Origin and Evolution of Insect Metamorphosis

Xavier Belles, *Institute of Evolutionary Biology (CSIC-UPF), Barcelona, Spain*

Advanced article

Article Contents

Insect metamorphosis can be classified into three modalities: ametabolan (no changes), hemimetabolan (progressive changes) and holometabolan (dramatical changes at the end of the cycle). The metamorphic changes are mainly regulated by two hormones: the moulting hormone, which promotes the moults, and the juvenile hormone (JH), which represses the transformation into the adult. The action of these two hormones is mediated by a number of transcription factors, and the molecular mechanisms regulating the expression of these and of the corresponding target genes are finally refined by the action of micro ribonucleic acids. Among the different transcription factors involved, those of the Broad-Complex group are especially interesting because they have a differential expression and JH dependency in holometabolans and hemimetabolans. Holometabolan metamorphosis probably evolved from hemimetabolan ancestors, although the mechanisms underlying such a transition are still obscure.

Introduction

One of the first formal theories related to the origin of insect metamorphosis was presented by William Harvey in 1651. He proposed that the insect egg contains so scarce nutrients that the embryo is forced to hatch before completing development. Then, during the larval life, the animal would accumulate enough resources to reach the pupal stage, which Harvey considered as the perfect egg. Soon after, however, Jan Swammerdam showed with their skilful dissections that the pupa is not a sort of egg but a transitional stage between the larvae and the adult. The careful studies of Swammerdam allowed him to classify insect metamorphoses into four main types. He categorised the first type for species that grow without transformation (lice were his example); in a second type, he included the species that develop the wings progressively and that transform into adults without any intermediate, quiescent stage (as crickets and cockroaches); a third type was represented by species whose wings develop under the larval cuticle and that undergo a quiescent pupal stage before transforming into adults (as in butterflies and beetles) and in a fourth type Swammerdam included the species that pass the pupal stage under the skin of the last larval stage (represented by flies).

ELS subject area: Evolution and Diversity of Life

How to cite:
Belles, Xavier (March 2011) Origin and Evolution of Insect Metamorphosis. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0022854

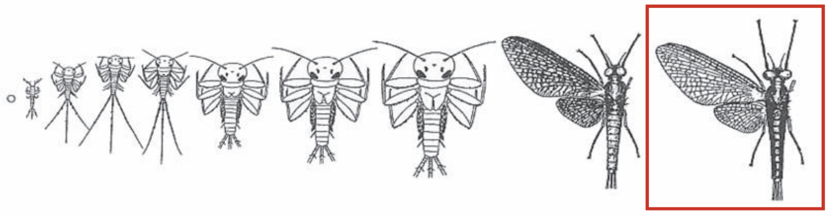
Although refined and completed with many examples, Swammerdam's categories are essentially the same used today. Indeed, contemporary textbooks classify the different modalities of insect metamorphosis into three main groups: ametabolans, which do not experience morphological transformations along the biological cycle; hemimetabolans, which hatch as nymphs with a morphology similar to that of the adult and grow progressively until the adult stage, which gain full flying wings and functional genitalia; and holometabolans, which hatch as a larva morphologically very different from the adult, then progressively grow through successive moults until the last larval instar, after which they moult into the pupal stage, often quiescent and similar to the adult, and then to the adult stage, with flying wings and fully formed and functional genitalia (**Figure 1**). These three categories correspond very closely to the three first types of Swammerdam, whereas the fourth type is a variant of the holometabolan modality, represented by the highly modified group of dipterans. **See also**: Insecta (Insects)

Types of Metamorphosis in a Phylogenetical Context

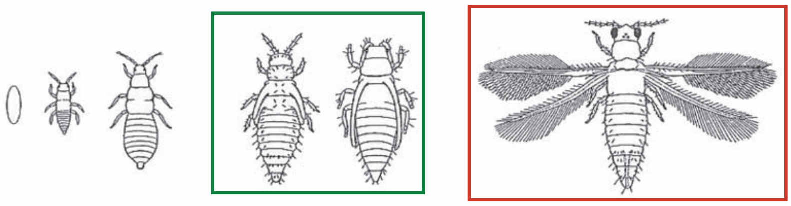
Among the three types of metamorphosis, the more modified is the holometabolan, which matches the monophyletic clade Endopterygota (= Holometabola, **Figure 2**). As aforementioned, holometabolan hatch as larvae

Ametabolan mode (Example: Archaeognatha)

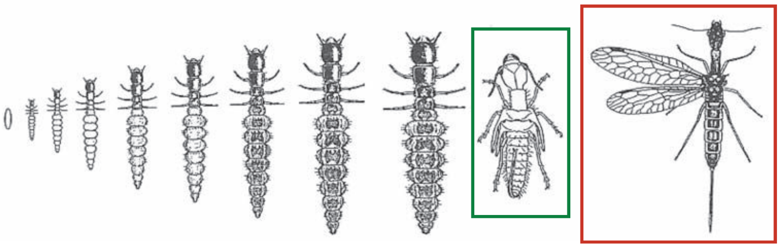
Hemimetabolan mode (Example: Orthoptera)



Prometabolan mode (Example: Ephemeroptera)



Neometabolan mode (Example: Thysanoptera)



Holometabolan mode (Example: Raphidoptera)

Figure 1 The main types of insect metamorphosis, ametabolan, hemimetabolan and holometabolan, and the subtypes of hemimetabolan, prometabolan and neometabolan. Quiescent stages are showed in a green square. Adult, reproductively competent stages are showed in a red square. In the ametabolans, the red square is open because the adult continues moulting. Modified from Sehnal *et al.* (1996). Published with permission from Elsevier.

morphologically different from the adult (**Figure 1**). In most cases, the primordia of adult organs (like wings, legs, eyes, genitalia and others) are placed within the larva as imaginal discs, and the complete development and eversion of them occur in the metamorphic transition (Svacha, 1992). Nevertheless, the diversity of situations concerning the type of imaginal discs and the moment in which they are first detectable is remarkable (Svacha, 1992), and the classification of such diversity would require studies in all representative groups before establishing generalisations. Among the holometabolans, the best known orders are Coleoptera, Hymenoptera, Lepidoptera and Diptera. In some groups, larvae undergo remarkable morphological changes in successive instars one or more times before reaching the pupal stage. It is the phenomenon known as hypermetamorphosis. The cases showing most dramatic changes are associated to very specialised predatory habits or with parasitic life styles. There are examples in a number of Neuroptera (Mantispidae), several Coleoptera (in Meloidae, Rhipiphoridae and some other families), in all Strepsiptera, in many Hymenoptera, in some Diptera and in few isolated examples of other orders of Endopterygota (**Figure 2**).

The hemimetabolans hatch in nymphal form, with a morphology similar to that of the adult, and grow progressively through a number of moults until the last nymphal instar, which is followed by the adult stage that differs from the nymph at least by the presence of

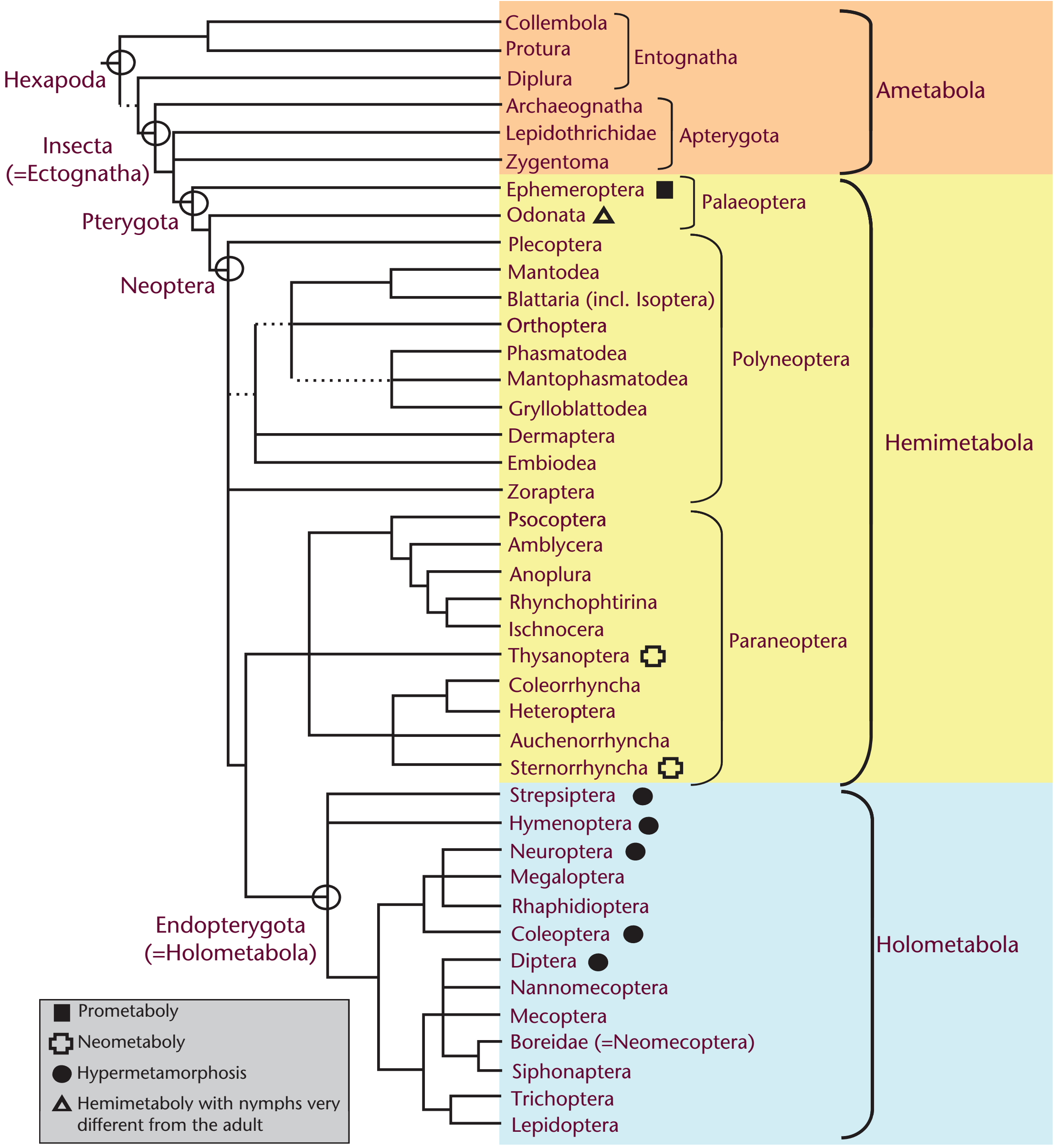


Figure 2 Hexapodan phylogeny, with indication of the types of metamorphosis in the different taxa. The phylogenetic reconstruction is based on Wheeler *et al.* (2001), Grimaldi and Engel (2005) and Kjer *et al.* (2006).

functional wings and genitalia (**Figure 1**). The polyneopteran and paraneopteran groups contain the most typical hemimetabolans (**Figure 2**), and familiar examples are those of the orders Orthoptera, Blattaria and Hemiptera. Particular groups of paraneopterans, as several Thysanoptera and Sternorrhyncha (Aleirodids and males of some Coccids), show an essentially hemimetabolan development but include one to three quiescent stages, which are reminiscent of the holometabolan pupal stage. This particular cycle has been distinguished as neometabolan development (Sehnal *et al.*, 1996) (**Figure 1**). Odonata and Ephemeroptera are two monophyletic groups whose metamorphosis is essentially hemimetabolan but with a number of particularities. The nymphs of Odonata are aquatic and morphologically quite different from the adult. The post-embryonic development of Ephemeroptera consists in a series of nymphal stages similar to the adult but without developed wings; the last instar nymph transforms into a subimaginal stage, with functional wings but without reproductive capabilities, which is followed by the perfect adult, winged and reproductively competent. This particular development has been categorised as prometabolan (**Figure 1**) (Sehnal *et al.*, 1996). Entognatha (Diplura, Protura and Collembola) and Apterygota (Archaeognatha, Lepidotrichidae and Zygentoma, **Figure 2**) are ametabolans; they hatch as nymphs with adult morphology and grow along their life through successive moults. After reaching the reproductively competent stage, they can continue moulting and growing (**Figure 1**), which is not the case of the holometabolans and hemimetabolans.

Endocrine Basis

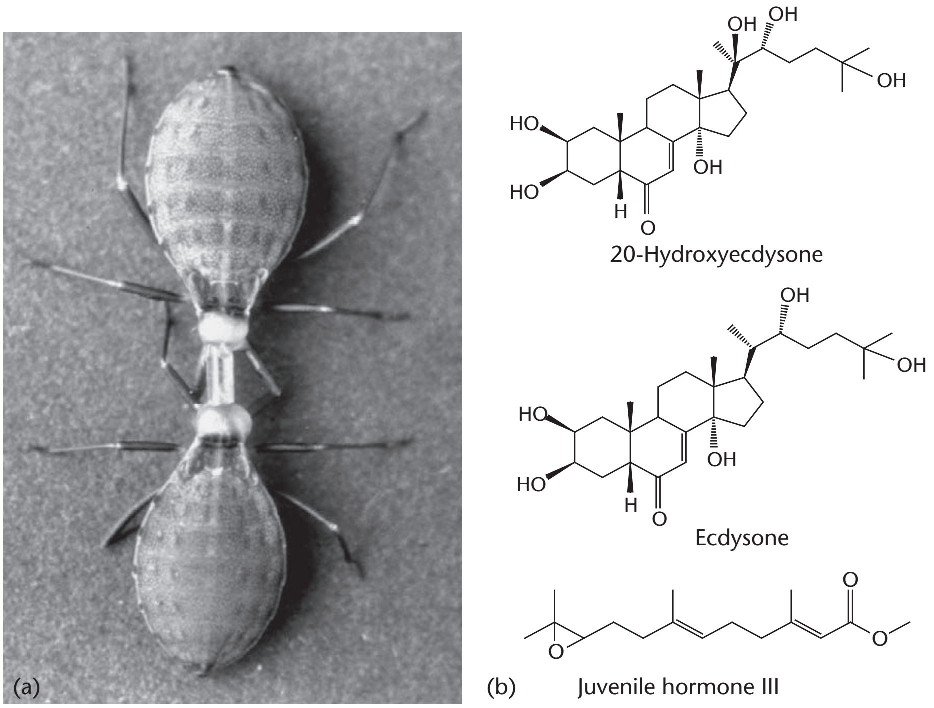
The basic information about the endocrine control of metamorphosis was established by the skilful experiments that Vincent B. Wigglesworth started in the 1930s, most of them involving parabiosis, that is, the physical connexion of two individuals that therefore share the haemolymph and the factors circulating in it. Typically, Wigglesworth connected two specimens at different developmental stages and one mediated the fate of the other thanks to the factors transported by the haemolymph, notably the moulting hormone and the juvenile hormone (JH, see discussion later, **Figure 3**) (Wigglesworth, 1954). Insects have a rigid exosqueleton and must moult to grow, and moulting is the basis for metamorphosis given that transformations take place through the moults. The moulting hormone has an ecdysteroidal structure (**Figure 3**), and during juvenile stages, it is synthesised by the prothoracic glands. Towards the end of each stage, ecdysteroid production increases rapidly, reaches maximal values and then decreases and remains low until the next moulting cycle (**Figure 4**). The increase phase is associated to the divisions that occur in the epidermal cells, then most of the ecdysteroid peak overlaps with the apolysis, that is, the separation of the old cuticle and the onset of secretion of a new one, and the final part of the peak coincides with the ecdysis, that is, the release of the old cuticle remnants or exuvia (Nijhout, 1994).

The other crucial endocrine factor in insect metamorphosis is the JH, which has a terpenoid structure (**Figure 3**) and is synthesised by the corpora allata. During the juvenile moults, JH levels are high, but in the pre-adult stage, they fall dramatically until being practically undetectable (**Figure 4**). JH has a repressor role upon metamorphosis, and its absence determines the metamorphosis (Nijhout, 1994; Riddiford, 2008).

At a higher level of regulation, the production of moulting hormone in the prothoracic glands is controlled by prothoracicotropic hormones, which have polypeptidic structure (Marchal *et al*., 2010), whereas that of JH in the corpora allata is regulated by allatotropic and allatostatic peptides (Weaver and Audsley, 2009). Also important are the peptidic hormones involved in regulating the ecdysis and emergence of the moulted specimen from the exuvia. Ecdysis is a complex process composed of pre-ecdysis, ecdysis and post-ecdysis behaviours, which are controlled by a cascade of peptide hormones from the Inka cells, and neuropeptides from the central nervous system. Inka cells produce pre-ecdysis and ecdysis-triggering hormones, which activate the ecdysis sequence initiated by the eclosion hormone through receptor-mediated actions on specific neurons (Zitnan *et al*., 2007).

Molecular Mechanisms

The most common moulting hormone is the ecdysteroid 20-hydroxyecdysone (**Figure 3**), and its effect is mediated by



(a)

(b) 20-Hydroxyecdysone

Ecdysone

Juvenile hormone III

Figure 3 (a) An experiment of parabiosis with the kissing bug, *Rhodnius prolixus*. Typically, VB Wigglesworth connected two specimens at different developmental stages and one mediated the fate of the other, thanks to the factors transported by the shared haemolymph. With these experiments, Wigglesworth demonstrated the existence of moulting and juvenile hormones. Photograph courtesy of the late Vincent B. Wigglesworth. (b) Structure of the main metamorphosis hormones in insects: ecdysone is the precursor of 20-hydroxyecdysone, the most common moulting hormone in insects; juvenile hormone III is the most common JH in insects.

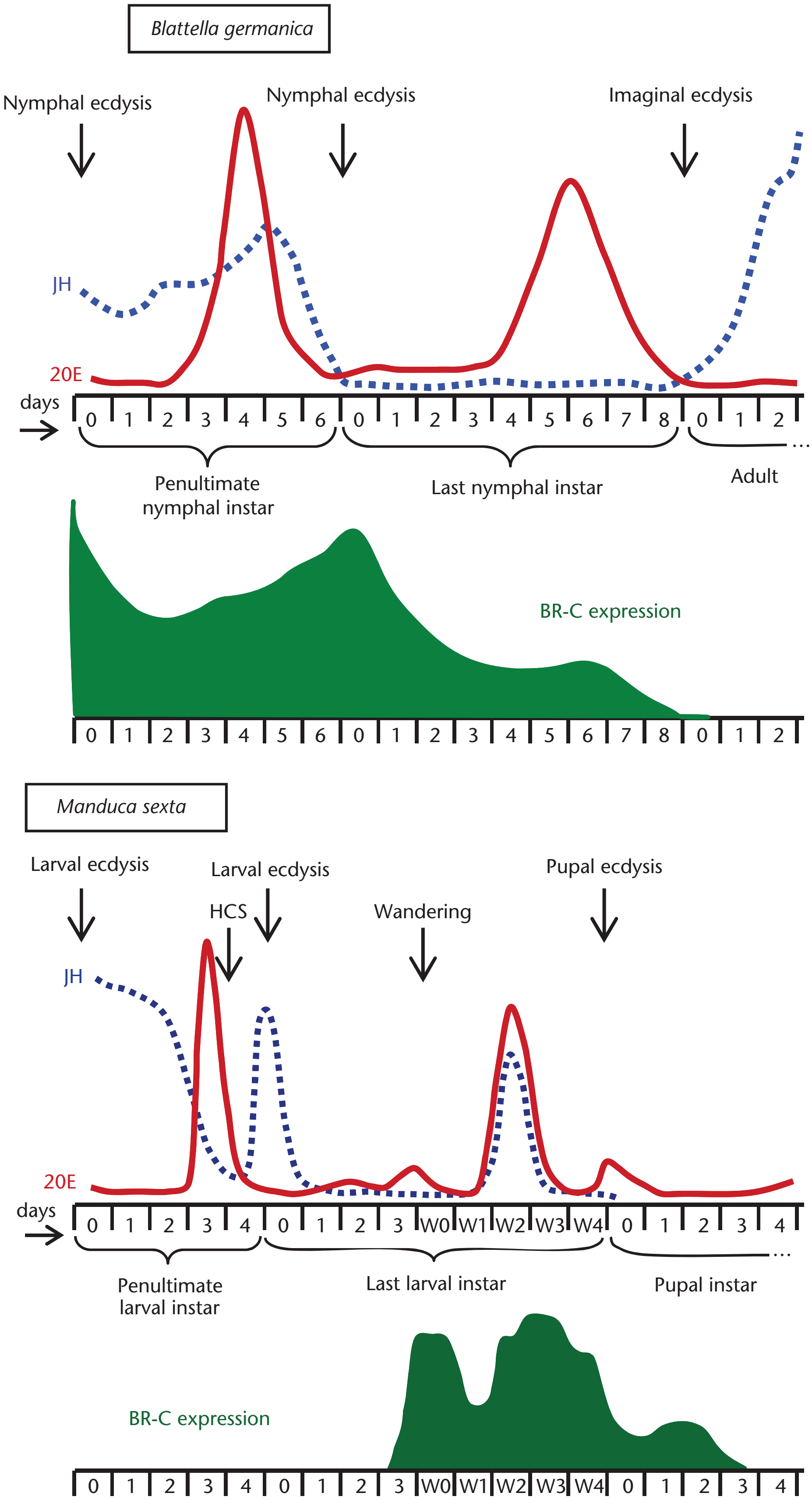


Figure 4 Diagram of the hormone titres and expression of Broad-Complex (BR-C) transcription factors in the last developmental instars in a hemimetabolan (the German cockroach, *Blattella germanica*) and a holometabolan (the tobacco hornworm, *Manduca sexta*). Hormone titres of *B. germanica* are from Romaña *et al.* (1995) and Treiblmayr *et al.* (2006), and BR-C data are from Belles X and Huang J-H (unpublished). Hormone titres and BR-C data of *M. sexta* are from Riddiford *et al.* (2003). HCS, head capsule slippage.

a genetic cascade of transcription factors. Upon binding to their heterodimeric receptor composed by two nuclear receptors, the ecdysone receptor (EcR) and the ultraspiracle (USP) or RXR, 20-hydroxyecdysone activate the expression of a hierarchy of transcription factors (HR3, HR4, HR39, E75, E78, FTZ-F1, etc.) that regulate the expression of the target genes underlying the cellular changes associated to moulting and metamorphosis (King-Jones and Thummel, 2005; Nakagawa and Henrich, 2009). Most of the reported data on this cascade of transcription factors refer to the higher Diptera *Drosophila melanogaster* and describe the mechanisms of 20-hydroxyecdysone not only in relation to moulting but also in the programmed cell death processes, which are associated to the metamorphic changes in this holometabolan (Thummel, 2002; Yin and Thummel, 2005). A number of studies carried out in the cockroach (Blattaria) *Blattella germanica*, a phylogenetically basal insect, indicate that the transcription factors involved in 20-hydroxyecdysone signalling are conserved, although details of the precise hierarchical and epistatic relationships between them may differ in hemimetabolans and holometabolans (Martin *et al.*, 2006; Cruz *et al.*, 2007, 2008; Mane-Padros *et al.*, 2008).

The molecular mechanisms underlying the action of the second main hormone involved in metamorphosis, the JH, are much less known (Riddiford, 2008). However, it has been unambiguously shown that the transcription factors Methoprene tolerant (Met) and Krüppel homolog 1 (Kr-h1) are involved in the transduction of the JH signal in the context of metamorphosis, at least in the holometabolans *D. melanogaster* and *Tribolium castaneum* (Coleoptera). Data suggest that Met might play the role of JH receptor (Riddiford, 2008), whereas Kr-h1 might be involved in early transduction of the JH signal, downstream of Met (Minakuchi *et al.*, 2008, 2009).

Recent experiments have shown the occurrence of a new level of regulation governed by micro ribonucleic acids (RNAs), which are RNAs of approximately 22 nucleotides that play a generally repressing action on messenger RNA (mRNA) stability and translation. Using RNA interference (RNAi) on *B. germanica*, Gomez-Orte and Belles (2009) silenced the expression of the enzyme dicer-1 (which is essential for the formation of microRNAs) in the last nymphal instar. This impaired the production of microRNAs, and the experimental insects instead of moulting to the adult stage transformed into supernumerary nymphs, which shows that microRNAs are crucial in insect metamorphosis, at least in hemimetabolans.

Broad-Complex Transcription Factors

Finally, an important group of transcription factors involved in metamorphosis is that known as Broad-Complex (BR-C). The BR-C family of transcription factors is especially interesting because they have different ways of action in hemimetabolans and holometabolans. Functional studies in *D. melanogaster* and the lepidopterans *Manduca sexta* and *Bombyx mori* have shown that BR-C factors are essential for the transformation of last instar larvae into pupae (Riddiford *et al.*, 2003; Uhlirova *et al.*, 2003; Zhou and Riddiford, 2002). In *T. castaneum* and the plecopteran *Chrysopa perla*, which are phylogenetically basal holometabolans, BR-C factors are also crucial for pupal differentiation (Konopova and Jindra, 2008). RNAi experiments carried out in the hemimetabolan *Oncopeltus fasciatus* (Heteroptera; Erezyilmaz *et al.*, 2006) suggested to these authors that BR-C transcription factors would govern progressive morphogenesis through the successive nymphal moults. What is clear from these data is that wing buds growth along nymphal stages of *O. fasciatus* depend on the expression of BR-C factors.

Interestingly, the hormonal contexts of these effects are different in holometabolans and hemimetabolans. In the hemimetabolans the situation is quite simple. Although with fluctuations possibly related to the successive bursts of moult-inducing ecdysteroids, BR-C factors are expressed all along the young nymphal stages, and expression only declines in the last nymphal instar, as observed in the milkweed bug *O. fasciatus* (Erezyilmaz *et al.*, 2006) and in the German cockroach *B. germanica* (Belles X and Huang J-H unpublished) (**Figure 4**), where six BR-C transcription factors have been reported (Piulachs *et al.*, 2010). During the last nymphal instar of *B. germanica*, however, there is a small peak of *BR-C* expression around the peak of ecdysone (**Figure 4**), which is produced in the absence of JH and that might be physiologically significant in the context of the adult moult.

In the post-embryonic development of the holometabolans *D. melanogaster*, *M. sexta* and *B. mori*, BR-C factors are expressed after a small peak of ecdysone produced in the absence of JH at the end of the last larval instar (**Figure 4**). Under these hormonal conditions, BR-C transcripts appear at the end of the feeding stage (beginning of wandering behaviour) in the epidermis of last instar larvae of *M. sexta*, and the insect becomes committed to pupal differentiation. Interestingly, exogenous administration of JH in this stage prevents the ecdysteroid-induced expression of *BR-C* (Zhou *et al.*, 1998). Later, BR-C transcripts levels decrease during the pupal stage (**Figure 4**) and the pupae transform into the adult. In pupae, exogenous JH induces the re-expression of *BR-C* and the insect undergoes a second pupal moult. These observations indicate that BR-C transcription factors determine the transition from larvae to pupae (Zhou and Riddiford, 2002). RNAi studies in *T. castaneum* (Konopova and Jindra, 2008; Parthasarathy *et al.*, 2008; Suzuki *et al.*, 2008) indicate that BR-C transcription factors are crucial for pupae formation also in this basal holomatabolan. Moreover, these studies suggest that BR-C factors are also important to prevent adult development, as individuals resulting from BR-C knockdown in larvae not only show features of larvae-pupae intermediates but also a number of adult characteristics.

The information available suggests that the hemimetabolans maintain the nymphal growth, especially wing buds development, with the high concentrations of JH that induce the expression of *BR-C*, and they moult into the adult stage when JH concentration and *BR-C* expression decrease in the last instar nymph. The small burst of expression observed in the last instar nymph of *B. germanica* (**Figure 4**) additionally suggests that BR-C transcription factors may still have a function in the adult moult. The holometabolans go through the larval stages in the presence of high concentrations of JH but with the expression of BR-C low or absent. Only at the end of the last larval instar, a small ecdysteroid peak in the absence of JH determines the onset of *BR-C* expression, and from this moment, the hormonal regulation is similar to that of the hemimetabolans: when JH levels decline in the pupae, the expression of BR-C transcription factors also declines, and the insect moults into the adult stage. Interestingly, *BR-C* expression shows a small peak in the first days of the pupal instar that is reminiscent of that observed in the last nymphal instar of *B. germanica* (**Figure 4**), and which could be relevant for the proper adult moult. The inhibitory properties of JH upon *BR-C* expression in the last instar larvae of *M. sexta*, when they become committed to pupal differentiation (Zhou *et al.*, 1998), seem specific of holometabolans. Indeed, these observations suggest that holometabolans maintain low levels of *BR-C* expression during larval life due to the high levels of JH, and from an evolutionary point of view, one can imagine that the inhibitory properties of JH upon *BR-C* expression in immature instars of holometabolans might have been a key innovation in the transition from hemimetaboly to holometaboly.

Functional Sense and Origin of the Metamorphosis

Between 45% and 60% of all living species are holometabolan insects (Hammond, 1992), which suggests that this type of metamorphosis is very efficient. Given that normally the resources exploited by juvenile stages are different from those used by the adult, juveniles and adults do not compete among them. This seems to confer an advantage to holometabolans, and might have been one of the evolutionary driving forces leading to the present diversity of forms and physiologies within the same group and even within the same species. **See also**: Insecta (Insects)

From a palaeontological point of view, the first winged insects appeared in the Palaeozoic. Carboniferous strata (approximately 350 Ma) already afford a remarkable diversity of fossil species with functional wings. The fossil remains indicate that the primitive Apterygota and the first winged insects were ametabolans. Towards the end of the Carboniferous and beginning of the Permian (approximately 300 Ma), practically all pterygotes showed a post-embryonic development divided into nymphal stages, showing slight metamorphic transitions between them and finishing in an adult stage, which indicates that hemimetaboly had already emerged. The first fossil insects that can be considered holometabolans appear in Permian strata (approximately 280 Ma; Kukalová-Peck, 1991; Labandeira and Phillips, 1996).

According to the latest phylogenetic studies (**Figure 2**) (Grimaldi and Engel, 2005; Kjer *et al.*, 2006; Wheeler *et al.*, 2001), the Endopterygota (= Holometabola) are monophyletic, which suggests that the innovation of holometabolan metamorphosis appeared only once. These studies also show that the sister group of the Endopterygota is that of paraneopterans, whose species are hemimetabolan but including a number of neometabolan groups (**Figure 1**) (Sehnal *et al.*, 1996). Therefore, the most parsimonious hypothesis is to consider that the holometabolans would have originated from hemimetabolan ancestors. Moreover, the neometabolan metamorphosis suggests that the pupa might not be such an exceptional innovation within the holometabolan. Thus, from a nymph relatively similar to the adult of the ancestor of Endopterygota + Paraneoptera, one can imagine a holometabolan evolution favouring those morphological and physiological modifications of the juvenile stages allowing the exploitation of new resources. For example, favouring those changes leading to the transformation of a nymph with legs and external wing primordia into a vermiform, apodous and apterous larvae, which would be more efficient for making galleries into a flesh fruit (like the maggot of a fruitfly), or to live within another insect (as the larvae of entomoparasitic hymenopterans). The adult, conversely, would optimise the reproductive capabilities: in addition to form the external genitalia, it would gain mobility becoming a flying insect, thus improving the possibilities for mate finding.

Current Theories on the Evolution of Metamorphosis

In 1883, John Lubbock took up again the hypothesis of Harvey and thoroughly reasoned that the origin and evolution of insect metamorphosis could be explained by the precocious eclosion of the embryo in the holometabolans. Thus, in the case of species in which the larvae look like the adult (hemimetabolan metamorphosis), the embryo completes all developmental stages ('protopod', 'polipod' and 'oligopod' stages) within the eggshell. Conversely, the species with vermiform larvae and pupal stage (holometabolan metamorphosis) would hatch before finishing the development observed in the hemimetabolans. Then, they would complete development in the pupal stage. Some authors, like Charles Pérez in 1902, qualified this hypothesis as outlandish, but Antonio Berlese reelaborated it in 1913, whereas Augustus Daniel Imms disseminated it among Anglo-Saxon readers from 1925 (Wigglesworth, 1954).

According to the precocious eclosion theory, the hemimetabolan nymphal stages would be equivalent to the holometabolan pupa. To the critics of this theory, as Howard Hinton (Hinton, 1948), post-embryonic development would be equivalent in hemimetabolans and holometabolans, thus the hemimetabolan last nymphal instar would be homologous to the holometabolan pupae. Between these two conceptions, other homologation systems were proposed, like that of Poyarkoff in 1914 or that of Heslop-Harrison in 1955 (Heming, 2003; Sehnal *et al*., 1996). Modern opinions on the evolution of metamorphosis still oscillate between the ideas of Berlese and those of Hinton, although the theory of the precocious eclosion has experienced an energetic revival with the contributions of James Truman and Lynn Riddiford (Truman and Riddiford, 1999, 2002), who focused on the endocrine aspects.

Under this approach, their original hypothesis postulated that the hemimetabolan would hatch after three embryonic 'moults', giving a nymph similar to the adult, whereas the holometabolans would hatch after two embryonic 'moults', giving a vermiform larva, very different from the adult. The hypothesis is appealing by its simplicity and high explicative power. However, ultrastructural studies based on a number of species representing a diversity of orders (Ephemeroptera, Odonata, Plecoptera, Neuroptera, Coleoptera, Lepidoptera, Mecoptera and Diptera) indicate that the embryo of all species produces three cuticle depositions (three 'moults'; Konopova and Zrzavy, 2005). The Diptera Cyclorrhapha (the most modified dipterans, like *D. melanogaster* upon which most of the experimental studies have been traditionally based) would be an exception, given that they show two embryonic cuticles, probably because the third one has been secondarily lost (Konopova and Zrzavy, 2005).

Moreover, it is important to note that the larvae of endopterygotes are very often more specialised than those of the exopterygotes. The maggot of a fruitfly, for example, cannot simply be envisaged as a vermiform and apodous creature that hatched in an early embryonic stage. On the contrary, it is an extremely specialised larva, secondarily but not primitively apodous. The mouth anatomy of most dipteran larvae is an example of this specialisation: the cardiostipes has become fused with the dististipes, as in some mosquitoes, and these remains of the maxilla are fused to the mandibles forming the typical mouth hooks of fly larvae. Therefore, a maggot, although apodous and vermiform, is more derived and specialised than the nymph of a cockroach, to put a characteristic hemimetabolan example.

Another important point in the Truman and Riddiford version of the precocious eclosion theory is the possible role of JH, whose production occurs earlier in the embryo of the studied holometabolan species (the tobacco hornworm *M. sexta* being used as example) than in the embryo of the studied hemimetabolan (the locust *Locusta migratoria* being used as example; Truman and Riddiford, 1999, 2002). These authors considered that morphogenesis of holometabolan became truncated as a result of the advancement of JH appearance during embryogenesis (see also Truman and Riddiford, 2007). However, treatment of embryos of the cricket *Acheta domesticus* (a typical hemimetabolan) with JH during the morphogenesis phase caused premature nymphal tissue maturation (Erezyilmaz *et al*., 2004), which did not clarify the above hypothesis of the truncation of development in the holometabolan embryo.

The modern version of the precocious eclosion theory has stimulated much additional research on the evolution of metamorphosis, especially focusing on hemimetabolan and phylogenetically basal holometabolan models, but it is still under debate. Indeed, textbooks in use today do not show a consensual view, and whereas some adhere to the theory (Grimaldi and Engel, 2005), others treat it more sceptically (Heming, 2003).

Perspectives

A first consideration that can be advanced is that establishing generalisations based on data obtained only on a handful species that are far from representing the morphological and functional diversity of insect metamorphosis may be unsafe. Especially, if the species on which we rely are a few holometabolan models remarkably modified, like lepidopterans and, especially dipterans.

Concerning the endocrine approach, it can afford valuable data, although they must be interpreted with due caution given that hormonal regulation is very plastic and prone to exaptations and convergences. In any case, pursuit of this approach would first require collecting basic information (especially quality data on ecdysteroid and JH levels in different stages, from the embryo to the last juvenile instar) from a greater diversity of species, in particular, in phylogenetically basal holometabolans (Coleoptera and Hymenoptera), in neometabolan and prometabolan hemimetabolans (Thysanoptera and Ephemeroptera) and in phylogenetically basal hemimetabolans, especially paleopterans like Odonata. Likewise, it would be convenient to compare the molecular mechanisms of hormonal action occurring in metamorphic and nonmetamorphic transitions in the present model species representing the hemimetabolans (Hemiptera, Orthoptera and Blattaria) and the holometabolans (Diptera, Hymenoptera, Coleoptera and Lepidoptera).

It is well known that some of the molecular mechanisms are conserved in holometabolans and hemimetabolans, but there are factors that behave divergently. Comparative studies of factors that have conserved the structure but whose functions and regulation became modified in the holometabolan evolution will afford new light to the mechanisms underlying the transition from hemimetaboly to holometaboly. An example of this is the aforementioned BR-C transcription factors. We have seen that the expression pattern, sensitivity to JH in different stages and specific functions of BR-C transcription factors in metamorphic changes are distinct in hemimetabolans and holometabolans (Erezyilmaz *et al*., 2006; Konopova and Jindra, 2008; Belles X and Huang J-H, unpublished). Of note, BR-C transcription factors contain a Broad complex-Tram track-brick a brack (BTB) domain, and such domain

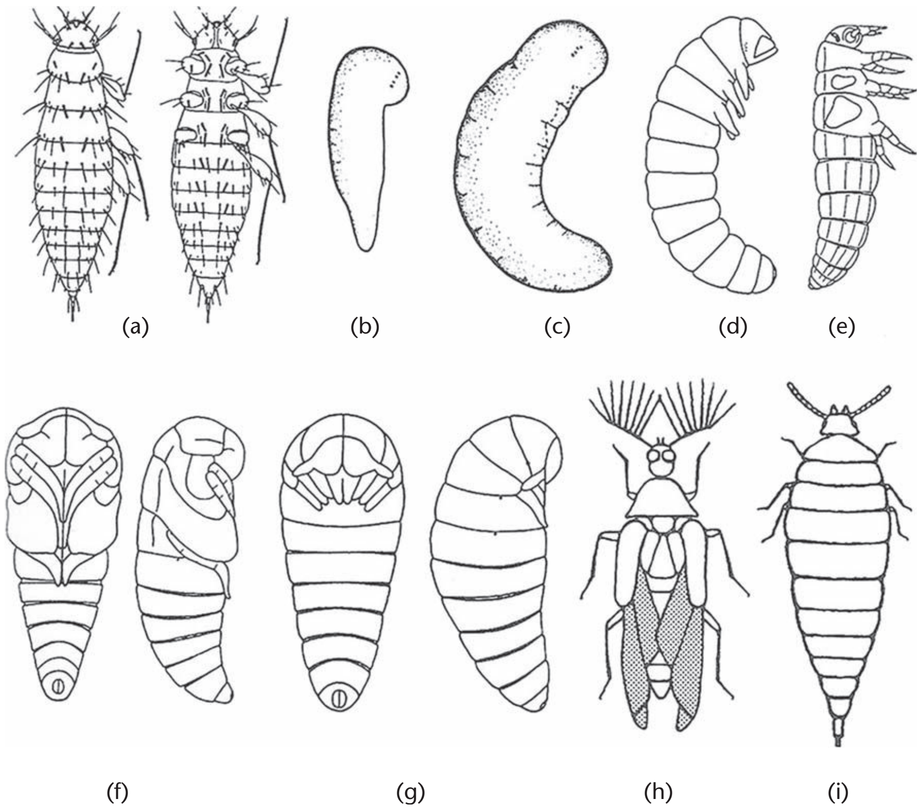


Figure 5 Successive stages in the biological cycle of the Ripiphorid beetle *Rhipidius quadriceps*, which is endoparasitic of cockroaches. (a) Triungulin larva, legged and very mobile (it searches the host and penetrates into it); (b–c) second larval type in different states of growth (which lives within the host); (d) third larval type (which also lives within the host); (e) fourth larval type (which leaves the host and pupates outside); (f) male pupae; (g) female pupae; (h) adult male and (i) adult female. Hypermetamorphic species like *R. quadriceps* can be key subjects to study the regulation and the evolution of insect metamorphosis. Drawings courtesy of Claude Besuchet.

mediates not only homo- and heterodimerisation, but also protein–protein interactions allowing the recruitment of corepressor complexes, including histone deacetylases, which cause chromatin deacetylation, thus contributing to transcriptional repression (Ahmad *et al.*, 2003; Albagli-Curiel, 2003; Costoya, 2007; Perez-Torrado *et al.*, 2006). This potential suggests that BR-C transcription factors might had acquired properties of regulators of metamorphic changes through evolution. Thus, they might have played a key role in the transition from hemimetaboly to holometaboly through changes of expression pattern, of sensitivity to hormones, especially to JH, and thanks to the acquisition of new morphogenetic roles.

In the context of complex metamorphic changes, the analysis of the endocrine correlations in the different stages of hypermetamorphic species might be practical in terms of experimental economy, given that hypermetamorphosis summarises in a single species most of the possible transformations occurring at the entire insect class. Taking an endoparisitic Ripiphorid beetle as an example (**Figure 5**) (Besuchet, 1956), it would be interesting to study how hormonally regulated are the successive transitions from the structurally complex triungulin (fully legged and mobile) larva to the subsequent extremely simplified apodous and vermiform second instar larva, then to a third larval type with a sort of leg primordia (a type that is maintained through a number of instars), then to a fourth larval type with long-segmented clawed legs, then to the pupae and finally to the adult (Besuchet, 1956).

In his book describing the journey in the *Beagle*, Charles Darwin narrates that when he arrived at San Fernando, in Chile, the authorities had arrested a man called Renous accused of witchcraft because he was capable of transforming disgusting worms into beautiful butterflies. The episode was only the persistence of the mystery of metamorphosis in a context of extreme ignorance in the middle of the nineteenth century. As we have seen in the earlier discussion, much progress has been achieved for unveiling this mystery, although the study of the evolution of metamorphosis still keeps key black boxes, the unveiling of which do not seem immediate. However, the molecular scales of observation and the new experimental tools will surely bring about new debates, hot and illuminating.

References

Ahmad KF, Melnick A, Lax S *et al.* (2003) Mechanism of SMRT corepressor recruitment by the BCL6 BTB domain. *Molecular Cell* **12**: 1551–1564.

Albagli-Curiel O (2003) Ambivalent role of BCL6 in cell survival and transformation. *Oncogene* **22**: 507–516.

Besuchet C (1956) Biologie, morphologie et systématique des *Rhipidius* (Col. Rhipiphoridae). *Mitteilungen der Schweizerische Entomologische Gesellschaft* **29**: 73–144.

Costoya JA (2007) Functional analysis of the role of POK transcriptional repressors. *Briefings in Functional Genomics & Proteomics* **6**: 8–18.

Cruz J, Martin D and Belles X (2007) Redundant ecdysis regulatory functions of three nuclear receptor HR3 isoforms in the direct-developing insect *Blattella germanica*. *Mechanisms of Development* **124**(3): 180–189.

Cruz J, Nieva C, Mane-Padros D, Martin D and Belles X (2008) Nuclear receptor BgFTZ-F1 regulates molting and the timing of ecdysteroid production during nymphal development in the hemimetabolous insect *Blattella germanica*. *Developmental Dynamics* **237**: 3179–3191.

Erezyilmaz DF, Riddiford LM and Truman JW (2004) Juvenile hormone acts at embryonic molts and induces the nymphal cuticle in the direct-developing cricket. *Development Genes and Evolution* **214**: 313–323.

Erezyilmaz DF, Riddiford LM and Truman JW (2006) The pupal specifier Broad directs progressive morphogenesis in a direct-developing insect. *Proceedings of the National Academy of Sciences of the USA* **103**: 6925–6930.

Gomez-Orte E and Belles X (2009) MicroRNA-dependent metamorphosis in hemimetabolan insects. *Proceedings of the National Academy of Sciences of the USA* **106**: 21678–21682.

Grimaldi D and Engel MS (2005) *Evolution of the Insects*. Cambridge: Cambridge University Press.

Hammond P (1992) Species inventory. In: Groombridge B (ed.) *Global Biodiversity*, pp. 17–39. London: Chapman and Hall.

Heming BS (2003) *Insect Development and Evolution*. Ithaca, NY: Comstock Publishing Associates.

Hinton HE (1948) The origin and function of the pupal stage. *Proceedings of the Royal Entomological Society of London. Series A* **38**: 77–85.

King-Jones K and Thummel CS (2005) Nuclear receptors—a perspective from *Drosophila. Nature Reviews. Genetics* **6**: 311–323.

Kjer KM, Carle FL, Litman J and Ware J (2006) A molecular phylogeny of Hexapoda. *Arthropod Systematics & Phylogeny* **64**: 35–44.

Konopova B and Jindra M (2008) Broad-complex acts downstream of Met in juvenile hormone signaling to coordinate primitive holometabolan metamorphosis. *Development* **135**: 559–568.

Konopova B and Zrzavy J (2005) Ultrastructure, development, and homology of insect embryonic cuticles. *Journal of Morphology* **264**: 339–362.

Kukalová-Peck J (1991) Fossil history and evolution of hexapod structures. In: *The Insects of Australia*, vol. 1, pp. 141–179. Carlton: CSIRO, Melbourne University Press.

Labandeira CC and Phillips TL (1996) A Carboniferous insect gall: insight into early ecologic history of the Holometabola. *Proceedings of the National Academy of Sciences of the USA* **93**: 8470–8474.

Mane-Padros D, Cruz J, Vilaplana L *et al*. (2008) The nuclear hormone receptor BgE75 links molting and developmental progression in the direct-developing insect *Blattella germanica*. *Developmental Biology* **315**: 147–160.

Marchal E, Vandersmissen HP, Badisco L *et al*. (2010) Control of ecdysteroidogenesis in prothoracic glands of insects: a review. *Peptides* **31**: 506–519.

Martin D, Maestro O, Cruz J, Mane-Padros D and Belles X (2006) RNAi studies reveal a conserved role for RXR in molting in the cockroach *Blattella germanica*. *Journal of Insect Physiology* **52**: 410–416.

Minakuchi C, Namiki T and Shinoda T (2009) Kruppel homolog 1, an early juvenile hormone-response gene downstream of Methoprene-tolerant, mediates its anti-metamorphic action in the red flour beetle *Tribolium castaneum*. *Developmental Biology* **325**: 341–350.

Minakuchi C, Zhou X and Riddiford LM (2008) Kruppel homolog 1 (Kr-h1) mediates juvenile hormone action during metamorphosis of *Drosophila melanogaster*. *Mechanisms of Development* **125**: 91–105.

Nakagawa Y and Henrich VC (2009) Arthropod nuclear receptors and their role in molting. *FEBS Journal* **276**: 6128–6157.

Nijhout HF (1994) *Insect Hormones*. Princeton, NJ: Princeton University Press.

Parthasarathy R, Tan A, Bai H and Palli SR (2008) Transcription factor Broad suppresses precocious development of adult structures during larval-pupal metamorphosis in the red flour beetle, *Tribolium castaneum*. *Mechanisms of Development* **125**: 299–313.

Perez-Torrado R, Yamada D and Defossez PA (2006) Born to bind: the BTB protein-protein interaction domain. *BioEssays* **28**: 1194–1202.

Piulachs MD, Pagone V and Belles X (2010) Key roles of the Broad-Complex gene in insect embryogenesis. *Insect Biochemistry and Molecular Biology* **40**: 468–475.

Riddiford LM (2008) Juvenile hormone action: a 2007 perspective. *Journal of Insect Physiology* **54**: 895–901.

Riddiford LM, Hiruma K, Zhou X and Nelson CA (2003) Insights into the molecular basis of the hormonal control of molting and metamorphosis from *Manduca sexta* and *Drosophila melanogaster*. *Insect Biochemistry and Molecular Biology* **33**: 1327–1338.

Romaña I, Pascual N and Bellés X (1995) The ovary is a source of circulating ecdysteroids in *Blattella germanica* (L.) (Dictyoptera, Blattellidae). *European Journal of Entomology* **92**: 93–103.

Sehnal F, Svacha P and Zrzavy JZ (1996) Evolution of insect metamorphosis. In: Gilbert LI, Tata JR and Atkinson BG (eds) *Metamorphosis. Postembryonic reprogramming of gene expression in amphibian and insect cells*, pp. 3–58. San Diego, CA: Academic Press.

Suzuki Y, Truman JW and Riddiford LM (2008) The role of Broad in the development of *Tribolium castaneum*: implications for the evolution of the holometabolous insect pupa. *Development* **135**: 569–577.

Svacha P (1992) What are and what are not imaginal discs: reevaluation of some basic concepts (Insecta, Holometabola). *Developmental Biology* **154**: 101–117.

Thummel CS (2002) Ecdysone-regulated puff genes 2000. *Insect Biochemistry and Molecular Biology* **32**: 113–120.

Treiblmayr K, Pascual N, Piulachs MD, Keller T and Belles X (2006) Juvenile hormone titer versus juvenile hormone synthesis in female nymphs and adults of the German cockroach, *Blattella germanica*. *Journal of Insect Science* **6**: 47.

Truman JW and Riddiford LM (1999) The origins of insect metamorphosis. *Nature* **401**: 447–452.

Truman JW and Riddiford LM (2002) Endocrine insights into the evolution of metamorphosis in insects. *Annual Review of Entomology* **47**: 467–500.

Truman JW and Riddiford LM (2007) The morphostatic actions of juvenile hormone. *Insect Biochemistry and Molecular Biology* **37**: 761–770.

Uhlirova M, Foy BD, Beaty BJ *et al*. (2003) Use of Sindbis virus-mediated RNA interference to demonstrate a conserved role of Broad-Complex in insect metamorphosis. *Proceedings of the National Academy of Sciences of the USA* **100**: 15607–15612.

Weaver RJ and Audsley N (2009) Neuropeptide regulators of juvenile hormone synthesis: structures, functions, distribution, and unanswered questions. *Annals of the New York Academy of Sciences* **1163**: 316–329.

Wheeler WC, Whiting M, Wheeler QD and Carpenter JM (2001) The phylogeny of the extant hexapod orders. *Cladistics* **17**: 113–169.

Wigglesworth VB (1954) *The Physiology of Insect Metamorphosis*. Cambridge, MA: Cambridge University Press.

Yin VP and Thummel CS (2005) Mechanisms of steroid-triggered programmed cell death in *Drosophila*. *Seminars in Cell & Developmental Biology* **16**: 237–243.

Zhou B, Hiruma K, Shinoda T and Riddiford LM (1998) Juvenile hormone prevents ecdysteroid-induced expression of broad complex RNAs in the epidermis of the tobacco hornworm, *Manduca sexta*. *Developmental Biology* **203**: 233–244.

Zhou X and Riddiford LM (2002) Broad specifies pupal development and mediates the 'status quo' action of juvenile hormone on the pupal-adult transformation in *Drosophila* and *Manduca*. *Development* **129**: 2259–2269.

Zitnan D, Kim YJ, Zitnanova I, Roller L and Adams ME (2007) Complex steroid-peptide-receptor cascade controls insect ecdysis. *General and Comparative Endocrinology* **153**: 88–96.