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Genotype to Phenotype: Physiological Control of Trait Size and Scaling in Insects[1]

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SYNOPSIS. For almost a century, biologists have used trait scaling relationships (bi-variate scatter-plots of trait size *versus* body size) to characterize phenotypic variation within populations, and to compare animal shape across populations or species. Scaling relationships are a popular metric because they have long been thought to reflect underlying patterns of trait growth and development. However, the physiological mechanisms generating animal scaling are not well understood, and it is not yet clear how scaling relationships evolve. Here we review recent advances in developmental biology, genetics, and physiology as they pertain to the control of growth of adult body parts in insects. We summarize four mechanisms known to influence either the rate or the duration of cell proliferation within developing structures, and suggest how mutations in these mechanisms could affect the relative sizes of adult body parts. By reviewing what is known about these four processes, and illustrating how they may contribute to patterns of trait scaling, we reveal genetic mechanisms likely to be involved in the evolution of insect form.

INTRODUCTION

The concept of ''allometry,'' or the scaling of body parts with body size, has a rich and distinguished history (Thompson, 1917; Huxley, 1932; Paulian, 1935; Wilson, 1953, 1971; Cock, 1966; Gould, 1966). Large individuals almost always have larger structures than smaller individuals. However, the precise relationship between adult trait size and body size can vary markedly among populations and /or species (Fig. 1).

Numerous comparative studies and laboratory selection experiments make it clear that properties of scaling relationships can and do evolve (reviewed in Emlen and Nijhout, 2000). In fact, evolutionary changes in the scaling of adult parts may underlie most of the dramatic morphological differences we observe among and within species. For example, changes in scaling contribute to variation in head sizes of ant soldier and worker castes (Wilson, 1953, 1971; Feener *et al*., 1988), head sizes of dimorphic bees (Danforth, 1991; Danforth and Neff, 1992; Kukuk, 1996; Danforth and Desjardins, 1999), eyestalk lengths in flies (Grimaldi and Fenster, 1989; Wilkinson and Dodson, 1997; Baker and Wilkinson, 2003), forceps lengths of earwigs (Simmons and Tomkins, 1996) and mandible and horn lengths of beetles (Huxley, 1931; Paulian, 1935; Otte and Stayman, 1979; Cook, 1986; Eberhard and Gutierrez, 1991; Kawano, 1995*a*, *b*; Emlen, 1996; Moczek, *et al*., 2002; Fig. 1).

Despite the fact that scaling relationships serve as important comparative tools in a wide variety of fields (*e.g.,* systematics, Dodson, 1975; physiology, McMahon, 1973; Calder, 1996; Schmidt-Nielsen, 1984; and population biology, Alberch *et al*., 1979; Lande, 1979; LaBarbera, 1989; Klingenberg, 1996), little is known about the developmental mechanisms that generate scaling (*i.e.,* the processes that coordinate final trait sizes with overall body size; Meyer *et al*., 1980; Nijhout and Wheeler, 1996; Galloni and Edgar, 1999; Stern and Emlen, 1999; Emlen and Nijhout, 2000; Miner *et al*., 2000). Even less is known about the changes in genetic mechanisms that underlie scaling relationship evolution (*e.g.,* shifts in the slope or y-intercept [Fig. 2]; Nijhout and Wheeler, 1996; Emlen and Nijhout, 2000).

Recent advances in developmental biology, genetics, and physiology bring us much closer to understanding the genetic mechanisms that control growth of adult body parts. However, these studies of mechanism almost never extend their findings to the population-level phenomena of scaling, and it is not yet clear how genetic variation influencing trait growth would affect the properties of trait scaling relationships. We begin to make this connection here, by showing how genes might influence the relative sizes of morphological structures.

In this paper we review four developmental and physiological processes known to influence the growth and final size of adult body parts in insects. These are 1) genetic specification of the rate of cell proliferation within developing structures; 2) nutritional regulation of the rate of proliferation of structures; 3) endocrine regulation of the duration of proliferation of structures; and 4) polyphenic reprogramming of the rate or duration of proliferation of structures. All four of these mechanisms are likely to influence the properties of scaling relationships through their effects on trait growth. By reviewing what is known about these four processes, and suggesting how they contribute to patterns of trait scaling, we provide critical first insights to the genetic mechanisms by which insect scaling relationships may evolve. Our primary goal is to illustrate how mutations in specific genes might alter the properties of existing scaling relationships—leading to evolutionary changes in the slope, y-intercept, or shape of these relationships. We also use this information to

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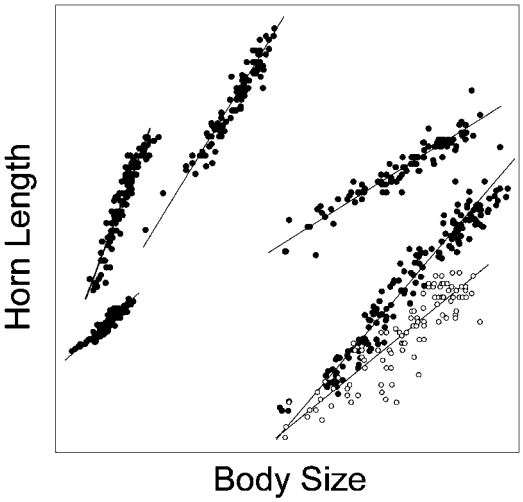


FIG. 1. Interspecific variation in scaling relationships. Horn lengths and body sizes are shown for static samples of adult males of six species of *Onthophagus* beetle (Coleoptera: Scarabaeidae); data for four of the species are provided courtesy of John Hunt. As recommended by Schmidt-Nielsen (1984) and LaBarbera (1989), we use the term "scaling" instead of "allometry." For this paper, "scaling relationships" refer to the covariation of trait size with overall body size, with no assumptions as to the slope or shape of the relationship.

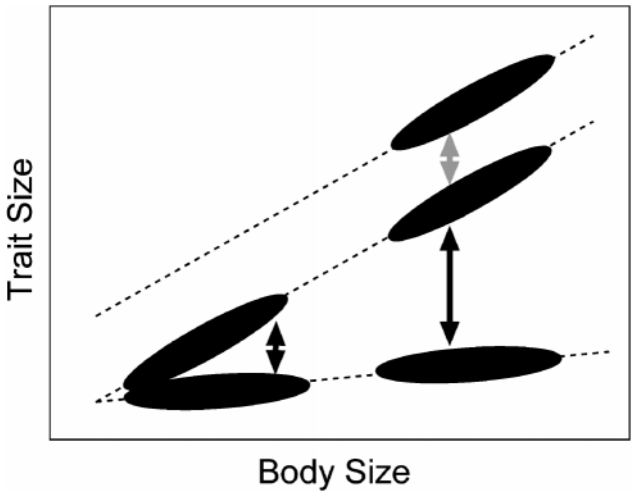


FIG. 2. Evolutionary modifications in scaling relationships can arise through genetic shifts in the y-intercept (gray arrow) or the slope (black arrows). Hypothetical populations are shown as black ovals.

identify likely candidates for the scaling mechanisms themselves. We focus on holometabolous (metamorphic) insects as a model system for understanding the evolution of scaling relationships. However, many of the mechanisms that we outline represent highly conserved developmental pathways. As a result, factors that affect scaling relationships in insects may also affect scaling across a wide variety of taxa.

BACKGROUND: INSECT METAMORPHOSIS, INSECT HORMONES, AND GROWTH OF IMAGINAL STRUCTURES

Historical perspective

Insects display an extraordinary variety of morphologies, life histories and behavior; they differ just as widely in the specifics of their development. Nevertheless, it is possible to identify basic endocrine and cellular processes that are shared by a breadth of insect taxa (*e.g.,* see reviews by Nijhout, 1994; Riddiford, 1994, 1996; Gilbert *et al*., 1996; Truman and Riddiford, 1999, 2002). In the following sections we briefly describe the life cycle of metamorphic insects, and the endocrine events that (in most cases) coordinate the morphological transformation from larva to adult. We focus on the subsets of cells that will form the adult structures (the "imaginal discs"; see below), because proliferation of these cells determines the final sizes of wings, legs, antennae, mouthparts, compound eyes and genitalia in the adults.

It is important to recognize at the outset that not all imaginal discs develop in the same way, and for this review we adopt the historical perspective of Truman and Riddiford (1999, 2002; see also Svácha, 1992). According to this view, the ancestral pattern of insect imaginal disc development is one of late growth of these structures, with cells commencing proliferation near the end of the final larval instar (similar in timing to the growth of wings and genitalia in hemimetabolous insect taxa; Truman and Riddiford, 1999, 2002). This pattern of late trait development appears to occur in all of the basal holometabolous orders of insects (*e.g.,* Megaloptera, Neuroptera, and Mecoptera), and in the more basal families of the Coleoptera, Diptera and Hymenoptera. However, at least six times in the history of the holometabola, imaginal discs have evolved the capacity to commence proliferation earlier in the developmental period—in some cases, much earlier (Truman and Riddiford, 1999, 2002). The most notable examples of this include the imaginal discs in *Drosophila* (only the abdominal histoblast cells display the ancestral developmental pattern in these flies), and the wing imaginal discs of Lepidopterans. We first describe the ancestral pattern of disc development, and then discuss specific exceptions to this pattern as appropriate.

Insect metamorphosis

The life cycle of most metamorphic insects includes several sequential larval instars followed by a molt to a pupa, and then a final molt to the adult (Fig. 3A). Once animals have emerged as adults, they do not molt again, and their size and external morphology are fixed.

Larvae are behaviorally, morphologically and physiologically specialized for feeding and growing (Wake and Hall, 1999; Truman and Riddiford, 2002). Most of the imaginal (*i.e.,* adult) structures are not visible or functional in larvae (*e.g.,* wings, compound eyes, genitalia, antennae), although it turns out that cells in these structures both commence, and often complete, their proliferative growth at this time. For most metamorphic insects, the last larval instar is the period when the final sizes of adult body parts are determined,

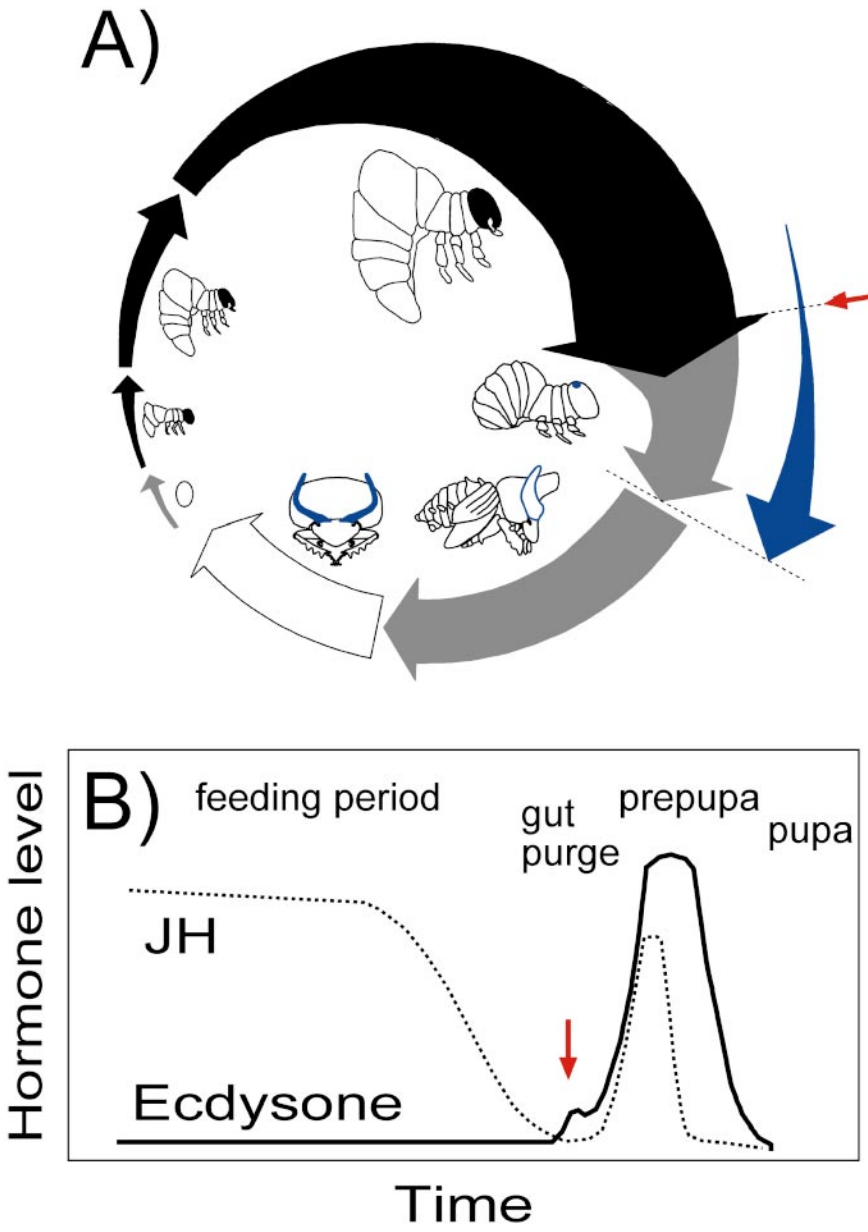


FIG. 3. (A) Life cycle of a metamorphic insect (*Onthophagus taurus*). After hatching, beetles pass through three larval instars before molting first into a pupa, and then into an adult. Black arrows indicate feeding periods; gray arrows indicate non-feeding periods. Arrow thickness approximates overall animal body size, and gaps between arrows indicate molting events. The final (third) larval instar can be divided into a feeding period, a gut purge (red arrow), and prepupal period. Imaginal discs begin proliferating near the end of the final larval feeding period, and have reached their final sizes by the end of the prepupal period (blue arrow). Drawings illustrate egg, first through third larval instars, prepupa (after the gut purge of the final larval instar), pupa, and adult. (B) Hormone profiles for final larval instar insects. Juvenile Hormone (JH) levels (broken line) are high during the first part of the larval feeding period, but drop as animals reach their largest body sizes. After JH levels have fallen, a small pulse of ecdysone (solid line) irreversibly initiates the onset of the gut purge and metamorphosis (red arrow in A,B). This small pulse of ecdysone is followed by a much larger ''molting'' ecdysone pulse that coordinates the formation and expansion of the pupal cuticle (prepupa period). A new pulse of JH also occurs at the beginning of the prepupa period.

and is therefore likely to be the period when the scaling of body parts to overall size occurs.

The final larval instar of metamorphic insects can be sub-divided into three physiologically and behaviorally discrete periods: the feeding period, the gut purge period, and the prepupal period (Fig. 3B). During the feeding period, animals feed and gain weight, and growth in overall body size occurs (Nijhout, 1994). Then, in response to a species-specific stimulus (*e.g.,* attainment of a critical body size, as in the tobacco hornworm *Manduca sexta*; Nijhout and Williams, 1974; Nijhout, 1975, 1994; D'Amico *et al*., 2001), animals stop feeding and begin to empty their gut of all contents in preparation for metamorphosis (the gut purge period). This physiological transition is often associated with additional behavioral changes:

larvae actively seek a pupation site (''wandering''), and/or form a protective pupal case.

After animals have completely purged their guts, the remaining days of the larval period are called the prepupal period. Larvae at this stage are relatively immobile, and many parts of the epidermis have detached from the outer larval cuticle (Hinton, 1958; Nijhout, 1994). Detached regions of epidermis grow rapidly at this time, and secrete new layers of cuticle. Because this new cuticle is formed inside the old larval cuticle, prepupal animals have two exoskeletons. Externally, they still resemble larvae, but they now have pupal cuticle folded up inside. This new pupal cuticle is soft, and remains folded until the animal molts, at which point it unfurls to take the pupal shape. Insect pupae have all of the major structures of the adult, and most of these structures have already reached their final dimensions.

Insect hormones

Both the molting process and the metamorphic transformation from larva to pupa are coordinated and regulated by hormones (reviewed in Nijhout, 1994; Riddiford, 1994, 1996; Gilbert *et al*., 1996). Two hormones in particular drive these fundamental processes: ecdysone and juvenile hormone (JH).

Ecdysone is a steroid hormone that exerts its effects by regulating downstream patterns of gene transcription (Lepesant and Richards, 1989; Andres *et al*., 1993; Cherbas and Cherbas, 1996). Cells are sensitive to ecdysone when they express receptors for this hormone, and variation in cell- or tissue-sensitivities can result from variation in the number or type of receptors expressed (Yao *et al*., 1992; Talbot *et al*., 1993; Fujiwara *et al*., 1995; Nijhout, 1994; Cherbas and Cherbas, 1996; Jindra *et al*., 1996; Riddiford, 1996; Champlin and Truman, 1998; Riddiford *et al*., 1999). Different tissues, and even the same tissues at different times, can have very different thresholds of sensitivity to ecdysone (Karim and Thummel, 1992; Emery *et al*., 1994; Riddiford *et al*., 1999). As a result, a gradually rising or falling ecdysone titer can sequentially trigger a series of time- and tissue-specific physiological events (Truman *et al*., 1974; Riddiford, 1985, 1994; Gilbert, 1989; Kremen, 1989; Nijhout, 1994; Champlin and Truman, 1998; Riddiford *et al*., 1999).

Juvenile hormone is a sesquiterpene secreted by the corpora allata, a pair of glands next to the insect brain (Bounhiol, 1938; Wigglesworth, 1940; Schooley and Baker, 1985; Tobe and Stay, 1985; Nijhout, 1994), and the level of JH present at times of ecdysone secretion can influence the specific pathways of gene transcription that are initiated (*e.g.,* ''larval'' *versus* ''pupal'' genes; Hiruma *et al*., 1999; Riddiford, 1996; Riddiford *et al*., 1999). Circulating levels of JH are influenced by larval feeding rate, larval food quality, and by individual growth (Johansson, 1958; Wang, 1965; Asencot and Lensky, 1976; Lenz, 1976; Velthius, 1976; Dogra *et al*., 1977; Goewie, 1978; deWilde and Beetsma, 1982; Ono, 1982; Rembold, 1987; Rachinsky and Hartfelder, 1990; Browder *et al*., 2001; Tu and Tatar, 2003). Consequently, JH serves as an important intermediary between the external environment of insects and internal processes such as gene transcription, cell proliferation, behavior and physiology (Nijhout and Wheeler, 1982; Nijhout, 1994, 1999; Gilbert *et al*., 1996; Riddiford, 1994, 1996; Dingle and Winchell, 1997).

Although ecdysone alone stimulates and coordinates larval-to-larval molts, it is the interaction between ecdysone and JH that coordinates the metamorphic transformation from larva to pupa (reviewed in Bollenbacher, 1988; Gilbert, 1989; Berger *et al*., 1992; Nijhout, 1994; Riddiford, 1994, 1996; Gilbert *et al*., 1996; Truman and Riddiford, 1999, 2002). Near the end of each larval instar, there is a large pulse of ecdysone (the ''molting pulse'') that regulates the formation and expansion of new cuticle. When this molting pulse of ecdysone occurs, the level of JH also present at that same time determines the identity of the following molt. If JH is present above a threshold level, the molt is from larva to larva. However, if JH is absent (or below threshold) when the molting pulse of ecdysone occurs, then the metamorphic transformation is initiated, and the animal molts from a larva into a pupa (Nijhout and Williams, 1974; Safranek *et al*., 1980; Bollenbacher, 1988; Rountree and Bollenbacher, 1986; Gilbert, 1989; Nijhout, 1994; Sehnal *et al*., 1996).

During the final larval instar, JH levels typically drop by the end of the feeding period so that JH is absent by the time the molting pulse of ecdysone occurs (Nijhout and Williams, 1974; Safranek *et al*., 1980; Hammock, 1985; Rountree and Bollenbacher, 1986; Nijhout, 1994; Fig. 3B). The first pulse of ecdysone that rises *in the absence of a background of JH* initiates the behavioral and physiological changes associated with metamorphosis (*i.e.,* the switch from feeding to gut purge; Dominick and Truman, 1985; Nijhout, 1994; Riddiford, 1994), and changes the identity of the subsequent instar from larva to pupa (Truman *et al*., 1974; Riddiford, 1978, 1981, 1982, 1985; Riddiford and Kiely, 1981; Kremen and Nijhout, 1998; Riddiford and Hiruma, 1998; Nijhout, 1994; Champlin and Truman, 1998). Once this transition has been initiated (red arrow in Fig. 3), metamorphosis is unstoppable and irreversible, and the rigid downstream sequence of physiological events is called the ''metamorphic endocrine cascade'' (reviewed in Nijhout, 1994; Gilbert *et al*., 1996). With this as a backdrop, we can now examine when and how adult body parts form.

Growth of imaginal discs

The clusters of cells that will form the imaginal structures (here called ''imaginal discs,'' but see Svácha (1992) and Truman and Riddiford (2002) for more precise definitions) behave as remarkably autonomous units. For example, the cells that will form the adult left foreleg are physically separated from cells that will

form the left forewing, the left midleg, or the right foreleg. In general, these clusters of imaginal cells remain dormant for most of the larval period, and then undergo rapid proliferation during the gut purge and prepupal periods (Williams, 1980; Fristrom and Fristrom, 1993; Milán *et al*., 1996; Nijhout and Wheeler, 1996; Truman and Riddiford, 1999, 2002; blue arrow in Fig. 3A). Since these developing structures are trapped inside the larval cuticle, they either invaginate into the larval body cavity, or they fold in on themselves as they grow, forming dense rings of epidermis (Gehring and Nothiger, 1973; Oberlander, 1985; Svácha, 1992; Kremen and Nijhout, 1998; Emlen and Nijhout, 1999; Miura and Matsumoto, 2000). After a period of proliferation, imaginal discs begin a process of cellular differentiation that involves changes in cell shape and unfolding of the new structure (*e.g.,* Fristrom *et al*., 1977; Fristrom and Fristrom, 1993). The unfolding of structures as the animal molts results in the sudden transformation from larval to pupal body form (*e.g.,* Fig. 6C).

Three properties of imaginal discs are central to discussions of genetic mechanisms that influence the relative sizes of body parts. First, growth of imaginal discs occurs primarily by cell proliferation (Meyer *et al*., 1980; Raff, 1996; Conlon and Raff, 1999; Weinkove and Leevers, 2000; Johnston and Gallant, 2002). Changes in cell size may also influence trait sizes (Conlon and Raff, 1999; Montagne *et al*., 1999; Verdu *et al*., 1999; Weinkove *et al*., 1999; Hodin and Riddiford, 2000); however, the majority of animal growth is thought to arise from cell proliferation, and we focus on this mechanism here (*e.g.,* a typical *Drosophila* wing disc grows from 50 to 50,000 cells in four days; Johnston and Gallant, 2002). Second, imaginal discs need not grow at the same time as the rest of the animal. Cell proliferation in the imaginal discs of many insects does not begin until around the time larvae stop feeding, and thus occurs after most growth in overall body size has ceased. Third, each of the different imaginal discs behaves as a relatively autonomous developmental unit.

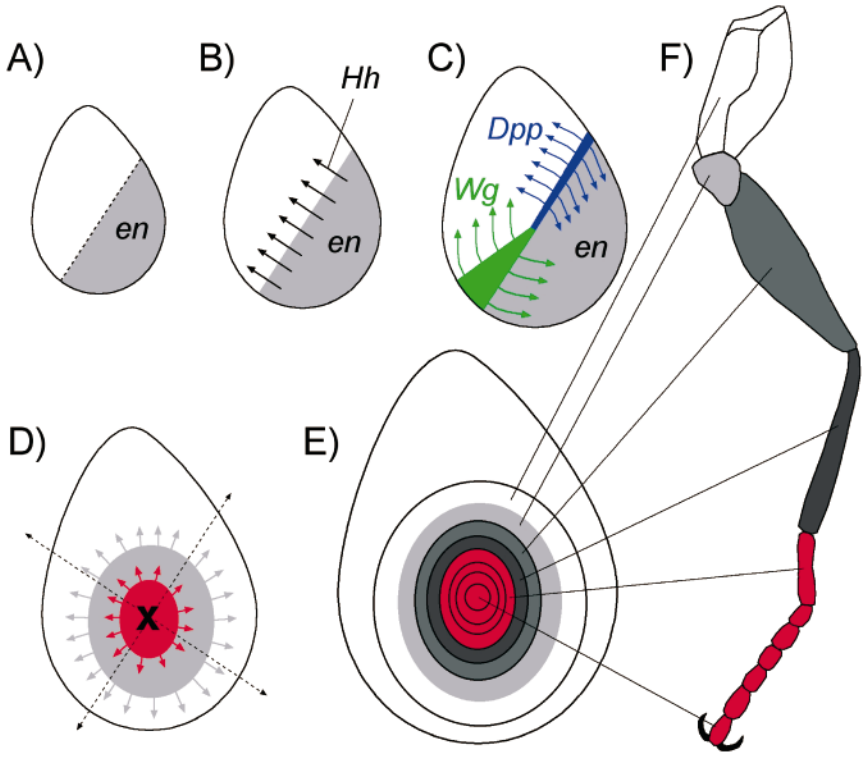
What factors determine the final sizes of adult body parts? Ultimately, the resulting dimensions of morphological structures will depend on how fast the cells in each imaginal disc proliferate (growth rate), and how long imaginal cell proliferation continues (growth duration). Each of the mechanisms described below influences the final sizes of adult body parts by determining either the rate or the duration of imaginal disc growth. Mechanism 1 operates by regulating the relative amounts of growth of territories, or domains, *within* each developing imaginal disc (specifying the proportions of different regions within a structure determines, to a large extent, the dimensions of the total structure). Mechanisms 2 and 3 regulate overall levels of proliferation of entire imaginal discs, by influencing the rate of protein synthesis in imaginal disc cells (Mechanism 2), or by specifying the duration of the physiological period permissive to imaginal cell proliferation (Mechanism 3). Our goal in reviewing these mechanisms is to highlight how genetic variation for aspects of development can influence animal morphology. Mutations in these mechanisms have the potential to affect the relative amount of growth of specific structures. As such, they may influence the relationship between trait size and body size, and they are of direct relevance to evolutionary biologists interested in the evolution of animal form.

MECHANISM 1: GENETIC REGULATION OF GROWTH RATE

As early as 1922, Kopec demonstrated that developing imaginal discs surgically excised from their natural physiological location retained their identity (and grew to a roughly appropriate final size) even when transplanted to another position in another individual (Kopec, 1922; see also Williams, 1961; Pohley, 1965; Garcia-Bellido, 1965). In fact, imaginal discs contain the genetic regulatory machinery needed to form an entire adult structure (reviewed in Cohen, 1993; Serrano and O'Farrell, 1997; Garcia-Bellido and Garcia-Bellido, 1998; Johnston and Gallant, 2002). During larval development, the epithelial cells within each of the imaginal discs become subdivided by a hierarchical sequence of spatially-explicit signals that diffuse from cell to cell (two important signaling molecules are Wingless [Wg] and Decapentaplegic [Dpp]; Cohen, 1993; Serrano and O'Farrell, 1997). These signals have been called "morphogens" (*sensu* Turing, 1952) because they direct the development of cells as they diffuse through the tissue (Lawrence and Struhl, 1996; Day and Lawrence, 2000). Partially overlapping gradients of morphogens specify unique domains within the disc epithelium, and cells at the intersections of these domains take active roles in coordinating both patterning and growth of the structure. Box 1 illustrates part of this process for the *Drosophila* leg.

Importantly, interactions between positional signals stimulate and coordinate cell proliferation locally within the disc (Peifer *et al*., 1991; Campbell *et al*., 1993; Struhl and Basler, 1993; Basler and Struhl, 1994; Diaz-Benjumea *et al*., 1994; Irvine and Wieschaus, 1994; Wilder and Perrimon, 1995; Zecca *et al*., 1995; Grimm and Pflugfelder, 1996; Johnston and Schubiger, 1996; Serrano and O'Farrell, 1997; Johnston and Gallant, 2002). For example, if a piece of a disc is surgically removed, cells at the wound edge no longer receive positional signals from their neighbors, and when these cells contact the opposite edge of the wound, the signals between them are inappropriate. This mismatch of cell signals stimulates proliferation in the wound region, and proliferation continues until the diffused pattern of signals is again complete (*e.g.,* Gibson and Schubiger, 1999). Thus the pattern itself dictates, to some extent, the proportions and final size of the structure.

Drosophila imaginal discs are important models for understanding the influence of genetic patterning networks on local proliferation within discs (reviewed in



Box 1: Pattern formation within *Drosophila* leg imaginal discs.

Developing imaginal structures become subdivided by a sequential and hierarchical sequence of spatially-explicit signals that diffuse from cell to cell (1,2). Partially overlapping gradients of these signals uniquely specify domains within the disc epithelium, and cells at the intersections of these domains take active roles in coordinating both patterning and growth of the structure. (A) Early in development, the gene *engrailed* (*en*) is expressed in cells in the posterior half of the leg imaginal disc (3). Each leg disc is now sub-divided into an anterior and a posterior domain by the boundary of expression of *engrailed*. (B) Cells containing engrailed are induced to produce and secrete the protein Hedgehog (Hh) which acts as a signal from posterior to anterior cells (4–8). This gene product diffuses through the epithelium and into the ''anterior'' domain of the disc. (C) Cells exposed to Hedgehog in the absence of engrailed (*i.e.,* ''anterior'' cells) are stimulated to begin expressing one of two locally-acting morphogens. Dorsal anterior cells begin secreting Decapentaplegic (Dpp), whereas ventral anterior cells produce and secrete Wingless (Wg; 9,10). Mutual repression between *Wg* and *Dpp* reinforces the distinction between dorsal and ventral cells, and minimizes overlap of these gene products (11). Discs are now divided along two orthogonal axes (dorsal-ventral and anterior-posterior), resulting in four molecularly unique domains. (D) A few cells at the intersection of these domains have high levels of both Dpp and Wg, and these critical cells form the distal tip of the developing appendage. These cells express *optimotor-blind* (*omb*; 12, 13), and then omb combined with high levels of Dpp and Wg stimulates expression of *Distal-less* (*Dll*), *arista-less* (*al*), and *epidermal growth factor* (*EGF*) (7, 14–17). Products of these latter three genes form a series of concentric gradients surrounding the distal tip of the developing leg. (E) Cells responding to positional information encoded in these overlapping gradients begin expressing still other patterning genes (18), and the result is an epithelial field subdivided along three axes: anterior/posterior, dorsal/ventral, and now proximal/distal. By the end of this process, the concentric rings of the proximal/distal axis correspond with each of the segments of the adult leg (F).

(1) Cohen, 1993; (2) Serrano and O'Farrell, 1997; (3) Lawrence and Morata, 1976; (4) Lee *et al*., 1992; (5) Mohler and Vanni, 1992; (6) Tabata *et al*., 1992; (7) Basler and Struhl, 1994; (8) Tabata and Kornberg, 1994; (9) Held, 1995; (10) Jiang and Struhl, 1996; (11) Abu-Shaar and Mann, 1998; (12) Wilder and Perrimon, 1995; (13) Grimm and Pflugfelder, 1996; (14) Campbell *et al*., 1993; (15) Diaz-Benjumea *et al*., 1994; (16) Galindo *et al*., 2002; (17) Campbell, 2002; (18) Bishop *et al*., 1999.

Cohen, 1993; Serrano and O'Farrell, 1997; Day and Lawrence, 2000; Johnston and Gallant, 2002), and this system provides evidence for several mechanisms by which the relative sizes of discs could evolve. For example, increased (or decreased) levels of *Wg* or *Dpp* expression by focal cells alters rates of cell proliferation within discs, and this can affect the final sizes of the corresponding adult structures (Nellen *et al*., 1996; Neumann and Cohen, 1996; Serrano and O'Farrell, 1997; Niwa *et al*., 2000; Adachi-Yamada and O'Connor, 2002; Martín-Castellanos and Edgar, 2002). Mutations in upstream factors that regulate *Wg* or *Dpp* activity (*e.g., Notch*, Go *et al*., 1998; *Ultrabithorax*, Stern, 2003*a*) also affect rates of cell proliferation within discs, as do mutations in receptors for Dpp (Johnston and Gallant, 2002), and mutations in the downstream signaling pathways regulated by Wg and Dpp (*e.g.,* Peifer *et al*., 1991; Johnson *et al*., 1995; Burke and Basler, 1996; Rauskolb and Irvine, 1999; Keisman and Baker, 2001; Keisman *et al*., 2001; Moreno *et al*., 2002). Thus, a variety of genetic elements interact to specify how spatial patterning is laid down within developing imaginal structures, and mutations in the involved gene-transcription pathways affect the relative amounts of proliferation of these structures. In fact, by specifying the shape and relative sizes of domains within imaginal discs, patterning networks may determine most of the overall dimensions (*i.e.,* size) of

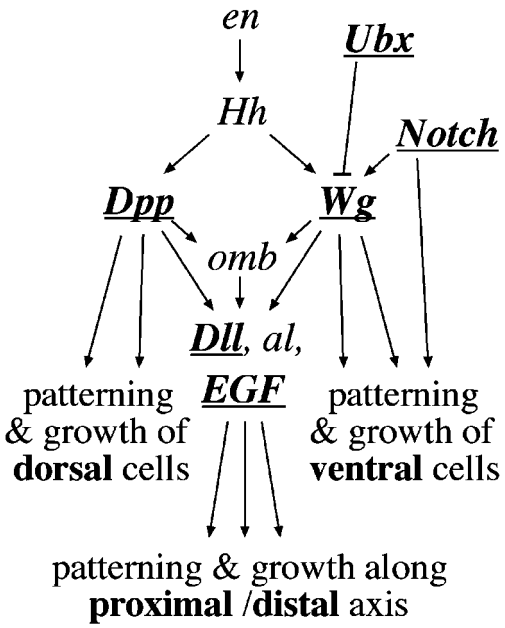


FIG. 4. Patterning network for *Drosophila* leg imaginal discs. Interacting signaling molecules and transcription factors divide the disc epithelium into a nested series of domains (see Box 1), and these genetic patterning interactions regulate local cell proliferation within the developing disc. Mutations in elements of this patterning network can alter rates of cell proliferation, and influence the final size of the structure (genes with demonstrated influences on the rate of cell proliferation are shown underlined). See Box 1 for references. *en, engrailed; Hh, Hedgehog; Ubx, Ultrabithorax; Dpp, Decapentaplegic; omb, optomotor-blind; Dll, Distal-less; al, arista-less; EGF, epidermal growth factor.*

each imaginal structure (*e.g.,* Day and Lawrence, 2000).

We summarize the genetic patterning network for *Drosophila* leg discs in Figure 4. This patterning network appears largely conserved between the different *Drosophila* imaginal discs (*e.g.,* wings: Cohen, 1993; Irvine and Wieschaus, 1994; Serrano and O'Farrell, 1997; antennae: Cummins *et al*., 2003; genitalia: Keisman and Baker, 2001; Keisman *et al*., 2001), and similar patterning networks direct appendage development in other insects (*e.g.,* butterflies: Weatherbee *et al*., 1999; beetles: Beermann *et al*., 2001; ants: Abouheif and Wray, 2002; crickets: Niwa *et al*., 2000; grasshoppers: Jockusch *et al*., 2000), arthropods (*e.g.,* spiders: Abzhanov and Kaufman, 2000), and vertebrates (Vogel *et al*., 1995; Pueyo *et al*., 2000; Campbell, 2002). In all of these examples, the basic elements of the patterning process are conserved, but details of the interactions vary. This suggests that evolutionary modifications in the shapes and sizes of body parts may arise in part from subtle alterations in these genetic patterning networks (*e.g.,* Niwa *et al*., 2000; Keisman *et al*., 2001; Cummins *et al*., 2003; Stern, 2003*a*).

MECHANISM 2: INSULIN-DEPENDENT REGULATION OF GROWTH RATE

Although morphogen signals and genetic patterning networks control local proliferation of different regions *within* each imaginal disc, additional, external factors control overall levels of proliferation of entire discs. These additional signals operate globally within the insect, and may influence the proliferation of all of the imaginal discs. Differences among discs in how they respond to these global signals (*e.g.,* through tissue-specific receptor activity) can lead to variation in the relative sizes of body parts, and may underlie the evolution of trait scaling relationships, and of animal proportions in general.

One of the most important "global" signaling mechanisms in metazoans is the insulin pathway. Cell proliferation requires high levels of protein synthesis, and in both vertebrates and insects this process is regulated by the insulin pathway (Edgar, 1999; Weinkove and Leevers, 2000; Ikeya *et al*., 2002; Johnston and Gallant, 2002; Nijhout and Grunert, 2003). Insulin, when bound to its receptor, activates an evolutionarily conserved signal transduction cascade that controls the activity of the protein translation machinery (Fig. 5).

In vertebrates, insulin cooperates with insulin-like growth factors to bind to its receptor (reviewed in Johnston and Gallant, 2002). A similar situation occurs in insects (Kawamura *et al*., 1999; Bryant, 2001; Nijhout and Grunert, 2003; Nijhout, 2003). This signaling mechanism has been best studied in *Drosophila*, where there are now seven identified insulin proteins: dILPs 1–7 (*Drosophila* Insulin-Like Proteins; Ikeya *et al*., 2002), and several types of growth factors (Britton and Edgar, 1998; Kawamura *et al*., 1999; Bryant, 2001; [Recently, both insulin, and growth factors have also been identified in the Lepidoptera: Nijhout and Grunert, 2003; Nijhout, 2003]). Three of the *Drosophila* dILP genes are expressed by insulin-producing neurosecretory cells in the brain during larval development (Rulifson *et al*., 2002), and these insulin proteins are secreted into the hemolymph where they act as circulating signals accessible to all developing tissues (Rulifson *et al*., 2002).

Importantly, levels of both insulin and growth factors are sensitive to larval nutrition (Kawamura *et al*., 1999; Bryant, 2001; Britton *et al*., 2002; Ikeya *et al*., 2002; Nijhout and Grunert, 2003). Expression of at least two of the dILPs is altered by perturbations in larval diet (flies starved for 24 hours show reduced levels of expression of dILP 3 and dILP 5; Ikeya *et al*., 2002), and production of growth factors responds to levels of larval nutrition (Kawamura *et al*., 1999; Bryant, 2001; Nijhout and Grunert, 2003). Levels of insulin and growth factors both affect overall rates of proliferative growth (*e.g.,* Kawamura *et al*., 1999; Bryant, 2001), and mutations in any of the components in this pathway mimic the effects of starvation—*i.e.,* they generate miniature, but normally proportioned animals (Edgar, 1999; Weinkove and Leevers, 2000; Brogiolo *et al*., 2001; Tatar *et al*., 2001; Britton *et al*., 2002; Ikeya *et al*., 2002; Johnston and Gallant, 2002). Combined, these results implicate the insulin-signaling pathway as an important centralized mechanism coordinating growth in insects. In fact, the insulin path-

way may be the primary mechanism by which insect growth is regulated in response to the external influence of larval nutrition (Edgar, 1999; Masumura *et al*., 2000; Bryant, 2001; Ikeya *et al*., 2002; Johnston and Gallant, 2002; Nijhout and Grunert, 2003; Stern, 2003*b*). For this reason, the insulin pathway is, at present, one of the most promising candidates for the elusive underlying mechanism of scaling itself (*i.e.,* for the basic process that coordinates proportional growth of body parts with nutrition-induced among-individual variation in overall body size). We return to this possibility at the end of this paper.

Although the insulin pathway clearly regulates overall growth of animals, several recent studies suggest that it may regulate *disc-specific* growth rates as well. Insulin receptors are expressed in imaginal discs (Chen *et al*., 1996; Brogiolo *et al*., 2001; Bryant, 2001), and discs produce their own growth factors that cooperate with insulin to stimulate local proliferation (Kawamura *et al*., 1999; Bryant, 2001). Manipulations of levels of either insulin or growth factors directly affects the rates of imaginal cell proliferation (Kawamura *et al*., 1999; Bryant, 2001; Zurovec *et al*., 2002; Nijhout and Grunert, 2003), and local over-expression of either the insulin receptor, or of one of the downstream kinases (*e.g.,* PIP_3K), leads to enlargement of specific imaginal structures (Leevers *et al*., 1996; Huang *et al*., 1999; Weinkove *et al*., 1999). We illustrate components of the insulin pathway with known disc-specific effects on proliferation in Figure 5. Because mutations in the insulin pathway have the potential to affect the relative sizes of specific adult structures, they also constitute plausible physiological bases for evolutionary modifications to animal form.

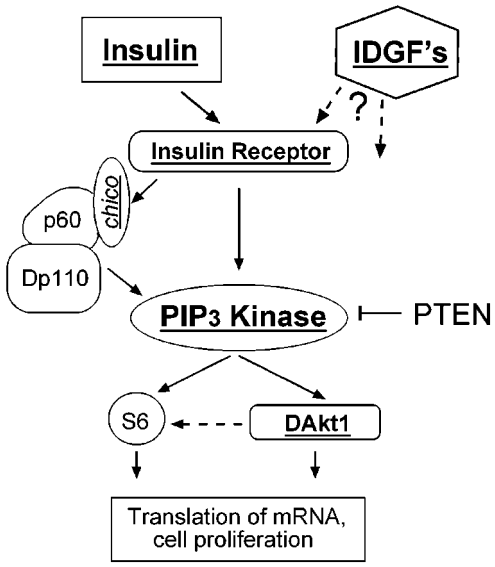


FIG. 5. Insulin signaling pathway in insects. Binding of insulin to insulin receptors activates signaling cascades that stimulate protein synthesis and cell proliferation, and this process is enhanced by the presence of growth factors. Insulin receptors are expressed in imaginal discs, and local modification to this pathway may regulate the rate of growth of specific imaginal discs. Components with demonstrated effects on rates of imaginal disc growth are shown underlined. Pathway modified from Edgar (1999) and Weinkove and Leevers (2000). IDGFs, imaginal disc growth factors; p60, a gene that serves as an adapter to the Dp110 gene, which is the *Drosophila* phosphoinositide 3-kinase; PIP_3 Kinase, phostphatidylinositol (3,4,5) triphosphate kinase; Dakt1, a *Drosophila* homolog of the mammalian kinase Akt (also known as protein kinase B); S6, ribosomal protein involved in mRNA translation.

MECHANISM 3: ENDOCRINE REGULATION OF GROWTH DURATION

The developmental hormones JH and ecdysone also act as global signals that can influence proliferation in imaginal discs. As already discussed, JH and ecdysone coordinate insect molting and metamorphosis. In many insects, they also coordinate the timing of proliferation in imaginal disc cells (Fig. 6). The wings and genitalia of hemimetabolous insects, and the imaginal discs of many holometabolous insects delay proliferation until the end of the larval period. This delayed onset of proliferation is mediated by hormones, and in particular, by an inhibitory effect of JH.[4] During early larval instars, and during the feeding period of the final larval instar, high levels of circulating JH combine with low levels of ecdysone to inhibit proliferation of imaginal cells (Wyss, 1976; Smagghe and Degheele, 1994; Kremen and Nijhout, 1998; Champlin and Truman, 1998; Truman and Riddiford, 1999, 2002; Miner *et al*., 2000; Oberlander *et al*., 2000).

When JH levels drop at end of the larval feeding period (Figs. 3B, 6), proliferation of imaginal cells begins (Svácha, 1992; Smagghe and Degheele, 1994; Monsma and Booker, 1996; Champlin and Truman, 1998; Truman and Riddiford, 1999). Imaginal tissues have genetically specified thresholds of sensitivity to JH (Fukuda, 1952; Milner and Dübendorfer, 1982; Ohtaki *et al*., 1986; Peel and Milner, 1992; red line in Fig. 6A), and when levels drop beneath this threshold, cell proliferation commences. This burst of proliferation continues through the gut purge period, while JH titers remain low.

At the onset of the prepupal period a new pulse of JH occurs simultaneously with very high levels of ecdysone (Figs. 3B, 6). This combination of hormones also stimulates proliferation of imaginal cells (Wyss, 1976; Kremen and Nijhout, 1998; Oberlander *et al*., 1998), and these cells continue to proliferate as long as this latter pulse of JH is above threshold. Near the end of the prepupal period, JH titers drop and cells are exposed to high levels of ecdysone *in the absence of JH*. This critical event triggers the end of imaginal cell proliferation, and initiates the metamorphic process of disc differentiation (Chihara *et al*., 1972; Oberlander

[4] This mechanism has been largely overlooked as a regulator of trait growth, probably because the majority of studies of imaginal disc development have focused on the discs of *Drosophila*, and on the wing discs of Lepidoptera, and these all are unusually insensitive to JH. However, even these discs are sensitive to interactions between JH and ecdysone during the prepupal period, and their proliferation is inhibited by JH during the larval feeding period if the concentrations are high enough (see text for references).

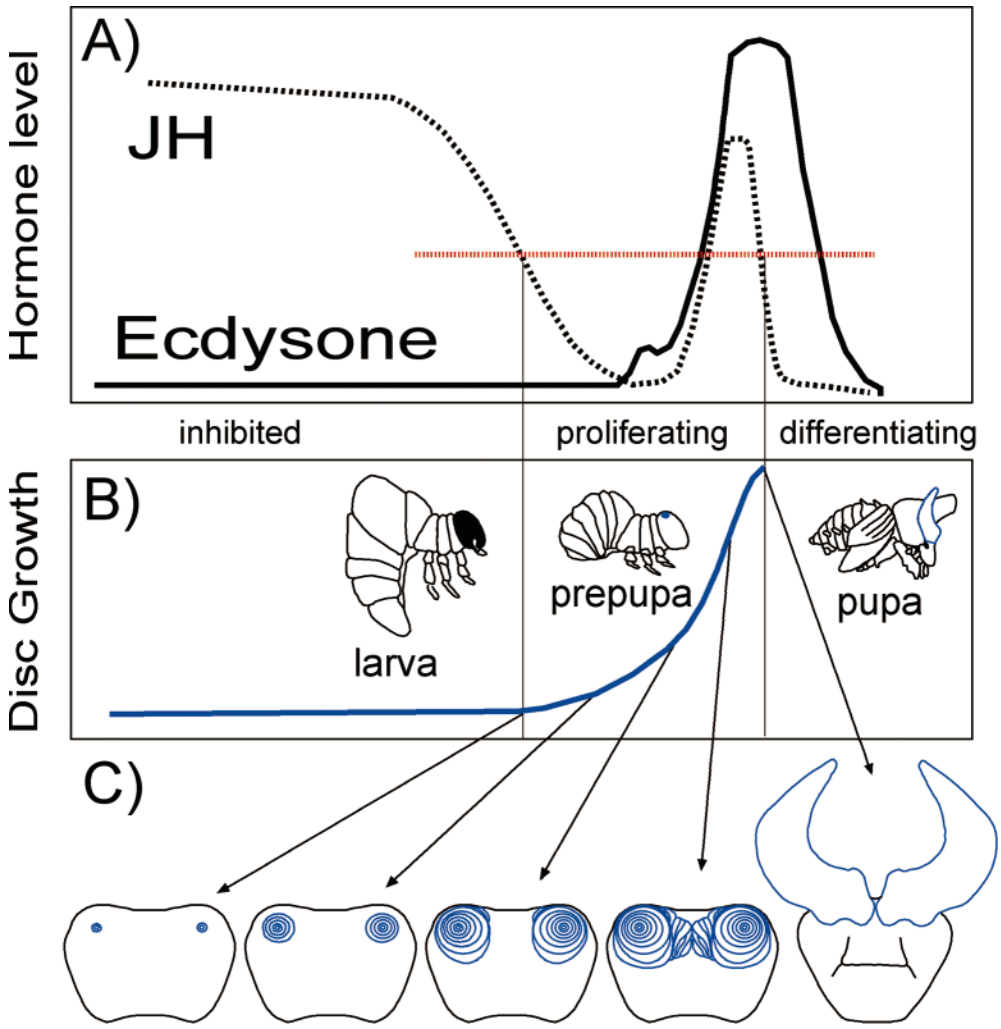


FIG. 6. Hormones control the onset and termination of proliferation in imaginal discs. (A) Hormone profiles as in Figure 3. Imaginal discs have genetically-mediated thresholds of sensitivity to JH (red line). (B) Proliferation in imaginal discs begins when JH levels drop below threshold, and continues until cells are exposed to very high levels of ecdysone in the absence of JH. The effect of JH on proliferation depends on the circulating levels of ecdysone: JH *inhibits* imaginal cell proliferation during the larval feeding period when ecdysone levels are low, but *stimulates* proliferation during the prepupal period when ecdysone levels are high. When levels of JH drop below threshold for the second time (during the prepupal period), high levels of ecdysone (now in the absence of JH) cause cells to stop dividing and begin a process of differentiation (see text for references). (C) Imaginal discs form as local invaginations of the larval epidermis (they are located beneath the larval cuticle); these structures fold inward as they grow, and unfurl to take their full shape at pupation. Shown for male horns of *Onthophagus taurus* beetles.

and Tomblin, 1972; Chihara and Fristrom, 1973; Wyss, 1976; Meyer *et al*., 1980; Milner and Dübendorfer, 1982; Peel and Milner, 1992; Smagghe and Degheele, 1994; Champlin and Truman, 1998; Cottam and Milner, 1998; Oberlander *et al*., 1998; Auzoux-Bordenave *et al*., 2002).

Consequently, both the onset and the termination of disc growth are determined by the joint action of ecdysone and juvenile hormone. These endocrine events define a window of proliferation for imaginal structures at the end of the final larval instar. For each imaginal disc, the duration of the growth period, and hence the final size of the adult structure, will depend on circulating levels of JH and ecdysone, and on imaginal disc-specific thresholds of sensitivity to these hormones. This latter factor is particularly relevant here, because genetically mediated hormone thresholds may provide another important mechanism for evolution of animal shape.

Genetic properties intrinsic to the imaginal discs determine their thresholds of sensitivity to JH and ecdysone, and mutations affecting these thresholds can contribute to evolutionary changes in the relative sizes of specific adult structures. For example, separate discs within a single animal often have very different hormone thresholds (Fukuda, 1952; Milner and Dübendorfer, 1982; Ohtaki *et al*., 1986; Peel and Milner, 1992; Kremen and Nijhout, 1998), and this can cause them to differ in the relative durations of their period of proliferation, and in their respective final sizes. In

the silkworm *Bombyx mori*, wing discs begin proliferating before leg discs, and both of these begin before the mandibles (Fukuda, 1952; Ohtaki *et al*., 1986).

Particularly extreme examples include the wing discs of Lepidoptera, and the wing and leg discs of *Drosophila*, which begin proliferating very early in the larval period (Svácha, 1992; Stern and Emlen, 1999; Truman and Riddiford, 1999, 2002). These imaginal discs have extraordinarily high thresholds of sensitivity to JH (Chihara *et al*., 1972; Chihara and Fristrom, 1973; Reddy *et al*., 1980; Cottam and Milner, 1998; Kremen and Nijhout, 1998; Miner *et al*., 2000), and insensitivity to JH permits them to commence growth early in the larval period (while JH levels are still quite high; Truman and Riddiford, 1999, 2002). Early onset of proliferation may be the mechanism by which these species evolved unusually large trait sizes (in the case of butterfly wings), or unusually rapid larval development (in the case of *Drosophila*), and in both cases, early proliferation probably evolved through shifts in the relative sensitivities of these imaginal discs to circulating levels of JH (Truman and Riddiford, 1999, 2002).

Each of the above examples involves disc-specific variation in sensitivity to JH. However, the period of imaginal disc growth can also be altered by changes in cell sensitivity to ecdysone. For example, eye discs of the tobacco hornworm moth, *Manduca sexta*, have an extended duration of proliferation that results from a very high threshold of sensitivity to ecdysone. In this case, the prepupal ecdysone pulse is not sufficient to terminate eye disc proliferation, even though JH levels drop. Thus, eye disc cells continue to proliferate until an even larger ecdysone pulse occurs during the middle of the pupal period (Monsma and Booker, 1996; Champlin and Truman, 1998).

Although it is now clear that both tissues within an individual, and individuals within populations (*e.g.,* Dingle and Winchell, 1997; Ayoade *et al*., 1999; D'Amico *et al*., 2001; Bertuso *et al*., 2002; Moczek and Nijhout, 2002) differ genetically for hormone thresholds, we still know very little about how changes in cell sensitivity to hormone levels arise. Mutations affecting levels of hormone receptor expression, or binding affinities of receptors to hormone, could all affect the concentrations of circulating hormone needed to elicit a physiological response, and for this reason we suggest that they constitute likely candidate mechanisms.

One recently characterized *Drosophila* gene appears especially promising: *headcase (hdc)*. Weaver and White (1995) showed that *hdc* is expressed specifically in imaginal cells, and that its pattern of expression exactly coincides with the period of imaginal cell proliferation. Mutations leading to loss of *hdc* function prevented imaginal cells from differentiating during the prepupal period (Weaver and White, 1995), and the authors suggest that this gene may be involved in hormone responsiveness during metamorphosis. Clearly, more studies will be needed to directly link patterns of gene expression within imaginal discs with cellular sensitivities to circulating hormones. Regardless of how they arise, genetic changes in the sensitivity of imaginal cells to either JH or ecdysone have the potential to alter the relative sizes of specific structures because they affect the duration of imaginal cell proliferation. As such, they constitute additional physiological bases for evolutionary modifications in animal form.

MECHANISM 4: POLYPHENIC REPROGRAMMING OF TRAIT GROWTH

Animal populations often exhibit more than one scaling relationship for the same trait. The most common example of this involves sexual dimorphism, where expression of a structure in males may differ strikingly from that of females (*e.g.,* mandibles in Lucanid beetles may be enlarged into exaggerated weapons in males, but not in females; Huxley, 1931; Otte and Stayman, 1979). Sexually dimorphic taxa are promising systems for explorations of the genetic control of scaling, because comparing gene expression in the imaginal discs of males and females has the potential to reveal mechanisms underlying evolutionary changes in trait scaling relationships, and in animal morphology.

In still other insect species, patterns of trait growth are altered facultatively, depending on the conditions individuals encounter as larvae. These 'reprogramming' events are typically mediated by hormones, and can involve local changes in patterns of gene expression that redirect the relative amounts of proliferation of structures (*e.g.,* Wheeler, 1991; Evans and Wheeler, 1999; Miura *et al*., 1999; Scharf *et al*., 2003). Reprogramming can result in large and small individuals within a population—even within the same sex—having very different trait scaling relationships, often separated abruptly by a critical threshold body size. These systems may be the most promising of all, because it is possible to induce animals with similar genotypes (*e.g.,* siblings) to produce divergent morphologies by manipulating environmental conditions in the laboratory. For example, in the male-dimorphic beetle *Onthophagus taurus*, males encountering favorable nutritional conditions grow larger than a threshold body size, and horn lengths in large males scale according to one relationship. Males encountering sub-optimal nutritional conditions do not reach this body size, and horn lengths in small males scale according to a different relationship (Emlen and Nijhout, 1999, 2001; Fig. 7).

Natural populations of this beetle include a mixture of both large and small individuals, and these populations are characterized by sigmoid, or broken scaling relationships between horn length and body size (Hunt and Simmons, 1997; Emlen and Nijhout, 1999; Moczek and Emlen, 1999; Fig. 8D). Similar scaling-relationship polyphenisms occur in other male dimorphic beetles (Huxley, 1931; Paulian, 1935; Eberhard, 1982; Cook, 1986; Eberhard and Gutierrez, 1991; Rasmus-

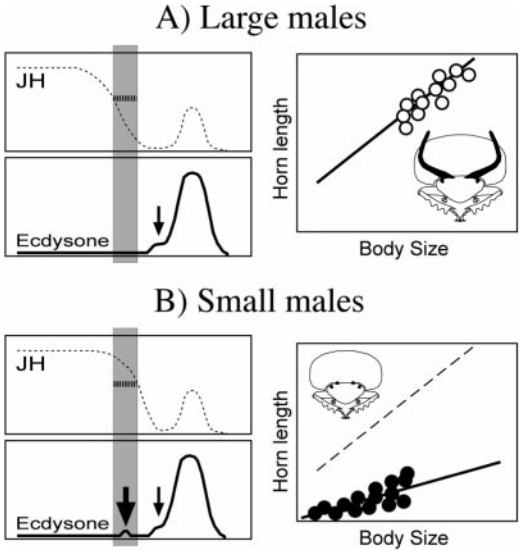


FIG. 7. Polyphenic reprogramming of trait scaling in the beetle *Onthophagus taurus*. Male beetles produce a pair of curved horns that extend from the base of the head, and horn lengths scale positively with body size in natural populations. However, horn length/body size scaling relationships differ for large and small males. The switch between patterns of horn scaling is mediated by hormones during a brief critical period near the end of larval feeding (vertical gray bar). (A) Large males have JH concentrations beneath a genetically-mediated threshold level, and horns in these animals grow according to a default (''wild-type'') pattern (open circles in right panel). (B) Small males have higher concentrations of JH during the critical period; JH concentrations above the threshold level appear to trigger a small pulse of ecdysone (large arrow) that reprograms horn growth in these males (closed circles in right panel). Small arrows indicate onset of metamorphic endocrine cascade; hormone profiles as in Figures 3B, 6. Modified from Emlen and Nijhout, 2001.

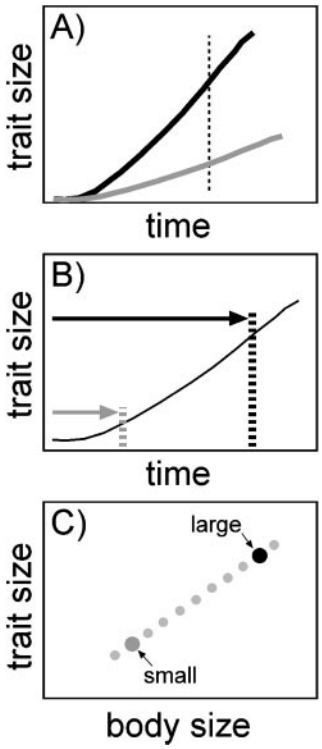


FIG. 9. Elements of a scaling mechanism. Scaling mechanisms coordinate relative amounts of proliferation in imaginal discs with the growth conditions (*e.g.,* nutrient environments) encountered by developing larvae. As such, the critical ingredient is that disc proliferation differs between large and small individuals. Body size-specific modification of imaginal disc growth can be accomplished in several ways: discs in large individuals may proliferate at a *faster rate* than similar discs in smaller individuals (A), or they may proliferate for a *longer duration* (B). In either situation, trait sizes in adult animals would scale positively with among-individual variation in overall body size (C). Growth curves of imaginal discs are indicated by solid lines; broken lines indicate the end of the period of proliferation; black = large individuals; gray = small individuals. Scaling could also result from variation in imaginal cell sizes (not shown), or from any combination of the above processes.

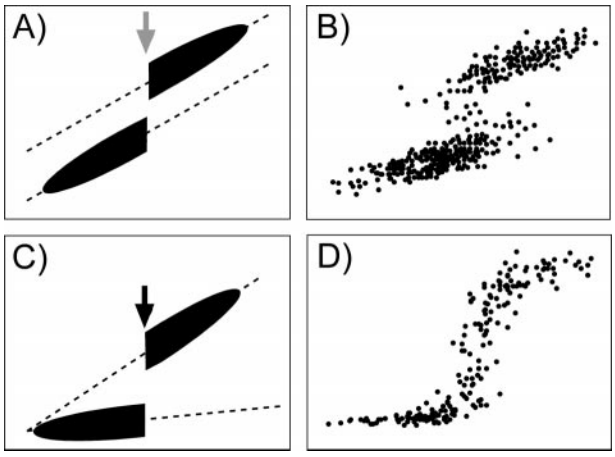


FIG. 8. Reprogramming of trait growth occurs when proliferation in imaginal discs is altered in response to conditions encountered during larval development. This can affect the y-intercept (A, B), and /or the slope (C, D) of trait scaling relationships. Arrows indicate reprogramming events (shading as in Fig. 2); data from measures of male horn lengths and body sizes in *Chalcosoma atlas* (B) and *Onthophagus taurus* (D).

sen, 1994; Kawano, 1995*a*, *b*; Emlen, 2000), male dimorphic bees (*e.g.,* Kukuk and Schwartz, 1987; Danforth, 1991; Hartfelder and Engels, 1992; Kukuk, 1996; Danforth and Desjardins, 1999), earwigs (Tomkins and Simmons, 1996), and in castes of aphids (Stern *et al*., 1996), bees (Rachinsky and Hartfelder, 1990; Hartfelder *et al*., 2000) and ants (Wilson, 1953, 1971; Wheeler and Nijhout, 1983; Feener *et al*., 1988; Wheeler, 1991; Diniz-Filho *et al*., 1994; Ward, 1997).

Unfortunately, little is known about how cellular reprogramming is achieved. These reprogramming events generally occur prior to the period of imaginal cell proliferation (*e.g.,* Wheeler and Nijhout, 1981, 1983; Rachinsky and Hartfelder, 1990; Emlen and Nijhout, 2001), are triggered by levels of juvenile hormone (*e.g.,* Wheeler and Nijhout, 1981, 1983), or by interactions between juvenile hormone and ecdysone (Rachinsky and Hartfelder, 1990; Emlen and Nijhout, 1999, 2001; Hartfelder *et al*., 2000), and they involve genetically-mediated thresholds of sensitivity to these

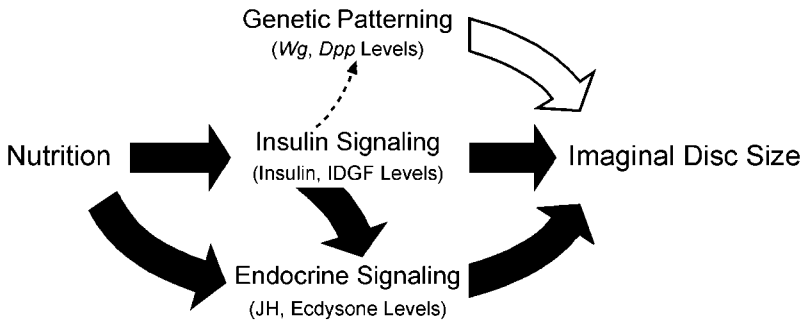


FIG. 10. Candidate mechanisms for scaling in insects. Genetic patterning, insulin signaling and hormones all affect cell proliferation in developing imaginal discs (Mechanisms 1–3; see text), and each of these mechanisms can influence the final sizes of adult body parts. Genetic patterning mechanisms operate autonomously within imaginal discs, and are not yet known to be sensitive to external environmental factors such as larval nutrition (although there is some evidence that levels of expression of the patterning genes *Wg*, *Dpp* and *Hh* are sensitive to elements of the insulin signaling pathway; Chen *et al*., 1996). Levels of insulin and growth factors are sensitive to variation in larval nutrition, they affect rates of imaginal cell proliferation, and they act as global signals within developing insects. For these reasons, the insulin-signaling pathway is the most likely candidate mechanism for the coordination of imaginal disc growth with nutrition and overall body size. Hormone levels (particularly JH) are also sensitive to external environmental variables, including larval nutrition, they also act as global signals within insects, and they influence the duration of proliferation of cells in imaginal discs. Consequently, endocrine signaling also is a candidate mechanism for scaling in insects. Interestingly, recent studies of insulin receptor mutants reveal connections between these latter two mechanisms: *Inr* and *chico* mutant animals produce significantly lower levels of JH and have altered patterns of Ecdysone secretion (Tu *et al*., 2002; Tu and Tatar, 2003). This suggests that insulin and endocrine signaling may interact to coordinate the relative growth of insect body parts.

hormones (Emlen, 1996; Moczek and Nijhout, 2002; Moczek *et al*., 2002).

Reprogramming may at first appear similar to Mechanism 3 (it involves the same hormones, and similar genetic thresholds of sensitivity to these hormones). However, several distinctions are worth noting. First, endocrine regulation of the duration of proliferation (Mechanism 3) affects all individuals in most, if not all, metamorphic insect species. In contrast, only a subset of these taxa incorporate the additional endocrine mechanism of tissue reprogramming, and in these taxa, only a subset of individuals (*e.g.,* those smaller than a threshold size) are affected. Second, reprogramming events occur at a different time than Mechanism 3, and although they may alter parameters relevant to Mechanism 3 (*e.g.,* they may shift thresholds of sensitivity to JH or ecdysone), they are not limited to this. In principle, reprogramming events could affect trait growth by altering the expression of genes from any of the above (or other) mechanisms.

Regardless of how it arises, polyphenic reprogramming of disc growth can profoundly affect the shape of trait scaling relationships (reviewed in Nijhout and Wheeler, 1982, 1996; Wheeler, 1991; Nijhout, 1999; Emlen and Nijhout, 2000). Because only a subset of animals are reprogrammed, natural populations contain a mixture of ''wild type'' and ''reprogrammed'' individuals, and these populations often have complex (*e.g.,* sigmoid or discontinuous) patterns of trait scaling (*e.g.,* Fig. 8). Interestingly, the exact properties of trait scaling that are altered by these reprogramming events can vary: depending on the species, complex scaling relationships can involve size-specific changes in slope, y-intercept, or both, suggesting that multiple mechanisms may be affected.

FROM PROLIFERATION TO SCALING: WHERE ARE WE NOW?

Scaling is a population-level phenomenon. Scaling relationships characterize trait development as it is expressed in individuals across a range of body sizes. These relationships provide meaningful descriptions of variation within natural populations, which is the raw material for natural and sexual selection. They also provide informative metrics for comparing patterns of trait expression among populations or species. Consequently, scaling relationships are a promising interface between developmental and evolutionary biology.

Remarkably, the developmental processes that actually generate scaling have hardly been explored, and no scaling mechanism has yet been identified for any insect. Describing a scaling relationship requires measurements of trait and body sizes for large samples of individuals (*e.g.,* Figs. 1, 8), and empirically assessing the effect of a mutation on trait scaling requires these measures for *both* mutant *and* wild-type populations. Unfortunately, none of the studies exploring mutational effects on trait size looked at the larger question of trait scaling. This may reflect differences in the methodologies of developmental and population biologists; it may reflect a lack of interest in the question by developmental biologists. Regardless, an understanding of the mechanism(s) of scaling is necessary before genetic studies of trait growth can be effectively integrated with population studies of morphological evolution. We end this review by proposing two candidate mechanisms for scaling in insects.

Scaling results from mechanisms that coordinate the relative amount of growth of imaginal discs with the final body size attained by each individual (Fig. 9). In insects, natural variation in body size is influenced by variation in larval nutrition: individuals reared under

favorable nutritive conditions mature at larger body sizes than genetically similar individuals reared under poor, or limiting nutritive conditions (*e.g.,* Wigglesworth, 1953; Chapman, 1982; Blanckenhorn, 1991; Hunt and Simmons, 1997; Iguchi, 1998; Imasheva *et al*., 1999; Emlen and Nijhout, 2000; Tu and Tatar, 2003). Yet, despite this pervasive source of environmental variation, adult trait sizes scale closely with body size in natural populations (reviewed in Stern and Emlen, 1999; Emlen and Nijhout, 2000).

Developmental mechanisms of scaling must be sensitive to variation in larval nutrition, and they must modify the growth of imaginal discs so that final trait sizes are appropriate for the nutritional conditions encountered, and the overall body sizes attained. Mechanisms 2 and 3 meet these criteria: both involve circulating signals that are extrinsic to the discs themselves (insulin and JH, respectively), the levels of these signals are sensitive to larval nutrition, and these signals affect the relative amounts of imaginal disc growth. Consequently, both insulin and endocrine pathways provide viable candidate mechanisms for basic patterns of trait scaling in insects, and these may operate alone or in combination to specify body size-dependent amounts of imaginal disc growth (Fig. 10).

In summary, although we are much closer to an understanding of physiological processes regulating the amount of growth of individual structures, we cannot yet extrapolate this information to population patterns of scaling, or to comparative studies of morphological evolution. Direct tests of these and other mechanisms will be needed before a more comprehensive understanding of the evolution of scaling is possible. Precise links between genetic variation in mechanisms regulating trait growth, and population patterns of trait scaling, are complex, and are likely to depend on the specific mechanisms generating scaling. Future studies will benefit if they integrate the approaches of both developmental and population biology. For example, genetic studies that perturb any of the four mechanisms reviewed here *and also* measure trait scaling relationships, will be able to bring laboratory studies of development into the realm of population biology and evolution. Only then will it be possible to directly link genetic variation in patterns of trait growth with one of the oldest and most widespread metrics of evolutionary biology: allometry.

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