be encoded autonomously within the developing imaginal discs
The autonomy of organ sizes was first revealed by work on the classic homeotic mutants (Lewis, 1978). When a haltere is homeotically transformed to a wing, the transformed wing is approximately the size of a normal wing, not a haltere. Likewise, imaginal discs transplanted to growth permissive environments, like the abdomen of an adult female, do not grow indefinitely. Instead, these discs stop growth at organ-appropriate sizes (Bryant and Simpson, 1984; Bryant and Levinson, 1985; Jursnich et al., 1990). Finally, unmanipulated discs stop growing at the appropriate point even when metamorphosis is delayed in larvae (i.e. even when the hormonal milieu is apparently permissive for further growth; Bryant and Simpson, 1984). These results all suggest that organ identity also includes at least some information on organ size.

Point 3. Final organ size is not dependent on cell number and may be regulated via fields of cells
Over the past seventy years various investigators have used the *Drosophila* wing to study how cell numbers contribute to organ size (Alpatov, 1930; Zarapkin, 1934; Robertson and Reeve, 1952; Robertson, 1959a,b; Delcour and Lints, 1966; Masry and Robertson, 1979; Cavicchi et al., 1985; Partridge et al., 1994; James et al., 1995; Stevenson et al., 1995; Guerra et al., 1997; McCabe et al., 1997; Pezzoli et al., 1997). The wing lends itself to such analysis since size can be convincingly estimated as a two-dimensional surface and cells can be rapidly counted by their individual trichomes (Dobzhansky, 1929). Although various experimental regimes were applied in these studies, only one

consistent pattern emerges: developmental temperature alters cell size and not cell number. All other treatments produced contradictory results or changes in both cell size and number. In particular, in natural populations, cell 'size' and number tend to show negative covariance, so that wings of a particular size with larger cells have fewer cells and vice versa (McCabe et al., 1997). These results suggest that under most conditions wing size is regulated independently of specific cell sizes or numbers.

Direct evidence that the final size and shape of wings are independent of the specific patterns of cell division or numbers of cells comes from several elegant experiments. Morata and colleagues (Garcia-Bellido, Ripoll and Morata, 1973; Morata and Ripoll, 1975) induced clones of cells within wing discs that divided faster than the surrounding cells in the disc. If the final size and shape of the wing depended on the patterns of cell divisions, then this treatment would have resulted in grossly misshapen wings. In all cases, however, the wings developed normally even though different parts of the tissue proliferated at different rates. In addition, Weigmann et al. (1997) performed a series of experiments that inactivated Cdc2, a gene that is required for entry into mitosis, in subsets of wing imaginal disc cells. This treatment resulted in cells that replicated their DNA and grew considerably larger, but failed to divide. Despite this treatment, wings developed a normal shape and size. Finally, Neufeld et al. (1998) performed complementary experiments, manipulating the rate of cell division to generate wings that varied five-fold in the numbers of cells. These wings also appeared normal in size and the experimentally induced over-proliferation was balanced by changes in cell size. All of these observations suggest that wing growth and patterning mechanisms do not depend on cell number (see also McCabe et al., 1997). Instead, these mechanisms produce the appropriate total apical surface area of the sum of cells composing a wing. Therefore, allometry cannot be understood as a simple function of cell numbers, but rather as a function of the behavior of a field of cells.

In summary, the imaginal discs contain information that specifies the identity of the organ, as well as its approximate final size, and organ size is not measured in units of cell numbers. It is possible that these processes are sufficient to generate an average-sized 'species-specific' organ. In this case, evolutionary allometries, allometry across species, may be explained in large part by changes in disc autonomous processes. But this is not the whole story, and the common assumption of autonomous control of disc growth has masked the unresolved mechanisms controlling static allometry: namely, how is approximate size *modified* in accord with body size?

PROCESS 2. SIZE-DEPENDENT MODULATION OF ORGAN GROWTH

We identify three possible models, which are not mutually exclusive, to explain static allometry. In the first model, each organ directly integrates resources circulating in the haemolymph at specific rates, thus resulting in organ-specific allometries. In the second model, resources and/or body size information is coordinated and translated into a signal that directs organ growth either continuously throughout development, or during discrete growth phases. In this model, there must be at least two steps. First, body size must be

assessed and, second, this information must be communicated to all of the growing organs. In the third model, growth of individual organs depends directly on nutrients, but the organs modulate each other's growth either through direct communication or via a centralized endocrine source.

Most published observations of organ growth can be accommodated by all of the models, or slight variations on the models. The first model may be considered a null model, since it requires no communication between tissues. We believe that there is sufficient evidence to reject this model as a complete explanation for static allometry. In addition, there is evidence that specifically supports the second and third models. We suggest that hormones may play an hitherto unsuspected role in the communication of size information. We therefore briefly review the currently understood role of hormones in development.

Endocrine regulation of insect metamorphosis

The endocrine control of insect metamorphosis has been recently reviewed (Nijhout, 1994; Gilbert et al., 1996), so we only briefly summarize the basic events. Three hormones are involved: the sesquiterpenoid juvenile hormone (JH), the neurosecretory peptide prothoracicotrophic hormone (PTTH) and the steroid molting hormone ecdysone (Fig. 4). During the first part of the final larval stage, JH is produced by the corpora allata at high levels. In response to some stimulus, such as the attainment of a critical larval body size, JH production ceases and circulating levels of JH drop. Sufficiently low levels of circulating JH stimulate the release of several brief pulses of PTTH, which then stimulate the prothoracic glands to secrete ecdysone. Ecdysone is released in two peaks. First, a small peak triggers behavioral and physiological changes, including
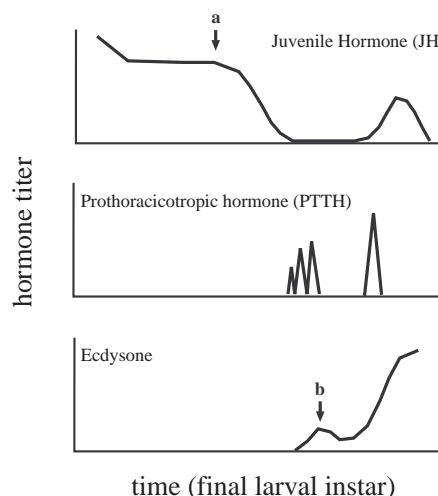

Fig. 4. Diagrammatic illustration of the endocrine cascade of holometabolous insect metamorphosis. Near the end of the final larval instar the corpora allata ceases production of JH (a; top). After JH levels in the insect haemolymph have declined, this absence of circulating JH triggers the release of the prothoracicotropic hormone (PTTH; middle), and the release of this hormone, in turn, initiates the release of the molting hormone, ecdysone (bottom). The first peak of ecdysone (b) leads to behavioral changes in larvae, including the cessation of feeding, and the active seeking of a location for pupation. The second pulse of ecdysone initiates the metamorphic molt. Modified from Nijhout (1994).

cessation of feeding by the larva, the purging of the gut contents and the active seeking of a location for pupation. This small peak also switches the commitment of the epidermis from larval to pupal development (Riddiford and Hiruma, 1990). A second, much larger peak of ecdysone starts the metamorphic molt (Fig. 4). This chain of events has been described as an endocrine 'cascade' (Gilbert et al., 1996), since each event in turn triggers the next, and since once the PTTH has been released, the metamorphic molt becomes irreversible.

Model two. A centralized body-size detection system

We propose that, in addition to their role in the temporal regulation of development, hormones communicate size information to all organs. For this mechanism to work, insects must be able to assess their own body size. The best evidence that insects can assess their body size is the observation that many species use the attainment of a critical size as a proximate trigger for initiating moulting and metamorphosis (Nijhout and Williams, 1974; Nijhout, 1975; Jones et al., 1981).

In reduviid bugs, abdominal stretch receptor neurons respond to distensions in the abdominal wall. Wigglesworth (1934) first recognized that large meals were required for molting (gradual feeding failed to induce the molt). He also showed that severing the ventral nerve cord prevented molting. Beckel and Friend (1964) induced molting by injecting saline into abdomens, confirming that abdominal stretch, and not nutrients, triggered the molt. Finally Anwyl (1972), Nijhout (1981, 1984) and Chiang and Davey (1988) identified specific neurons coupled to the endocrine system, which produce sustained action potentials proportional to the degree of stretch. Sufficient discharge stimulates the release of PTTH, and thus ecdysone, inducing the molt (Nijhout, 1984, 1994). In other insects the mechanism of size-assessment is not understood. In *Manduca sexta*, for example, larvae utilize critical body sizes (e.g. Nijhout and Williams, 1974; Nijhout, 1975; Jones et al., 1981), but detection of abdominal stretch does not appear to be involved (Nijhout, 1994).

Although the existence of critical sizes provides convincing evidence that insects can assess their size (Wigglesworth, 1953; Allegret, 1964; Nijhout, 1975, 1979; Jones et al., 1981), the critical size for metamorphosis is not likely to be used by organs to regulate static allometry because it is not a good predictor of the *final* adult size. The main problem is that larvae continue to feed after the attainment of a critical body size until the first pulse of ecdysone (between a and b in Fig. 4; Nijhout, 1975, 1981). Since individuals vary in their feeding and growth rates, they may mature at different final body sizes even when they initiate metamorphosis using the same critical body size. In summary, unknown mechanisms provide the relevant measurements, either of total body size or of stored and circulating nutrients, for use in regulating static allometry.

Evidence from polyphenisms

The best evidence for hormonal coding of body size information comes from experimental studies of polyphenisms. Polyphenic insects facultatively adopt one of several discrete developmental outcomes in response to environmental conditions (e.g. the soldier and worker castes in ants, or the queen vs. worker morphology in bees) and these developmental decisions are mediated by hormones. For example, differential feeding leads to quantitative differences
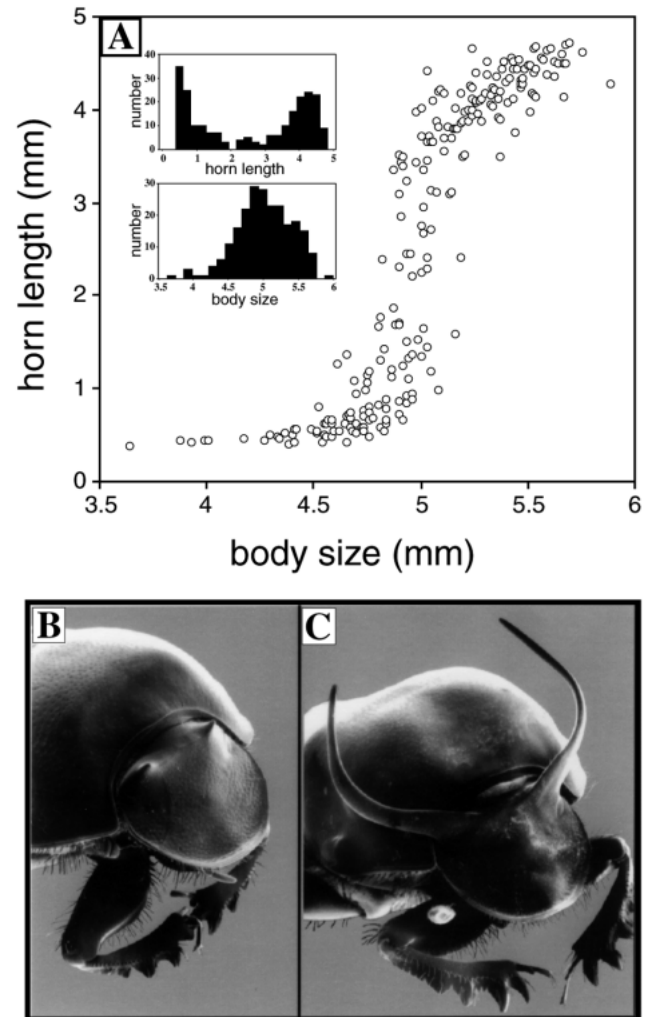

Fig. 5. Horn polyphenism in male dung beetles (*Onthophagus taurus*). (A) Males growing larger than a threshold size (approximately 5 mm prothorax width) produce fully developed horns, whereas males below this size do not. This results in co-occurrence of two discrete male morphologies within populations (B,C). From Emlen and Nijhout (1999).

in JH titers in honeybees, mediating caste production (Rachinsky and Hartfelder, 1990), and different photoperiods produce quantitative variations in ecdysone level in butterflies, mediating wing morph determination (Rountree and Nijhout, 1995). These variations in hormone levels typically alter development during a sensitive, or critical, period (Wirtz, 1973; Hardie, 1980; Wheeler and Nijhout, 1983; Endo and Funatsu, 1985; Pener, 1985; Koch and Büchman, 1987; Zera and Tiebel, 1988; Tanaka, 1994; Emlen and Nijhout, 1999).

A subset of these polyphenisms, those linked to total body size, are especially relevant to this review. For example, haemolymph levels of JH or tissue-specific sensitivities to JH (e.g. receptor densities) mediate the size-related shifts from worker to soldier development in ants during short critical periods (Wheeler, 1991). Similar body size-specific differences in JH levels regulate male horn development in beetles, where large males produce horns and smaller males do not (Fig. 5; Emlen and Nijhout, 1999). Small beetles can be induced to

produce large horns by treating them with JH during a critical period after larvae have ceased feeding (Fig. 6; Emlen and Nijhout, 1999), suggesting either that JH is produced at higher levels in larger beetles or that larger beetles are more sensitive to JH.

JH has been implicated in all of the studied insect polyphenisms in which the switch involves individual differences in body size (e.g., queen versus worker determination in honeybees, Wirtz, 1973; Rembold, 1987; Rachinsky and Hartfelder, 1990; stingless bees, Velthius, 1976; and bumblebees, Röseler, 1976). This role for JH is further supported by evidence from studies on ants, bugs, honeybees and stingless bees that suggest that both the size of the organ that secretes JH (the corpora allata), and the levels of circulating JH, are influenced by feeding rate, by food quality and by individual growth (Johansson, 1958; Wang, 1965; Asencot and Lensky, 1976; Lenz, 1976; Velthius, 1976; Goewie, 1978; de Wilde and Beetsma, 1982; Ono, 1982; Rembold, 1987). Although all of these developmental

Fig. 6. Endocrine control of horn production in *Onthophagus taurus*. (A) Topical applications of JH during a short time window can cause small, typically hornless, males to produce horns. Large and small males were produced by overfeeding or underfeeding, respectively. (B) The size-dependent production of male horns is regulated by JH during a brief time interval very late in the final larval instar (after the animals have ceased feeding, stage III). From Emlen and Nijhout (1999).

responses to JH incorporate a threshold, or 'all or none', response to the hormone, we suggest that this is a reflection of the choice of studied traits (polyphenisms are feasible, dramatic targets of study), rather than an indication that all responses to JH involve thresholds. For example, graded variations in levels of JH regulate vitellogenin (yolk) production by insect fat bodies (Hagedorn and Kunkel, 1979; Nijhout, 1994).

Other evidence

There is also evidence that ecdysteroids are involved in the continuous regulation of cell division in growing imaginal discs. Four studies have reported that low levels of ecdysone promote growth and cell division, in contrast to ecdysone's 'traditional' role as a moulting hormone. Bodenstein (1943) demonstrated that, in *Drosophila,* imaginal discs grew in adult abdomens only when co-transplanted with larval ring glands, an endocrine complex producing both ecdysone and juvenile hormone (Nijhout, 1994), and more ring glands, up to a limit, promoted more growth. He further demonstrated that the probability of growth and differentiation was dependent on both the number of ring glands and the identity of the imaginal disc; under the same assay conditions, leg discs grew and partially differentiated whereas genital discs showed little growth and failed to differentiate. Madhavan and Schneiderman (1969) showed that, in the wax moth (*Galleria melonella*), high levels of ecdysone promoted the transition to the pupal stage, whereas lower levels of ecdysone promoted regeneration of experimentally damaged imaginal discs. Damaged discs regenerated only in the presence of ecdysone. Postlethwait and Schneiderman (1970) reported that, in *Drosophila melanogaster,* imaginal discs implanted into adult abdomens only grew when low levels of ecdysone were repeatedly injected. Recently, Champlin and Truman (1998), working with in vitro cultures of *Manduca sexta* optic lobes, reported that moderate levels of ecdysone stimulated proliferation, whereas high levels triggered a maturational response involving a wave of apoptosis. In addition, Hodgetts et al. (1977) found that, other than the two large ecdysone peaks associated with metamorphosis, ecdysone is detectable at low levels throughout development in *D. melanogaster* from at least the second larval instar (the earliest time point measured). All of these results suggest that low levels of hormones are required for organ growth, that graded differences in hormone level, up to a limit, lead to graded differences in organ growth and that intrinsic differences between organs (e.g. leg versus genital) may cause them to respond differently to the same hormonal signal.

Recently, direct evidence for non-autonomous growth regulation has emerged from studies of *D. melanogaster.* Britton and Edgar (1998) reported experiments on the effect of nutrition on the progression of the cell cycle in larval cells, which undergo successive rounds of DNA synthesis but do not divide (endoreplication), versus cells that undergo mitosis during larval growth, such as the imaginal cells and the mitotic neuroblasts. They demonstrated that the endoreplicating and mitotic cells enter the cell cycle in response to different signals, for example when larvae were transferred from a nutritious to a minimal diet, endoreplicating cells ceased growth whereas mitotic cells did not. However, their findings of greatest relevance to our discussion concern the translation of larval
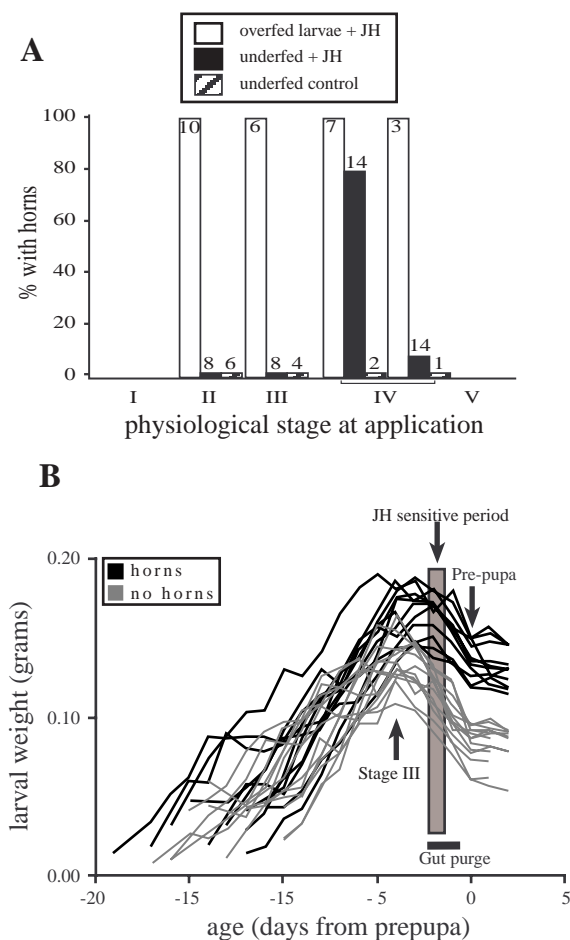
nutrition into growth. They first demonstrated that larval growth, including DNA replication in all organs, was dependent on the presence of a full complement of dietary amino acids, but that amino acids were not sufficient to produce autonomous growth in vitro. That is, in larvae, the presence of amino acids must be translated into a signal that triggers growth in other tissues. They identified the fat body as the source of a diffusible factor promoting growth in the mitotic cells (see also Davis and Shearn, 1977; Fain and Schneiderman, 1979; Shearn et al., 1978), although this factor does not promote growth of endoreplicating cells. There must be at least two sources of non-autonomous signals in growing larvae that promote growth. These exciting results elucidate the multiplicity of factors that may be involved in regulating growth and support our claim that growth of discs is not completely autonomous.

Further, Kawamura et al. (1999) reported the discovery of a new family of Imaginal Disc Growth Factors (IDGFs) from *Drosophila* that are expressed in the fat body at high levels and enhance growth of imaginal cells in vitro. It is possible that the IDGFs correspond to the mitogenic factors discussed above. These growth factors act synergistically with insulin, in vitro, and the *Drosophila* insulin receptor is required in vivo for normal growth (Chen et al. 1996). The receptor(s) for the IDGFs are unknown.

One caveat regarding the recent work in *Drosophila* is warranted. Although these studies demonstrate the action of non-autonomous signals required for growth, it is not yet clear if these are the same signals that modulate *relative* growth (static allometry) and the assays currently in use are not appropriate to detect relative growth. We predict that techniques utilized by population biologists, such as sampling broadly across size variation and quantitative comparisons of organs, will prove fruitful for discovering whether these molecules are involved in allometry.

Taken together, (1) the regulation of hormones by environmental and size-related cues, (2) the regulation of organ growth and particularly final organ size by such hormones, and (3) evidence for multifarious non-autonomous controls of cell division support a model where nutritional or size information is translated by centralized systems into hormonal cues that regulate organ sizes.

Model three: feedback between growing organs

In a third model, there is no centralized translator, but the growing imaginal tissues themselves translate available nutrients into (1) their own growth, (2) signals that regulate the endocrine system, which in turn regulates growth and developmental decisions as reviewed earlier, and (3) signals that regulate the growth of other imaginal tissues.

There is substantial evidence that the imaginal discs regulate the endocrine system. Growing imaginal discs prevent the progression of an animal to metamorphosis; if wounded discs are transplanted to the abdomen of a host individual, metamorphosis of the host is delayed until the implanted disc regenerates, and the length of this delay corresponds to the amount of regenerative growth (Ursprung and Hadorn, 1962; Pohley, 1965; Madhavan and Schneiderman, 1969; Rahn, 1972; Dewes, 1973, 1979; Simpson et al., 1980). Likewise, mutants that cause discs to continue growing beyond their normal size also lead to long delays in the metamorphosis of these animals (Bryant, 1987; Jursnich et al., 1990; Sehnal and Bryant, 1993).

These effects appear to result from the growing discs preventing the endocrine cascade described above and, in particular, preventing the release of ecdysone (Sehnal and Bryant, 1993). The repression of ecdysone production by growing discs may be an important feedback mechanism coordinating development between all of the growing tissues (Sehnal and Bryant, 1993).

Three experiments also provide evidence that imaginal discs negatively regulate the growth of other discs. First, Madhaven and Schneiderman (1969) working with the wax moth, *Galleria mellonella,* reported that injured discs stopped the growth of other discs until the injured discs had regenerated to approximately the same size as the unoperated discs. This repression may be mediated by ecdysone, since both the regenerating and normal discs resumed growing after ecdysone injection.

Second, Pohly (1965) surgically removed wing imaginal discs from the moth *Ephestia kühniella*, with the primary aim of studying regeneration of the discs. He reported that the size of the regenerated wings depended on whether only a single disc or two discs was removed and allowed to regenerate. Regenerated wings were always smaller than uninjured control wings. However, the control wing on an animal with a single regenerated wing was smaller than a normal wing on an unoperated animal, suggesting that the regenerating disc slightly repressed the final size of the normal wing. In addition, when two wing discs were removed and regenerated simultaneously, the resulting wings were smaller than normal, but *larger* than the regenerated wing on an animal with only a single regenerate. This suggests that the unoperated wing in the first experiment actively repressed growth of the regenerate.

Finally, more detailed quantitative studies of disc extirpation without regeneration performed by Nijhout and Emlen (1998) also support the hypothesis of negative regulation between discs (see also Klingenberg and Nijhout, 1998). They found that removal of a single disc results in slight overgrowth of other discs, and removal of more discs results in even more
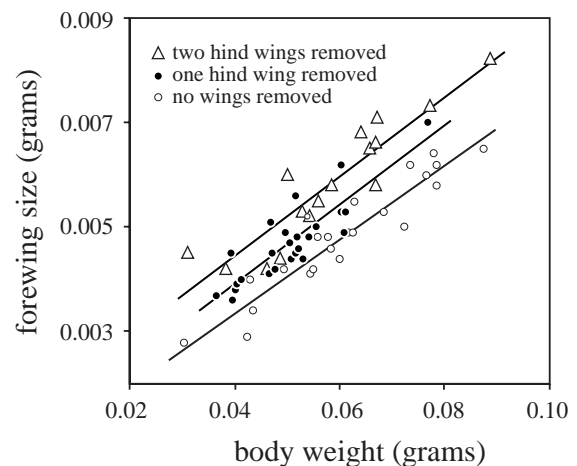

Fig. 7. Negative feedback among different imaginal discs. When a hindwing imaginal disc was surgically excised from caterpillars of the buckeye butterfly *Precis coenia* (filled circles), the remaining forewings grew disproportionately large (i.e. larger than similar forewings on individuals with both hindwings intact; open circles). When two hindwings were surgically removed, the remaining forewings grew even larger (triangles). This suggests that the presence of growing imaginal discs inhibits the growth of other growing discs. From Nijhout and Emlen (1998).
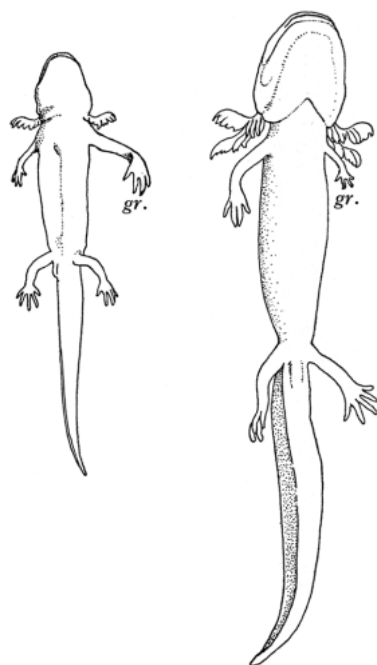
Fig. 8. Vertebrate limbs contain autonomous size information. The left forelimbs of *Amblystoma punctatum* (left) and *A. trigrinum* were reciprocally transplanted at the tail-bud stage and assayed 50 days later. The grafted limbs (gr) grew at rates, and to a final size, more similar to the control limb of the donor species (Twitty and Schwind, 1931; drawing from Huxley and De Beer, 1934).

Fig. 9. Antler allometry in red deer. Log-log plot of antler versus body weight. Redrawn from Huxley (1932).

growth in the remaining discs (Fig. 7). The precise measurements from this study elucidate further details. They effectively observed a shift in the intercept of the static allometry; organs were too large for their body size, or put another way, they were the correct size for animals of a larger body size. This indicates either that the allometric growth of organs was reprogrammed, or that the discs interpreted the loss of other discs to mean that the organism was larger than it really was. This experiment may provide a glimpse of one mechanism by which evolutionary shifts in allometries could result from alterations in direct or indirect communication among discs, or between discs and body size.

In summary, the growth of body parts appears to be only partly autonomous and is regulated by mechanisms involving either a centralized system for translating nutrition or body size into a growth signal, and/or by communication between imaginal tissues and the rest of the body. Unraveling this second phase of allometry may also provide insights into the evolution of animal shape. For example, if different organs vary in the density of receptors for a 'body size' signal, leading to differences in the 'sensitivity' of each organ to body size, then organ-specific allometries could evolve by changes in the distribution of these receptors. Such a mechanism may underlie genetic variation in static allometry within populations (e.g. Weber, 1990; Wilkinson, 1993; Emlen, 1996), as well as sexual dimorphisms.

PARALLELS WITH VERTEBRATES

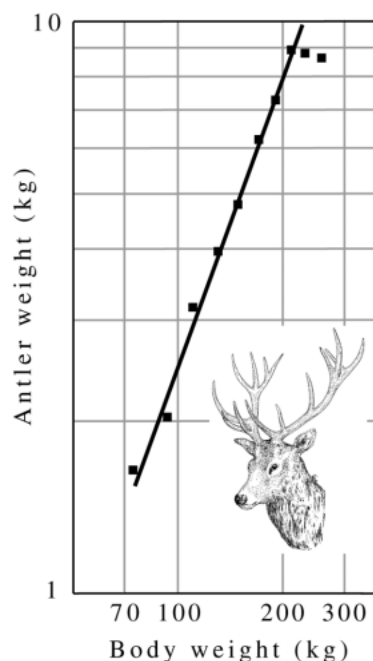The mechanisms proposed above for growth control in insects

are likely to sound familiar to vertebrate biologists. For example, it has long been known that circulating hormones and growth factors regulate cell growth and body size in vertebrates (Jenkin, 1970). In addition, there are striking parallels between insect allometry and limb development in vertebrates. Vertebrate limbs, like imaginal discs, arise from morphogenetic fields of cells that contain an autonomous 'identity'; limb fields transplanted to different regions of an embryo produce the correct 'donor' limb (reviewed in Huxley and De Beer, 1934; Gilbert, 1997). As in imaginal discs, limb identity appears to result from a spatial map, with cells along boundaries acting as signaling centers (reviewed in Tickle, 1996; Johnson and Tabin, 1997). Vertebrate organs also contain autonomous information that enables growth to the correct (at least) approximate final sizes (Fig. 8). However, this growth requires non-autonomous signals. For example, the initial growth of limb buds requires growth factors produced by the underlying mesonephros (Stephens et al., 1991; Geduspan and Solursh, 1992, 1993). Control of later limb growth has not, to our knowledge, been extensively investigated.

Similar to size-dependent growth regulation in insects, some vertebrate organs also grow relative to overall body size. For most organs, this process would be difficult to detect, as both organ size and overall body size increase continuously during development. However, some organs, such as ungulate antlers, are periodically regrown and subsequent versions of the regenerated organ scale to increasing body sizes. Antlers, as distinct from horns that grow continuously throughout adult life, are regrown each year during a brief and rapid bout of localized growth (Goss, 1983). In large species, such as moose and elk, antler length increases at greater than one centimeter per day. In addition, the final size of each years' antlers is, up to a maximum antler size, allometrically proportional to the size of the individual (Fig. 9; Huxley, 1932). Therefore, the

antlers grow relative to the actual size of the animal, illustrating that the phenomenon that we have explicated in insects is present in vertebrates. To our knowledge, the molecular mechanisms of this regulation are unknown.

In 1964, Goss considered these issues, particularly with regard to regeneration and developed the Functional Demand Theory to explain how organs grow to the appropriate size (see also pp. 431-436 of Huxley and De Beer, 1934). In essence, he proposed that organs continue to grow until they produce enough of some function, say detoxification by the liver, for the body in which they grow. While Goss argued persuasively for internal organ growth regulated by functional demand, the theory, in its pure form, appears an unlikely explanation for growth of other organs, such as limbs. We can easily imagine, however, that particular functions could be replaced by symbolic signals, such as absorption of hormones, to regulate organ size.

THE SHAPE OF ALLOMETRY IN THE FUTURE

Organ growth in insects is largely, but not entirely, autonomous to the organs themselves (also see Bryant and Simpson, 1984). The imaginal discs autonomously contain patterning information that specifies their shape and perhaps their approximate size independently of the precise number of cells. But disc growth is also modified both by the overall growth of the animal and by the growth of other discs. We suggest that future studies of the developmental basis of allometry must focus on size differences among individuals. This entails a break from 'standardized' laboratory conditions, since it is the variations in size and shape resulting from growth under varied conditions that constitute the raw material of scaling relationships in natural populations. We predict that the interface between the methods of population biology and the techniques of developmental biology and endocrinology will prove most informative. The developmental problem that to Thompson and Huxley was so intuitively appealing, and so apparently amenable to quantitative analysis, appears to present a novel paradigm that requires new ways of thinking and new experimental approaches.

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