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Origin of caterpillars: Distant hybridization vs. descent with common ancestry

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Abstract

Since the 'theory of natural selection' proposed by Darwin, the study of kind and diversity of life got a whole new perspective under the light of evolution, but there is a lack of shreds for evidences regarding the origin of complicated developmental strategies among organisms. Insects are not an exception as they include a sheer number of taxa with diverse life histories. Insects having 'caterpillar' or 'larva' like immatures completely different from the adults arrived late in the evolutionary timescale, whereas their preceding ancestors had immatures almost similar to the adults, known as 'nymphs'. This review summarizes two recently available contrasting hypotheses and evidences regarding the origin of caterpillars, did they evolve as descents with modification or as the products of distant hybridization? It also aims to provide endocrinological and molecular overview of insect metamorphosis and how it differs between insects with caterpillars and those without caterpillars.

Keywords: Barnacle, caterpillar, evolution, hybridization, larval transfer, metamorphosis

Introduction

Evolution has forged life into diverse forms since the appearance of first self-replicating nucleic acids. In the process of evolution millions of species have been created, from tadpole to T-Rex and viruses to elephants. It was always compelling for naturalists to decipher the complicated developmental mysteries of organisms. In this biosphere of heterogeneous lives, insects constitute the largest group of fauna and they have always amused naturalists by their remarkable ecological and physiological patterns of life. One such mystery that haunted entomologists for so long is how the 'caterpillar' evolved in holometabolous insects (insects with three post embryonic life stages *viz.* larva, pupa and adult) when their hemimetabolous and ametabolous sisters (insects having juveniles similar to adults in gross morphological traits) do not have such a creepy, crawling and nasty (as most people think) creature in their life cycle! Evolution of three post embryonic life stages from ancestor two (or one) post embryonic life stages was a significantly successful biological adaptation that helped to radiate the holometabolous insects into a sheer number of species ^[1]. This particular group of insects having a 'caterpillar' like stage in life not only outnumbered the sister taxa but became more numerous than all the existing animal, plant and fungi species together ^[2]. In this review we are going to share the available knowledge of insect metamorphosis based on endocrinological and molecular data as well as comparative morphological and anatomical data from related taxa like the Crustacea and the Onychophora.

Defining a caterpillar

'Caterpillar' is a general term mostly used for the worm like crawling immatures of insects, especially butterflies and moths. There is one or the other form of 'caterpillar' in the orders of holometabolous insects ^[3] *viz.* Lepidoptera, Raphidioptera, Neuroptera, Coleoptera, Hymenoptera, Mecoptera, Megaloptera, Psiphonoptera, Strepsiptera and Trichoptera which are termed as 'scarabeiform', 'eruciform', 'campodeiform' and 'apodous' based on their distinct morphology ^[4] but united under a common scientific term 'larva'. Basically the caterpillar is a juvenile post embryonic stage of the aforesaid insect orders and characterized by one pair of jointed legs in each of the three thoracic segments and pair of unjointed prolegs in all or some of the abdominal segments ^[3, 5]. It hatches out of the egg and occupy a distinct ecological niche that differ from the habitat of the adult form ^[6]. Often there is a huge difference between the food habit of larva and the adult. The modified characters like loss of legs and ocelli, reduced sclerotization in head, spines and hairs on integument evolved independently several times in

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different holometabolous taxa [3]. In holometabolous insects, caterpillars or larvae do not show any external rudiments of wings or genitalia, which appears in the relatively immobile ‘pupal stage’ just before transforming into adult, whereas in

hemimetabolous insects, the ‘nymphs’ (a term used for their juveniles) after hatching from eggs, gradually develops the adult organs like wings and genitalia through successive instar [6, 7] (fig.1).



Fig 1: Left: Photograph of a typical caterpillar of holometabolous insect (from www.pexels.com) Right: SEM image showing the successive nymphal instars and adult of a typical hemimetabolous insect, a barklouse (Psocoptera); adapted from Grimaldi and Engel²

Distant hybridization

Distant hybridization may be defined as the crosses between distant species, genera or higher ranking taxa in contrast to close hybridization, which occurs between varieties of the same species or subspecies [8]. It facilitates combination of two gene pools giving rise to a new genotype. Interspecific hybridization can lead to a sudden and long lasting change in interbreeding species when compared to mutation and genetic recombination which are the raw material for natural selection [8, 9]. As the process of distant hybridization creates variation in population compressing the evolutionary timescale it commands for further investigation into the operation of natural selection [9]. That is why ‘distant hybridization’ was described as ‘natural laboratories for evolutionary studies’ [9, 10]. Since Darwin mentioned hybridization in the context of speciation, many evolutionary biologists started a quest to understand this phenomenon in extant and extinct flora and

faunas. The studies during 1930s and ‘40s by some eminent botanists revealed that genetic information can be exchanged between species (introgression) and it is not at all ‘too rare’ among plants [9]. In case of animals earlier it was suggested that interspecific hybridization is very insignificant but during last 80 years’ number of hybrids recorded with respect to total species described increased rapidly covering significant groups of animals including insects (fig. 2). Although the estimation of hybridization among animals was just 1% the occurrence of occasional hybridization may lead to change in genomic architecture of species and create significant impact on origin and fate of evolutionary lineages [9]. Based on this belief Donald I. Williamson from University of Liverpool came with an all new hypothesis of evolution of caterpillars from onychophorans by distant hybridization or hybridogenesis [3].

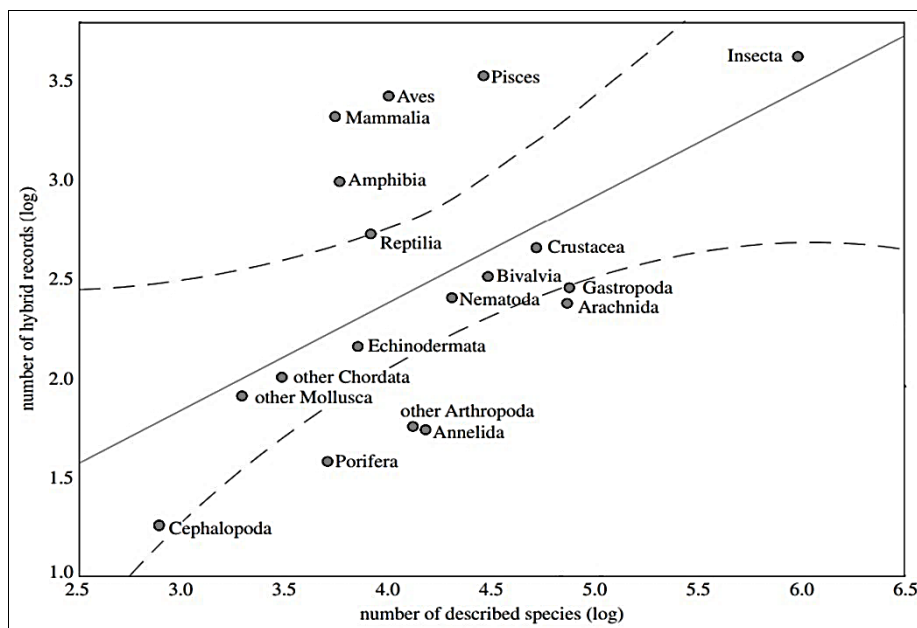


Fig 2: The log of hybrid reports as a function of the log of described species in the respective taxon. Dashed lines represent 95% confidence limits; adapted from Schwenk *et al.* [9]

Theory of larval transfer, assumptions and evidences

For so long it was believed that caterpillars and adults are originated from a common ancestor and they are products of a single genome until Williamson suggested that larvae are originally some animals added to life history of some other animals in evolutionary timescale through 'larval transfer mechanism' [3, 11, 12]. Basically, he suggested a process of hybridization between two distant species producing an animal that would resemble one parent, but would metamorphose into a form resembling the other parent [11].

To impose the theory of larval transfer on insects, Williamson drew the analogy from the developmental biology of an unusual crustacean arthropod, the rhizocephalan barnacle [3]. Rhizocephalan barnacles are highly evolved parasitic barnacles grouped in superorder Rhizocephala. Along with the two other superorders Thoracica and Acrothoracica they are ranked in the infraclass Cirripedia (Cirri-feather like; Pedia-foot). Like other normal barnacles they have similar larval stages *viz.* naupili and cyprid larva [13] but instead of transforming into a 'shelled adult' cemented to a substrate

(general habit of barnacle adults) it finds a decapod (crab, shrimp, prawn etc.) host and transforms into something very unusual which from nowhere look like a barnacle or a crustacean or an arthropod [14, 15]. The cyprid larva of Rhizocephalans injects a mass of cells inside the haemolymph of decapods where they multiply into root like tissues (interna) that grows inside virtually through all parts of the body. As the parasite gets matured, it produces a sack of eggs outside the carapace of the host termed externa [15, 18].

Williamson hypothesized that rhizocephalans were not originally crustacean arthropods but acquired the 'cirriped like larva' by one or more larval transfer *i.e.* hybridization with one or more cirriped taxa³. Williamson's idea was turned down when phylogenetic analysis with 41 morphological characters and molecular data comprised of 6244 sites from 18S, 28S and H3 genes (expressed both in larva and adults) showed that rhizocephalans are not only crustacean arthropod but they form a clade with the other two groups of barnacles *i.e.* Thoracica and Acrothoracica [19, 20].

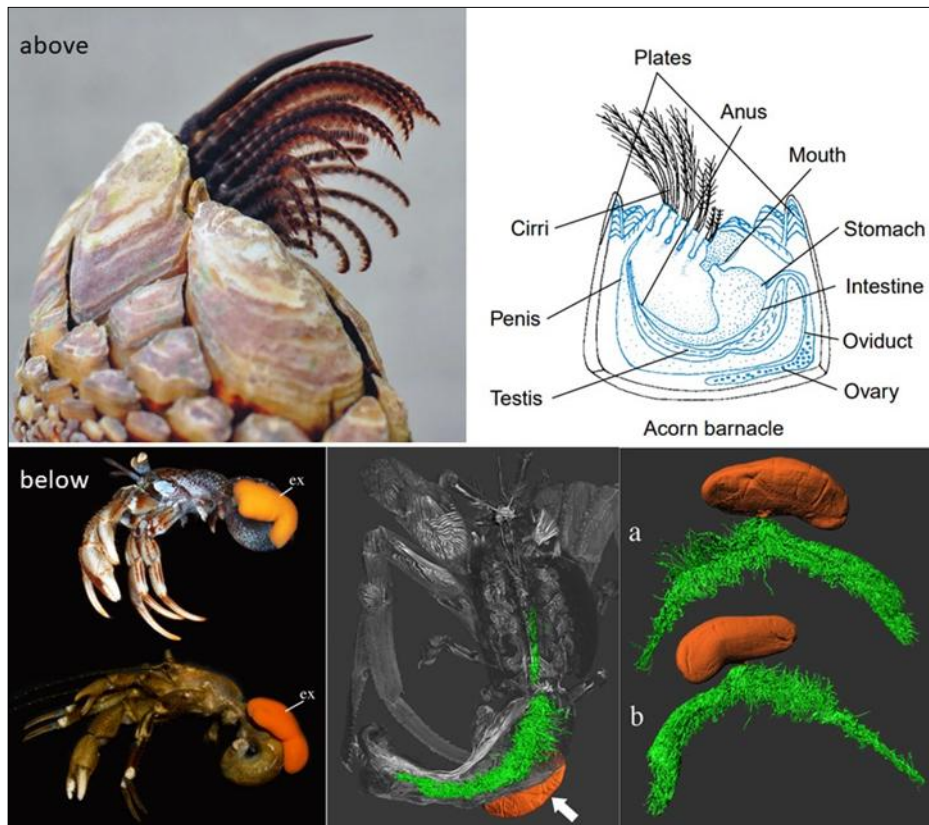


Fig 3: Above: An adult acorn barnacle and its schematic morphology showing well differentiated body regions [16]; Below: A 3D representation of rhizocephalan barnacle without any arthropod like segmentation, ex-externa (in orange), green root like tissue represents the interna, a-right side view, b-left side view; adapted from Noever *et al.* [17]

Larval transfer from velvet worms to insects: speculation and verification

The primitive Lepidoptera larvae, especially those belong to family Micropterigidae like the larva from genus *Micropterix* has unusual architecture when compared to other familiar insect larvae. The moth *Micropterix* has a caterpillar which resembles velvet worm: it has a pair of antenna, lack clear distinction between thorax and abdomen, lack jointed legs, but possess hydrostatically movable fleshy legs ending in conical claws on almost all the post-cephalic segments [3, 11] (fig. 4). Williamson suggested that in the Upper Carboniferous period insects acquired caterpillar-like larva by hybridizing

with velvet worms (Onychophorans) [11]. Many of the hybrids were born perished, but very few survived and remodified the body plan in the evolutionary timescale to suit their ecological niche and that is why there are varieties of caterpillars today across the holometabolous orders. These hybrids contained an integrated genome: an Onychophoran genome and an insect genome [11]. The onychophoran genome was expressed in early phases of the life cycle as caterpillar whereas the insect genome was expressed in the adult stage, a hypothesis termed as 'sequential chimera' [3, 11]. Regarding the origin of pupal stage, Williamson suggested as the caterpillars had onychophoran tissues and organs which were quite different

from adult organs and tissues, these insects evolved to commit ‘start-again metamorphosis’ through an immobile ‘factory-reset’ stage termed as the pupa [11]. Williamson predicted that molecular studies can unravel the onychophoran genome from holometabolous insects and at the same time holometabolous insects should have larger genome size compared to that of hemimetabolous insects which lack a larval stage in their life history [3].

The earlier and most recently published Phylogenetic studies [21, 22] including phylogenomic analyses [23] of arthropods show

that insects are more closely related to Branchiopod crustaceans and onychophorans form a sister group of the clade Eurarthropoda (Hexapoda + Crustacea + Chelicerata + Pycnogonida + Myriapoda) which clearly shows that insects do not contain any onychophoran genome and they have very negligible probability to hybridize with the onychophorans or velvet worms [19, 23] (fig. 5). Available genome size data (C-value) of major hemimetabolous and holometabolous insects from ‘Animal Genome Size Database’ also provide strong evidence against Williamson’s predictions [19, 24] (fig. 5).

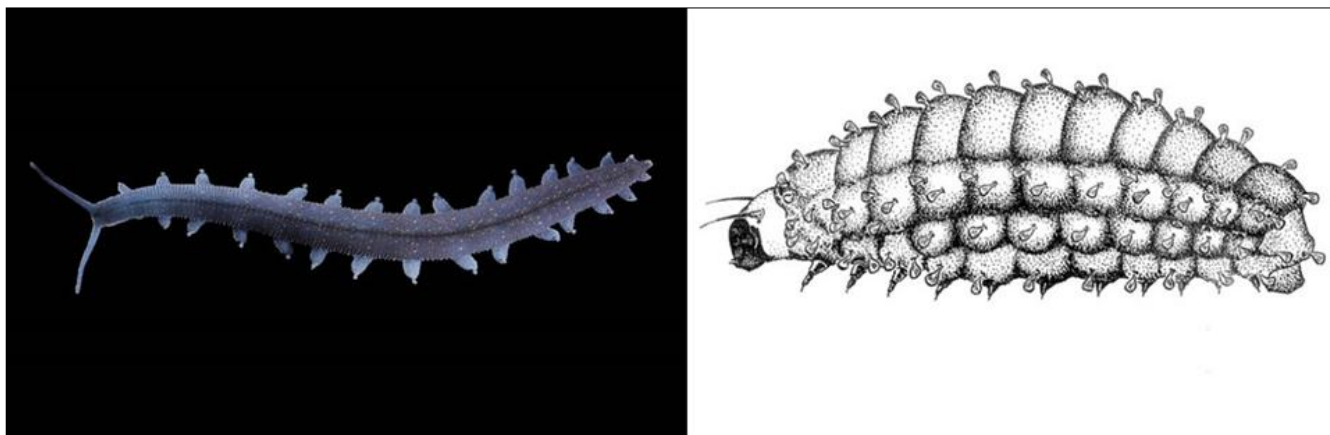


Fig 4: Left: An onychophoran adult, adapted from Muller *et al.*, 2017 Right: Larva of *Micropherix calthella* by Lorenz, 1961

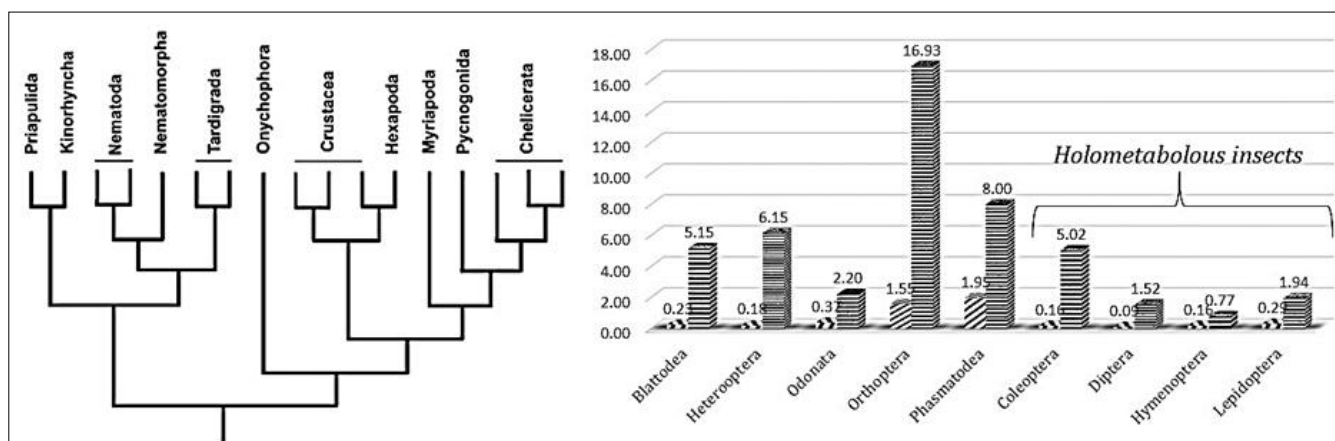


Fig 5: Left: Phylogeny of Ecdysozoa showing Onychophora as a sister group of Eurarthropoda, adapted from Edgecombe, 2009. Right: Comparison of genome sizes between major orders of insects showing less C-value for holometabolous insects, horizontally patterned bars show maximum C-value, whereas diagonally patterned bars indicate minimum C-value of respective group.

Endocrinology of insect metamorphosis

Metamorphosis in insects is governed by two major class of hormones *viz.* juvenile hormones (JH) and ecdysteroids (20E: the activated form of ecdysone hormone) secreted to hemolymph from corpora allata and prothoracic glands respectively. As the titre of 20E increases, insect moults into next instar through the shedding of old cuticle and production of a new one but morphology of the instar (whether nymph or larva) is determined by the titre of JH [25, 26].

During the embryonic growth of hemimetabolous insects, JH drops down before the first embryonic moult (E1) and comes back after E1 has moulted into a ‘pronymph’ (during 48-60% of embryogenesis) which is characterized by distinct ultrastructure of cuticle, lack of sclerotization, absence of wing buds and a sensory nervous system equivalent to holometabolous larva [7]. The first nymphal cuticle is secreted during 75-85% of embryogenesis. This first nymphal stage hatches out of the egg shell shedding the old pronymphal

cuticle [7] (fig. 6). The titre of JH remains high from hatching to pre adult nymphal moult and drops down to an undetectable titre during moulting into adult in case of hemimetabolous insects [6, 7]. Once JH binds to its putative receptor methoprene-tolerant (*met*) it regulates the expression of Kruppel homolog-1(*Kr-h1*) and Broad-Complex (*BR-C*) genes [6, 25, 26]. Expression of *Kr-h1* causes ‘status quo’ moults which results into a new instar retaining morphology of the former [29]. Expression of *Br-C* governs progressive morphogenesis as wing bud development through the successive nymphal instars [6, 30]. Both of these genes are downregulated when JH declines during moulting into adult [31, 32] (Fig. 7).

Holometabolous insects do not commit 3 pre-hatching moults inside the egg shell as seen in the case of hemimetabolous insects. Instead of moulting into a pronymph, the E1 in holometabolous insects moult into a larva during 45-50% embryonic growth, which corresponds with the pronymphal

cuticle formation in hemimetabolous insects [7]. Larva itself hatches out of the egg without moulting into a 3rd pre-hatching stage (Fig. 6). In holometabolous insects this hormonal cross talk is quite different from hemimetabolous insects as *BR-C* is regulated by Ecdysteroids and JH produce an inhibitory effect on *BR-C* expression during juvenile instars to prevent adult tissue differentiation until the commitment peak of ecdysteroid during larval-pupal moult [7, 31, 33]. At the end of larval development, 20E in the absence or negligible titre of JH activates a group of early genes (*BR-C*, *E74* and *E75*) which in turn activate a large group of late genes during the pupal peak of 20E. JH can no longer be able to prevent programmed histolysis and adult tissue differentiation. Differentiation to an adult occurs in the presence of high 20E titre, in the absence of JH and of *BR-C* activity [33] (fig.7). The striking phenomenon is *BR-C* is expressed only during larval-pupal moult and again, it is

downregulated by the inhibitory action of 20E as expression of *BR-C* during pupal-adult moult may lead to another pupal instar. In hemimetabolous insects *BR-C* is expressed only after the pronymphal peak of ecdysteroids during blastokinesis which provides an evidence for downregulation of *BR-C* prior to pronymphal peak. From these evidences it can be suggested that: i) Commitment peak in holometabolous insects is equivalent to the pronymphal peak of hemimetabolous insect (fig.8); ii) Larval stage of holometabolous insects is equivalent to the pronymphal stage of hemimetabolous insects. iii) Pupal stage of the holometabolous insects is similar to nymphal stage of the hemimetabolous insect as the hormonal trajectories at initiation and termination of the nymphal stage (comprising all the nymphal instars) are comparable to that of the pupal stage (fig.8).

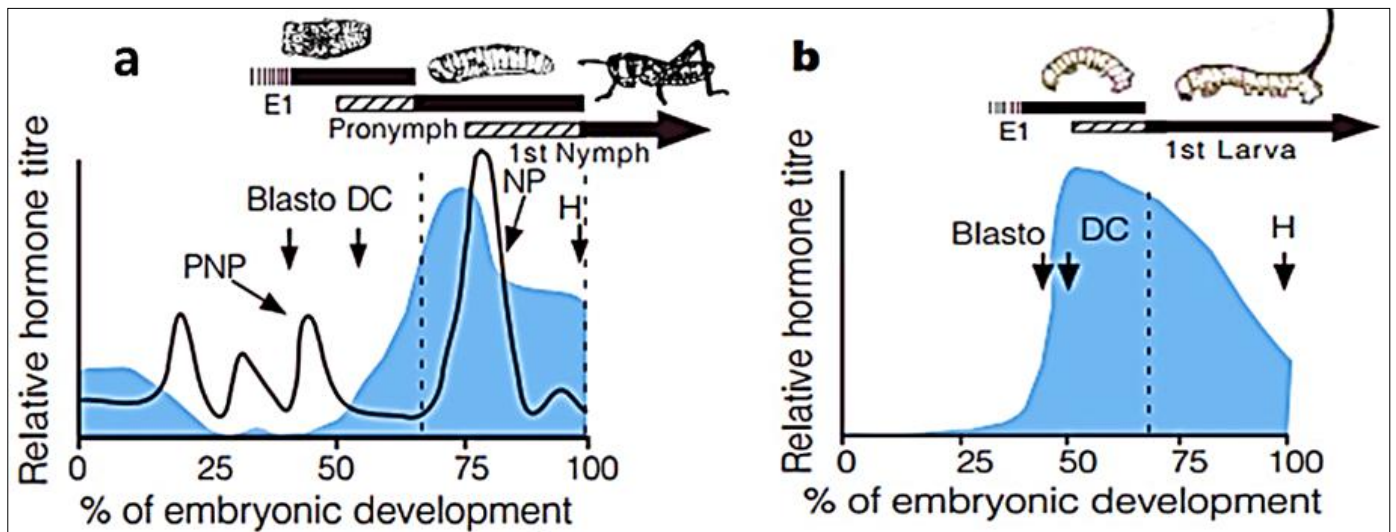


Fig 6: comparison of embryonic titres of ecdysteroid (black) and juvenile hormone (blue) for a) hemimetabolous insect *Locusta migratoria* and for b) a holometabolous insect *Manduca sexta*; Blasto- blastokinesis, DC- dorsal closure, E1- first embryonic instar, H- hatch, PNP- pronymphal peak, NP- nymphal peak; adapted from Truman and Riddiford [7]

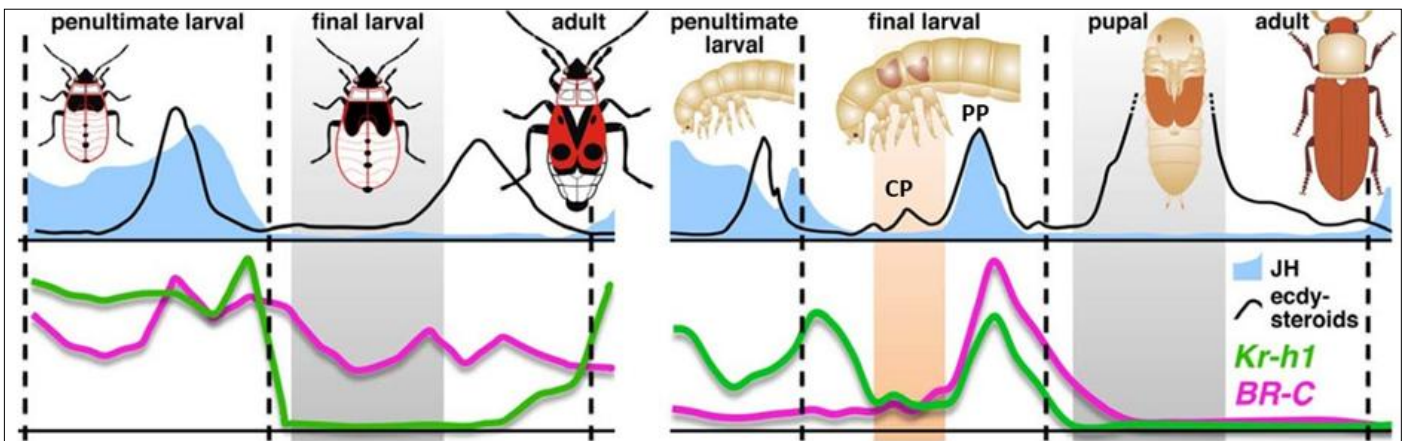


Fig 7: Comparison of hormonal titres and molecular expressions between late post embryonic stages of hemimetabola (indicated by the cartoon of *Pyrrhocoris apterus*) and holometabola (indicated by the cartoons of *Tribolium castaneum*); CP- commitment peak; PP- pupal peak; adapted from Konopova *et al.* [27]

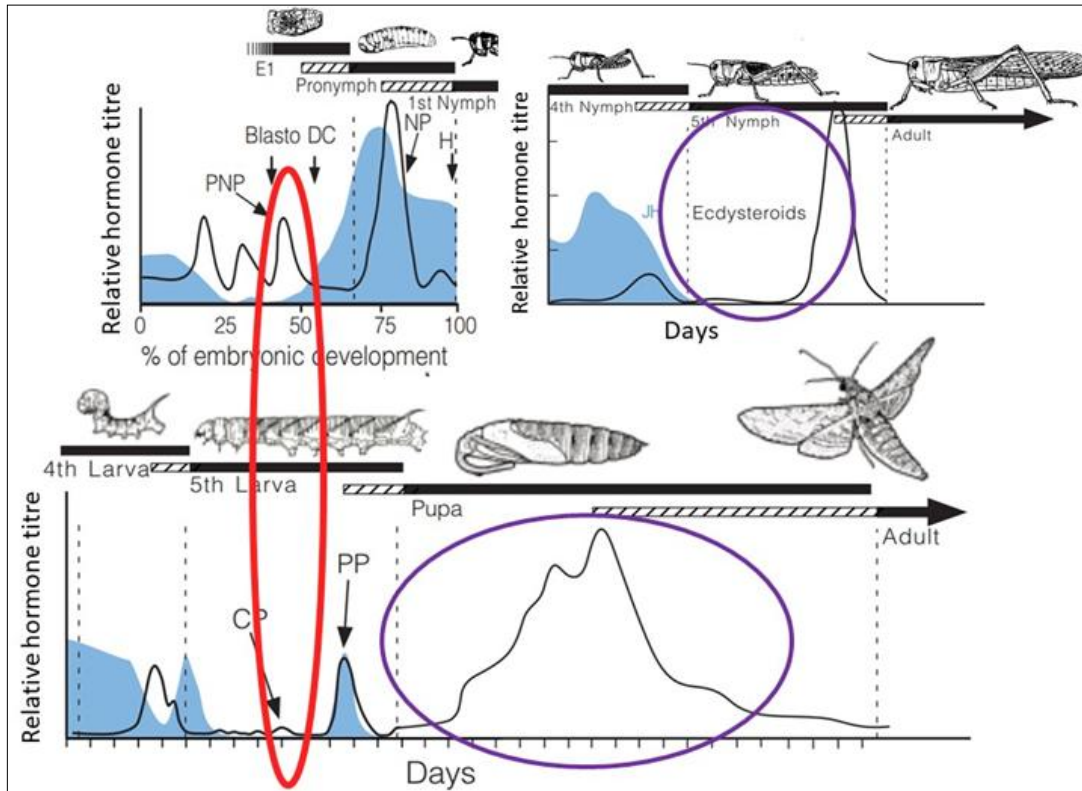


Fig 8: A comparison between hormonal trajectories during embryonic and postembryonic developmental stages of hemimetabolous insect and that of postembryonic developmental stages of holometabolous insects. There is a clear similarity between pronymphal peak and commitment peak. The last nymphal-adult moult is also similar to pupal-adult moult of holometabolous insects. The red and purple ellipses show similarity in hormonal trajectories; adapted from Truman and Riddiford [7]

The pronymphal hypothesis of caterpillars’ origin

Based on molecular and endocrinological evidences Truman and Riddiford postulated the comprehensive steps [7] in evolution of insect metamorphosis which in turn explains the origin of caterpillars in holometabolous insects (fig. 9):

Step 1: Basal insects in the evolutionary timescale had three distinct developmental stages viz. pronymph, nymph and adult. Pronymph was a non-feeding stage confined within the egg shell.

Step 2: Pronymph acquired the feeding habit inside the egg shell as it started consuming extra mass of yolk which was not enclosed during dorsal closure of the embryo. Such a feeding pronymphal stage is termed as ‘protolarva’.

Step3: The females of primitive insects having ‘protolarva’ started laying eggs in noble habitats (in soil, under bark, inside plant tissue) which were free from risk of predation. This adaptation facilitated premature hatching of protolarva and utilization of food resources encountered in this protected habitat. Due to an abundance of resources and their efficient utilization by protolarva caused ‘natural selection’ to maintain this form through successive instars. Hormonal trajectories were also changed in coordination with these new adaptive stages. The shift in timing of embryonic JH secretion i.e. early secretion and continuous presence thereafter helped to maintain the wormy protolarva through several instars [7] (fig. 6). Now the crawling worm can no longer be considered as a ‘protolarva’ rather it must be considered as the first true larval stage.

Step 4: The nymphal and adult stages had a similar ecological niche which caused competition among them, resulting in reduction of nymphal stages as only adult had the morphological machineries for reproduction.

Step 5: The nymphal stages were no longer needed for food

acquisition and growth as these tasks have been shifted to larval stages and at the same time adult became more focused with mating and multiplication. As a result, selection favoured the nymphal stages to get condensed into a single, relatively immobile non-feeding pupal stage, which could serve as a transitional stage by larval tissue dissolution followed by adult tissue differentiation.

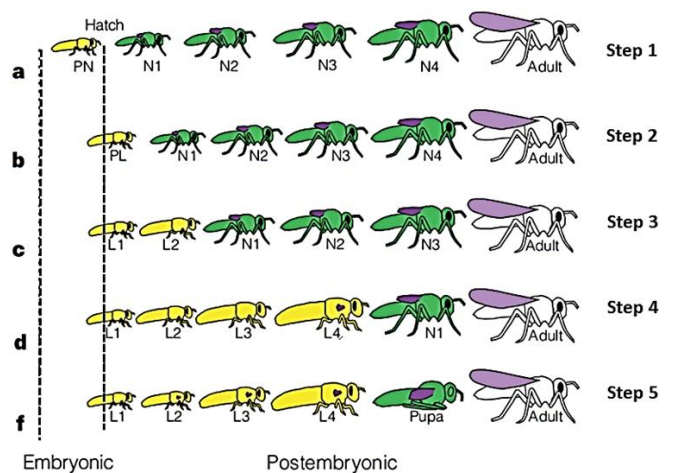


Fig 9: Evolutionary steps in origin of larva and pupa in holometabolous insects; modified from Truman and Riddiford [7]

Conclusion

Naturalists through ages tried to understand and explain the complicated phenomenon of insect metamorphosis in a number of ways in which ‘Berlese theory of dembrionization’ and ‘Hinton’s theory of metamorphosis’ were most celebrated schools of thought [35, 36]. None of these hypotheses were unambiguous as they were postulated without physiological

and molecular evidences. Till now Truman and Riddiford's idea of the pronymph of hemimetabolous insects evolving into the caterpillar of holometabolous insects in the evolutionary timescale is most positively accepted hypothesis among the entomologists [30, 37, 38]. At the same time, one should remind that nature is a celebration of imperfection. If nature was so perfect in creating life, it would never let the life to diversify because perfection would demand the creation of an exact clone repeatedly from the existing life which is very rare (some asexual organisms). Distant hybridization is such an unusual natural phenomenon that is very difficult to trace back in time but it occurred several times in nature across plant and animal kingdom. We cannot turn down this perspective on the origin of some forms of life on earth as it may be a process of evolution that was later subjected to natural selection to create organisms with complicated life histories [11]. We still do not have a whole genome sequence for all the invertebrate taxa but so far the maximum sequence has been compared which revealed strong evidence in support of monophyletic origin of the Insecta (includes all hemimetabolous and holometabolous insects) and no close relationship with onychophorans [23]. Future researches focused on a thorough understanding of the comparative functionality of molecular switches and endocrine tuners across the invertebrate taxa can enlighten the mysteries of metamorphosis in a far better way.

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