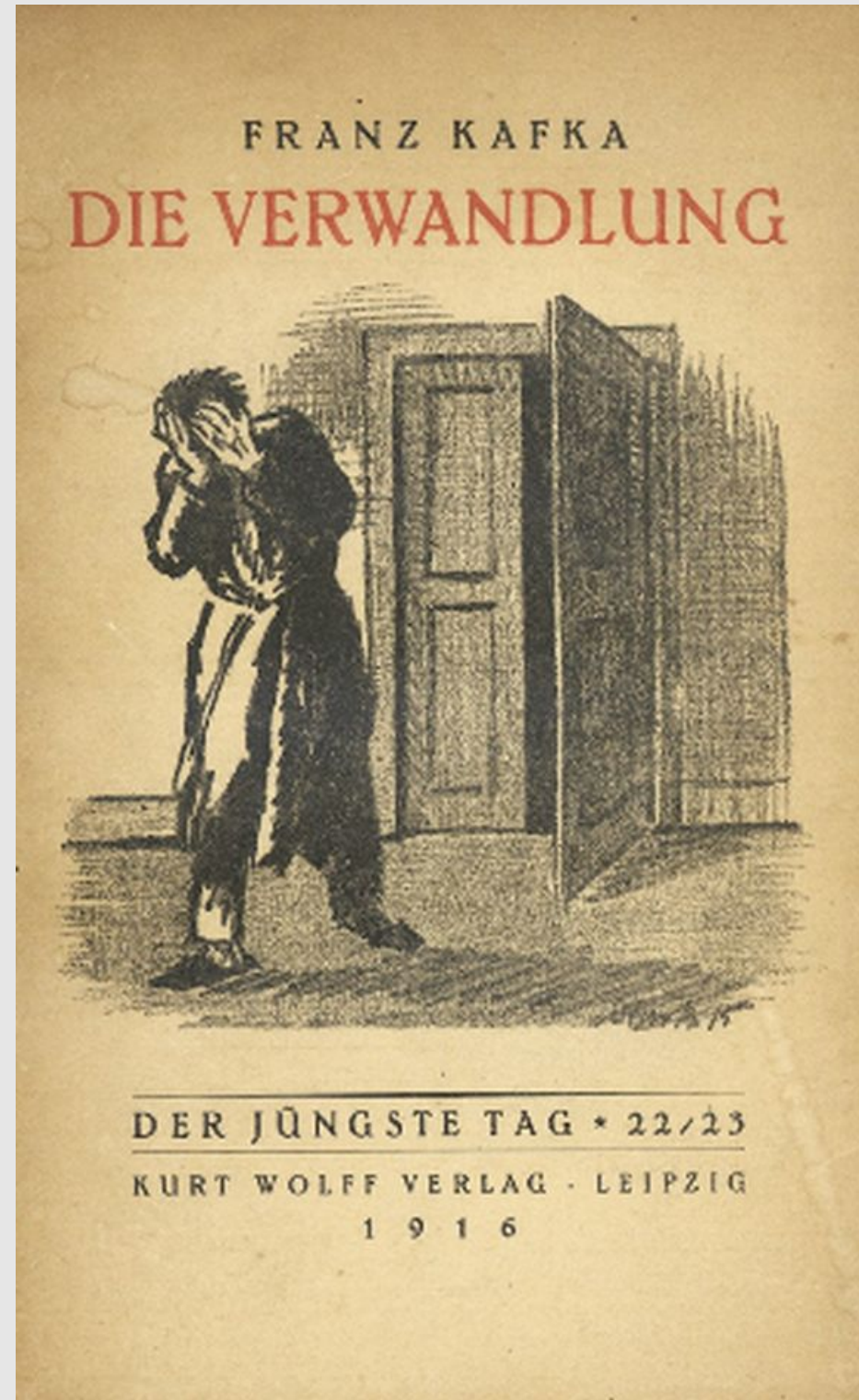


Insect Metamorphosis

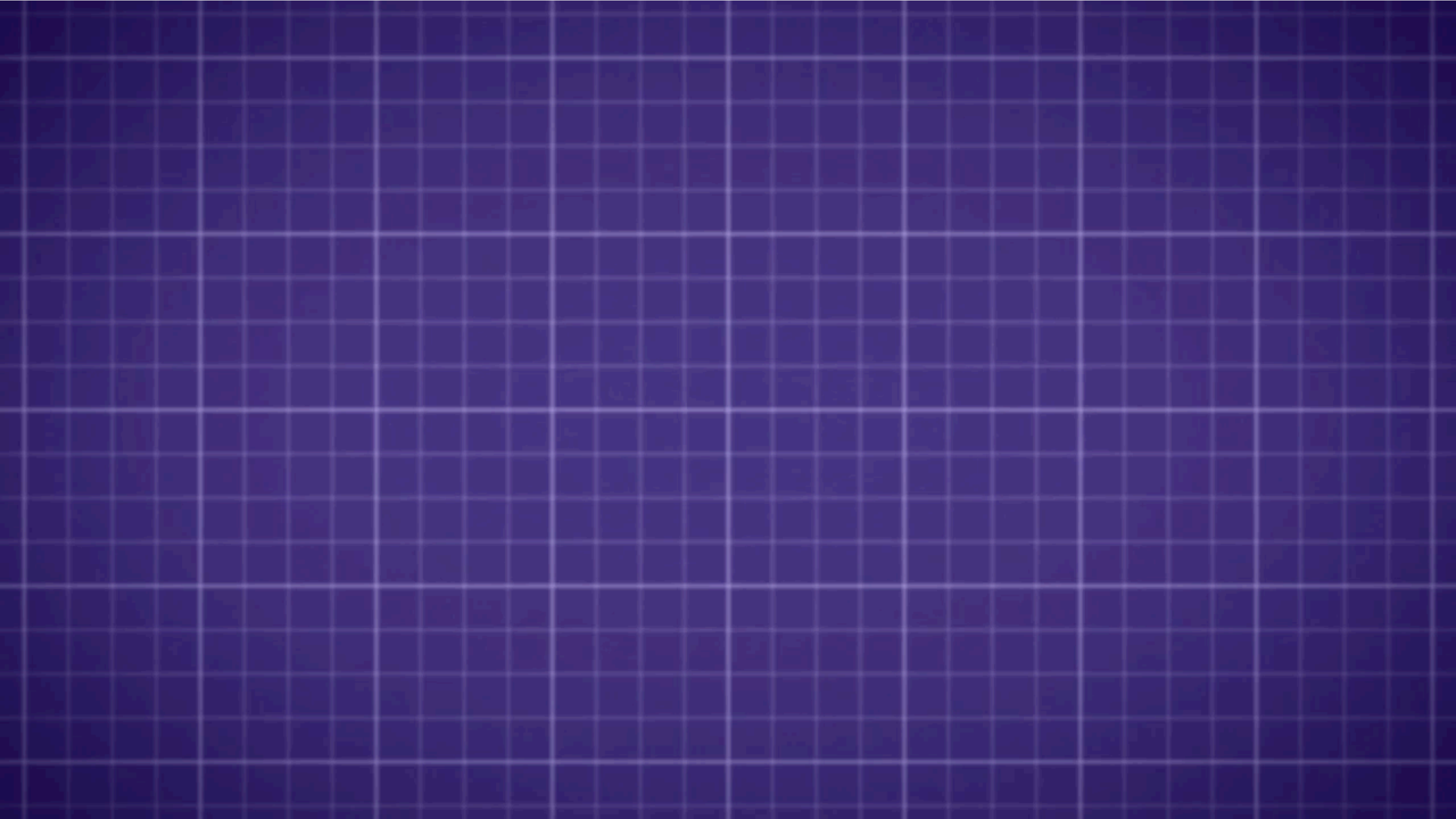


Brian O'Meara
EEB464 Fall 2019

Learning objectives

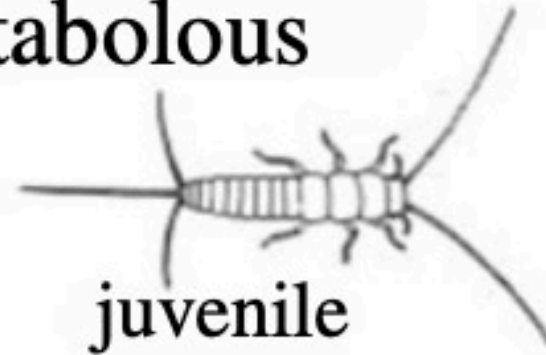
- Understand details of metamorphosis
- Understand a hypothesis for how this developed
- Understand how competing ideas may be evaluated



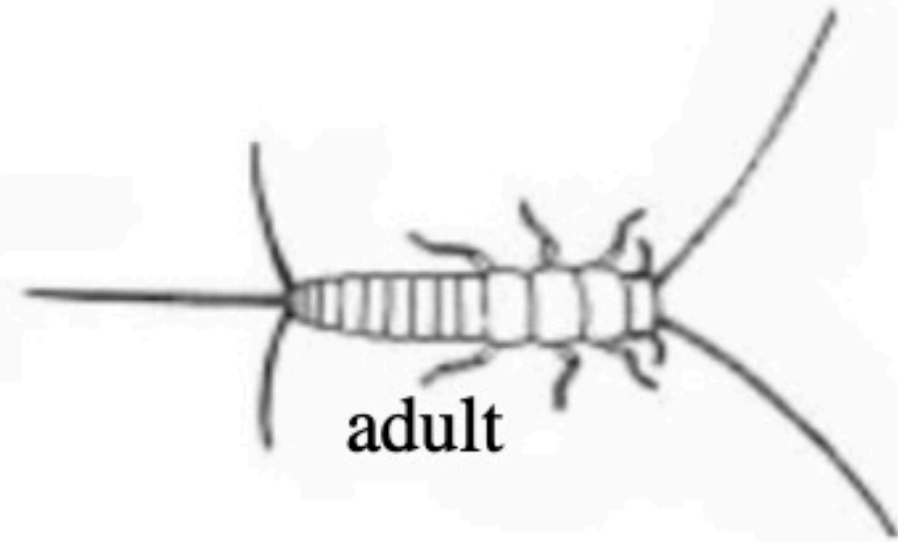


(a)

ametabolous



juvenile



adult

hemimetabolous



pronymph



nymph



adult

holometabolous



larva



pupa

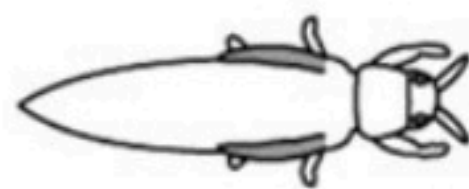


adult

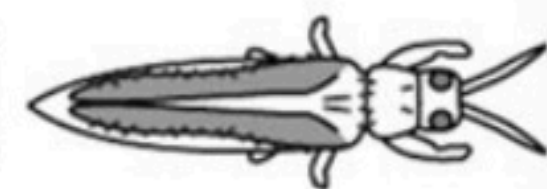
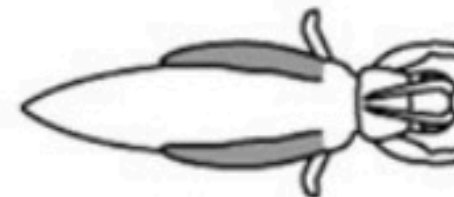
neometabolous



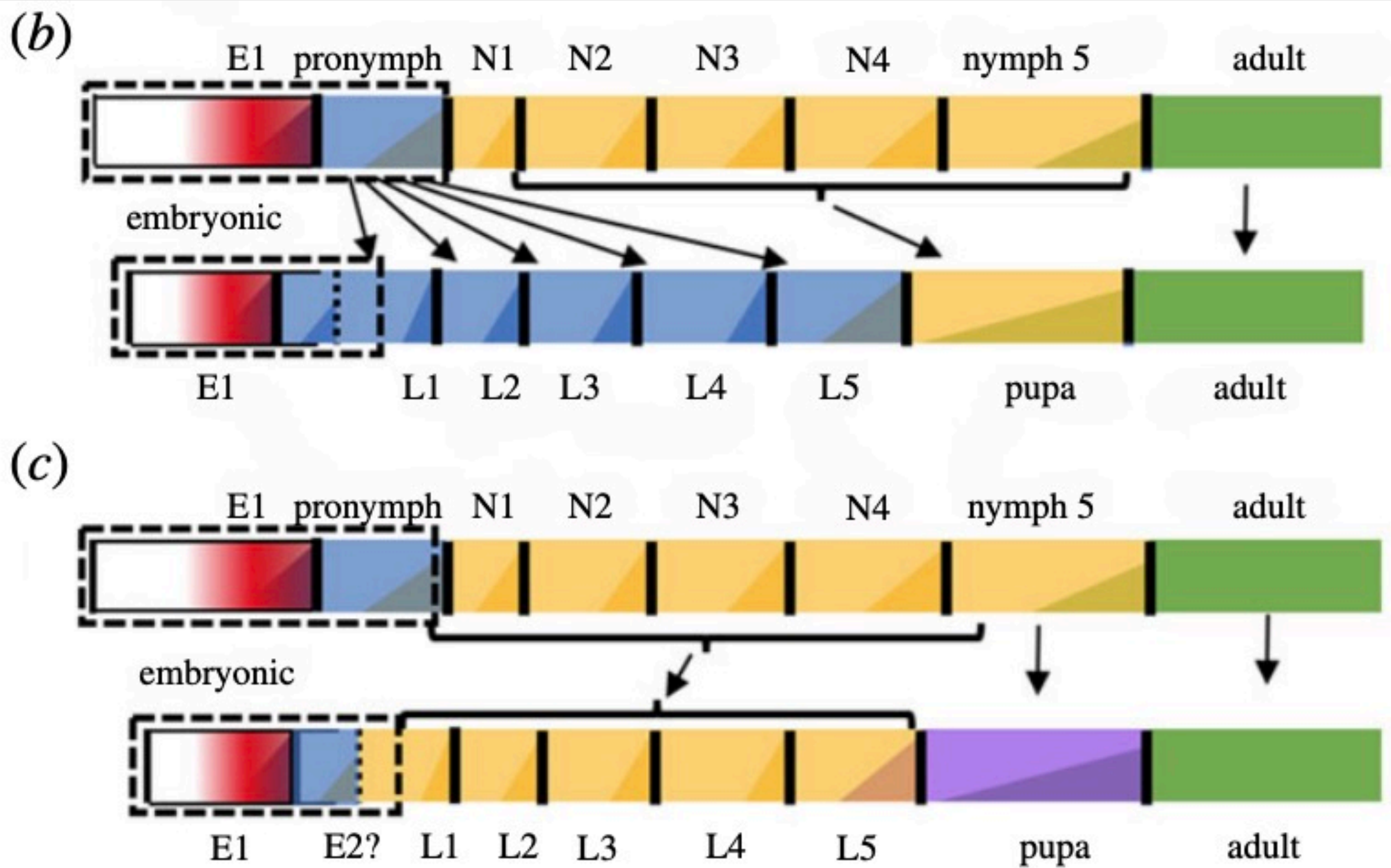
larva



propupa/pupa



adult



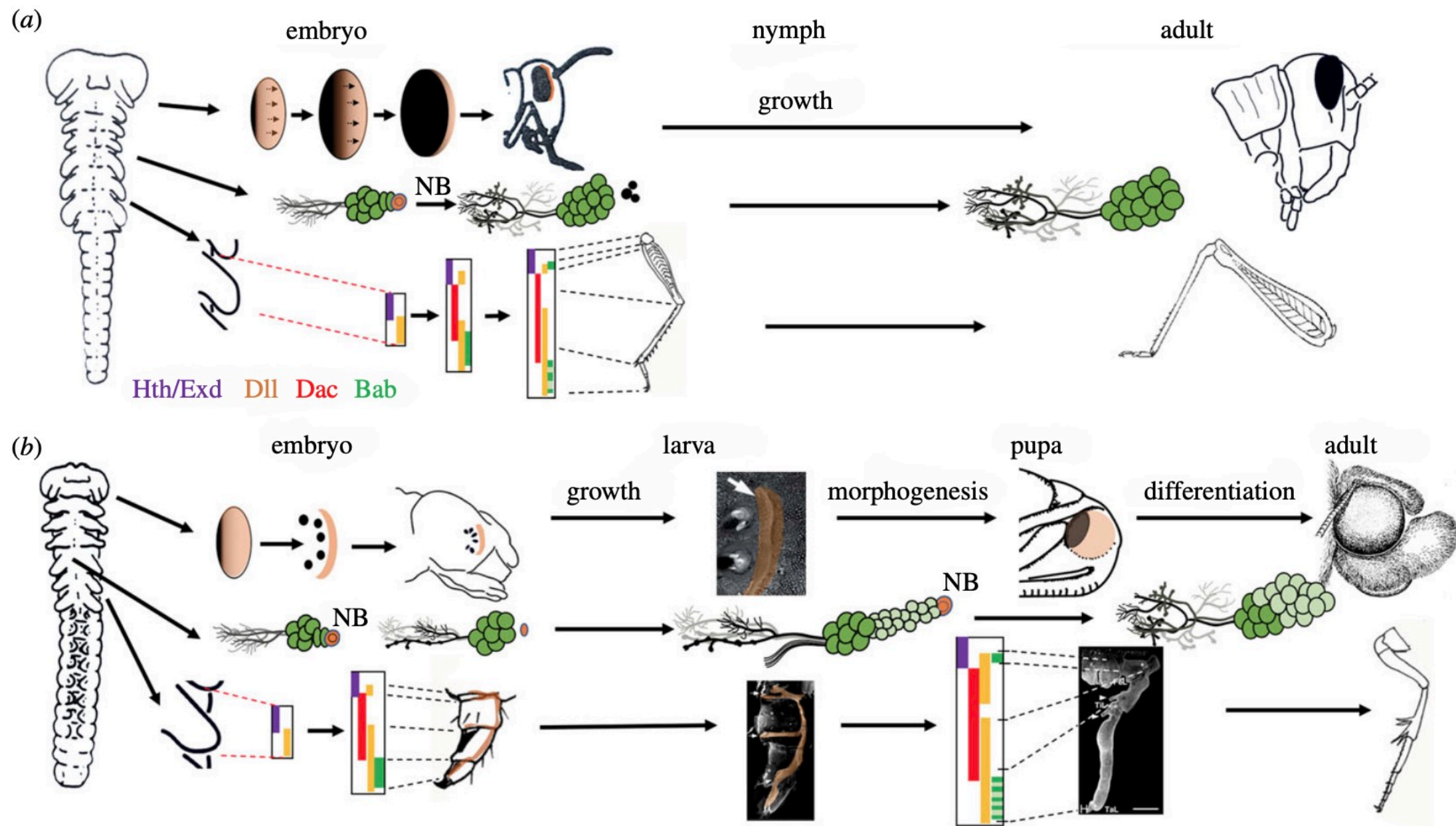


Figure 2. Comparison of embryonic and postembryonic development of a generalized hemimetabolous insect (cricket/grasshopper) with a holometabolous insect (moth). (a) Orthopteran development showing progressive patterning of the eye primordium and leg bud. Rows of ommatidia in the eye form as a wave of differentiation (arrows) moves anteriorly across embryonic primordium. CNS neuroblasts (NB) die late in embryogenesis after producing all of their neurons. The leg bud transforms into the leg by the recruitment of a sequence of proximal–distal patterning genes that determine the leg segments. These structures increase in size during nymphal life with little new additions except for ommatidia at the anterior margin of the eye. Based on [15–20]. (b) Development in the moth embryo does not progress as far as that in the Orthoptera. Partially patterned systems serve as the basis of larval structures, but persisting embryonic centres (light orange) are carried into the larva and become the imaginal primordia that generate the adult structures. Based on [16–18,21–24]. Hth, Homothorax; Exd, Extradenticle; Dll, Distal-less; Dac, Dachshund; Bab, Bric-a-brac. See text for more details.

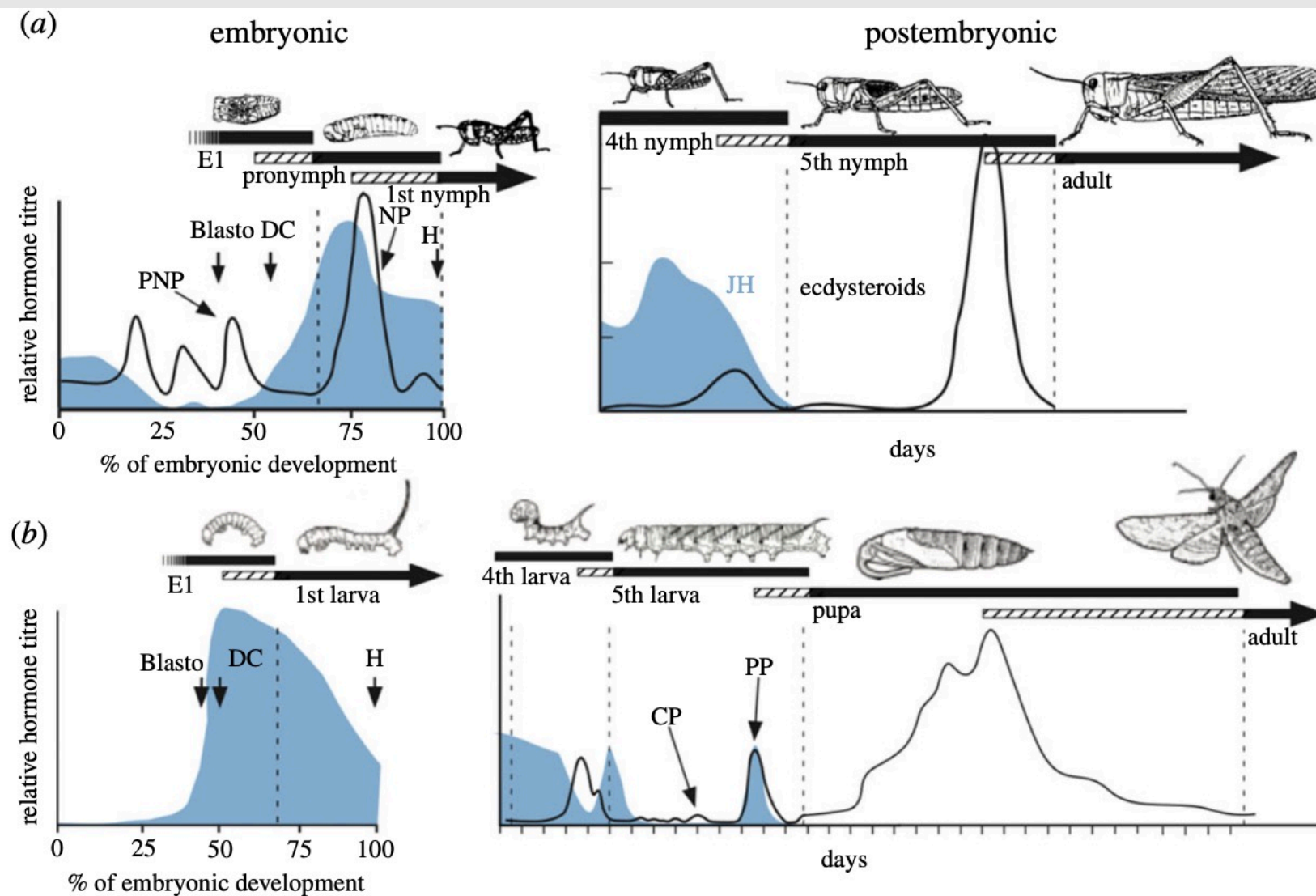


Figure 4. Comparison of the embryonic and postembryonic titres of ecdysteroids (black) and JH (blue) for (a) hemimetabolous insects, the grasshoppers *L. migratoria* (embryonic) and *Schistocerca gregaria* (postembryonic) and for (b) a holometabolous insect, *M. sexta*. The bars relate the presence of the respective cuticles to the hormone titers (cross-hatching represents pharate periods). Ecdysteroid titres are not available for *Manduca* embryos. Vertical dashed lines: times of ecdysis; Blasto, blastokinesis; DC, dorsal closure; E1, covered by the first embryonic cuticle; H, hatch. Ecdysteroid peaks: CP, commitment peak; PP, prepupal peak; PNP, pronymphal peak; NP, nymphal peak. Reprinted from [4]. (Online version in colour.)

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Caterpillars evolved from onychophorans by hybridogenesis

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Communicated by Lynn Margulis, University of Massachusetts Amherst, Amherst, MA, July 24, 2009 (received for review May 15, 2009)

I reject the Darwinian assumption that larvae and their adults evolved from a single common ancestor. Rather I posit that, in animals that metamorphose, the basic types of larvae originated as adults of different lineages, i.e., larvae were transferred when, through hybridization, their genomes were acquired by distantly related animals. "Caterpillars," the name for arachnids with thoracic and abdominal legs, are larvae of lepidopterans, hymenopterans, and micropterans (scorpionflies). Grubs and maggots, including the larvae of beetles, bees, and flies, evolved from caterpillars by loss of legs. Caterpillar larval organs are dismantled and reconstructed in the pupal phase. Such indirect developmental patterns (ontamorphosis) did not originate solely by accumulation of random mutations followed by natural selection; rather they are fully consistent with my concept of evolution by hybridogenesis. Members of the phylum Onychophora (velvet worms) are proposed as the evolutionary source of caterpillars and their grub or maggot descendants. I present a molecular biological research proposal to test my thesis. By my hypothesis 2 recognizable sets of genes are detectable in the genomes of all insects with caterpillar grub- or maggot-like larvae: (i) onychophoran genes that code for proteins determining larval morphology/physiology and (ii) sequentially expressed insect genes that code for adult proteins. The genomes of insects and other animals that, by contrast, only lack larval, not recognizable sets of genes from single animal common ancestors.

hybridization | insect evolution | interphyletic crosses | larval transfer | metamorphosis

Darwin (1), Haeckel (2), and most zoologists assume that any larva and its adult evolved from a single common ancestor. My larval transfer hypothesis, by contrast, assumes that the basic form of all larvae were transferred as adults of other taxa. Larvae originated when adult genes were acquired by other different animals probably primarily by sexual hybridization (3, 4). The dual genomes of the merged lineages are expressed in a temporal sequence: first from the genome of one animal of another species can hatch as a larva that resembles one parent (5). Later metamorphosis occurs when the genome of the other species (indistinguishable from the second parent. Larvae were later added to established animal phylogenies and merged lineages continue to evolve. My idea is that metamorphosis represents an evolutionary legacy. A change in genetic expression during development from one taxon to another, and it is testable.

The prevalent common ancestor assumption of monophyly and my larval transfer hypothesis are contrasted here as relation to the evolutionary origins of caterpillar larvae. Although caterpillars as acquired larvae were mentioned earlier (3–5), descriptions of caterpillars were not considered. This exposition that includes new evidence from microsatellite analysis and a Cambrian lobopod presents the concept for testability with molecular biological methods. This specific example of my larval transfer hypothesis is the evolutionary origin of caterpillars is now amenable to verification or to disproof by genome analysis.

Haeckel's "biogenetic law" (2) developed from the common ancestor assumption, it postulates that larvae represent ancestral

adults and ontogeny is a short, rapid recapitulation of phylogeny. Garstang (6) proposed that modern larvae represent ancestral larvae rather than adults. He posited that adults in one taxon that resemble larvae in another are "persistent larvae," i.e., forms that were originally larvae but now mature without metamorphosis. I regard his persistent larvae as relatives of adult animals that were sources of larvae in distant lineages.

"Even the illustrious Cuvier did not perceive that a barnacle was, as certainly is, a crustacean, but a glance at the larva shows this to be the case in an unmistakable manner" wrote Darwin (ref. 1, p. 420), and barnacles provide a clear example of the differences between Darwin's attitude to larvae and mine. Even the illustrious Cuvier did not perceive that a barnacle was, as certainly is, a crustacean, but a glance at the larva shows this to be the case in an unmistakable manner" wrote Darwin (ref. 1, p. 420), and barnacles provide a clear example of the differences between Darwin's attitude to larvae and mine. Even the illustrious Cuvier did not perceive that a barnacle was, as certainly is, a crustacean, but a glance at the larva shows this to be the case in an unmistakable manner" wrote Darwin (ref. 1, p. 420), and barnacles provide a clear example of the differences between Darwin's attitude to larvae and mine.

The 2 contrasting concepts to explain rhizocephalans can be experimentally distinguished. If rhizocephalans are parasitic barnacles, i.e., adults that lost all barnacle morphology by reduction yet their larvae retain virtually all features of larval barnacles, their genomes should be typical of cirripedes. Alternatively, if, as I suggest, rhizocephalans are not arthropods but acquired arthropod larvae by hybrid transfer, at least 3 genomes should be detected. Those that code for nauplius and cypris larvae should be similar to those in cirripedes, while the third "adult" genome should differ distinctly from that of cirripedes.

Caterpillars. Caterpillar larvae are found in many insect orders. They usually have a pair of 3-jointed legs on each of the 3 thoracic segments and paired unjointed prolegs on some or all of the 10 abdominal segments. The prolegs are attached by hydrostatic pressure. Such larvae occur in some species of the order Lepidoptera (butterflies and moths), Hymenoptera (ants, bees, wasps, and sawflies), and Microptera (scorpion flies and hanging flies). Caterpillars present many variations.

Author contributions: D.I.W. designed research, performed research, analyzed data, and wrote the paper.

Supplemental material is available for this article.

Reprints: Dr. Donald I. Williamson.

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clusters of species of modern caterpillars (Fig. 2). No corresponding structures exist in extant onychophorans.

Discussion

Terrestrial onychophorans most likely evolved from aquatic lobopods by means of desiccation resistance. A succinct statement of my testable hypothesis is that "An onychophoran was the evolutionary ancestor of caterpillars." Modern velvet worms in this view, are surviving relatives of the onychophoran that hybridized with an insect, which, as a result, acquired caterpillar larvae, perhaps in the late Carboniferous period, as suggested by Labandeira and Phillips (20) and Shear and Kukavica Ick (19). This hybridization event occurred before lepidopterans became extant in Mesozoic (Fig. 2*A*), probably the most archaic of extant lepidopterans (21).

Neither Haeckel (2) nor Garstang (6) discussed insect larvae, but, under Haeckel's recapitulation theory, holometabolous insects would have evolved from adult caterpillars, and under Garstang's variant of it, holometabolous insects would have evolved from animals with caterpillar larvae. The sudden appearance of fully formed caterpillars is inexplicable under either theory. Onychophorans cannot be accepted as an example of Garstang's persistent larvae, because they evolved from marine lobopods that predated insects by hundreds of millions of years. Many corollaries of my hypothesis are testable. If insects acquired larvae by hybrid transfer, the total base pairs of DNA of eurypterids insects that lack larvae will be smaller than those of eurypterids (holometabolous) species that have both larvae and pupae. Genome sequences are known for the fruitfly, *Drosophila melanogaster*, the honeybee, *Apis mellifera*, the maize rootworm, *Anthonomus grandis*, the red flour beetle, *Tribolium castaneum*, and the silkworm, *Bombyx mori*; holometabolous species with marked metamorphoses. I predict that an outgroup (Microptera Megaloptera), an earwig (Dermaptera), a cockroach (Diptera), or a locust (Orthoptera) will have larvae, (a) of a dated animals without larvae, and (ii) of a dated of the previous adult source of the larval form.

The hypothesis of the onychophoran origin of caterpillar larvae and their apodous descendants provides a clear testable example of my larval transfer concept. Component transfer, by my hypothesis, also resulted from sexual hybridization and was an essential feature of the Cambrian explosion (11, 31). Both larval transfer and component transfer exemplify evolution by merger of genomes. So does the origin of new cystic cells by symbiogenesis represent an example of acquisition of foreign genomes in the microbial world and its larger descendants (5, 32). These salutary evolutionary processes differ from "Darwinian gradual descent with modification followed by natural selection," but they are additions to and not replacements of Darwin's great insight into the history of life and the generation of its diversity.

ACKNOWLEDGMENTS. I wish to thank Carlos A. Ackema, Barthold Beutels, Martin Bräuer, Andrew P. Butler, Peter P. Butler, Lynn Margulis, James MacArthur, Frank P. Ryan, Robert Steinberg, and Sergio Vicens for constructive comments.

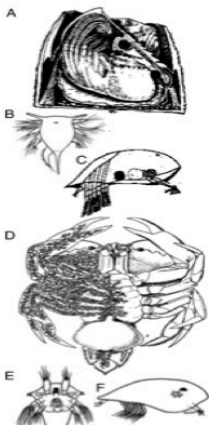


Fig. 1. A barnacle, a rhizocephalan, and their larvae. (A) The barnacle *Balanus tintinnabulum* in longitudinal section. (B) Nauplius and (C) cypris larvae of *Balanus*. (D) The rhizocephalan *Sacculina carpi* infecting the crab *Gecarcinus lateralis*. Right side of crab shown transparent to illustrate infection. (E) Nauplius and (F) cypris larvae of *Sacculina carpi*. (G) Larva of *Sacculina carpi*. (H) Larva of *Sacculina carpi*. (I) Larva of *Sacculina carpi*.

Larvae of Trichoptera (caddisflies), Coleoptera (beetles), and some Hymenoptera lack abdominal prolegs. Nearly all butterflies and moths (Lepidoptera) have caterpillar larvae (Fig. 2*A* and *D*), but larvae of the superfamily Neptulioidea are apodous. Typical lepidopterans caterpillars have prolegs on abdominal somites 1–6 and 10, each with a flattened tip armed with a series of crochets. In the Geometridae, however, prolegs occur on somites 6 and 10 only, and in the Micropteridae, the 3 pairs of thoracic appendages and 9 pairs of abdominal appendages of *Microptera* (Fig. 5*A*) are similar prolegs, each ending in a single claw. The abdominal appendages are vestigial in other micropterids, such as *Ephemera* (12).

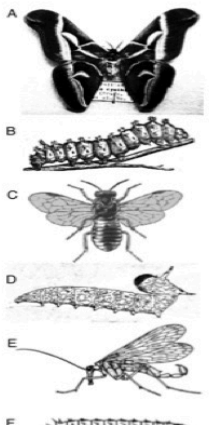


Fig. 2. Insects with caterpillar larvae. (A) and (B) Lepidoptera. (A) Adult of the moth *Sarcophaga*. (B) Larva of *Sarcophaga*. (C) and (D) Hymenoptera. (C) Larva of *Sarcophaga*. (D) Larva of *Sarcophaga*. (E) and (F) Microptera. (E) Larva of *Sarcophaga*. (F) Larva of *Sarcophaga*.

Hymenopterans of suborder Apoecriti, which includes wasps, bees, and ants, have larvae that lack legs, prolegs, and ocelli. Larvae of suborder Symphyta, which includes saw flies, horntails, and woodwasps, have large compound eyes, 3 pairs of thoracic legs, and 6–10 pairs of abdominal prolegs without terminal crochets (Fig. 2*C* and *D*). Compound eyes also occur in the 3 types of micropteran larvae. Caterpillar-like larvae, with abdominal prolegs, occur in the micropteran families Panorpidae (Fig. 2*E* and *F*) and Bimidae. Larvae of the Panorpidae lack abdominal prolegs and are scarabaeiform, i.e., they resemble the larvae of scarab beetles. The aquatic larvae of the Nannochorini

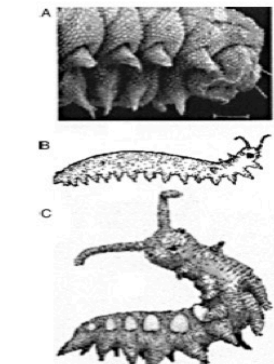


Fig. 3. A caterpillar, an onychophoran, and a lobopod. (A) Caterpillar of the moth *Sarcophaga*. (B) Larva of *Sarcophaga*. (C) Larva of *Sarcophaga*. (D) Larva of *Sarcophaga*. (E) Larva of *Sarcophaga*. (F) Larva of *Sarcophaga*.

retidae look like the wingless larvae of dark beetles (Elateridae). The Mecoptera and Eimeroptera probably have no larvae (13).

From these specific examples, it can be seen that a range of larvae exists from those with appendages on all thoracic and abdominal segments, through examples in which varying number of appendages have been lost, to maggots, without appendages. Loss of appendages, apparently independently, evolved several times, such that maggots are typical larvae of both social hymenopterans (bees and ants) and cyclophorid dipterans (some flies). This scenario suggests that caterpillars first appeared with their full complement of appendages; they did not gradually evolve from legless ancestors. This favors larval genome acquisition (larval transfer) over the assumption of Darwinian gradual descent with modification.

Metamorphosis. Insects mature in one of 3 ways. Ametabolous species develop gradually, without metamorphosis. Hemimetabolous species hatch as aquatic nymphs, which metamorphose directly into winged adults. Holometabolous species pass

through a pupal phase of limited mobility between the last larval stage and the winged adult, and all insects with caterpillar larvae or derivatives fall in this category. In the pupa, the inner tissues and organs of the larva disintegrate to form a structureless "soup." In most holometabolous insects, the soup, legs, wings, and nerves of the imago develop from imaginal discs formed in the last larval stage. The adult gut, digestive gland, and other internal organs grow from the pupal soup of dedifferentiated cells, i.e., cells that have returned to the stem cell stage. The imaginal discs of *Drosophila* and other cyclophorid dipterans differ from those of most holometabolous insects in general shape and arrangement, and nearly all of the adult head and thorax is formed from them. Cyclophorid dipterans uniquely have histoblasts, formed during embryonic life, which remain undifferentiated until the pupal phase, when they develop into most of the adult abdomen (14).

Metamorphosis in holometabolous insects is an example of "start again metamorphosis." This term was originally applied to marine bryozoans (most animals), in which all larval tissues and organs revert to stem cells, and the juvenile (miniature adult) grows from those stem cells (3). In holometabolous insects, imaginal disc and histoblasts play no part in larval development, and larval tissues undergo histolysis and cytotoxicity to produce "pupal soup." No larval components or organs contribute directly to adult components or organs. A life history that involves dismantling a complex larva then starting again to differentiate an adult is consistent with the "descent with modification" assumption. I question the assumption that holometabolous insects evolved solely by larval descent. Rather my hypothesis of larval transfer, i.e., that 1 or more hybridizations transferred caterpillar larvae to insects (3, 4), is more consistent with evidence of sequentially expressed insect genes in their apodous descendants differed too dramatically from adult insects to which they develop for gradual metamorphosis to have evolved by accumulation of mutations. Rather the pupal phase permitted metamorphosis by cellular differentiation and re-differentiation.

Onychophorans and lobopods. Onychophorans or velvet worms are terrestrial "worms with legs," and they show a combination of features of arthropods and annelids. The thin cuticle consists of chitin and various proteins, and they molt like arthropods. The appendages, however, are unjointed and are extended by hydrostatic pressure, as in parapodia of annelids. The excretory system and the musculature of onychophorans also resemble those of annelids. All species have 1 pair of antennae and a pair of oral papillae. In all species, there are 13 to 41 pairs of legs. Specimens gain a pair of feet at the first molt in some species, and in others the molted feet are smaller than the feet of the next molt. *Onychophora* (Fig. 3*B*) is a Tasmalian genus with 21 pairs of feet in both sexes. Onychophorans occur today in Central and South America, West and South Africa, East Asia, and Australasia, but specimens from Eocene amber suggest that they previously had a wider distribution with modification.

Lobopods, e.g., *Microdictyon* (Fig. 3*C*), resembled onychophorans. While et al. (17) gave a table of known fossil lobopods from Lower Cambrian to Eocene, and they point out that "Heterotremus and specimens found from the Cretaceous period have been interpreted as Onychophora." *Heterotremus* was described from the Upper Carboniferous beds of Macon Creek, Illinois (18). Macon Creek has yielded a mixture of marine, freshwater, and terrestrial fossils (19), implying that it may have been an estuary, where lobopods could have made the transition from aquatic to terrestrial life.

The segmental dorsal sclerites of *Microdictyon* (Fig. 3*C*) may be homologous to those of *Onychophora*, which metamorphose directly into winged adults. Holometabolous species pass

10. Briggs O (1980) The morphology, mode of life, and affinities of *Conodonta perfoliata* (Crustacea: Phylloporidae), Middle Cambrian, Burgess Shale, British Columbia. *Phil Trans R Soc Lond B* 281:409–487.

11. Bräuer M (2009) Darwin's lost World: The Cambrian Explosion. Oxford: Oxford Univ Press, Oxford.

12. Davis GH (1987) Lepidoptera. In: Invertebrates, Vol. 1, ed. Bräuer M (Oxford Univ Press, Oxford).

13. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

14. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

15. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

16. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

17. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

18. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

19. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

20. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

21. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

22. Bräuer M (1988) Invertebrates. Princeton Univ Press, Princeton.

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Williamson 2009, PNAS

The evolution and loss of distinctive larval forms in animal life cycles have produced complex patterns of similarity and difference among life-history stages and major animal lineages. One example of this similarity is the morphological forms of Onychophora (velvet worms) and the caterpillar-like larvae of some insects. Williamson [(2009) *Proc Natl Acad Sci USA* 106:15786 –15790] has made the astonishing and unfounded claim that the ancestors of the velvet worms directly gave rise to insect caterpillars via hybridization and that evidence of this ancient “larval transfer” could be found in comparisons among the genomes of extant onychophorans, insects with larvae, and insects without larvae. Williamson has made a series of predictions arising from his hypothesis and urged genomicists to test them. Here, we use data already in the literature to show these predictions to be false. Hybridogenesis between distantly related animals does not explain patterns of morphological and life-history evolution in general, and the genes and genomes of animals provide strong evidence against hybridization or larval transfer between a velvet worm and an insect in particular.

Hart & Grossberg 2009, PNAS