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ENDOCRINE INSIGHTS INTO THE EVOLUTION OF METAMORPHOSIS IN INSECTS

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■ **Abstract** This review explores the roles of ecdysone and juvenile hormone (JH) in the evolution of complete metamorphosis and how metamorphosis, in turn, has impacted endocrine signaling. JH is a key player in the evolution of metamorphosis because it can act on embryos from more basal insect groups to suppress morphogenesis and cause premature differentiation, functions needed for transforming the transitional pronymphal stage of hemimetabolous insects into a functional larval stage. In the ancestral condition, imaginal-related growth is then delayed until JH finally disappears during the last larval instar. In the more derived groups of the Holometabola, selective tissues have escaped this JH suppression to form early-growing imaginal discs. We discuss how complete metamorphosis may have influenced the molecular aspects of both ecdysone and JH signaling.

CONTENTS

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INTRODUCTION

The evolution of complete metamorphosis resulted in an explosive diversification of the insects, making them the dominant group of terrestrial animals in their size range. Their pathway toward complete metamorphosis began with their evolution of the ability to fly. Flight allowed insects to evade predators, disperse rapidly, and find new food resources. It also initiated some fundamental changes in their life histories. The earliest of the flying insects, such as the Paleodictyoptera, appeared to be ametabolous, in that they lacked metamorphosis (23, 71). Fossil series of nymphs of these insects show that the immature forms had proportionately scaled-down "winglets" that were articulated and had venation, but they were held out to the side and likely of no use aerodynamically. The transformation of these growing wings into immovable wing pads folded over the back protected the developing wings from harm and ushered in the "incomplete metamorphosis" that we see in hemimetabolous insects.

Because they typically maintain a similar body plan throughout their life, the immature and adult stages of most hemimetabolous insects live in the same habitat and utilize the same food. Consequently, there is the potential for competition between the needs for growth and the needs for reproduction. Obviously, there are notable exceptions to this generalization, such as the mayflies and dragonflies. Even constrained with a hemimetabolous life style, these groups have evolved quite different nymphal and adult stages, thereby allowing each to exploit a different habitat. Nevertheless, the evolution of complete metamorphosis, with its distinctive larval stage, provided an unprecedented ability for insects to separate the needs for growth from those for reproduction. This innovation resulted in a rapid radiation of insects and the production of the 10 holometabolous orders that are living today (70).

The insects provide a unique opportunity to examine the mechanisms that underlie the evolution of complex life histories. The holometabolous insects are clearly a monophyletic group (70, 152) and have generated a rich diversity of life history strategies. Moreover, we have extant examples of ametabolous and hemimetabolous insects, representing grades of life history complexity that the insects had to traverse during the evolution of complete metamorphosis. This review examines the evolution of insect metamorphosis from the perspective of how endocrine signaling may have been involved in this innovation and how it may have changed as a result.

BACKGROUND

Over the years, hypotheses for the evolution of complete metamorphosis in insects have involved attempts at establishing homologies between the life stages of hemimetabolous and holometabolous insects. The basic problem has been to

transform the two-stage life history of hemimetabolous insects (with the nymph and adult stages) into the three-stage life history pattern of larva, pupa, and adult of holometabolous insects. The major hypotheses have differed in trying to establish a correspondence between the stages.

One view initially proposed by A. Berlese (13) and later elaborated by A.D. Imms (59) makes a clear distinction between the hemimetabolous nymph and the holometabolous larva. They proposed that the larva was an innovation that arose from a premature completion of embryonic development. They noted similarities between various larval forms (e.g., apod, polypod, and oligopod larvae) and the changes in morphology seen in embryos of primitive insects during the course of embryogenesis. They proposed that the various larval forms were generated by terminating embryogenesis at various points in this progression. As the larva became the major feeding stage, the number of nymphal instars was reduced, eventually to the single instar that became the pupa.

An opposing view was originally proposed by Poyarkoff (100) and later extended by Hinton (52). They held that the nymphal and larval stages were equivalent. The start of niche separation between the immature and mature forms brought about a widening gap in morphology that eventually had to be bridged by the evolution of the pupa as a transitional stage. This could have occurred through the progressive modification of the last nymphal stage or by the division of the nymphal-adult molt into two discrete molts, first to the pupa and then to the adult. The Poyarkoff-Hinton view is in vogue today (128) and is seen in the common usage of the term larva for the immature stages of both hemimetabolous and holometabolous insects.

As we have discussed previously (147), we think that recent endocrine and developmental data are more consistent with some of the views of Berlese. Hence, throughout the review we retain the use of the term nymph for the immatures of hemimetabolous insects and the term larva for those of holometabolous insects. Moreover, we propose that hemimetabolous insects actually have two distinct immature stages rather than one. The first stage, which we call the pronymph, is typically overlooked but appears to be the basis for the evolution of the holometabolous larva (147).

EMBRYONIC DEVELOPMENT AND THE GENERATION OF THE LARVAL STAGE

For most animals the time of birth or hatching is a critical event that marks the transition from the relatively predictable environment within the egg shell or the womb to the unpredictable environment of the outside world. This transition, though, does not necessarily occur at a fixed time in development as seen for examples of precocial versus altricial young in both birds and mammals. Arthropods also show a great range in the capacity of the newly hatched individual to function in the outside world. An altricial style of development is seen in most arachnid orders,

in that the instar that hatches is typically nonfeeding, lacks pigmentation, has poor locomotor capacity, and continues to utilize its yolk stores (149). It is truly the continuation of embryonic development but outside the shell. In scorpions, the nonfeeding hatchlings are carried for up to two weeks on the back of the mother. In spiders, two nonfeeding instars are passed in the safety of the egg sac. In both cases the young disperse and start feeding only after they have molted to the first juvenile stage. A distinctive hatchling stage is also evident in myriapods (57) and in the terrestrial crustaceans, the woodlice, in which the hatchling instar is carried in the marsupium pouch of the mother (48). The morphological differences between the hatchling and subsequent juvenile stages are most extreme in the nauplius and zoea larvae of aquatic crustaceans. These have evolved modifications to facilitate dispersal in the plankton. These larvae may or may not feed and may progress through a number of morphologically distinct instars before their metamorphosis to the juvenile stage.

A specialized hatchling stage is also seen in the basal orders of insects. There is no general name given to this stage, so we have selected the name used in the dragonfly literature (30) and have called it the pronymphal stage (147).

The Pronymphal Stage of Insects

In the basal groups of insects, the pronymph is a highly specialized stage that differs in many ways from the subsequent nymphal stage (14). It is usually unpigmented and has a reduced set of bristles. Its abdominal cuticle lacks sclerites, and its cuticular ultrastructure (121) differs from that of the nymph. As seen in Figure 1, it has unique morphology and body proportions. As in the hatchling stages of other arthropods, the insect pronymph is typically nonfeeding and subsists on its store of yolk.

In the two ametabolous orders, the bristletails (Archeognatha) and the silverfish (Zygomenta), the pronymph shows only subtle morphological specializations (Figure 1), but its cuticle lacks sclerites and it does not feed. This initial stage lasts for 3–4 days in *Petrobius maritimus* (33) and a similar period in *Ctenolepisma longicaudata* (76), *Thermobia domestica*, and *Lepisma saccharina* (119). Consequently, the ametabolous insects are similar to other arthropods in that their hatchling instar persists for a number of days after hatching. Their pronymphs, however, are not helpless like those of arachnids but have good locomotor abilities like those of crustaceans.

In the hemimetabolous pteryogote insects, the pronymphal stage has a more specialized morphology than seen in apterygotes. Its legs are often bent at locations that do not correspond to normal joints. This morphology would be useless for walking but it insures the proper development of the limbs within the confined space of the egg shell. In the hemimetabolous orders, the production of the first nymphal cuticle has been telescoped back into the late stages of embryogenesis (21, 37, 77, 82, 88, 123; Figure 2). Hence, the insect that hatches is not a true pronymph, as seen in the apteryogotes, but is a pharate first instar nymph still covered by the pronymphal cuticle.

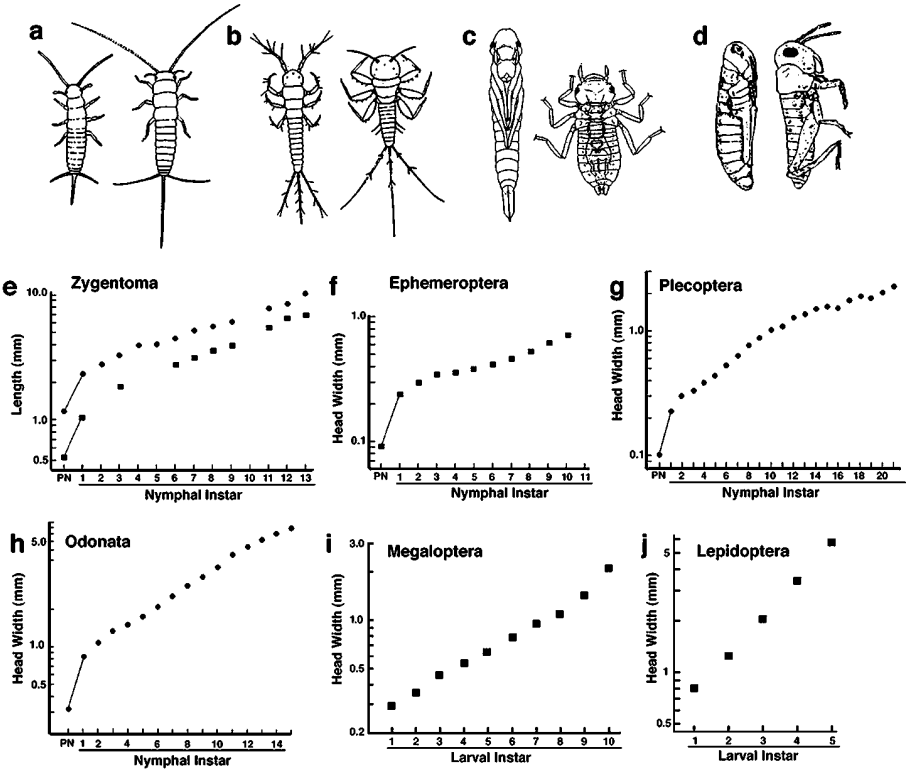

Figure 1 Comparison of the early immature stages of ametabolous (*a, e*), hemimetabolous (*b–d, f–h*), and holometabolous (*i, j*) insect species. (*a–d*) Drawings of the pronymph (*left*) and first instar nymph (*right*) of (*a*) a silverfish *Ctenolepisma longicaudata*, (*b*) mayfly *Stenonema interpunctatum*, (*c*) dragonfly *Epiophlebia superstes*, and (*d*) *Locusta migratoria*. (*e–j*) Comparison of the dimensions of the head or body parts of the immature instars of various insects during postembryonic life. In the ametabolous (*e*) and hemimetabolous (*f–h*) species, there is a marked shift in body proportions between the pronymph (PN) and the first nymphal instar. Typically, no such shift is seen in the immature series of holometabolous species (*i, j*). (*e*) Cercus (*squares*) and antenna lengths (*circles*) for the silverfish, *Ctenolepisma longicaudata*; (*f*) head width of the mayfly *Stenonema interpunctatum*; (*g*) head width of a stonefly, *Neoperla clymene*; (*h*) head width of the dragonfly *Anax imperator*; (*i*) head width of the alderfly *Sialis rotunda*; and (*j*) head width of the tobacco hornworm, *Manduca sexta*. Figure from 147.

In many hemimetabolous insects, such as cockroaches, lice, and true bugs, the pronymph stage is passed completely within the egg shell. The pronymphal cuticle is shed during hatching and the insect emerges from the shell as a first stage nymph (131). In these instances, the form of the pronymph is likely determined solely by the need to adapt to the shape of the egg, although it may also have

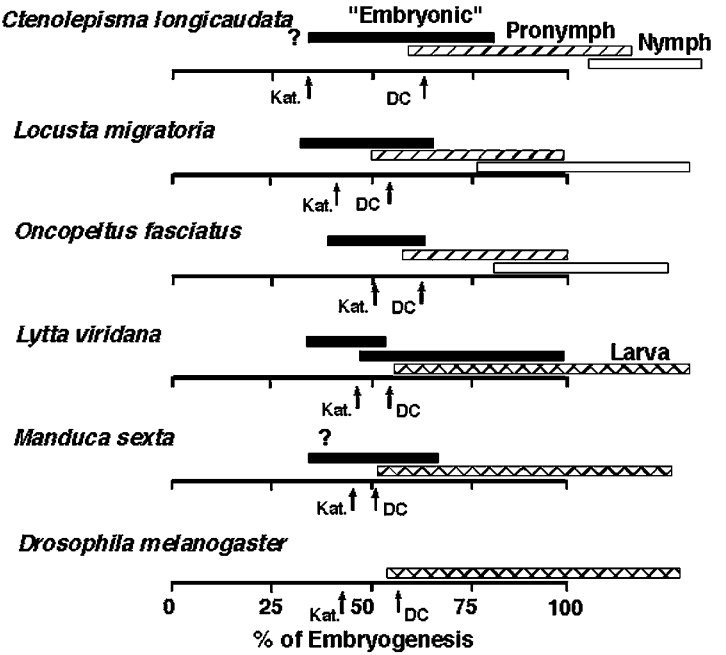

Figure 2 The timing of the production of pronymphal, nymphal, and larval cuticles during embryogenesis of ametabolous (*Ctenolepisma*), hemimetabolous (*Locusta*, *Oncopeltus*), and holometabolous (*Lytta*, *Manduca*, *Drosophila*) insects. Black bars, early embryonic cuticles; hatched bars, pronymphal cuticle; double-hatched bars, larval cuticle; and white bars, nymphal cuticle. DC, dorsal closure; Kat., katatrepsis. Hatching occurs at 100% of embryogenesis. Data from 18, 22, 37, 49, 74; J.W Truman, unpublished.

some specializations, such as egg bursters, that aid in rupturing the chorion. Other hemimetabolous insects have a more modified pronymph because the hatchling escapes from the oviposition site in its pronymphal rather than nymphal form. Consequently, these pronymphs have evolved locomotor adaptations to facilitate in this escape. The pronymph of grasshoppers, for example, is incapable of walking but it can dig through the soil quite effectively (14). By contrast, after the pronymphal cuticle is shed and the insect assumes its nymphal form, it can walk but no longer dig. Similarly, some dragonflies lay their eggs in plant tissue near water, and the newly hatched pronymph may fall onto soil rather than into water. The pronymph uses body flexion to hop over the soil in search of water (31). Once in water, the pronymphal cuticle is shed and the insect then shifts into the locomotor mode characteristic for dragonfly nymphs—walking and jet propulsion.

The Relationship of the Pronymph to the Holometabolous Larva

The portion of the Berlese-Imms hypothesis that different larval forms were generated by curtailing embryogenesis at different phases has been rejected as being too simplistic. For example, many instances of the appearance of abdominal prolegs on larvae have nothing to do with the transient abdominal "appendages" that appear during embryogenesis (51, 91). But discarding this specific hypothesis does not invalidate the general idea that the larva was derived from an embryonic stage found in more basal insects. A comparison between the state of development of a larva and hemimetabolous embryos can best be made for the central nervous system (CNS). The neurons in each segmental ganglion arise from a stereotyped set of neuronal stem cells, the neuroblasts (NBs), each of which produces a characteristic lineage of neurons (7). Each NB in the segmental array is uniquely identifiable based on position, and with only minor changes, the same set of individual NBs are found in ametabolous (*Ctenolepisma*) (146), hemimetabolous (grasshopper) (7), and holometabolous insects (*Drosophila*) (35, 142). In the first two groups, the NBs generate all their progeny during embryogenesis, and the insect hatches with a complete set of neurons in its segmental ganglia. In holometabolous insects, in contrast, the NBs begin proliferation at the appropriate time in embryogenesis but then become quiescent after producing an initial set of neurons for the larva. The quiescent NBs then reactivate during larval life and generate the remainder of the neurons needed for the adult nervous system (145). Indeed, in terms of the numbers and types of neurons, *Drosophila* and *Manduca* larvae are more similar to a mid-stage grasshopper embryo than they are to a nymph.

The pronymphal instar is appropriately placed during embryogenesis to have been coopted to become the larva. The pronymphal stage begins after katatrepsis, the morphogenetic movements that bring the embryo to its final position in the egg. The pronymphal cuticle begins to be deposited about the time of dorsal closure (Figure 2). It is the first cuticle to be made after the insect has an intact body surface and also the first cuticle that is substantial enough to be actively shed (rather than simply fragmenting). In embryos of holometabolous insects, the cuticle deposited at the corresponding stage in development is that of the first instar larva (Figure 2). A late round of cuticle production corresponding to that of the nymph is not seen in holometabolous embryos.

Similarities between the pronymph and the larva are also evident in the peripheral sensory system. At the start of the pronymphal stage, the body wall of grasshopper embryos already has its full set of proprioceptive neurons, including stretch receptors and chordotonal organs (81). These early-born neurons establish the main sensory nerve branches, and large numbers of mechano- and chemosensory neurons follow these pathways into the CNS during the subsequent formation of the nymph. The body wall of newly hatched larvae of *Drosophila* and *Manduca* have the proprioceptive neurons corresponding to those of the pronymph but relatively few sensory bristles (81). This set of neurons constitutes the entire larval

sensory system for *Drosophila* (62), whereas *Manduca* adds additional sensory bristles during subsequent larval molts (46). In the legs, mouthparts, and antennae of the pronymph, a few early-born neurons likewise pioneer the pathways to the brain and ventral ganglia (8). It is likely, although not yet demonstrated, that the stereotyped arrays of sensilla on the antennae and palps of larvae were also derived from the corresponding pioneer neurons of the pronymph.

These similarities support the hypothesis that the holometabolous larva is derived from the pronymphal stage. Although the latter stage usually involves only a single instar, as the larva it has a number of instars with a relatively stable morphology within the series (Figure 1). The morphological shift corresponding to the pronymph-nymph transition has been deferred until the end of larval life, when the larva transforms into a pupa. There are obvious exceptions to a morphologically stable larval stage, as most dramatically shown in the hypermetamorphosis of meloid beetles, strepsipterans, and mantispids. These insects have an active triungulin stage that hatches from the egg and later molts into a grub-like larva. Hypermetamorphosis, however, is a highly derived condition, related to a parasitic lifestyle. The earliest holometabolous insects likely had a rather uniform series of larval instars.

Scenario for the Evolution of the Larval Stage

A possible scenario for the transformation of the pronymph into the nymph has been presented before (147), so we only briefly touch on the salient points. We think that a necessary preadaptation for this transformation was having a pronymphal stage capable of feeding. This is not unprecedented. For example, in arachnids, the larval stage of ticks and mites has evolved into an active feeding stage. For hemimetabolous species in which the female hides her eggs in soil, plant material, or under bark, the pronymph has evolved novel locomotor adaptations for moving through this microhabitat and escaping from it. These types of adaptations, coupled with an extended pronymphal stage, similar to that still found in ametabolous insects, would place the pronymph in a novel microhabitat not available to the nymph or the adult. If the pronymph could feed, it could exploit resources that could not be used by either the nymph or the adult. The availability of good-quality resources would favor pronymphs with extended development in this stage and with adaptations for more efficient locomotion and food gathering. It is interesting that among the earliest fossil holometabolous larvae are those found in plant galls (73), a situation that might arise if females initially hid their eggs in plant tissue.

In this scenario, there initially would have been two stages, the pronymph (now the larva) and the nymph, that were both involved with feeding and growth. The larva would not compete with the adult for resources, whereas the nymph and the adult would still be potential competitors. If selection favored separating the resources utilized for growth from those used for reproduction, then the larval form would be favored and all growth would eventually occur in the larval form. As originally proposed by Berlese (13), we think the nymphal stages were relegated

to a series of nonfeeding instars at the end of larval growth. In hemimetabolous insects, the number of nymphal instars can be quite plastic, depending on the amount of growth that has to occur (92). With the amount of growth reduced to nil, the nymph would be reduced to a single instar, which was later modified to be the transitional stage between the larva and the adult.

It is interesting that this pathway to metamorphosis may have been attempted independently by the thrips (Thysanoptera). These insects accomplish their growth during the three larval instars but then have 2–3 nonfeeding pupal instars before the final molt to the adult (86).

THE TIMING OF IMAGINAL PROLIFERATION: THE ORIGIN OF IMAGINAL DISCS

In addition to the generation of a novel larval form, the evolution of insect metamorphosis also required that the adult form eventually be generated at the end of larval life. Much of our general picture of the development of the adult comes from higher flies such as *Drosophila*. For most of its organ systems, there is a complete separation between larval and adult tissues, and cellular carry-over from one stage to the next occurs in only a few organs such as the nervous system and the Malpighian tubules. The larva carries sets of diploid imaginal cells—the imaginal discs and imaginal nests (16a, 29). These are tucked away in its body and contribute little or nothing to the functioning of the larva. They typically proliferate during larval life and at metamorphosis differentiate into an adult organ to replace their larval counterpart. This strategy of the early sequestration and formation of imaginal discs is typical for most imaginal structures of higher Diptera and for the wing discs of Lepidoptera, both of which have been popular experimental systems over the years (95).

This type of development, however, does not represent the way that imaginal structures form in all holometabolous larvae (139). In many larvae, the future imaginal tissues play a functional role in the larva (rather than living as a virtual parasite). An example is seen in the development of the wing imaginal disc in the mealworm beetle, *Tenebrio molitor* (102). Prior to the last larval instar, there are no wing imaginal discs, and all of the epidermal cells in the lateral thorax produce larval cuticle during each larval molt. A few days after the onset of the last larval instar, cells in the lateral margins of the meso- and metathorax become columnar, detach from the overlying cuticle, and begin to proliferate. As the wing imaginal disc forms, it first in-pockets into the body cavity but then evaginates into the space that forms between the epidermis and the overlying cuticle. It is interesting that among larvae that wait until the last larval instar to start their wing growth, some invaginate to form a wing disc, whereas others evaginate in the space between the epidermis and the larval cuticle to form a wing bud (144).

For the remainder of the review, we use the following terminology as we define it here. An epithelium that has locally detached from the cuticle and begun

proliferation is an imaginal disc (regardless of whether it forms early as in *Drosophila* or late as in *Tenebrio*). In the case of larvae with late-forming discs, a specialized region of the epidermis that gives rise to the imaginal disc is an imaginal primordium. The cells of the primordium participate in larval functions, such as the production of the larval cuticle, but they are special because of their eventual fate. A primordium can often be identified in earlier instars because it lacks specialized features such as bristles and muscle insertions, and its cells are relatively small and diploid.

The Ancestral Pattern of Imaginal Growth

Given the different patterns of timing of formation of imaginal discs, which condition is the ancestral one? An analysis of wing disc formation in a variety of holometabolous larvae (128, 147) suggests that the ancestral condition is to defer imaginal growth until the end of larval life. Late-forming imaginal discs are found in basal holometabolous orders of Megaloptera, Neuroptera, and Mecoptera. Also, the more basal families in the Coleoptera, Diptera, and Hymenoptera all delay their imaginal disc formation until the last larval stage. The crown groups in the latter orders, though, show discs that arise early in larval life. Overall, the evolution of early-growing imaginal discs has occurred at least six times in the Holometabola (147).

Two major benefits have arisen from the evolution of complete metamorphosis: resource partitioning between larva and adult and the evolution of rapid life cycles. These two changes have made holometabolous insects the consummate exploiters of ephemeral food sources such as fruit, flowers, carrion, and dung. These two innovations, though, appear to have arisen sequentially in insect evolution (128). The ancestral condition is one in which adult-related growth begins only at the end of larval life and, consequently, an extended prepupal period is needed to accommodate this growth. The more basal holometabolous insects have gained resource partitioning but at the price of extending the length of their life cycle. The subsequent shortening of the life cycle only came later with the evolution of early-forming imaginal discs that allowed adult growth to begin early in larval life.

It should be stressed that simply because one adult structure, such as the wing, arises according to the derived pattern does not mean that all of the discs in a larva originate in the same manner. In Lepidoptera, such as *Manduca*, the wing imaginal disc grows according to the derived pattern of early disc formation, whereas the imaginal discs for the legs, antennae, and mouthparts all follow the ancestral pattern of appearance in the last larval stage. In contrast, in higher flies, almost all of the adult epidermal structures arise according to the derived pattern with only the abdominal histoblasts showing an ancestral pattern of late proliferation (78, 79). Consequently, during the evolution of larval forms in a given group, the option for early growth can be made on an organ-by-organ basis, presumably by balancing adaptive needs with existing developmental constraints.

The selective pressures for the early formation of an adult-specific structure such as the wing are relatively easy to understand because this structure has no function in the larva, and the few cells that represent the wing primordium can be invaginated and formed into a disc without affecting larval function. The issues become more complex in the case of structures that may be used in both the larva and the adult, such as the legs and antennae. The relationship between the larval and adult form of an appendage can be nicely seen for the antenna of *Bombyx mori* (139). In the larval structure, specialized olfactory sensillae are located on the distal regions of the second and third antennal segments, but the more proximal regions are relatively unspecialized and represent the adult antennal primordium (Figure 3). Early in the last larval stage, the primordium detaches from the cuticle and begins rapid proliferation to form a prominent antennal disc (139).

The situation is topologically more complicated in the case of the leg primordium in Lepidoptera. This primordium has a complex morphology because the femur, tibia, and tarsus of the larval leg all contribute to the corresponding regions of the adult leg (67). In the last larval instar, the cells of the leg primordium detach and begin rapid growth as a complex leg imaginal disc. Given the constraint that each major segment of the larval leg contributes to part of the leg primordium, the logistical problems of forming an early-developing imaginal disc in the confined regions of the larval leg appear to be formidable. Consequently, there may be developmental compromises that have to be established relating to the needs of the larva having a complex leg versus the overall need for a rapid life cycle. We clearly need more information about how complicated structures like legs and mouthparts are transformed during metamorphosis in a range of holometabolous insects.

Lessons from Prothetely

Although detailed comparative data are lacking for the metamorphosis of complex appendages, we can glean some information from the descriptions of cases of prothetely reported for holometabolous larvae. Prothetely is the appearance of precocious pupal structures in the larva (153) and can sometimes be elicited experimentally in larvae by implantation of the corpora allata or by application of juvenile hormone (JH).

In the case of larvae of the butterfly *Precis coenia*, the structures that form after treatment with JH mimics in the final instar are grotesque structures that are a mosaic of larval and pupal cuticle (69). Pupal cuticle is secreted by cells of the growing imaginal disc, whereas larval cuticle is remade by the epidermis outside of the primordium region. The extent of the grotesque transformation depends on the timing of JH application. In the leg, for example, treatment with JH early in the last larval stage before the leg primordium has detached results in a perfect supernumerary larval leg. If the treatment is delayed until the primordium has detached and transformed into an imaginal disc, then a larval-pupal mosaic is produced. The extent of the pupal region is determined by how much the leg

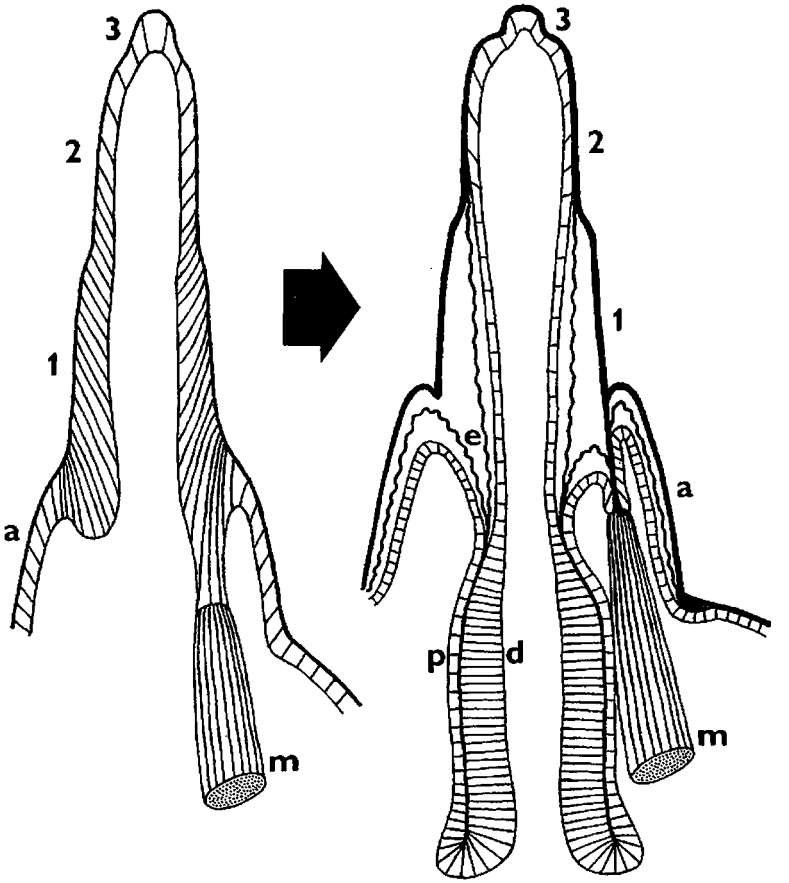

Figure 3 Schematic representation of the formation of the antennal imaginal disc during the last larval stage of *Bombyx mori*. The larval cells remain attached to the tip of antennal segments 2 and 3, but the more proximal epidermis detaches to form the antennal disc. a, articulating membrane; d, antennal disc; e, ecdysial membrane; m, muscle; p, peripodial membrane (139).

disc has grown prior to the treatment with JH. Hence, the ability of the leg to form a true mosaic is due to the presence of a discrete primordium in the leg. Unlike lepidopteran larvae, larvae of the flour beetle, *Tribolium confusum*, that show prothetely do not produce a mosaic leg. Rather their legs show intergrades between the larval and the pupal state (90). The lack of mosaicism may mean that these larvae may not possess discrete imaginal leg primordia.

Figure 4 summarizes the different relationships that may exist between the larval and adult forms of a structure such as the leg. In the simplest condition (which is likely ancestral), the cells that make the larval leg are polymorphic cells that can

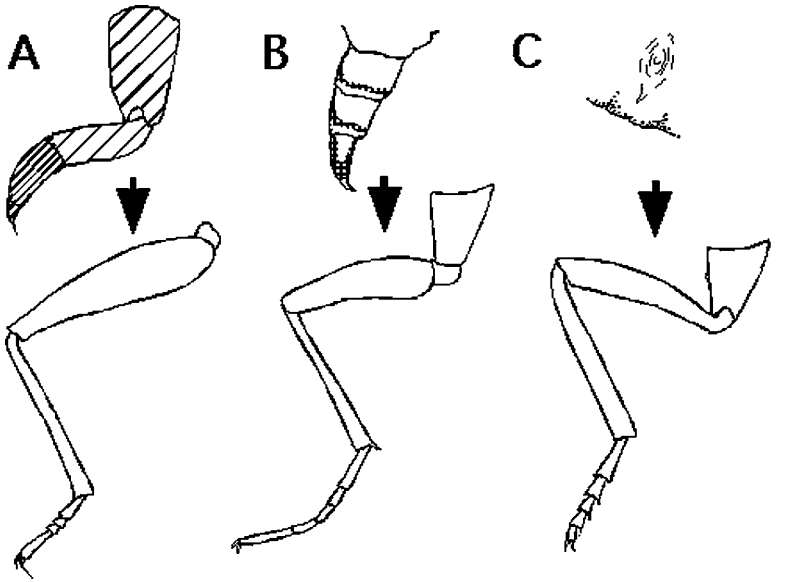

Figure 4 Different grades in the relationship of larval and adult tissue in the leg. (*A*) There is no primordium and the cells of the larval leg are polymorphic (*hatched*) and subsequently form the adult leg. (*B*) Although all epidermis of the larval leg forms larval cuticle, some regions (*cross-hatched*) constitute a primordium that forms most or all of the adult leg. Most of the specialized larval region (*white*) does not survive metamorphosis. (*C*) The imaginal primordium is transformed into an invaginated, early-growing imaginal disc that has no function in the larva. The larval tissue is complete replaced.

make larval, then pupal, then adult cuticle as the leg undergoes its metamorphic transformation. This would make the beetle leg similar to the well-documented case of the abdominal epidermis of Lepidoptera, in which the same cells make the cuticle for the larva, pupa, and adult (109). A more derived condition is represented by the lepidopteran leg in which there has been a restriction of cell populations into a primordium region versus the remainder of the leg. The primordium cells make larval cuticle during the molts but then can transform into an imaginal disc that subsequently makes the pupal and adult structures. An interesting question is whether the larval cells outside of the primordium are terminally specialized and are capable of making only larval cuticle. The most extreme condition is then represented by *Drosophila*, in which the larval structure is no longer found and the cells of the leg primordium no longer play a functional role in the larva. Instead, they invaginate to form an early-growing imaginal disc (29).

Naturally occurring prothetely has been reported in basal holometabolous groups such as the Raphidioptera (5) and the Megaloptera (39) and is often associated with

exposing the larvae to abnormal temperature regimens. In the snakeflies (Raphidioptera), the protheletic larva shows precocious formation of wing pads, compound eyes, and genital pads (5). The remaining larval structures, such as the mouthparts, antennae, and legs, appear to be unaffected. In the alderfly *Sialis lutaria*, the precocious structures are pupal wings and compound eyes, whereas the rest of the animal remains larval (39). This variation suggests that in these basal holometabolous insects, imaginal primordia may be involved in making only wings, eyes, and genitalia. The more extensive use of primordia, for legs, antennae, and mouthparts, may have come about as larval appendages were simplified and diverged further from their adult counterparts.

ENDOCRINE MECHANISMS ASSOCIATED WITH THE EVOLUTION OF METAMORPHOSIS

The main developmental hormones in insects are the family of hydroxylated steroid hormones, the ecdysones, and the family of sesquiterpene hormones, the JHs (92). In general, the ecdysones are molting hormones, with periodic surges causing the events involved in molting, whereas JHs are status-quo hormones that maintain the insect in its current form as the insect is responding to the molting surge of ecdysone.

In immature insects, the ecdysones are typically produced by the prothoracic glands or their anatomical equivalents (e.g., the ventral glands of Orthoptera) (104). In beetle larvae, however, oenocytes (118) and even general and disc epidermis (37, 83) are also thought to synthesize and release ecdysones. Although the traditional product of the prothoracic gland is α-ecdysone, that of *Manduca* secretes a mixture of α-ecdysone (αE) and 3-dehydroecdysone (3DE), with the latter rapidly converted to α-ecdysone by blood enzymes (43). Peripheral tissues, especially the midgut, then metabolize the E to 20E. This biochemical conversion can be either rapid or, as in the adult molt of many Lepidoptera, delayed for a number of days. Because, as described below, the two ecdysones differ in their range of biological actions, the shift in the composition of circulating ecdysteroids has important implications for the timing of developmental switches.

The inactivation of 20E comes about through two different pathways (104). Irreversible inactivation occurs through further hydroxylation of 20E to 20,26 dihydroxyecdysone. Alternatively, the hormone may be inactivated by sequestration in the form of polar conjugates, such as 20E-phosphate. The polar conjugates can then be excreted or stored. During oogenesis, 2-deoxyecdysone and ecdysone conjugates are put into the egg, presumably to be used in development before the embryo's own endocrine system becomes functional.

The JHs are expoxymethyl farnesoate (JH-III) and a number of related structures derived from homomevalonate (125). JH-III appears to be the most common JH in most insects, but Lepidoptera rely on JH-I and JH-II, whereas some of the higher Diptera have JH-III-*bis*-epoxide as well as JH-III. The synthetic source of JH is

the corpora allata. The metabolic inactivation of JH involves an attack on either the ester or the epoxide group (116). A curious developmental modification is seen in *Manduca* early in metamorphosis, at which time the corpora allata secrete JH acid, the normally inactive metabolite of JH (15). Imaginal discs then locally convert the JH acid to JH using methyl transferase (137). There is a local requirement for some imaginal discs to be exposed to JH during the larval-pupal transition to prevent them from undergoing precocious adult differentiation (66, 154). There are also some indications that JH acid may be important in the determination of competence for metamorphosis (60, 61).

JH is found only within the insects, but in crustaceans the mandibular gland makes the related methyl farnesoate (56). This compound has a clear role in reproduction but has little effect on the morphological changes that accompany adult reproduction. Methyl farnesoate has also recently been found in barnacle cyprids, and exposure of cyprids to exogenous methyl farnesoate causes precocious metamorphosis (134). It is interesting that only high concentrations had this effect. Low concentrations, comparable to those seen in the day 0 cyprid, inhibited metamorphosis.

Endocrine Changes During Embryogenesis

Although most attention has focused on the role of the developmental hormones during postembryonic life, they also have a complex role during embryogenesis (38, 55, 75). We think that shifts in the endocrinology of embryogenesis have been important for the evolution of the larval stage. Two changes had to occur to transform the pronymph into the larva. First, the trend in the hemimetabolous orders is for the pronymph to be a transitional stage, with tissue maturation occurring as the nymph is formed. There had to be advancement in tissue maturation into the pronymphal stage so it could cope with conditions in the outside world. Second, there had to be a suppression of tissue patterning processes that originally led to the adult body plan, and these modified to generate the novel form of the larva.

In accordance with some of the ideas of the late V. Novák (94), we think that JH has been an important agent in modifying embryonic development of hemimetabolous insects. Figure 5 illustrates the ecdysone and JH titers during embryogenesis of the locust, *Locusta migratoria*. Peaks of ecdysones occur periodically during embryogenesis, coinciding with the production of the serosal cuticle and the three embryonic cuticles. JH is present in the newly laid egg as a result of its gonadotropic role during oogenesis, but then is metabolized by specific esterases as embryonic development begins (115, 116, 141). Consequently, JH is absent in midembryogenesis, when the embryo is undergoing most of its morphogenesis and growth. It then reappears prior to the beginning of the nymphal molt (141) and in association with the final histodifferentiation of the embryonic tissues. The delay in the appearance of JH until just prior to the nymphal molt is also seen in the cockroach *Nauphoeta* (58), and the bug *Oncopeltus fasciatus* (36).

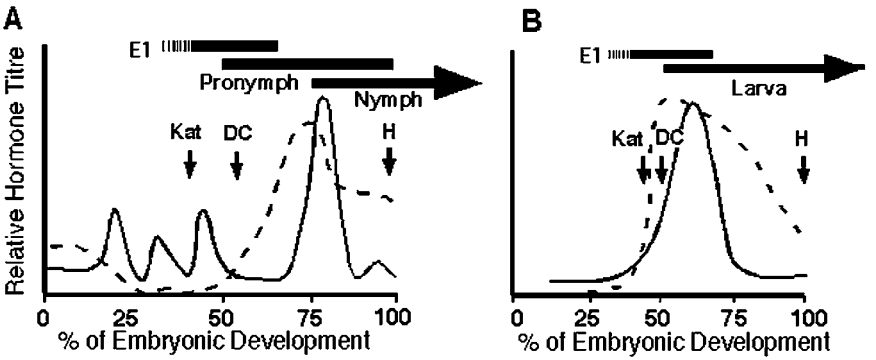

Figure 5 Comparison of the titers of ecdysones (*solid line*) and juvenile hormones (*dashed line*) during embryogenesis of (*A*) the grasshopper *Locusta migratoria* and (*B*) the lepidopterans *Manduca sexta* (JH) and *Heliothis virescens* (ecdysones). DC, dorsal closure; H, hatching; Kat, katatrepsis. Data from 12, 42, 74, 141.

The importance of a JH-free period during insect embryogenesis has been explored by the application of JH or JH mimics at various times during embryonic development. Slama & Williams (132) first discovered the ovicidal actions of JH on the eggs of the linden bug *Pyrrhocoris apterus*. These studies were followed by studies on the moth *Hyalophora cecropia* (105, 114), the locust *Schistocerca gregaria* (93), and *Drosophila melanogaster* (135) as well as studies on many pest species (138). A consistent feature of the response to applied JH is an interference with the process of katatrepsis so that the embryos assume abnormal positions in the egg. Effects on the growth and morphogenesis of the embryo itself, though, are extremely varied. JH applied to the eggs of Lepidoptera (105, 114) have relatively little effect on growth, whereas JH treatment of the eggs of the locust, *S. gregaria* (93; J.W. Truman, L.M. Riddiford & E.E. Ball, unpublished data), and the firebrat, *Thermobia domestica* (117; JW Truman & LM Riddiford, unpublished data), results in miniature embryos with reduced appendages.

The most detailed analysis of the effects of JH mimics on the embryos of lower insects is that by Novák (93) on *Schistocerca*. He observed a range of dose-dependent effects including extremely small nymphs with apparently a reduced number of abdominal segments at high doses. Not only was embryonic growth reduced, but also certain nymphal characters, such as tanned mandibles, appeared earlier in the JH-treated embryos. Sbrenna-Micciarelli (122) later showed that these embryos secreted only two cuticles rather than the three that normally form during embryogenesis. In the JH-treated embryos, the second cuticle had bristles and the ultrastructure of nymphal cuticle rather than that of pronymphal cuticle. Indeed, this second molt became the terminal molt of embryogenesis, even though the embryo was only 10–20% of its normal size. Similarly, in the cricket *Achaeta*, early JH treatment prevented the appearance of the labral "teeth" used by the

pronymph to rupture the egg shell and caused the premature formation of sclerotized mandibles characteristic of the nymph (D. Ereyilmaz, L.M. Riddiford & J.W. Truman, unpublished data).

Although it is possible to evoke early differentiation in embryos of lower insects by treatment with JH, the complementary result of suppressing nymphal differentiation by blocking JH production late in embryogenesis has been harder to achieve. The best results have been reported for the cockroach, *Nauphoeta cinerea*, in which embryos were treated with the allatocidal compound, precocene (19). These treated embryos apparently developed into nymphs, although there was a suppression of the final differentiation of some internal tissues such as the gut. In grasshoppers, in contrast, chemical allatectomy via precocene had little effect on embryogenesis, although the insects later showed precocious metamorphosis (97). The variability in the effectiveness of this treatment may relate to the fact that the chemical allatectomy requires that the corpora allata become synthetically active so that the precocene can be converted into its cytotoxic metabolite. It is not known how much JH is produced before the glands are destroyed.

Experimentally, then, JH produces the types of effects that would facilitate the evolution of the larval stage. It induces premature differentiation of the embryo, with an accompanying alteration of morphology. One would then expect the appearance of JH to be relatively advanced in embryos of holometabolous insects. At this time, detailed information on the titers of JH during embryogenesis is available for only one order in the Holometabola. For the lepidopterans *H. cecropia*, *Manduca sexta*, and *B. mori* (12), JH is already at its highest levels at the time of katatrepsis, prior to the secretion of the first larval cuticle (Figure 5). If the Lepidoptera are characteristic of the Holometabola in general, there has indeed been a heterochronic advancement in the appearance of this hormone.

As described above, the development of embryos of holometabolous insects is less affected by exogenous JH compared with insects from the more basal groups. For example, in *H. cecropia* (114), *Bombyx*, and *Manduca* (L.M. Riddiford & J.W. Truman, unpublished data), the application of JH early in embryogenesis blocks katatrepsis, but it does not suppress growth or cause marked premature differentiation. This reduced effectiveness of JH treatment on embryonic growth and differentiation is consistent with a scenario in which the processes ancestrally suppressed by JH no longer occur in the embryo and have been deferred to metamorphosis. What remains is a basic developmental core that is JH insensitive.

In summary, an advancement in the time of appearance of JH during embryogenesis may have been a key development in the transformation of the pronymphal stage into a functional larva. In both grasshoppers and *Thermobia*, the experimental application of JH causes precocious differentiation to occur during the pronymphal period. Moreover, the differentiation of appendages can occur before they are completely patterned. Hence, JH has the ability to disrupt the formation of the adult body plan to force the differentiation of structures based on incomplete patterning information. This modified patterning information might then have provided the basis for producing the novel form of the larva. A crucial question for the future

is the way in which JH might disrupt the functioning of the patterning genes involved in the formation of the appendage such as *decapentaplegic*, *wingless*, and *hedgehog* (24, 29, 32).

The embryonic effects of JH are complex and not well understood. A consistent feature of the action of this hormone across all insect groups is an interference with katatrepsis, but embryos vary widely in how JH treatment affects their growth. In embryos of more basal insects, most of embryonic growth occurs after katatrepsis, whereas this is not the case for most holometabolous insects. Consequently, we do not know whether JH suppresses the late embryonic growth directly or if its interference with katatrepsis (41) indirectly results in growth suppression. Also, during the molt to the pronymph, the main active steroid is αE and an unknown ecdysteroid, whereas 20E becomes prominent only during the molt to the nymph (58, 74). In postembryonic life, 20E is associated with differentiation programs, whereas αE is more associated with proliferative growth and patterning (25, 53, 112). It is not known whether the strong differentiative effects of JH are due to the direct action of this hormone or due to alterations in the composition of the steroids that are then secreted.

Postembryonic Modifications in Endocrine Signaling

From an endocrine perspective, nymphs and larvae are similar to each other in that their status-quo molts are caused by ecdysone acting in the presence of JH. These groups of insects, however, differ markedly in the patterns of endocrine secretion that coordinate metamorphosis (Figure 6; 108). In hemimetabolous insects, the JH titer drops to undetectable levels at the onset of the last nymphal stage, and the next ecdysone surge then causes the formation of the adult stage. In the Holometabola, in contrast, JH declines at some point in the last larval stage. There follows a small surge of ecdysone that terminates larval feeding, promotes premetamorphic behaviors such as cocoon spinning, and commits larval tissues to pupal development (92, 106). The cuticle of the pupal stage is produced later in response to a second large surge of ecdysteroids. This ecdysone surge is associated with the transient reappearance of JH. A similar reappearance of JH at the onset of metamorphosis is lacking in hemimetabolous insects. JH is then eliminated just before pupal ecdysis so that the subsequent surge of ecdysone in the pupa causes adult commitment and differentiation. Here we deal with the possible origins of the holometabolous endocrine pattern and the manner by which tissues have evolved to cope with this new endocrine landscape.

ENDOCRINE CONTROL OF IMAGINAL GROWTH How do the modified endocrine titers in the holometabolous insects relate to the deferred growth and differentiation of the imaginal tissues? In the derived imaginal discs found in higher Diptera, such as *Drosophila*, the rate of cell addition during larval growth is apparently constant without reference to the periodic steroid surges that cause the larval molts (20). In the Lepidoptera, such as *B. mori*, there are quantitative changes in cell division

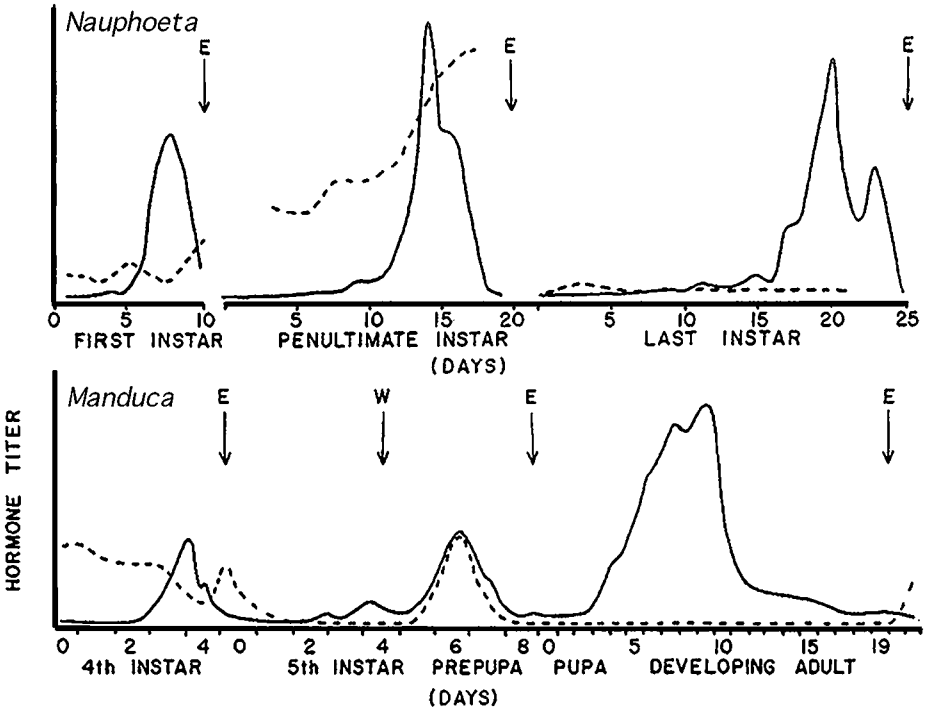

Figure 6 Schematic of juvenile hormone (*dotted line*) and ecdysone (*solid line*) titers in (*top*) the cockroach *Nauphoeta cinerea* and (*bottom*) the moth *Manduca sexta*. E, ecdysis; H, hatching; W, wandering. Data summarized in 75a and 108.

in the wing discs associated with ecdysone and JH but no absolute control (72). In the imaginal tissues that show the ancestral pattern of growth, however, the developmental hormones play a major role in regulating growth.

The adult compound eye in *M. sexta* arises according to the ancestral pattern of development (25, 84). The adult eye primordium is a crescent of diploid cells that lie just anterior to the larval simple eyes (the stemmata). About 36 h after ecdysis into the last larval stage, the primordium begins its transformation into the eye imaginal disc. A series of in vitro and in vivo experiments have indicated that the transformation of the primordium into the eye imaginal disc is signaled by the removal of JH (D.T. Champlin, L.M. Riddiford & J.W. Truman, unpublished data). For example, when the eye primordium area was excised from penultimate stage larvae and maintained in culture, it detached and began proliferation within about 24 h if cultured in the absence of JH, but it remained dormant when physiological concentrations of JH were present. Therefore, the presence of JH during larval life tonically suppresses the eye primordium and prevents it from transforming into the eye imaginal disc.

The role of JH in repressing imaginal disc formation in the larva is consistent with its actions in the embryo to repress aspects of morphogenetic growth that would normally produce the adult form (147). The continuing presence of JH during larval growth maintains this repression, which is finally removed in the last instar when the JH disappears. Morphogenetic growth is free to resume but in the context of forming an imaginal disc.

If the regulation of growth of the eye disc in *Manduca* is a general model for the ancestral pattern of growth control of imaginal tissues, then imaginal discs that begin growth in early larval instars where JH is continuously present (108) must somehow have escaped its suppressive action. Possible mechanisms could involve the selective loss of JH receptors or the local production of JH-catabolic enzymes to reduce the JH levels in the particular tissue. At this time, the JH receptors have not yet been identified. The role of local metabolism of JH has some support in the report that wing discs of the lepidopteran *Galleria mellonella* acquire high levels of JH esterase activity early in the final larval instar, prior to the enzyme appearing in the hemolymph to wipe out the circulating JH (103). Whatever the mechanism, this escape has occurred at least six times in the holometabolous insects (147); it will be of interest to determine if the premature growth of imaginal discs arose in different ways.

An interesting feature of the metamorphosis of *Drosophila* is that, like other higher flies, this insect is quite refractory to treatment with exogenous JH (107, 111). The tissues that form from the imaginal discs make normal pupal and adult structures regardless of the presence of high exogenous JH during the final two larval instars. This is not surprising because it was likely a loss of sensitivity to JH that allowed these discs to begin growth early during larval life (79). The only epidermal tissues in *Drosophila* that are still responsive to JH treatment at the onset of metamorphosis (pupariation) are the abdominal histoblasts (99, 107). These histoblasts do not begin to divide until 3 h after pupariation (78) and consequently are the only epidermal derivatives in the fly to show an ancestral-like pattern of proliferation control.

THE ENDOCRINOLOGY OF THE LARVAL-PUPAL TRANSITION The larval-pupal transition of holometabolous insects shows endocrinological features that have no counterpart in the postembryonic development of hemimetabolous insects (Figure 6). One unique feature is the commitment peak of ecdysteroids that terminates larval feeding, initiates premetamorphic behaviors, and commits larval tissues to a pupal fate (92, 109). The second feature is the reappearance of JH (or JH acid) during the prepupal peak of ecdysone (6). This JH is critical for the normal larval-pupal transition, although species vary dramatically in their response to this hormonal cue, as seen in the effects of removal of the corpora allata during the final feeding stage. The most extreme responses known are in *H. cecropia*, in which allatectomized last stage larvae transform into pupae that have extensive adult characters including the compound eyes, antennae, and wings. (154). In *Manduca*, major effects are seen in the developing compound eye and a patch

of tissue around the wing hinge, but the other imaginal structures are fully pupal (66). In *B. mori* and *G. mellonella*, the insects are fully pupal externally (17, 98). What is the difference between these species in the context of the requirements for JH in the larval-pupal transition?

It is informative to examine the function of these various hormonal signals from the perspective of a tissue that shows an ancestral growth pattern such as the eye imaginal disc in *Manduca*. Although the proliferation in the growing imaginal disc can be suppressed by JH, this JH control is lost after the tissue is exposed to the commitment peak of ecdysone (D.T. Champlin, L.M. Riddiford & J.W. Truman, unpublished data). Proliferation continues for the next two days in the committed disc but in a JH-independent fashion. The rising titer of the prepupal peak of ecdysteroids begins the patterning of the eye imaginal disc (25, 84). This process starts in the posterior margin and gradually moves anteriorly as a distinct furrow that progresses across the disc. The cells in the wake of the furrow acquire cell fates and organize themselves into proto-ommatidial units. The pupal molt occurs relatively early in this patterning process, and cells both anterior and posterior to the furrow produce pupal cuticle. If JH is removed, however, the undetermined cells anterior to the furrow still make pupal cuticle, but the determined cells behind the furrow differentiate into their adult structures such as rhabdomes, screening pigments, and crystalline cones. These observations suggest that during the larval-pupal transition, JH is especially important for cells that have adopted their final fates prior to the production of pupal cuticle. These decisions may not occur at a constant time for all species. Hence, the lack of an adult overshoot in allatectomized *Bombyx* and *Galleria* may simply reflect that, in their imaginal tissues, the cells have not reached the point of cellular determination by the time the prepupal ecdysone peak forces the production of cuticle. In allatectomized *H. cecropia*, in contrast, many cells may have made this decision by the time the prepupal peak occurred.

In holometabolous insects, the reappearance of JH during the larval-pupal transition may be a replay of an ancestral relationship between JH and the development of embryonic tissues. For example, during embryogenesis in more basal insects, JH is absent during the early growth of appendages but then appears as they undergo their nymphal differentiation. In holometabolous insects, much of this embryonic growth has been deferred to the last larval stage after JH titers have declined, but JH then reappears during the larval-pupal transition as the newly grown tissue differentiates to its pupal (nymphal) state.

MOLECULAR MODIFICATIONS ASSOCIATED WITH METAMORPHOSIS

At the molecular level, we know the details of the action of the developmental hormones quite well for selected holometabolous insects. It is unfortunate that we have relatively little comparative data from hemimetabolous or ametabolous species to allow us to draw evolutionary relationships and patterns. Nevertheless,

there are some molecular trends that are worth discussion and some areas that will be fruitful for future study.

Evolution of the Ecdysone Receptor Complex

The ecdysone receptor complex, consisting of the ligand-binding ecdysone receptor (EcR) and its heterodimeric partner ultraspiracle (USP), is conserved through the arthropods (47, 112) and also found in some parasitic nematodes (133). EcR has two to three isoforms that have been reported in holometabolous insects including *Drosophila*, *Tenebrio*, *Manduca*, and *Bombyx*, but thus far only one isoform has been found in *Locusta* (112). At the onset of metamorphosis, there are different receptor profiles in larval versus imaginal tissues (64, 140, 148). USP has a single isoform in *Drosophila*, but there are multiple isoforms of USP in *Manduca* and *Aedes*. There is a shift in USP isoforms during molting in *Manduca*, as dictated by the hormonal milieu (54, 63), and in *Aedes* during vitellogenesis (151). Consequently, there may be special usage of the EcR and USP isoforms associated with metamorphosis. Indeed, analyses of mutants in *Drosophila* lacking particular EcR isoforms indicate that there are functional differences among the isoforms (11, 127) The lack of information about whether there are multiple EcR and USP isoforms in hemimetabolous insects prevents us from speculating about the possible evolution of the isoforms and their function at present.

USP is an ancient nuclear receptor whose ortholog, RXR, is found in vertebrates and partners with diverse receptors such as the thyroid hormone receptor and the retinoic acid receptor (80). RXR is found throughout the metazoans, including the jellyfish (47, 112). For most of the animals, both DNA and ligand-binding domains of the protein show patterns of divergence that are expected considering the phylogenetic distances of the animals. However, there has been a rapid divergence of the ligand-binding domain within the Lepidoptera and the Diptera. The abrupt change in this domain is intriguing but its significance is unclear. One possibility is that RXR may have had a ligand that constrained its evolution and that the ligand was lost or changed in the Lepidoptera and Diptera (47). Alternatively, USP may have gained a ligand. *Drosophila* USP binds JH with low affinity (65), but the recent structural analysis of its ligand-binding domain (27) and that of *Heliothis* (16) shows that neither JH nor methoprene can displace the bacterial phospholipid sequestered by the recombinant protein in its ligand-binding pocket. JH acid, however, conceivably could fit into this pocket (16). More work is necessary to resolve the role of ligand in the evolution of USP from RXR.

Evolutionary Aspects of Ecdysone Action

Outside the Holometabola, we are ignorant of the status of many genes thought to be the main target of ecdysone action. Work on the puffing response of the giant polytene chromosomes of *Chironomus tentans* (28) first indicated that ecdysone acted to directly control gene transcription and identified some of the sites where these genes might reside. Subsequent studies on *Drosophila* established a scenario for the interaction of these genes (3) and led to their eventual cloning and

demonstration that many were indeed transcription factors that regulate themselves and sets of downstream target genes. The interaction of these genes has been the topic of a number of reviews (50, 143) that should be consulted for details. The target genes that were first described in *Drosophila* are also expressed during molting responses in *Manduca* (112, 113) and *Tenebrio* (85). The temporal expression of these genes has been examined in the context of ecdysone-induced molting and found to be similar across this range of species. Clearly, this basic pathway is conserved through the Holometabola and is likely a feature of insects as a whole. Indeed, the role of some of these genes in coordinating molting processes may predate the insects because in the nematode *Caenorhabditis elegans*, the orthologs of DHR3 (68) and βFTZ-F1 (2, 45) are also associated with the production of new cuticle. Although *C. elegans* lacks both EcR and USP orthologs, some of the parasitic nematodes have both proteins, and exogenous ecdysteroids can cause molting in these species (133).

The genes of the ecdysone cascade are beautifully adapted for coordinating a complex process such as molting because it requires sequential batteries of genes to be activated in a complex coordinated fashion. Not all of ecdysone action, though, is mediated through such a cascade. This is evident from an examination of the regulation of eye development in *Manduca* (25). As described, the cells in the eye imaginal disc begin to adopt particular fates at the beginning of the prepupal ecdysone peak as the morphogenetic furrow moves across the disc. This patterning process requires the tonic presence of either αE or 20E but at levels below that needed to activate the ecdysone cascade. If the levels of 20E are high enough to activate the cascade, the tissue patterning and cell determination processes stop and the tissues begin to mature their ommatidia unless JH is present.

In insects in the basal groups, such as the apteryogote orders, the processes of tissue patterning and morphogenesis are features of embryonic development and little occurs during postembryonic life. Although ecdysone is clearly present in these embryos, we do not know if this hormone is actually required for these processes to occur in the embryo. Indeed, early embryonic grasshopper legs cultured with yolk undergo growth and the sequential production of pronymphal and nymphal cuticles without the addition of exogenous steroids (88). In the derived condition of metamorphosis, however, these processes have been delayed into postembryonic life, thereby becoming firmly under the control of the ecdysone signaling system. An intriguing question, then, is how has the ecdysone signaling system been able to "capture" these patterning networks? A possible insight into how this capture may have occurred comes from experiments on the wing discs of *Drosophila*, in which patches of cells are generated that lack the USP component of the ecdysone receptor complex (126). In the wing disc, the determination of cells to become sensory neurons normally requires exposure to 20E, but in the USP null patches these processes can occur even in the absence of steroid. This result indicated that for some processes the ecdysone receptor complex acts as a repressor and that ecdysone serves to release this repression. The evolution of repressor response elements in key genes in patterning networks may have provided a way for the ecdysone system to gain control over these patterning pathways.

The role of ecdysone in the molting process is a feature of all arthropods and may extend to the entire clade of molting animals, the Ecdysozoa (1). The involvement of the genes of the ecdysone cascade in the molting response is also similarly ancient. The involvement of ecdysone in regulating tissue morphogenesis and patterning may be a more recent acquisition that occurred as these developmental processes were suppressed during embryogenesis and shifted out into postembryonic life. The importance of steroid-dependent derepression may have accompanied this shift.

The Actions of Juvenile Hormone

The main message of this review is that JH secretion and the way that tissues respond to it have been important forces in the evolution of insect life histories. A major problem in examining the molecular aspects of potential change is that there is still not a clear consensus about the nature of the JH receptor. As discussed above, USP is one candidate; yet, it does not fit the criterion of high-affinity binding of the hormone usually considered a hallmark of a hormone receptor.

Another candidate suggested for the JH receptor is the product of the *Methoprene-tolerant* (*Met*) [formally known as *Resistance to Juvenile Hormone* (*Rst(1)JH*)] gene in *Drosophila* (156). Loss-of-function *Met* mutants show reduced sensitivity to the juvenilizing effects of JH III or its mimics and reduced intracellular JH-binding activity (129). Yet null *Met* mutants show no defects in embryonic or larval development or metamorphosis, only reduced vitellogenesis (155). This gene encodes a protein with similarity to the aryl hydrocarbon receptor nuclear translocator (4). The aryl hydrocarbon receptor nuclear translocator is a cytoplasmic protein that complexes with the dioxin receptor (arylhydrocarbon receptor) when it binds dioxin or other xenobiotics. The complex translocates to the nucleus where it activates genes involved in the metabolism of the xenobiotic (124). Indeed, the Met protein is found in the nuclei throughout larval development (101). Exactly how this type of action may be related to JH action is unknown, although dioxin binding in a *Helicoverpa zea* extract is competitively inhibited by methoprene or JH I (87).

A third candidate for the JH receptor is the JH-binding protein JP29 that was isolated from *Manduca* epidermis and is found primarily in the nucleus (26, 96, 130). Like USP, this protein binds JH specifically but with low affinity. Unlike USP, it has no DNA-binding domain but instead belongs to a family of JH-binding proteins found in both hemolymph and tissues that includes the Takeout protein of *Drosophila* thought to be involved with the circadian control of feeding behavior (120, 136). JP29 is found in *Manduca* epidermis during larval life and then disappears as the epidermis is being pupally committed by ecdysone acting in the absence of JH at the end of the feeding stage (130).

None of these proteins has all the characteristics and developmental expression pattern one would expect for a JH receptor modulating the action of ecdysone during larval life and preventing its action of switching gene expression that must occur for metamorphosis to proceed. We do, however, have some insights

into some key genes that appear to be involved in this switch that are regulated by JH.

The *broad* gene in *Drosophila* encodes four different protein isoforms of a transcription factor called the Broad-Complex (BR-C) in the *bric-a-brac-tramtrack-broad* (BTB) family that includes several known chromatin-altering proteins (10). These isoforms share the common BTB protein interaction domain and differ only in the cysteine-histidine (C_2H_2) zinc finger DNA-binding domain. Mutants that remove the entire complex in *Drosophila* develop normally until metamorphosis but then cannot proceed. The various *broad* transcripts are first induced by ecdysone at metamorphosis, with some appearing before others in a tissue-specific manner (40). Molecular data from salivary gland genes show that BR-C acts to repress larval genes and to activate glue protein genes required for metamorphosis apparently by DNA binding (150). BR-C also activates a specific fat body gene *fbp1*, but in this case protein-protein interactions are likely the primary mechanism (89). Thus, *broad* appears to be a key gene for the entry into metamorphosis.

The *broad* gene has also been found in *Manduca*, where its expression is associated with pupal commitment of tissues at the time of wandering (157, 158). It is important that in the context of the epidermis, the expression of *broad* is clearly controlled by JH, and the addition of JH represses the appearance of this gene. It is interesting, however, in the wing discs, where BR-C appears earlier in parallel with their commitment for metamorphosis as the JH titer declines, exogenous JH can only delay but not suppress its appearance (157). In the developing eye primordia, *broad* expression is not seen until the disc is formed and is committed to the ecdysone-induced movement of the morphogenetic furrow (D.T. Champlin, L.M. Riddiford & J.W. Truman, unpublished data). Thus, one of the first molecular correlates of commitment to pupal differentiation is the appearance of transcripts for BR-C. These transcripts are normally induced by ecdysone in the absence of JH, but in the derived wing discs, pupal commitment occurs during the decline of JH in the apparent absence of ecdysone (H. Yoshida & L.M. Riddiford, unpublished data).

Examination of the expression of BR-C in both *Manduca* and *Drosophila* shows that this gene is prominently expressed during the formation of the pupal stage but not during the formation of the adult (159). When JH is given to a newly ecdysed pupa, it molts to another pupa rather than to an adult (110), and *broad* transcripts are found during this molt (159). These transcripts also reappear in pupal wings cultured with 20E and JH for 24–96 h but not in wings cultured only with 20E. Although the situation is straightforward in *Manduca*, complexity arises in *Drosophila* because of its partial loss of sensitivity to JH. When newly pupariated individuals are treated with JH or its mimics, they form a normal pupal stage (99, 111). The imaginal discs in the head and thorax then form normal adult cuticle, but the abdominal epidermis, derived from the late-dividing histoblasts, makes a second pupal cuticle, evidenced by re-expression of two pupal cuticle genes and the suppression of an adult cuticle gene (159). In these treated animals, *broad* mRNA is reinduced in the abdomen and is associated with the formation of this second pupal cuticle. However, the head and thorax show neither the reinduction

of *broad* expression nor pupal cuticle gene expression (159). Rather than giving JH if one ectopically expresses one of the BR-C isoforms during the time of cuticle formation in adult development, the fly will form pupal cuticle in both the abdomen and the head and thorax! Thus, by the misexpression of this transcription factor, one can cause the cells determined to produce adult cuticle to re-express pupal cuticle genes.

Hence, an important pathway by which JH prevents the larval-pupal transformation is via its regulation of *broad* expression. For the epidermis, the BR-C expression is necessary for both pupal commitment and for the production of pupal cuticle. This pathway leading from JH regulation, through *broad* expression, to pupal cuticle formation is used in *Manduca* for all of the body regions. In the case of *Drosophila*, however, the abdomen maintains this relationship, but in the head and thorax the link between JH and suppression of *broad* has been broken but the relationship between *broad* and pupal cuticle formation remains. As suggested, the loss of this linkage to JH may have occurred when the imaginal discs had to escape suppression by JH in order to start growth early in larval life.

Thus far, *broad* had not been reported from any hemimetabolous or ametabolous insect. We would predict, however, that if it were present in these, one should find its expression first during the pronymphal-nymphal molt in the embryo. It is at this time that the JH-regulated ecdysone switch should first occur. It should also be present during all the nymphal molts and disappear during the final adult molt. Alternatively, *broad* may have been an invention of complete metamorphosis where a transcription factor controlling the switch to the pupal state was needed to prevent precocious adult differentiation.

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CONTENTS