

Origin of insect metamorphosis based on design-by-contract: larval stages as an atavism

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Abstract

The steps leading to the insertion of a larval stage during the evolution of holometabolous insects remain largely unknown and appear to be irreducibly complex. The main problem is that an introduction of a new body plan would disrupt existing developmental processes and would require many sequential steps that have to be implemented simultaneously in order to be functional. In the evolution of insect metamorphosis, this would have required the parallel evolution of set aside cells, of a new functional larva and of the induction of metamorphosis. In this article, the engineering paradigm design-by-contract is used to unravel the potential evolutionary steps for metamorphosis. In modelling on design-by-contract, evolution is seen as a continuously expanding system of functional modules which are separated by conserved interfaces. A scenario in which the larval stages were developed before the adult body plan, is relatively simple since it will keep existing developmental interfaces intact. It is therefore proposed that the larval stage of holometabolous insects reflect the *reintroduction* of an ancient, existing body plan and therefore represents an atavism. This scenario can be based on a developmental switch by a differential expression of Hox genes that can be induced by changes in hormonal timing. Thus, design-by-contract as a predictive evolutionary framework may provide an alternative explanation for the origin of insect metamorphosis.

Introduction

Holometabolous insects show a separate larval life stage that undergoes complete metamorphosis to yield the morphologically different adult phase. For example, in the life cycle of a butterfly, the embryo that grows within the egg hatches into the larval caterpillar stage, and undergoes metamorphosis in the pupal stage to finally emerge as an adult butterfly imago. Similarly, the life cycle of *Drosophila* involves a maggot-like larval stage from which adult flies are hatched. Since the ancestral condition for embryogenesis in insects appears to be direct development, holometabolous insects are believed to have acquired new larval life stages during evolution. This process would have been driven by the evolutionary advantages that different requirements for food sources between larval and adults stages would bring (Truman and Riddiford, 1999; 2002). However, the *de novo* evolution and insertion of the new larval body plan into an existing insect developmental plan would require many functional intermediates with unclear evolutionary advantages and as such would represent a classical 'irreducibly complex' (Behe, 1996) scenario. The evolutionary advantage of food partitioning between larva and adult in holometabolous insects will only be effective after the complete evolution of the feeding larva and can therefore not be the drive for the development of the larval stage. Short of gradual molecular developmental scenarios for the theory that the larval stages were *de novo* developed late in evolution, the origin of insect metamorphosis remains a mystery.

The crucial steps in the evolution of metamorphosis are the initiation of molecular steps towards the development of the first functional larva, and the concomitant obligatory development of metamorphosis to the reproductive adult. In all insects, the insect fate map is established early in development during the formation of the cellular blastoderm. After the formation of the blastoderm, the embryo starts to differentiate under the influence of the Hox genes until the fundamental body plan is mapped out. In holometabolous insects, two distinct separate developmental pathways are established in this blastoderm stage. One will give rise to the larval stages via the well-described pattern formation in the early embryo, the other pathway leads to the setting-aside of the imaginal cells that will later form the adult (or imago) during metamorphosis. In this process, imaginal disc cells give rise to most of the adult body structures, while apoptosis destroys most of the larval body (Gilbert, 2003). Since the development of holometabolous insects requires the precise execution of sequential steps, each which is dependent on the correct execution of earlier steps (Akam, 1995; Kmita and Duboule, 2003), it is difficult to envision a scenario in which modern insects have evolved the evolution of the set-aside cells, the developmental sequence of the larval body plan, and metamorphosis without severe interruptions to existing developmental processes.

Design-by-contract as a framework for evolution of metamorphosis

Recently, it has been argued that genome evolution can be modelled on the design-by-contract methodology (de Roos, 2005; 2006a; 2006b). This design paradigm for software development describes an abstract architecture for complex systems that allows for a robust evolving system that is flexible and extensible (Meyer, 1997). In this methodology, a software system is viewed as a set of communicating modules whose interaction is based on precisely defined interfaces. The effect of designing interfaces across modules is a reduction of the interdependencies across modules or components and a reduction of the risk that changes within one module will create unanticipated changes in other modules. Evolution is modelled in this approach as a continuously expanding system of functional modules, or an evolving molecular machine, where the constancy of the interfaces is enforced by the dependencies of vital functions on this interface. In a sequence of events, for instance, the last event is ultimately dependent on the correct implementation of all previous events and a change in one of the interfaces between the functional modules will affect all downstream processes. In this article, this design-centric approach will be used to study the evolution of insect metamorphosis, a process that relies upon the activation of distinct developmental pathways.

Reactivation of ancient body plan during evolution

In an evolutionary scenario based on design-by-contract, a sequence of developmental events leading to a certain body plan cannot be changed since established developmental interfaces have to remain intact. Holometabolism is a late feature in the arthropod tree and therefore, larval stages were introduced while the complex adult insect body plan was already established. In order to leave the development sequence intact, a logical solution for the introduction of the larval stages is that the larval stages have evolved early in evolution and the adult stages later in evolution. Since ancestral insects do not show larval stages, it is proposed that the *reactivation* of an ancient larva-like stage in the evolution of holometabolous insects explains the origin of metamorphosis. (Fig. 1). First, a simple body plan was gradually developed based on a basic segmented body plan, resulting in larva-like organisms that would for instance resemble maggots, sawfly larvae or caterpillars. Second, the more complex euarthropod body plan with joint appendages diverged from this ancestor arthropod, leading to primitive crustaceans and ametabolous insects. Third, holometabolous insects evolved when the ancient body plan was introduced in parallel with the existing body plan. The larval features can thus be seen as a recurring ancient feature in arthropod evolution and would represent a true atavism.

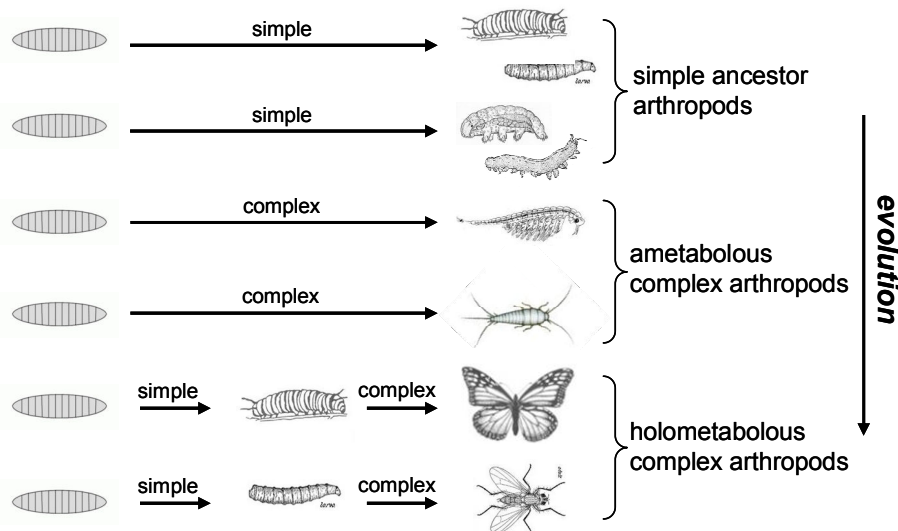


Fig. 1 Proposed steps in the evolution of holometabolous insects. The evolution of the simple ancestor arthropods such as the onychophores and tardigrades or larva-like ancient body plans (*upper panels*) was followed by a transition to the first true complex arthropod body plan, such as the ancient *Artemia* or silverfish (*middle panels*). The reintroduction of the ancient ancestor body plan can then be implemented by re-invoking the ancient simple body plan during development, followed by the normal metamorphosis to a complex insect body plan (*lower panels*).

The scenario that the larval stages did not have to be developed *de novo* but were reinstated in evolution, can be reconstructed from embryonic development. The formation of the larval body is already determined in the syncytial blastoderm stage, where a gradient of hormones determines the segmental pattern. The differentiation of the complex body plan in holometabolous insects starts later in development in the cellular blastoderm when the imaginal discs are set aside in a dormant state while the rest of the embryo develops into the larva. The formation of set-aside cells during evolution is similar to the migration of precursor cells in direct developing insects (Stone and French, 2003). Thus, the evolution of metamorphosis can be made on the basis of a switch between body plans in development. The ancestral simple body plan was developed first as a sequence of events starting from a syncytium and leading to the ancestral larva-like body plan, comparable to *Drosophila* larval development (Fig. 2). The development of a complex body plan later in evolution could be based on the differentiation of a part of the embryo and involved migration of cells to form organs and limb buds as in direct-developing insects. A reactivation of the ancient developmental pathway, combined with a temporary inhibition of direct development, would introduce the larval phase in the normal developmental scheme. In this switch in development, the entire sequence of events leading to the adult body plan as well as to the ancestral plan are still intact, in line with the design-by-contract modeling.

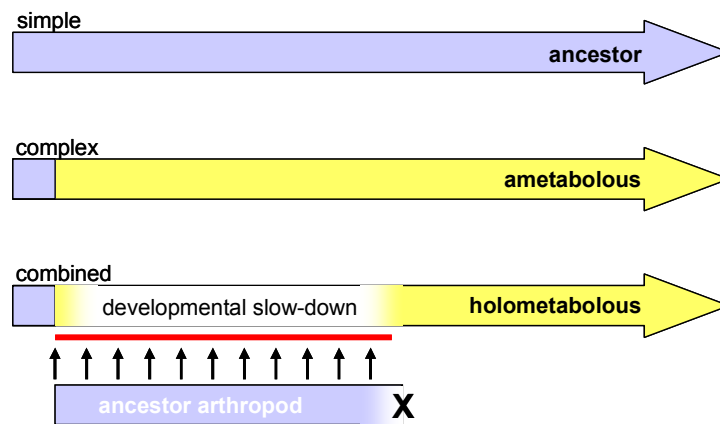


Fig. 2 Basic evolutionary switch in body plans. The default developmental sequence to a simple ancestor arthropod body plan (*upper panel*), could have been later changed into another direction leading to the ametabolous, complex insect body plan (*middle panel*). A temporary reactivation of the ancestral developmental plan and a parallel negative feedback of the adult body plan, would allow the growth of the ancestor simple body plan; a subsequent deactivation of larval stages and a concomitant loss of inhibition of the complex body plan, would then lead to the adult stage of the holometabolous insects (*lower panel*).

Molecular determinants of body plan

Hox genes can be considered the master switches controlling segmentation and body plan (Akam, 1995; Averof and Akam, 1995) by acting as stable binary switches that direct lineages of cells to adopt alternative developmental fates (Akam, 1995; Averof and Akam, 1995). Mutations in Hox genes are for instance involved in the transition from crustacean-like arthropod ancestors with multiple limbs to six-legged insects (Gallant and Carroll, 2002; Ronshaugen et al., 2002). Therefore, the reactivation of the larval body plan in evolution could also be based by switching to an ancient Hox expression pattern. A disruption of Hox genes result in homeotic transformations that generate less complex body plans and mimic inferred ancestral states (García-Bellido, 1977), showing that changes in Hox gene expression can indeed induce ancient pathways in insects. The proposed switch in early embryological development can therefore be based on a change in Hox gene expression in evolution leading to redirection of body plan development. Since all arthropods share the same basic set of Hox genes, the larval and adult body plan can be based on a differential use of these Hox genes (Akam, 1995; Averof and Akam, 1995) and would not need the evolution of new genes. Hox genes and their effectors may thus represent the molecular implementation of the switch to an ancient body plan in evolution.

The formation of adult structures in holometabolous structures by the growth and eversion of the imaginal discs is controlled by juvenile hormones (JH). In insects with partial or no metamorphosis, the absence of JH during embryo formation and development allows the embryo to become a miniature version of the adult, while in embryos of insects with complete metamorphosis there is an early appearance of JH that promotes formation of the larval stage.

The disappearance of juvenile hormones allows the growth of imaginal discs, which will give rise to the complex adult structures. A simple reactivation of these precursor cells by a reduction of JH levels could restart the differentiation of the imaginal discs leading to the adult organism, a process similar to organogenesis in direct-developing insects. Since the hormone that induces the larval developmental sequence also acts to suppress (Truman and Riddiford, 1999) growth of the imaginal cells, changes in the timing of JH expression could act as the control of the evolutionary switch in development. In this way, JH can be regarded as the ancestral hormone since it would induce the simple ancestral larval body plan, which is consistent with the hypothesis that the ancestral developmental role of JH is the regulation of embryogenesis (Truman and Riddiford, 1999). The disappearance of JH in evolution could have been one of the steps that would allow development of other body plans.

Larvae as an ancient life form

The simple morphology of many larvae already suggests their ancient origin, which is especially clear for the vermiform maggots that show a simple segmented morphology with no appendages or complex sensory organs. It could be speculated that the different larvae of holometabolous insects represent different stages in arthropod evolution, e.g. the legless maggots, the sawfly larvae with only prolegs, the caterpillars with prolegs as well as jointed legs and a single armoured head plate, or grubs that have jointed legs and a body armour. There are also much simpler body plans in the arthropod tree, for instance the onychophores (velvet worms) and tardigrades (water bears) that are closely related to arthropods based on molecular phylogenetic analysis but that do not have the general arthropod adult body plan (Giribet et al., 2001). However, the putative ancestor may not be limited to the arthropod tree, but could potentially also be found in the Ecdysozoal line. Both the morphology of ancient arthropods and the simpler body plans of extant ancient arthropods are in line with the proposition that larval stages represent an ancient life form. It is interesting to note that organisms exist that are capable of reproducing while in the larval form. This occurs in the females of certain progenetic organisms like beetles, Strepsiptera, bagworms, and gall midges and is a strong indication for the existence of once fertile independent larva-like organisms.

Another indication for an ancient origin of insect larvae can be found in the developmental processes. The syncytial development of the larva can be considered an ancient design pattern for acquiring multicellularity while maintaining polarity. Starting with a unicellular organism with some form of polarity in the form of a hormone gradient, the step to multicellularity can then be made by an inhibition of cytokinesis. This will result a syncytium in which the original gradient that existed in the single cell can be maintained in the syncytium. A late induction of cytokinesis will lead then to a multicellular organism, in which the individual nuclei of the embryo have been exposed to the gradient. Not only all insects show a syncytial development (Davis and Patel, 1999), but also basic hexapod orders such as the Collembola (e.g. the springtail) that are believed to belong to a separate evolutionary line that predates even the separation of insects and crustaceans (Nardi et al., 2003), show syncytial cleavages, further substantiating the ancestral syncytial state. This would be similar to the steps taken in development in *Drosophila* development in the sequence fertilized egg, syncytial blastoderm to cellular blastoderm.

Design-by-contract and evolution

Design-by-contract can be seen as an additional set of constraints to a basic modular design. By subdividing the developmental system into functional modules that are separated by constant interfaces, one can unravel the logical sequence of events for its evolution. This design-by-contract approach sees Life as an evolving molecular machine that can add functionality with every developmental life cycle. The principle of functional continuity in evolution would enforce the constancy of the interfaces, allowing only extensions to the existing molecular machine. This results in evolutionary scenarios that are essentially similar to the 'ontogeny follows phylogeny' paradigm. For a developmental sequence of events, design-by-contract predicts that the chain of events that is seen in development reflects the path that was followed in evolution. The reintroduction of larval phases can be seen as an extension of the direct-development of insects since it leaves existing pathways intact, thereby allowing the independent evolution of the larval stages. This engineering approach has been proven fruitful in proposing alternative scenarios for the origin of introns (de Roos, 2005), the origin of the nucleus (de Roos, 2006a) and the origin of life (de Roos, 2006b). Design-by-contract may therefore provide a predictive evolutionary framework by giving the engineering principles and guidelines that help to unravel the path taken in evolution.

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