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Puberty & Metamorphosis Related to Reproduction: Metamorphosis, Insects

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Introduction

Although insects are often regarded as negative to our economy because of their phytophagous nature or their ability to act as vectors of diseases, mankind would have difficulties surviving without pollinator species, such as bumblebees and bees. Insects are the most abundant and diverse group of organisms on earth. Having maintained this position for over 400 million years, they have witnessed the rise and fall of the dinosaurs and survived planet-wide extinctions. Despite our best efforts at eradication of numerous pest insect populations, this very speciose group of animals continues to thrive. Insects share a unique combination of attributes that has enabled their unparalleled success. This includes a small body size, the ability to fly, high reproductive potential, the presence of an exoskeleton, their developmental plasticity and ability to undergo metamorphosis. The changes that take place during this process can be extreme, allowing juvenile and adult insects to take advantage of different food sources and adapt in an ever-changing environment.

Insect Life Cycles

Insects do not have an internal skeleton as vertebrates do, instead, they are surrounded by a rigid armor, called the exoskeleton. This exoskeleton or cuticle protects them from desiccation and mechanical injury, but also gives the insect shape and provides a base to which muscles are attached. Although the rigidity of this cuticle protects them, it makes the apparently 'simple' process of growing extremely complex. For the insect to increase in size, the cuticle must be shed periodically and a larger one must be synthesized. During this process of molting a new cuticle is synthesized under the old one, part of the old cuticle is digested and resorbed and finally the remaining old cuticle is shed in a behavioral process called ecdysis.

In this way, the life cycle of an insect is characterized by several successive stages, each separated by a molt. Most insects hatch from eggs into a first juvenile stage. When the immature insect grows, it will pass through a defined number of instar stages (exact number varies depending on the insect species), before completing metamorphosis into the (winged) adult reproductive stage. Three major modalities of metamorphosis can be found within the diversity of insects. These include no changes or ametaboly; partial or incomplete metamorphosis known as hemimetaboly; and complete metamorphosis or holometaboly.

Metamorphosis is a marked change in body form, through which the adult gains distinct features. Ametabolous insects, such as silverfish (order Thysanura), do not undergo metamorphosis. In this design, the immature stages resemble the adult stage, except from the presence of mature gonads and functional genitals. At each molt these insects increase in size and their gonads and genitals develop progressively, until the animal is able to reproduce. Unlike hemimetabolous or holometabolous insects, Ametabola can continue to molt and grow after reaching the reproductively competent stage. Hemimetabolous insects, such as locusts (Orthoptera), cockroaches (Dictyoptera) and true bugs (order Hemiptera), undergo incomplete metamorphosis. The adult morphology of these insects closely resembles that of the instar stages (also called nymphs), but only differs by the presence of functional wings, gonads and genitals. Throughout their life cycle these insects undergo a gradual transformation with a sharp discontinuity at the adult molt, undergoing metamorphosis during a single molt (Fig. 1(A)). Since they maintain a similar body plan throughout their life, juvenile and adult stages of most hemimetabolous insects live in the same habitat and use the same food. Consequently, competition may exist between the needs for growth and the needs for reproduction. Notable exceptions are the mayflies (Ephemeroptera) and dragonflies (Odonata), which evolved quite different nymphal and adult stages, thereby allowing each to exploit a different habitat. Holometabolous insects, such as butterflies (order Lepidoptera), beetles (order Coleoptera), wasps, ants and bees (order Hymenoptera) and flies (order Diptera), undergo complete metamorphosis. In this design, the body plan of the instars (also called larvae) differs drastically from that of adults. These animals are therefore able to populate different niches and separate the needs for growth from those for reproduction. To allow complete metamorphosis, Holometabola go through a non-feeding pupal stage (Fig. 1(B)). Upon hatching, the primordia of the adult organs (such as wings, legs, eyes, genitals, etc.) are already present within the larvae as so-called imaginal discs (Section Holometabola). These imaginal discs will only differentiate to adult organs during metamorphosis in the pupal stage. This innovation resulted in a rapid diversification of insects and the establishment of the holometabolous orders that are living today. According to the 'pronymph hypothesis' (Truman and Riddiford, 1999), the pupal stage in Holometabola corresponds to the nymphal stages in Hemimetabola, while the holometabolous larval stages correspond to the pronymphal stage in Hemimetabola. This pronymphal stage is the period during which hemimetabolous embryos are enveloped by a cuticle, which is shed upon hatching.

Control of Metamorphosis

Independent of the design of the life cycle, postembryonic development is highly timed and choreographed through the action of different factors. Here, we will focus on the action of the lipophilic insect hormones: juvenile hormones and ecdysteroids,

Fig. 1 Hemimetabolous and holometabolous life cycles and hormone titers. (A) Life cycle of the desert locust, *Schistocerca gregaria*, a typical hemimetabolous insect, containing 3 stages: egg, instars (or nymphs) and adult. (B) Life cycle of the tobacco hornworm, *Manduca sexta*, a typical holometabolous insect, containing 4 stages: egg, instars (or larvae), pupa and adult (moth) (Reproduced from Verlinden, H., Vleugels, R., Zels, S., et al., 2014. Chapter three – Receptors for neuronal or endocrine signalling molecules as potential targets for the control of insect pests. In: Cohen, E. (Ed.), Target Receptors in the Control of Insect Pests: Part II, Advances in Insect Physiology. Burlington: Academic Press, pp. 167–303. Available at: <https://doi.org/10.1016/B978-0-12-417010-0.00003-3>). (C–D) Changes in the juvenile hormone (dotted line) and ecdysteroid (full line) titers regulating molting and metamorphosis in (C) the desert locust and (D) the tobacco hornworm (Reproduced from Chapman, R.F., 2012. The Insects: Structure and Function, fifth ed. Cambridge: Cambridge University Press.).

neuropeptides and insulin-like peptides (ILP) during metamorphosis, a process that requires a strictly regulated balance between cell death of the juvenile tissues and proliferation of the adult ones.

Hormonal Control of Metamorphosis

Post-embryonic development of insects is highly dependent upon different types of hormones. The classic insect hormones are the lipophilic ecdysteroids and juvenile hormones (JHs). In several insect model species, such as the fly *Drosophila melanogaster* and the moth *Manduca sexta*, ecdysteroid biosynthesis is initiated through the action of prothoracicotropic hormone (PTTH). PTTH is secreted by a few neurosecretory cells in the brain that terminate in the corpora allata (CA), a pair of highly specialized neuroendocrine organs in the insect's head (see below). Other factors will also influence the ecdysteroidogenic activity of the PG and are discussed by Marchal et al. (2010). The secretion of PTTH is highly timed (Section Growth and Critical Weight) and occurs prior to each molt. PTTH will bind to its receptor Torso, which is present on the membrane of a specialized endocrine organ called the prothoracic gland (PG) (Rewitz et al., 2009). These glands express the so-called *Halloween* genes encoding cytochrome P450 enzymes responsible for ecdysone (E) biosynthesis. The release of E into the hemolymph surges prior to each molt. E will be converted to its active metabolite, the molting hormone 20-hydroxyecdysone (20E), in several non-endocrine tissues. 20E will bind to a nuclear receptor complex of ecdysone receptor (EcR) and ultraspiracle/retinoid-X-receptor (USP/RXR), thereby initiating transcription of different pathways and eventually triggering a strictly regulated cascade of neuropeptides, which induces the typical ecdysis behavioral sequence. For a detailed review on the neuropeptidergic regulation of ecdysis, the reader is referred to Žitňan and Adams (2012). The sesquiterpenoid JH is produced and secreted by the CA. The nature of the molt – larval to larval or larval to adult – is defined by the presence or absence of JH during this critical period. A surge in 20E titer in the presence of JH will ensure juvenile characteristics, resulting in a larval to larval molt. While a peak of 20E in the absence of JH results in a metamorphic molt. Because of this action, JH is also sometimes referred to as the 'status quo' hormone. JH exerts its function through methoprene-tolerant (Met), a basic-helix-loop-helix (bHLH)/Per-Arnt-Sim (PAS) protein. In several insect species, a steroid receptor co-activator (Taiman/SRC) has been identified as a functional partner of Met. JH binding to Met will ensure heterodimerization of Met with Taiman/SRC, allowing the complex to bind E box-like motifs present in the regulatory regions of downstream genes, such as the transcription factor Krüppel-homolog 1 (Nation, 2015).

Titers of these two critical hormones have been studied in a variety of hemimetabolous and holometabolous insect species. The changes in titers during metamorphosis of a representative hemimetabolous and holometabolous insect species will be discussed below.

To successfully fulfil their role in insect development, it is obvious that concentrations of both 20E and JH in the hemolymph must be strictly regulated and important cross talk exists. These concentrations are not only regulated by their biosynthesis, but also by their degradation and secretion. For a detailed review on the factors that regulate the ecdysteroid and JH titers in immature insect stages, the reader is referred to [Niwa and Niwa \(2016\)](#) and [Hiruma and Kaneko \(2013\)](#), respectively.

Hemimetabola

The JH and ecdysteroid titers throughout the final two instar stages of the desert locust, *Schistocerca gregaria*, are shown in [Fig. 1\(C\)](#). During the penultimate instar stage (4th instar) the JH titers are high during the critical period of molting. Consequently, the ecdysteroid peak precedes a nymphal to nymphal molt. On the other hand, in the last instar stage (5th instar) the lack of JH during the critical period results in a nymphal to adult molt. Experimental application of JH during this critical stage in last instars would lead to an extra nymphal stage.

Holometabola

Similar to Hemimetabola, high JH titers during the critical periods of molting result in a larval to larval molt ([Fig. 1\(D\)](#)). The larval-pupal transition in Holometabola is characterized by changes in hormone titers that have no counterpart in postembryonic development of hemimetabolous insects. In the penultimate instar stage (4th instar) of the tobacco hornworm, *M. sexta*, JH titers decline moderately prior to molting. However these titers are still high enough to produce a new larval cuticle and prevent the development of imaginal discs ([Section Holometabola below](#)). In the last larval stage, JH titers drop below detectable levels or reach trace levels right before a small peak in ecdysteroid titer. This first, small ecdysteroid peak in the absence of JH is called the commitment peak and is not sufficient to initiate molting, instead it results in an irreversible commitment to metamorphosis. Consequently, the insect will stop feeding and enters what is called the wandering stage, during which it will seek an appropriate place to pupate. It will also cause the epidermis to switch its genetic program so that it can no longer produce a larval cuticle, but only a pupal cuticle when exposed to the subsequent, 10 times higher, ecdysteroid peak. This second ecdysteroid peak eventually results in the secretion of the pupal cuticle and shedding of the larval one. Moreover, this second peak is accompanied by a peak in JH titer, which regulates the development of the imaginal discs. Due to the absence of the JH receptor in the epidermis at the time of this second ecdysteroid peak, a larval molt is prevented. When environmental conditions are suitable during the pupal stage, the adult molt will be initiated by a peak in ecdysteroid titer. As JH is absent during this surge in ecdysteroids, due to a rise in the level of JH esterase (which metabolizes JH), the adult cuticle can be produced. Application of JH during this ecdysteroid peak, would result in a pupal to pupal molt, indicating that the JH receptor is again present in the epidermis.

Growth and Critical Weight

Metamorphosis is a complex and tightly regulated process that concludes larval growth and results in the transformation to the adult stage. Since growth only occurs in immature stages, adult body size is determined by the size the larva attained when it stopped feeding and initiated metamorphosis. Control of size is therefore tightly connected to the mechanism that controls metamorphosis. Size determination has been well-studied in model insects, such as *M. sexta* and *D. melanogaster*. When the larvae of these holometabolous insects attain their critical weight, growth ceases and the above mentioned endocrine cascade and subsequent metamorphosis is initiated. Critical weight is defined as the weight of the insect at which initiation of metamorphosis can no longer be delayed by starvation, i.e., when last instar larvae have reached a certain body size after a period of feeding and intrinsic growth, the path to metamorphosis is inherently switched on. As long as the larva does not obtain its critical weight, its brain will be unable to release PTTH, the primary inducer of ecdysteroid biosynthesis in the PG ([Section Hormonal Control of Metamorphosis](#)). The attainment of critical weight provides a checkpoint that ensures that larvae have stored enough nutrients to make it through the non-feeding pupal stage, during which these nutritional stores will fuel the development of adult tissues and structures. Larval growth is therefore crucial for effective completion of metamorphosis and the success of reproduction in the adult stage. The mechanism that initiates PTTH and 20E secretion prior to the metamorphic molt, when JH is absent, will control the final body size and can be quite diverse in different insect species.

The attainment of critical weight is controlled by both environmental and genetic factors. The principal genetic regulators of growth are ILP and ecdysone, together with their receptors and signalling pathways. The biosynthesis and secretion of these hormones are in their turn regulated by complex interactions of other endocrine and molecular mechanisms, by environmental factors, such as nutrition, and by physiological mechanisms that sense body size. Also oxygen levels appear to be involved in critical weight sensing. For a detailed review, the reader is referred to [Nijhout \(2015\)](#).

Maturation of the Reproductive System in Immature Stages

The changes that take place during transformation of the juvenile to adult insect depend on the design of their life cycle, hemimetabolous or holometabolous. Nevertheless, molting of the insect to the adult stage inherently entails the development of functional gonads and genitals. Here, we will discuss changes that take place in hemimetabolous and holometabolous insects during metamorphosis with a focus on the reproductive organs. While only gradual changes take place in hemimetabolous insects, holometabolous insects go through a stage with very marked changes. Throughout post-embryonic development growth of internal

organs may result from an increase in cell size and/or cell number. In general, tissues that are destroyed upon metamorphosis undergo cell enlargement, while those that persist undergo cell multiplication.

Hemimetabola

Hemimetabola gradually develop adult features or instar features just resemble those of adults. Two examples of gradually developing traits are the wing buds and the genitalia. At each molt, the wing buds will increase in size, until they form functional wings in the adult stage (**Fig. 1(A)**, wing buds and wings in black). Similarly, the genitalia develop throughout post-embryonic development, but are only functional in the adult stage. Also the gonads are already present after hatching and gradually grow. In many insects, sperm production is already initiated during the last instar, although spermatogenesis continues in the adult stage. Also oogenesis can start in last instars, but vitellogenesis is a typical adult feature. On the other hand, the musculature of instars resembles that of adults, even the flight muscles are already present in instars. Although, these flight muscles still increase in size by an increase in both fiber size and the amount of fibers. Some muscles that are only used during ecdysis disappear in adults. Consequently, also the neuronal supply of these muscles dies. Other changes in the neuronal system are found in the sensory neuronal pathways, where some neurons change their role towards adult behavior.

Holometabola

In holometabolous insects the degree of modifications during metamorphosis depends on the resemblance between the larval and adult stage. Relatively small modifications take place in beetles (Coleoptera), while in flies (Diptera) and butterflies (Lepidoptera) tissues are almost completely rebuilt after hydrolysis and phagocytosis. The pupal stage allows the transition between the larval and adult form. During the pupal molt and subsequent pupal stage, all larval organs, except the central nervous system and reproductive organs, are broken down and major internal reconstruction takes place. In the pupal stage all adult features become recognizable, although they are not fully expanded to their adult form. As eclosion only takes place under the appropriate environmental conditions, metamorphosis may already be completed a few hours, days or weeks before eclosion.

Details on the developmental changes during metamorphosis vary between insect species and organs. In general there is an extensive reconstruction of the musculature and the central nervous system (CNS). For instance, flight muscles will be formed and muscles will be attached to the cuticle during the pupal stage. As the adult insect is characterized by a larger brain, compound eyes and larger antennae, the CNS is also thoroughly remodeled. Furthermore, the digestive system of most holometabolous insects undergoes drastic changes due to the different larval and adult diets. Larval stages often feed on leaves, while the adult stage often has a fluid diet. As some organs are remodeled, others are newly formed. These organs develop from imaginal discs, undifferentiated groups of ectodermal cells that will grow into adult tissues only under appropriate hormonal control during pupation. During the larval stages the cells of these imaginal discs only increase in cell number by mitotic division. Ecdysteroid secretion and falling JH levels in the final instar of *D. melanogaster* will signal the imaginal discs to undergo rapid differentiation into adult structures during the pupal stage. The best known and most studied imaginal discs are those of the wings, legs and genitalia. While the imaginal discs of wings and legs are paired, the genital disc is unpaired and bilaterally symmetrical. The genital disc is located in a medial position at the posterior end of the insect. Genital discs are sexually dimorphic, i.e., their morphology and growth are sex-dependent. Genital discs will give rise to the hindgut, analia and internal and external genitalia, but not to the gonads. These organs (testes and ovaries) are formed during the embryonic stage and will slowly develop throughout the larval stages (**Fig. 2(B)**, green structures). Also the genital discs are formed during the embryonic stage and will increase in cell number and differentiate into a male or female disc throughout the larval stages (**Fig. 2(A)**). However, it is only in the pupal stage that these genital discs will develop into the previously mentioned organs (**Fig. 2(B)**). The embryonic genital disc contains three regions, the male genital primordium (MGP), the female genital primordium (FGP) and the anal primordium (AP). During larval development the sex-determination genes and homeotic genes determine the proliferation of the different regions. In female larvae the MGP is brought into a 'repressed state', called the repressed male genital primordium (RMP). In male larvae the FGP will be repressed and is therefore called the repressed female genital primordium (RFP). For a detailed review on the genetic control of genital disc formation and differentiation, the reader is referred to [Estrada et al. \(2003\)](#). In the prepupa the external genitalia (vulva, vaginal plates of female; penis apparatus, clasper of male) start to develop. The genital ducts (oviducts, uterus, vagina, seminal vesicles, ejaculatory duct and sperm pump) and accessory structures (seminal receptacle, spermathecae, accessory glands and paragonia) on the other hand only start development in the early pupa. After development of these reproductive tissues, the testes will make contact with the seminal vesicles, while the ovaries fuse with the oviducts. The adult eventually emerges with a fully formed reproductive system.

Depending on the insect species the freshly emerged adult will be reproductively competent or it will have to go through a period of maturation before being able to reproduce. The time frame in which spermatogenesis occurs, is species dependent. In most insects spermatogenesis already starts in the larval stages and formation of spermatids, and subsequent spermiogenesis, is often delayed until the last larval or pupal stage. In species that do not feed as adults, the whole process of spermatogenesis (incl. spermiogenesis) is completed before adult eclosion. However, in most insect species spermatogenesis is only completed in the adult stage. Often the production of new sperm continues throughout adult life. For a long time, spermatogenesis in insects was not considered to be under hormonal control. However, it has now become clear that in a wide range of insect species, both Hemimetabola as well as Holometabola, ecdysteroids synthesized in the testes can stimulate spermatogenesis ([Nation, 2015](#)). Similarly to spermatogenesis, oogenesis is very species dependent. In general, oocytes get stuck in the prophase or metaphase of

Fig. 2 Development of the reproductive organs in *Drosophila melanogaster*. (A) Schematic overview of the development of the genital discs in both sexes (from embryo to larva) (adapted from Sánchez, L., Guerrero, I., 2001. The development of the *Drosophila* genital disc. *BioEssays* 23, 698–707. doi:10.1002/bies.1099). (B) Schematic drawings of the development of the adult reproductive system from genital discs and gonads (ovaries or testes) (reprinted with permission from Atlas of *Drosophila* Development by Volker Hartenstein, Society for Developmental Biology's Web Server. Available at: <https://www.sdbonline.org/sites/fly/atlas/00atlas.htm>). acg, accessory glands; AP, anal primordium; de, ejaculatory duct; FGP, female genital primordium; gd, genital disc; L3, third instar larva; MGP, male genital primordium; ov, ovaries; ovd, oviducts; pa, penis apparatus; pg, paragonia (male accessory glands); rec, rectum; RFP, repressed female genital primordium; RMP, repressed male genital primordium; sp, sperm pump; spt, spermatheca; src, seminal receptacle; te, testes; ut, uterus; va, vagina; vs, seminal vesicles.

the first meiotic cycle, and a period of maturation in the adult stage is required before ovulation and resumption of meiosis take place. This maturation includes the incorporation of lipids and yolk into the growing oocyte. The yolk precursor proteins are produced by the fat body in a process called vitellogenesis. In most insects, vitellogenesis is considered an adult feature. However, in some Lepidoptera, which do not feed as adults, vitellogenesis is initiated during the last larval or pupal stage and yolk accumulation is completed prior to adult eclosion. Ovarian growth, vitellogenesis by the fat body and uptake of vitellogenin by the developing oocytes is under hormonal control of both JH and ecdysteroids, as well as additional hormonal factors including ILP and neuropeptides (Nation, 2015).

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