

Cinderella's new shoes – how and why insects remodel their bodies between life stages

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Metamorphosis in insects is a remarkable phenomenon where the larva undergoes a striking morphological reorganization to give rise to the adult. Over the years, various physiological factors and pathways that govern metamorphosis have been discovered, and at the same time, some understanding about the origins of this phenomenon has also emerged. This review summarizes the current state of knowledge of both the mechanisms underlying metamorphosis, as well as the theories put forward to explain its evolution.

Keywords: Evolution, hemimetaboly, holometaboly, insects, metamorphosis.

ABOUT 400 million years ago in the swamps of the primitive Devonian world evolved a group of animals that are found today in almost every ecological nook and corner. With millions of species, the insects exhibit an evolutionary savoir faire unequalled by the rest of the animal kingdom. Within 100 million years of their origin, insects acquired various adaptive features that led to their large-scale ecological supremacy – small size, wings and a remarkable specialization of their life cycle called metamorphosis¹. In our childhood, many of us may have experienced the wondrous moment of witnessing the change undergone by insects during metamorphosis. On finding caterpillars, children often put them in jars with a few leaves and twigs. The caterpillar feeds on the leaves and gradually grows big, and then one fine day it disappears and in its place a small lump (sometimes beautiful), the pupa or cocoon, hangs from the twig. Days go by and then one morning an elegant creature emerges from the cocoon – a colourful butterfly or a somber moth, fluttering about, beating its wings against the walls of the jar. It reminds us of the fairy tale of Cinderella, the girl who would change every evening from a ragged, miserly maid to a gracious lady, her rags turning into a beautiful and fashionable dress, complete with glass shoes, yet changing back to her former self at the stroke of midnight. The transformation of a caterpillar to a butterfly is indeed strikingly amazing and the wondrous, awestruck gaze of a child befits the phenomenon, rather than a cold accepting gaze of an adult!

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How does an insect remodel itself so completely from one life stage to another so as to appear like an entirely different creature and why does it do so? The question pertains to metamorphosis (Greek: 'meta' change + 'morphé' form), the complex morphological changes that take place during transition from larval stage to adult stage, generally associated with changes in habitat and feeding biology as well^{2,3}. Scientific studies of metamorphosis can be traced back to Aristotle, whose interests included observing the change in the form of insects⁴. Global transformations take place during metamorphosis, involving differentiation and growth in cells as they form new organs, as well as cell death leading to loss of old organs. Since the insect body is covered by a chitinous exoskeleton, this prevents it from growing and hence the exoskeleton has to be shed and regrown periodically during the growth phase to accommodate the growing body – a process known as molting or ecdysis⁵. The final molt is usually the most dramatic and results in emergence of the adult, which is strikingly different from the earlier instars. The process is under endocrine regulation and demands an understanding of the developmental processes and mechanisms that govern such remarkable phenotypic plasticity. At the same time, why such divergent life histories of larvae versus adult arose in the first place, resulting in metamorphosis as a process to bridge the two modes of life remains an intriguing evolutionary question.

Types of metamorphosis

Insects display a fair variety in metamorphosis. The basal groups (wingless insects) like Collembola (springtails), Protura and Diplura – grouped together as Entognatha, and Archaeognatha (bristletails) and Zyegntoma (silverfish), show a gradual increase in size throughout their development, without showing any sudden changes, and are considered as ametabolous or ametamorphic, i.e. they do not show any metamorphosis. These insects continue to grow and molt even after attaining sexual maturity, i.e. adult life⁶ (Figure 1). The winged insects (Pterygota) terminate molting on reaching the winged adult stage (except for Ephemeroptera, i.e. mayflies, that have two winged instars – the non-reproducing subimago and the sexually mature imago)⁶. The pterygotes broadly show

two kinds of metamorphosis – hemimetaboly and holometaboly. Insects belonging to orders Hemiptera (true bugs, aphids, cicadas and scale insects), Odonata (dragonflies and damselflies), Ephemeroptera (mayflies), Orthoptera (grasshoppers and crickets), Mantodea (praying mantises), Dermaptera (earwigs), some Blattodea (cockroaches and termites), Phasmatodea (stick insects), Phthiraptera (sucking lice) and Plecoptera (stoneflies) have juveniles called nymphs or larvae (rarely called naiad in aquatic forms). Here, beginning from an early point in postembryonic development, the adult organs like wings and genitalia start developing gradually. These insects are considered to have incomplete metamorphosis and are called hemimetabolous. However, the change between the last larval instar and adult is considered as significantly greater than the changes between the different larval instars (Figure 1). On the other hand, in insects considered to show complete metamorphosis or holometabolous insects – orders Diptera (flies and mosquitoes), Coleoptera (beetles), Lepidoptera (butterflies and moths), Hymenoptera (bees, wasps and ants), Neuroptera (lacewings), Mecoptera (scorpionflies), Megaloptera, Raphidioptera, Siphonaptera (fleas), Strepsiptera and Trichoptera (caddisflies), the difference between juvenile

(larva) and adult stage is much more distinct and conspicuous, and larvae and adults have different habitats as well. The holometabolous larvae never show any external rudiments of wings or genitalia, which sometimes develop internally in the form of imaginal discs (invaginated groups of undifferentiated embryonic cells). Between the last holometabolous larval instar and adult is found a non-feeding instar of limited or completely suppressed locomotory ability, which usually remains hidden or somehow protected by an external covering. This instar is called the pupa, and undergoes massive tissue degradation and rebuilding. The pupa shows external rudiments of wings and genitalia⁶ (Figure 1). Apart from these two kinds of metamorphosis, mayflies (Ephemeroptera) show the presence of two winged instars – the non-reproducing subimago and the sexually mature imago, and are said to show prometaboly, which is often considered as the state ancestral to development of hemimetabolism or holometabolism⁶ (Figure 1). In some thrips (Thysanoptera), whiteflies (Aleyrodomorpha) and males of scale insects (Coccomorpha), another kind of metamorphosis with several wingless larvae, one to three partly or fully quiescent pre-adult instars (with or without external wing rudiments), and finally the adult, has evolved independently and is referred to as neometaboly⁶ (Figure 1). Both prometabolous and neometabolous insects do not show the degree of difference between larval and adult habits as shown by holometabolous insects, and can be considered as similar to hemimetabolous groups in this respect.

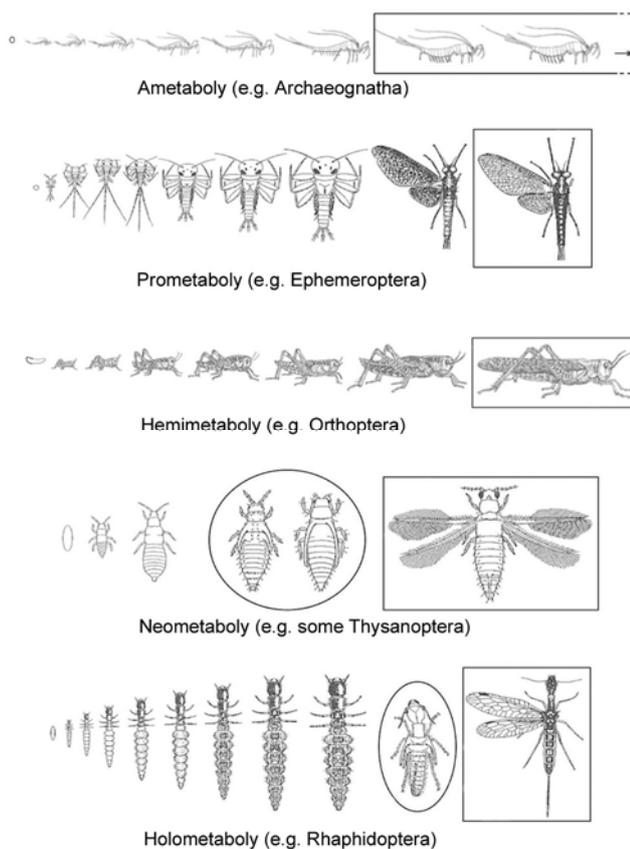


Figure 1. Five different types of metamorphosis shown by present-day insects. Resting stages (pupa) are encircled, adults are boxed (not to scale; adapted from Sehnal *et al.*⁶, with permission from Elsevier).

Physiological mechanisms

Wingless insects continue to molt after attaining sexual maturity and there is no predetermined final instar. This is considered as a primitive character. In advanced insects, i.e. pterygotes (excepting mayflies), there is always only one flying instar (unless wings have been secondarily lost or reduced) that never molts again. The greatest morphological change occurs between the last larval and adult stages in hemimetabolous insects, and in two steps – larva to pupa and pupa to adult in holometabolous insects, i.e. always towards the end of development. Three major events occur during this period: (i) structural metamorphosis (reorganization of morphology), (ii) obligatory termination of molting cycles (attainment of predetermined final instar) and (iii) achieving reproductive competence (reaching adult stage or sexual maturity). Originally there may have been some temporal difference in attaining these three events, but in modern insects it is difficult to distinguish between them, especially with respect to endocrinological regulation of metamorphosis. It is possible that mechanisms associated with structural metamorphosis may have been primarily connected with reproductive maturity or termination of molting, and have

secondarily been involved in morphological changes⁶. Majority of studies investigating physiological bases of metamorphosis have focussed on either hemimetabolous or holometabolous insects, and information is largely lacking on ametabolous insects.

Development in ametabolous insects

Amongst wingless insects, the firebrat *Thermobia domestica* (Zygentoma) has been found to produce juvenile hormone JH III⁷ and ecdysteroids similar to pterygotes⁸. It was found that JH secretion by the corpora allata was highest in the first and second instars, declined in the third instar and then rose again, and corresponding changes in volume of corpora allata cells were also observed⁹. It was inferred that the decline in JH in the third instar allowed the appearance of integumental scales that are absent in earlier instars and appear for the first time in the fourth instar – possibly an effect of absence of JH during molting. This suggests that the occurrence of scales is controlled by JH in a process similar to larva–adult metamorphosis in pterygotes. It has also been found in the same species that the haemolymph shows a peak in ecdysteroids concentration during each molting cycle that can be correlated with apolysis (separation of epidermis from the old cuticle) and the onset of new cuticle deposition⁸. This is analogous to the cyclic increase in ecdysteroids during molting in hemimetabolous and holometabolous insects. Unfortunately, the physiological bases of molting remains largely unexplored in ametabolous insects.

Nymph to adult transformation in hemimetabolous insects

JH and ecdysteroids are the two key hormones regulating metamorphosis in insects. The origin of the name ‘juvenile hormone’ arises from its inhibitory effect on metamorphosis, i.e. its role in retention of larval characteristics, or restraining development toward the adult form. JH titre remains high from hatching till the last nymphal molt, i.e. during the juvenile molts, and its level falls abruptly in the pre-adult stage finally becoming almost undetectable¹⁰ (Figure 2). This may be a primitive situation in metamorphic pterygotes¹¹. JH is secreted from the corpora allata, and its production declines rapidly during the last nymphal instar, a low JH level being a crucial requirement for metamorphosis¹². On artificially increasing the JH titre by implanting corpora allata or by application of exogenous JH, metamorphosis can be prevented, and conversely, on reducing the JH level by removing corpora allata from the penultimate larval instar, precocious metamorphosis can be induced to give rise to miniature adults⁶. Production of JH is regulated by stimulatory allatotrophins and inhibitory allatostatins, that are neuropeptides that act on receptors in corpora

allata and corpora cardiaca¹³. Their mode of action can depend on the age and intrinsic level of JH production of the individual, but the exact mechanisms of how these peptides are regulated and how in turn they regulate JH production remains to be explored¹⁴. Although the major focus on endocrinology of metamorphosis has been through the study of holometabolous insects, recent work has unravelled some of the molecular mechanisms of mode of action of metamorphosis hormones in hemimetabolous insects. JH appears to act through its putative receptor methoprene-tolerant (*Met*) to regulate *Kruppel homolog 1* (*Kr-h1*) and *Broad-Complex* (*BR-C*) genes^{15,16}. *Met* conveys the signal induced by JH to maintain high expression level of *Kr-h1*, and knocking down *Met* or *Kr-h1* by RNAi induces the precocious appearance of adult coloration, wings and genitalia. *Kr-h1* expression falls naturally during the last nymphal instar, permitting adult development. Thus *Met* and *Kr-h1* serve as JH-dependent repressors of metamorphic changes. JH may further act by modifying the expression of genes activated by ecdysteroids, or may also directly influence gene expression through positive and negative genetic control¹⁷. However, such evidences from hemimetabolous insects are lacking. *BR-C* transcription factors also appear to govern progressive morphogenesis through successive nymphal molts, as wing bud growth through different stages can depend on *BR-C* factors¹⁸. *BR-C* factors are expressed throughout all nymphal instars and the expression falls in the last nymphal stage^{10,18}. Thus hemimetabolous insects undergo growth (especially wing bud development) in the nymph stage with high JH level, which may induce the high expression of *BR-C*, and molt into the adult stage after both JH and *BR-C* levels decline in the last nymphal instar (Figure 2).

Another key regulatory factor for successful metamorphosis is the ecdysteroid titre. Ecdysteroids are typically produced from the prothoracic gland, and the ecdysteroid level increases rapidly towards the end of each instar stage, reaching a molt-inducing peak, and then again decreases and remains low till the next molting cycle^{6,10} (Figure 2). The increase is first associated with epidermal cell division, then with apolysis, i.e. separation of the old cuticle from the body, and finally with ecdysis, i.e. exfoliation of the exuvia or remnants of the old cuticle¹⁹. Ecdysteroids are secreted mostly by the prothoracic gland, or sometimes by the integument^{20,21}. 20-Hydroxyecdysone is the more active form of ecdysteroids and it binds to a heterodimeric nuclear receptor (formed by two receptors, EcR and RXR/USP), in turn regulating a cascade of nuclear hormone receptor genes (found to be *BgE75*, *BgHR3*, *BgHR4* and *BgFTZ-F1* in cockroach *Blattella germanica*) that display regulatory crosstalk to regulate molting. Different phases of the ecdysteroid cycle – increase, peak or decrease, activate or inhibit different players of this network of nuclear receptors²². Other peptidic hormones, especially neurohormones, may also

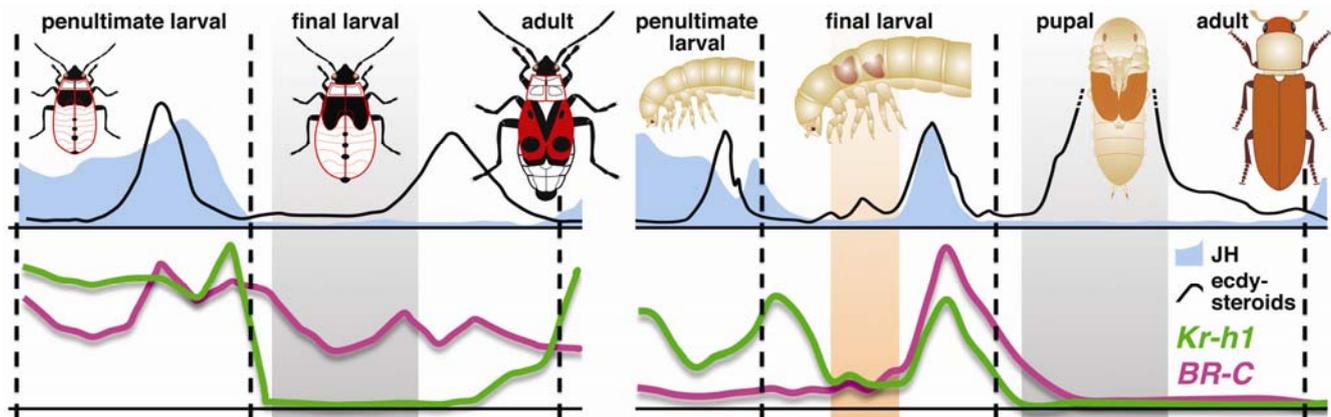


Figure 2. Hormonal and molecular regulation of metamorphosis. Left and right cartoons signify the similarities and differences in hormonal and molecular pathways associated with hemimetaboly and holometaboly respectively. *BR-C* and *Kr-h1* expression profiles are for *Pyrrhocoris apterus* (bug) and *Tribolium castaneum* (beetle), and JH and ecdysteroid titres are for *Blattella germanica* (cockroach) and *Manduca sexta* (moth) (reprinted from Konopova *et al.*¹⁵).

be important for regulating ecdysis, but these have not been studied properly in hemimetabolous insects²³. Recent studies have revealed the role of a new level of regulation in hemimetabolous insects – microRNAs (miRNA), which are small RNA molecules around 22 nucleotides long, generally playing a role in repressing action of messenger RNA (mRNA). Experimental down-regulation of miRNA production prevents molting into adults and instead causes insects to retain nymphoid features, suggesting that miRNAs may perhaps regulate genes that are JH or ecdysteroid targets²⁴.

Larva to pupa to adult transformation in holometabolous insects

The role of JH and ecdysteroids in inhibiting and stimulating metamorphosis respectively, remains conserved in holometabolous insects. Peaks of ecdysteroids trigger molting, whereas the presence of JH during molting maintains the juvenile characters. Thereby broad JH titre peaks may be found overlapping with the molt-inducing peaks of ecdysteroids⁶ (Figure 2). When JH level remains low during molting, metamorphosis to adult state ensues¹⁶. In some insects like honey bees (*Apis*: Hymenoptera), a derived condition is found where JH-levels remain at a steady level from embryos till early last larval instar in worker larvae, and in queen larvae it goes up to several folds higher than worker larvae. The dependence of sexual maturation on decrease in JH and increase in ecdysteroids may be an ancient characteristic, as similar control of previtellogenesis (egg yolk formation) has been found in the ametabolous insect *T. domestica*²⁵. As in hemimetabolous insects, JH (or sometimes carboxylic acids of JH) are secreted by the corpora allata and JH secretion can be governed by neurosecretory cells releasing allatoprotrophic peptides and amines²¹. The JH acids are sometimes regarded as prohormones and may be converted to

JH, especially in future adult organs like the imaginal discs²⁶. JH titres in haemolymph may be a result of balance between JH secretion and its catabolism by JH-specific epoxide hydrolases and esterases, and it may be protected from degradation by binding with JH-binding proteins²¹ (Figure 3). An important function of JH in holometabolous insects is to modulate the action of ecdysteroids, and the lowering of JH level prior to pupation induces inhibition of feeding and the onset of wandering, where the larva looks for a suitable location to undergo pupation²⁷. Like in hemimetabolous insects, JH acts through *Met* and *Kr-h1* to regulate JH-inducible genes, and in *Drosophila* activates *FTZ-F1* after binding with *Met* and *Tai* to initiate regulation of nuclear genes²⁸. The antagonistic effect of JH on metamorphosis is through *Kr-h1*, which suppresses ecdysteroid-induced switching on of *BR-C*, in turn permitting larval growth and development²⁷ (Figure 3). Thus the interplay between JH and *BR-C* is quite different in hemimetabolous and holometabolous insects, and the acquisition of an inhibitory effect of JH on *BR-C* expression in juvenile instars may have been a key step in the evolutionary transition from hemimetaboly to holometaboly¹⁰. Since *BR-C* permits changes in gene expression required for change in morphology between instars, it plays a vital role in regulating metamorphosis.

Ecdysteroids have numerous functions in holometabolous insects – regulation of neuronal growth, reproductive roles, stimulation of growth and development of imaginal discs, initiation of breakdown of larval structures during metamorphosis, elicitation of deposition of cuticle by epidermis, etc.²¹. Ecdysteroids are produced in the prothoracic gland of holometabolans, which typically secretes ecdysone, which in turn converted to the principal molting hormone 20-hydroxyecdysone (20E) by *P₄₅₀* monooxygenase in mitochondria of peripheral tissues. The production of ecdysone is controlled by the protho-

racicotrophic hormone (PTTH) secreted by corpora allata²¹ (Figure 3). 20E binds to a heterodimeric nuclear receptor composed of the ecdysone receptor (EcR) and ultraspiracle (USP) or RXR, in turn activating a hierarchy of transcription factors that regulate the expression of target genes governing cellular changes associated with molting and metamorphosis. Some of the nuclear receptors described earlier for *B. germanica*, that ultimately modulate *βFT-Z1* and *HR4* remain conserved in both hemimetabolous and holometabolous insects, but their regulatory interplay differs. *HR4* appears to be involved in all postembryonic transitions in hemimetabolous insects, which may be the ancestral function, but it appears to have acquired a new holometabolous-specific role in coordinating growth and maturation in the last larval instar²². There appears to be some crosstalk between JH and 20E in modulating various transcription factors and genes that are crucial for execution of metamorphosis with JH sometimes facilitating and sometimes inhibiting 20E-induced gene expression¹⁷ (Figure 3). Along with various other genes, the genes associated with apoptosis (*diap2*, *rpr*, *hid*) are expressed in the pupal stage and this is important for breakdown and cell death of larva-specific structures. Changes in ecdysone titres translate into waves of gene expression during holometabolous

metamorphosis. Indeed in *Drosophila*, probably the most well studied holometabolous insect, about 872 target genes have been found to change their expression during metamorphosis, out of which around 200 are induced in the larva, more than 400 in the prepupa and around 350 in the pupa. Interestingly, the different sets of genes can be modulated by the same set of master regulatory genes, like the *Hox* genes²⁹. Recently, it has been found that some transcription factors like E93 can act as a temporal identity determinant, bestowing responsiveness to other signalling pathways on the target genes in a time-dependent manner³⁰. Thereby, the same genes and signalling pathways that were employed in a different way during embryonic development can be redeployed in another way during metamorphosis to bring about large-scale morphological changes.

Some peptidic hormones are involved in regulating the process of molting or ecdysis, and emergence of the molted insect from the exuvia. Ecdysis itself is a complicated process comprising pre- and post-ecdysis behaviours, controlled by a cascade of peptide hormones from Inka cells and neuropeptides of the central nervous system. Inka cells in tracheas produce pre-ecdysis and ecdysis-triggering hormones that activate ecdysis initiated by the molting hormone, acting through receptors on specific neurons²³.

Comparing hemimetaboly and holometaboly

When compared with hemimetabolans, the holometabolous penultimate larval instar has evolved additional characteristics to facilitate transition to the pupa¹⁶. The first characteristic is a safety mechanism, wherein JH prevents release of ecdysteroids from the prothoracic glands during the last larval instar, thereby preventing precocious molting³¹. The inhibitory effect does not arise prior to the last larval instar. JH titres decline during the last larval instar in response to acquiring a critical size or weight, i.e. the minimum size at which metamorphosis cannot be delayed^{19,32}. The mechanism by which JH production from corpora allata ceases remains unknown. Finally the acquisition of critical weight gives rise to ecdysone-induced changes. A small peak of ecdysone in the absence of JH in the last larval instar (Figure 2) induces stopping of feeding and onset of wandering behaviour, resulting in the larva searching for a suitable place to undergo metamorphosis. It also induces a switch in epidermal cell fate, whereby the epidermal cells become committed to form pupal cuticle in place of larval cuticle¹⁶. A similar size-based safety mechanism may also exist in hemimetabolans, where the small rise in ecdysteroids occurs in the final instar, after decline in JH levels, immediately prior to molting to adult instar (Figure 2). Thus low ecdysteroid levels may regulate a turnover in cell fate in hemimetabolans as well. This

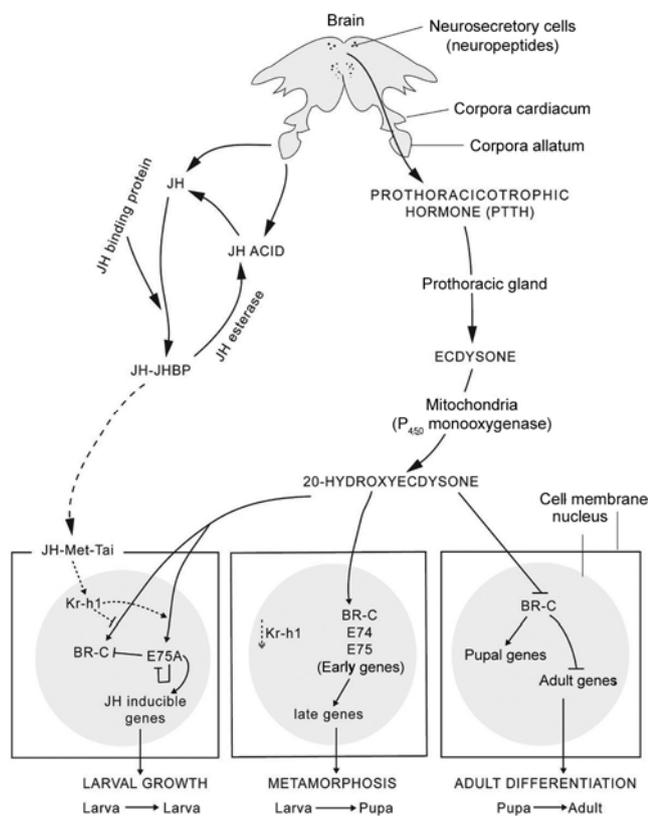


Figure 3. Physiological pathways controlling metamorphosis in holometabolous insects (adapted from Gilbert *et al.*²¹ and Dubrovsky¹⁷, with permission from Elsevier).

similarity between hemimetabolans and holometabolans suggests that the mechanisms that govern transformation from nymph to adult in hemimetabolans have been temporally shifted in the holometabolans to facilitate the transition from pupa to adult. This may be a key step in the evolution of holometaboly. Another difference between hemimetabolans and holometabolans is that in holometabolans, a resurgence of JH occurs prior to the molt to the pupal stage (Figure 2). The function of JH at this stage, after pupal commitment has taken place, appears to be preventing precocious adult development. The mechanism that enables reappearance of JH remains unknown, and there is some evidence that JH action may be mediated through the inactive prohormone JH-acid at this stage, which may result in a tissue-specific regulation of enzymes that are important for the evolution of the pupal stage¹⁶.

How and why did metamorphosis evolve?

Various theories proposed starting from the late nineteenth century to explain the evolution of insect metamorphosis claimed that holometaboly could be a result of precocious eclosion of the embryo from the egg³³. In hemimetabolans, the embryo supposedly completes all developmental stages before hatching, whereas in holometabolans the embryo may hatch out before completing development, which would be completed later in the pupal stage. Such a theory was elaborated by Berlese in 1913, and subsequently taken up by other authors³⁴. According to this theory, the hemimetabolan nymphs would be similar to the holometabolan pupa. The Berlese theory was first successfully criticized by Hinton³⁵, who postulated that postembryonic development is similar in hemimetabolans and holometabolans, thereby rendering the last nymphal instar of hemimetabolans equivalent to the pupa of holometabolans. Interest has been revived in the theory of precocious eclosion by the pronymph theory that postulates that hemimetabolans have three postembryonic stages – pronymph, nymph and adult, comparable to the larva, pupa and adult of holometabolans³⁶. Hemimetabolans are postulated to undergo three ‘embryonic molts’ before hatching from the egg, resulting in a nymph similar to adults, whereas holometabolans undergo only two such molts, thereby resulting in a larval life stage homologous to the pronymph. This theory draws support from morphological and anatomical similarities of the larva and pronymph, and there is also some endocrinological support. As described earlier, ecdysteroids induce the production of new cuticle during a molt, whereas JH regulates the nature of the molt. For both larvae as well as nymphs, molts in the presence of JH are ‘status quo’ molts, resulting in instars that retain the morphology of the previous stage, whereas molts in the absence of JH result in metamorphosis. In hemimetabolans, the first

molt after the decrease of JH titre results in the adult, but in holometabolans the situation is more complicated. Following decline of JH for the first time, a small peak of ecdysteroids causes commitment of tissues to pupal differentiation. The pupal molt however occurs later, in response to a large peak of ecdysteroids with simultaneous reappearance of JH (Figure 2). JH again disappears before the large ecdysteroid surge that induces adult differentiation. Analogous to the post-embryonic endocrine milieu, peaks of ecdysteroids and JH are also seen during embryonic molts. The simultaneous peaks of JH and ecdysteroids seen during transformation of pronymph to nymph (i.e. nymphal peak) may have undergone a heterochronic shift and are found during the transformation of larva to pupa in holometabolans, resulting in the prepupal peak following the commitment peak of ecdysteroids (Figure 4). There may also be a heterochronic shift in JH production resulting in early JH secretion in hemimetabolans followed by fall in JH levels that may give rise to several ecdysteroid peaks prior to blastokinesis and these may be equivalent to the commitment peak of ecdysteroids in holometabolans, thereby facilitating the shift from pronymph to nymph during hemimetabolan embryogenesis (Figure 4). This shift in the timing of JH and ecdysteroid peaks may provide a simple mechanism for the difference in hemimetabolan and holometabolan development. The increased divergence between the pronymph and larva over evolutionary time may have further resulted in various mechanistic differences between hemimetabolan and holometabolan development. The criticism against the pronymph theory is that ultrastructural studies on representative species from a large number of orders show that all species undergo three cuticle depositions (molts) in the embryonic stage³⁷, refuting the claim that hemimetabolans undergo three embryonic molts and holometabolans undergo two such molts, and thus the theory still remains debated.

Another key player in the evolution of metamorphosis appears to be *BR-C*. In holometabolans, prior to the last larval instar, *BR-C* is inhibited by JH resulting in prevention of metamorphosis (Figure 3), whereas no such effect has been found to exist in hemimetabolans, where *BR-C* levels can remain unaffected by rise or fall in JH levels throughout nymphal life (Figure 2). However, in holometabolans, the inhibitory effect of JH on *BR-C* is lost following the commitment peak of ecdysteroids in the last larval instar (Figure 2). Interestingly, it has been found that such an inhibitory effect of JH on *BR-C* may be present in the hemimetabolan embryonic stage, as *BR-C* expression starts getting manifested following the pronymphal peak³⁸ (drop in JH and elevation in ecdysteroids around blastokinesis; Figure 4). Thereby, it has been suggested that the pronymphal peak of hemimetabolans may be equivalent to the commitment peak of holometabolans¹⁶. Holometaboly may thus have evolved from hemimetaboly by delaying the first drop in JH production till the

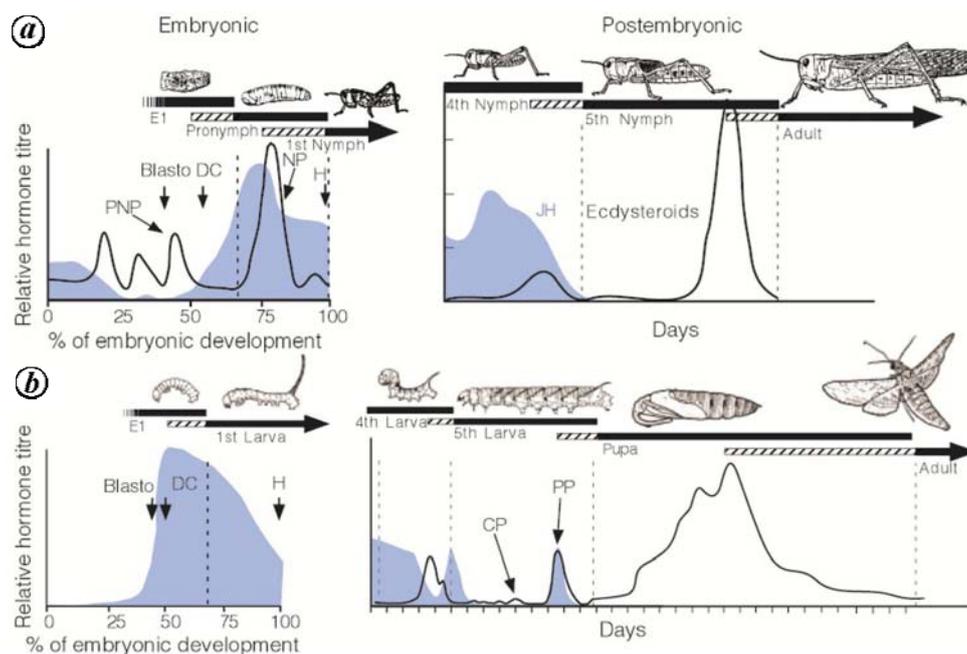


Figure 4. Endocrinology of embryonic and postembryonic insect development. Comparison of the embryonic and postembryonic titres of ecdysteroids (black) and juvenile hormone (blue) for (a) hemimetabolous insects, the grasshoppers *Locusta migratoria* (embryonic) and *Schistocerca gregaria* (postembryonic) and (b) a holometabolous insect, the sphinx moth *Manduca sexta*. The bars over the hormone titres show the times when the cuticles of the particular instars are present; cross-hatching represents the pharate stage when the insect is still covered by the old cuticle of the preceding stage. Detailed ecdysteroid titres are not available for embryos of *Manduca*. Vertical dashed lines show the times of ecdysis (shedding of the old cuticle). Blasto, Blastokinesis; DC, Dorsal closure; E1, First embryonic instar; H, Hatch. Ecdysteroid peaks: CP, Commitment peak; PP, Prepupal peak; PNP, Pronymphal peak; NP, Nymphal peak (reprinted from Truman and Riddiford³⁶, with permission from Nature Publishing Group).

penultimate juvenile instar, thereby giving rise to a prolonged larval life. Another interesting way in which *BR-C* may modulate the difference between hemimetabolism and holometabolism is through its action on wing formation. In hemimetabolans *BR-C* expression remains high throughout all nymphal stages and this induces progressive differential growth of wings throughout the juvenile phase, whereas in holometabolans *BR-C* is expressed only in the last larval and pupal stages, correlated with a bout of differential growth of adult structures³⁹. Thereby, variation of the same underlying genetic and physiological mechanisms can give rise to evolution of two different kinds of metamorphosis.

Metamorphosis in insects appears to us as a dramatic and spectacular phenomenon, arousing our curiosity regarding the evolution of such divergent life-history traits within the lifetime of an organism. However, the existence of larval stages that are markedly different in morphology and habit from the adult is widespread in the animal kingdom, and perhaps the abundance of insects among terrestrial animals that facilitates our observation gives rise to the notion of insect metamorphosis as a special case. Presence of morphologically distinct larval and adult stages is common in all invertebrate phyla, and can be seen in some vertebrates as well (e.g. amphibians)⁴⁰. Since the majority of invertebrate taxa are marine and thereby relatively more difficult to study or observe, they

have attracted less attention than terrestrial taxa. The adult body plan is believed to be the ancestral state and evolved earlier, evident in sharing of patterning genes among different body plans in different phyla, and the larvae are believed to have evolved by co-option of adult genes into independently evolved larval forms, as evident in distinct patterning genes despite morphological convergence among different larval forms⁴¹. The primary reason for development of a small motile larva in invertebrates is considered as developing a mechanism of dispersion. Dispersion is crucial in colonizing new habitats, and should be of great importance to species where adults are sedentary or of limited motility. It also helps maintain populations of species which live on temporally unstable habitats, and thereby must disperse regularly in search of suitable habitat. In some taxa, the larva is also considered as a feeding stage whose purpose is to build up reserves for an adult form, that will enable or enhance reproductive abilities of the organism⁴⁰. The biphasic metazoan life cycle is considered to have evolved under two different selection optima – for dispersal (with small larvae), and for reproduction (with larger adults)⁴².

In most insects, however, the winged adult is more motile than the larva and thus the roles of habitat selection and dispersal can be best considered to have been transferred to the adults. The origin of metamorphosis in insects appears to be connected to the evolution of wings

and flight, as this is the most significant difference between ametabolous wingless insects and metamorphic pterygotes⁶. There is some palaeontological evidence suggesting that Palaeozoic insects showed gradual wing development, without any particular metamorphic instar, with several flying instars⁴³. Wing precursors are likely to have a function like short-distance flight or gliding, to avoid predators or move from one plant to another, before long-distance flight evolved. With the perfection of long-distance flight, the winglets of non-flying juveniles probably underwent negative selection, to facilitate easier locomotion and molting. There could also have occurred a transition of juveniles to aquatic environment, which contributed towards loss of wings in juveniles, as interestingly, ancient insect taxa like Ephemeroptera, Odonata, Megaloptera and Plecoptera possess aquatic larvae. The reduction of wings in juveniles probably contributed to their success in rendering them less vulnerable and capable of invading new microhabitats. Following this, the diversification between early and late instars gave rise to the origin of metamorphosis⁶. Further modifications like establishment of a predetermined final instar, and fixing reproductive activities to this instar have led to the situation found in modern insects. Some fossils along with the development of modern Ephemeroptera (two-winged instars – sexually immature sub-imago and mature imago) suggest that structural metamorphosis to a flying stage can precede sexual maturation by at least one molt. This suggests that prometaboly was probably the ancestral state of modern insects from which hemimetaboly evolved (Figure 5). Indeed, some Ephemeroptera exhibit hemimetaboly where the original adult instar is believed to have been lost, and the function of reproduction transferred to the sub-imago, providing evidence for evolution of hemimetaboly from prometaboly. Later, holometaboly evolved from hemimetaboly (Figure 5). The holometabolan pupa is considered as a premetamorphic instar, and the adult instars may have originated as a reproducing first postmetamorphic sub-imago. Difficulty in molting may have favoured the molt restrictions in flying forms, and once a predetermined final instar was established, the final adult instar could undergo morphological modifications rendering it incompatible with further molting,

but enhancing and perfecting the ability to fly instead⁶. It is interesting to note that secondary loss of wings in adults of various insects has never resulted in the restoration of adult molting. This is how metamorphosis probably evolved from an ancestral wingless ametabolous condition.

Concluding remarks

Charles Darwin narrated in *The Voyage of the Beagle*⁴⁴, a story about a German collector in natural history named Renous, residing in San Fernando, Chile, who had been arrested on charges of heresy because he could allegedly change disgusting looking caterpillars into beautiful butterflies! Such was the mystery of metamorphosis in the backdrop of ignorance and religious xenophobia of the mid-nineteenth century! Since then, considerable progress has been made over the years to unravel this mystery. Many of the underlying physiological factors that govern metamorphosis are known now, and this has also resulted in developing and understanding the difference between hemimetaboly and holometaboly. Some understanding on how metamorphosis evolved has also emerged, and the recent development of the pronymph theory has generated renewed interest in investigating the origins of metamorphosis. However, most of the information on the physiology of metamorphosis comes from studying *Manduca sexta* (moth) and *Drosophila melanogaster* (fruit fly), and a few other hemimetabolous insects. Inclusion of other insects, especially taxa that show other types of metamorphosis like prometaboly, will throw light on the origin of hemimetaboly from prometaboly. Development of apterous ametabolous insects also needs to be studied in more detail to better understand the origins of the physiological pathways that govern metamorphosis. Although much of the mystery of how ‘disgusting looking caterpillars’ can turn into ‘beautiful butterflies’ has been cleared, aspects of this amazing transformation still remain waiting to be unravelled.

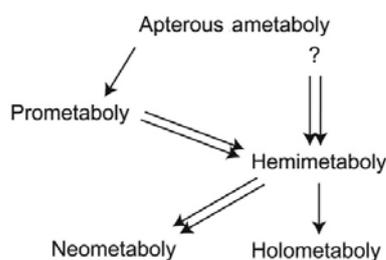


Figure 5. Presumed evolutionary relationships of different kinds of insect development. Single arrow – single origin, double arrow – multiple origins (adapted from Sehnal *et al.*⁶, with permission from Elsevier).

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