

Alessandro Minelli · Carlo Brena · Gianluca Deflorian ·
Diego Maruzzo · Giuseppe Fusco

From embryo to adult—beyond the conventional periodization of arthropod development

Received: 11 October 2005 / Accepted: 4 April 2006 / Published online: 3 May 2006
© Springer-Verlag 2006

Abstract The traditional framework for the description of arthropod development takes the molt-to-molt interval as the fundamental unit of periodization, which is similar to the morphological picture of the main body axis as a series of segments. Developmental time is described as the subdivision into a few major stages of one or more instars each, which is similar to the subdivision of the main body axis into regions of one to many segments each. Parallel to recent criticisms to the segment as the fundamental building block of arthropod anatomy, we argue that, while a firm subdivision of development in stages is useful for describing arthropod ontogeny, this is limiting as a starting point for studying its evolution. Evolutionary change affects the association between different developmental processes, some of which are continuous in time whereas others are linked to the molting cycle. Events occurring but once in life (hatching; first achieving sexual maturity) are traditionally used to establish boundaries between major units of arthropod developmental time, but these boundaries are quite labile. The presence of embryonic molts, the ‘gray zone’ of development accompanying hatching (with the frequent delivery of an immature whose qualification as ‘free-embryo’ or ordinary postembryonic stage is arbitrary), and the frequent decoupling of growth and molting suggest a different view. Beyond the simple comparison of developmental schedules in terms of heterochrony, the flexible canvas we suggest for the analysis of arthropod development opens new vistas into its evolution. Examples are provided as to the origin of holometaboly and hypermetaboly within the insects.

Keywords Arthropoda · Molting · Heterochrony · Holometaboly · Hypermetaboly

Arthropod development: the cuticular view

Two different frameworks are currently employed to divide the temporal duration of arthropod development. For the first segment of embryonic development, a periodization—dividing ontogeny into meaningful and comparable temporal units—is adopted similar to that in use in describing embryonic development of other metazoans. Periodization can either be based on the time elapsed from egg laying, or expressed as percentage of total embryonic development under standard conditions, or with reference to a predictable series of events such as the cellularization of the blastoderm, gastrulation, katanepsis, etc. Later development, however, is usually described as a sequence of temporal segments of an ontogeny delimited by molts. In the entomological literature, these segments are usually (but not universally) referred to as stages and instars, whereas students of crustaceans often prefer to speak of phases and stages, respectively. Terminology is indeed far from fixed, thus the equivalence between stages uniformly called, for example, a prelarva, a larva, or a nymph in distantly related arthropod clades is far from granted.

At any rate, irrespective of terminology, traditional periodization based on molting is most obviously applied to postembryonic development, but may also extend to advanced embryonic development, to the extent that this part of ontogeny is also punctuated by molts (cf. Konopová and Zrzavý 2005).

The current description of arthropod late embryonic and postembryonic development is dominated by a cuticular view. This description has the double shortcoming of diverting attention from a host of other potentially important processes and of focusing on a largely discontinuous process that often masks parallel, largely continuous processes.

In this paper, we argue that an emphasis on the sequence of molts constrains our view of arthropod development and

Communicated by guest editors Jean Deutsch and Gerhard Scholtz

A. Minelli (✉) · C. Brena · G. Deflorian · D. Maruzzo ·
G. Fusco
Department of Biology, University of Padova,
Via Ugo Bassi, 58 B,
35131 Padova, Italy
e-mail: alessandro.minelli@unipd.it
Tel.: +39-049-8276303
Fax: +39-049-8276230

especially hinders perspectives on the evolution of developmental schedules. In the first part of the paper, we will show how limited is the coupling between the molting cycle and other important events in the development of an arthropod. In the second part, we provide some examples of how to approach the problem of the origin of novelties in the developmental evolution of arthropods within a framework less constrained by the current molt-centered perspective. We also briefly discuss the question of the homology between ontogenetic stages and stage sequences.

Aligning developmental schedules

When comparing the developmental schedules of two arthropods, two reference points are taken for granted, without even discussing in which sense or how far they are actually equivalent. These two points are the hatching from the egg and the molting to the adult. The period between these two points is subdivided by molts into a series of instars (from less than a handful up to a few dozens) which are generally grouped into a small number of stages, such as the larva, pupa, and imago of holometabolous insects, or the nauplius and the copepodids of copepods, or the larva, protonymph, deutonymph, tritonymph, and adult of many mites. As already mentioned, two or three molts also punctuate late embryonic development, but in this paper, we will deal with them only tangentially.

Close parallels can be drawn between this descriptive framework for developmental time and that currently adopted to describe arthropod architecture as deployed along the animal's main body axis. Between two main morphological reference points (the problematic 'acron' and 'telson' of traditional comparative anatomy), a series of segments is identified, which are more or less distinctly clustered to form body regions, or tagmata. More often, just two or three tagmata are recognized, more rarely four. It has even been suggested (Minelli 1996, 2003) that there is a correlation between the structural articulation and complexity of the temporal axis of an arthropod's development

and the structural articulation and complexity of the main body axis of the same animal.

We will focus in this study on development, beginning with a short discussion of the two conventional ends of postembryogenesis: hatching and molting to the first, or only, adult instar.

The conceptual framework we have adopted in our recent efforts to downgrade the traditional value of the segment as a fundamental unit of description of arthropod body architecture (Minelli and Fusco 2004; but see also Budd 2001; Minelli 2004; Fusco 2005) provides a blueprint for a comparable effort presented in this study in analyzing the inadequacy of the traditional elements of description of arthropod developmental schedules.

Postembryonic development: hatching, the starting point

The events surrounding hatching are a gray zone of development: the limit between embryonic and postembryonic conditions is defined in arbitrary ways. The question may seem purely semantic or taxonomic, but we will demonstrate that this is not so. For example, the way we interpret the stages immediately before and after hatching has a strong relevance to our views on the possible origin of holometaboly. Konopová and Zrzavý (2005), for example, contend that exopterygotes and endopterygotes hatch at equivalent stages because their embryos all shed three embryonic cuticles. The exception is cyclorrhaphous flies, with only two embryonic cuticles. In the opinion of Konopová and Zrzavý (2005), however, peculiarities of cuticulogenesis would suggest that in the transition from apterygote to pterygote (compare, e.g., the silverfish with a mayfly or a dragonfly), the first postembryonic (larval) instar of apterygotes has become embryonized in pterygotes. That is, the link between hatching and molting is not fixed, even within the insect lineage. Among the centipedes, the epimorphic species, for which all segments are formed during embryonic development, hatch at a stage

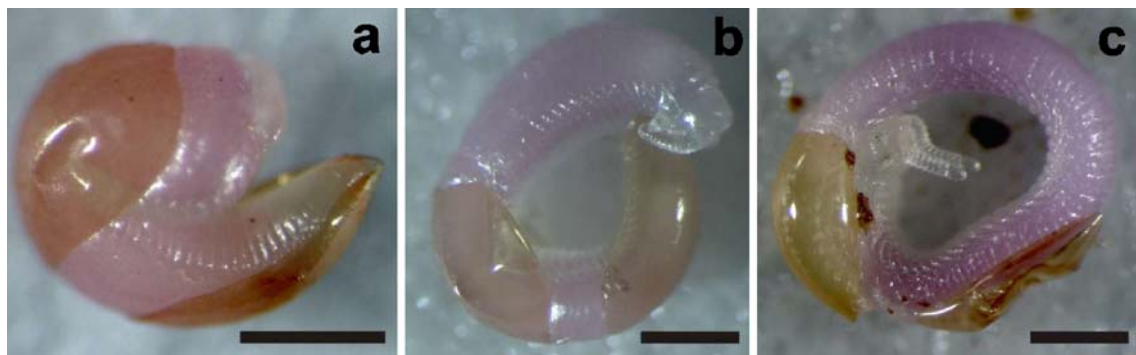


Fig. 1 **a** Last embryonic instar, **b** first postembryonic instar (peripatoid), and **c** second postembryonic instar (fetus) of the geophilomorph centipede *Strigamia maritima*. When the chorion splits open, exposing the last embryonic instar, the animal does not abandon it. Limb buds are barely visible and the antennae are unsegmented. The peripatoid has more developed limb buds and

segmented antennae, but the muscular system is poorly developed and the animal is not able to move. The fetus has segmented limbs, sparsely setose antennae, and is capable of 'writhing' movements. It will take another molt (to adolescence I) before the animal abandons the brood. Bars 0.5 mm

that does not correspond to the first postembryonic stage of anamorphic species, whose posterior segments are added during postembryonic development, in a double and somehow contradictory way. If the hatchlings of epimorphs are more advanced than the hatchlings of anamorphs because the former express all segments at hatching, they are however more embryo-like (Fig. 1) and well deserve the current names of peripatoid and fetus.

Most pantopods hatch as a protonymphon, a free-swimming, feeding larva, but in Callipallenidae and in some Nymphonidae, the hatchling is filled with yolk and remains, without any feeding, attached to the cephalic appendages of the male through several further molts (Bain 2003).

As for the Arachnida, Moritz (1993) noted that in their ground plan, there is one postembryonic stage that can be anticipated in the egg. This stage is generally called a prelarva, but terminology is far from uniform. Much diversity indeed is found in the condition of the animal just before and/or just after hatching. For example, in many mites, an immobile prelarva is found within the chorion. This prelarva is sometimes still unchanged when the chorion splits open. The same is true for the whipscorpions (Uropygi), where the prelarva molts to a larva within the opened chorion. In pseudoscorpions, a molt accompanied by the rupture of the chorion turns the embryo (more precisely, embryo I) into what is still called an embryo II, despite the fact that the little arachnid has hatched. The end of the embryonic phase of spiders is signaled externally by rupture of the chorion, which contains an immobile prelarva with incompletely articulated and clawless legs. In some basal spider clades, one more molt, to a prelarva II, takes place within the chorion. In all spiders, the prelarva molts to a slightly mobile larva with completely articulated legs, but one or two more molts are still required to reach the first mobile instar (nymph I). In many harvestmen (Opiliones), a 'larva' contained within the chorion molts to nymph I just as the chorion breaks, but in some families, (e.g., Sironidae), this larva lives an independent life for some time after hatching before molting to a nymph.

In millipedes (Diplopoda), the hatchling is a pupoid with unarticulated anlagen of antennae and legs. In the pill millipede *Glomeris*, it is covered by two cuticles (blastodermal and embryonic), but in other diplopods, there seems to be no blastodermal cuticle.

Some diversity also is found among the Pauropoda. In *Pauropus*, the hatchling is a pupoid, which molts into the first active instar. However, in *Gravieripus*, there are two pupoid instars, the first of which lacks external appendages, whereas the second has anlagen of antennae and two pairs of legs.

In Symphyla, the first stage outside the chorion is a prelarva with nonfunctional mouth parts.

In geophilomorph centipedes and in julid millipedes, the animal is not ready to abandon the chorion as the latter splits. As the next molt does not follow very soon, the last embryonic instar is, in some way, the same as the first postembryonic instar (Fig. 1).

Many decapod crustaceans, supposed to have only a zoea as first postembryonic instar, actually hatch as prezoa (Gore 1985), which is a nonfeeding and nonswimming stage.

Among hexapods, the first instar is sometimes active and quite similar to later instars, as in springtails and butterflies, but in others, such as dragonflies and grasshoppers, the hatchling is a prelarva covered by an embryonic cuticle, comparable to the pupoid of millipedes. In silverfish, the first free instar still depends on yolk in its midgut; and in mayflies, it differs from the next stage in gross morphological traits such as in the absence of gills.

Summing up, there is no reliable phylum-wide correlation between hatching and any of the following: (1) molting, (2) degree of morphological differentiation of the hatchling, and (3) body segmentation. Thus, hatching is far from being an unquestionable reference point for the comparison of ontogenetic schedules.

Postembryonic development: the end point

Let us now move toward the other end of the developmental sequence. Is the molt to the adult stage, whichever way the adult is defined, characterized any better than hatching? As we will show, this is hardly so.

First, there is no precise correspondence between the molt to adult and the production of mature gametes. Sometimes, as in the cellar spider *Pholcus phalangioides*, sperm are mature a couple of weeks before the molt to adult (Michalik and Uhl 2005). Even within the limits of pterygote insects, adults of mayflies (Ephemeroptera), stoneflies (Plecoptera), and butterflies and moths (Lepidoptera), where sperm and eggs are ready before the molt to the adult stage, may be compared to those of midges and flies (Diptera) or beetles (Coleoptera), where the presence of mature sperm and eggs in the adult is generally obtained later and depends on food intake by the adult. However, even this broad generalization at the level of individual insect orders is far from firm. Among dipterans, for example, maturity can be ascribed to the nonfeeding pharate adult (the adult when still within the pupal cuticle), as in the case of the parthenogenetic chironomid midge *Paratanytarsus grimmi* (Langton et al. 1988). Among the Diptera, again, and the Coleoptera are also found the few known instances of pedogenesis, i.e., reproduction in the larval stage (a condition other than reproduction by larviform adults which have undergone complete metamorphosis, as in several fireflies and other insects). For a better assessment of the relevance of these cases of pedogenesis, it may be pertinent to mention that in all cases thus far known (*Micromalthus debilis*, Coleoptera Micromalthidae; *Heteropeza*, *Miastor*, Diptera Cecidomyiidae), pedogenetic reproduction by parthenogenetic larvae alternates with amphigonic reproduction by adults.

Another critical feature is that more than one instar may be sexually mature; many arthropods have adult-to-adult molts (AAMs). The phylogenetic distribution of taxa with one adult instar suggests that this condition is derived

within large groups but does not rule out the possibility of character reversal. Among the chelicerates, horse-shoe crabs (*Xiphosura*) have AAMs, but scorpions do not have. AAMs are probably a plesiomorphic feature in the clade of whip spiders (*Amblypygi*) plus spiders (*Araneae*) (with advanced spiders only having one adult instar) and of insects proper. AAMs are known for many bristletails (*Archaeognatha*) and silverfish (*Zygentoma*), and one AAM (subimago to imago) survives in most representatives of the mayflies, a basal pterygote clade. As for crustaceans, AAMs are common in malacostracans, have been reported for a branchiopod (Ferrari and Grygier 2003), but are not known for any copepod. It is interesting to note that AAMs do not occur in some clades of anamorphic arthropods, such as proturans, or polydesmoid and chordeumatoid millipedes. A derived form of AAM is present, however, in at least some juloid millipedes. This is periodomorphosis, i.e., the occurrence of two or more molts, following the molt to the first mature instar, during which a regular alternation of sexually mature and sexually disabled condition occurs, defined by the cyclical gonadal development accompanied by an alternation between fully developed and partially regressed sexual appendages (Sahli 1990). A phenomenon similar to julid periodomorphosis has been also reported for some crayfishes (Hobbs 1981).

Molts are thus clearly decisive for the development (and, occasionally, regression) of features such as copulatory structures, gonopods, and other organs providing specific mating recognition systems. These skeletal structures can only change through molting but may be only loosely correlated with gonadal development.

Arthropod molts as a developmental timer

Let us now briefly discuss how far molts represent a reliable or even an obligate timer for most of the developmental events in an arthropod's life history.

The molting cycle of insects begins with the secretion of the prothoracicotropic (PTTH) hormone from neurosecretory cells in the brain. Mechanisms that control the secretion of PTTH will therefore control the timing and frequency of the molting process (Nijhout 1994). However, although the molting process of insects is in general associated with growth, 'the liaison between feeding and PTTH liberation is obscure in most insects' and different species apparently do not use the same cue for body size (Sehnal 1985).

On the coarsest level of analysis, molts are often described as the (necessary) events at which most of size increase is accomplished. In many instances, this is far from true, even if the vast majority of arthropods behave in this respect quite differently from nematodes, where the increase in length obtained with the (usually four) molts of the worm often accounts for only 10% of the total, and sometimes less than that (Nigon 1965). In this respect, reference is made in textbooks to the case of highly fecund females, such as those of some termites, whose abdomen may swell to a truly extraordinary degree. Quite remark-

able, however, is also the degree to which the soft cuticle of the trunk of many holometabolous larvae may extend during the intermolt interval. This explains why they can have few larval instars with very high intermolt growth increments (Nijhout 1994). Some poorly sclerotized larval appendages of decapods also grow more or less continuously (Gore 1985).

Overall changes unaccompanied by molts are not necessarily limited to size increase but can also include conspicuous changes in shape, as in several siphonostomatoid copepods (Kabata 1979) that are similarly characterized by a poorly sclerotized exoskeleton (Ivanenko et al. 2001).

On the opposite side of a likely continuum of relationships between molting and growth are the frequent examples where molts are not accompanied by an increase in size and may even be marked by a slight decrease. Decapod larvae may enter a sequence of molts (occasionally more than 30) with little or no growth and little or no morphological change. Following this sequence of 'mark-time molts' (Gore 1985), the larva may complete its development. In collembolans, very numerous AAMs are often observed (Krool and Bauer 1987), frequently unaccompanied by changes in size. When larvae of the dermestid beetle *Trogoderma glabrum* are starved, they continue to molt, though at a much reduced frequency, and such individuals actually become smaller with each molt (Beck 1971). In the case of many insects, there appears to be less need for a specific molting stimulus as the time since last molt increases.

Molts may also occur between two nonfeeding or even immobile stages, as in the case of the hypermetabolous blister beetles or Meloidae (see below) or the thrips (Thysanoptera). The latter are quite puzzling because their postembryonic development consists of only two active nymphal instars, followed by an immobile prepupa and by one (Terebrantia) or two (Tubulifera) equally immobile pupal instars. External morphological differences between the prepupa and the (first or only) pupa are evident, although not dramatic, but the two pupal instars of the tubuliferans are really very similar, a circumstance that adds to our difficulty in interpreting the origin and significance of these molts between immobile instars.

A second set of remarks will take into account the relationship between the cuticular aspects of arthropod development, as obviously punctuated by molts, and the (internal) morphogenetic processes whose time course may be limited to intervals shorter than a molting cycle or may span more than one instar without being obviously affected by the molting event. There is no need to summarize the complex and often dramatic processes occurring during the pupal stage of holometabolous insects but it may be worth mentioning that very extensive morphological changes occur also in the second half of the fourth (last) nymphal stage in the aleyrodid hemipterans ('whiteflies').

Different species of lithobiomorph centipedes have been described to exhibit different schedules of 'segment addition' across the five stages of the anamorphic phase of their postembryonic development (Andersson 1979). We

recognize newly added posterior segments as new articulated terga and sterna or additional pairs of more or less fully developed limbs appear at the rear end. However, the timing of the process of segmentation at the level of internal organs, for example the ventral nerve cord, is only loosely correlated with segmentation at the level of the external integument. In *Lithobius forficatus*, neuromeres are differentiating continuously during the intermolt (Fig. 2), and there is sizeable individual variation in the degree of segmentation of the terminal part of the nerve cord completed at the time of a specified molt (M. Chiodin, G. Deflorian, and G. Fusco, unpublished data). Staging the development of the central nervous system is thus best described in terms of its internal descriptors (segmentation included) rather than with reference to the molting events. The same choice in favor of a noncuticular periodization will be certainly of value for the development of many other organ systems. A study on the evolutionary change of lithobiomorph anamorphosis cannot be based exclusively on the idiosyncratic grid provided by the molt cycle.

Homology of postembryonic stages and instars

The molt during which an insect larva turns into a pupa and the next molt to an adult are in some way different from the previous larva-to-larva molts. Corresponding differences are found in other arthropods, whenever molts separating conspicuously different stages are contrasted with molts

separating instars with similar organization. This point is not trivial, and its significance is not so obvious.

In contrasting between two or more types of molts, one should be able to distinguish between two components: (1) differences, if any, in the molting process proper; and (2) differences in other aspects of the animal's development, which are only visible at (after) the molt but have little or nothing to do with the molting process itself.

However, important physiological changes along the postembryonic schedule are not necessarily expressed by conspicuous qualitative differences between stages or instars. Intraspecific variation in the number of postembryonic instars may reveal those critical points. For example, in those females of the grasshopper *Chorthippus brunneus* that have one more nymphal instar than the usual four, the extra instar occurs between the normal instars II and III, as revealed by its intermediate morphology (Hassall and Grayson 1987). The molt between nymphs II and III marks a specific transition corresponding to a characteristic reorientation in the insertion of the developing wings (Uvarov 1966). A similar intercalation of an extra instar in a precise position along the postembryonic developmental schedule has been recorded in other species (*Chorthippus mollis*, Thorens 1991; the earwig *Labidura riparia*, Caussanel 1966).

That some molting cycles are developmentally more important than others is an old observation.

The largest systematic effort to identify such molts in an arthropod's postembryonic schedule and to use them as reference points to align the sequence of stages and instars of different taxa was centered around the concept of *stase*. This term was introduced by the acarologist Grandjean (1938) to define the developmental segment between two molts, provided that these are accompanied by discontinuous changes in external characters. This definition of *stase* does not require the occurrence of major morphological changes such as between caterpillar, chrysalis, and butterfly, but it is also more strict than the generalized concept of instar as simply the interval between any two molts (see also André 1988, 1989). The number of stases is apparently fixed for any major clade of mites. This periodization of postembryonic development is probably adequate for mites, but it becomes too fine-grained and thus less interesting, in comparative terms, in other arthropod groups.

Aligning arthropod developmental schedules, by either stage or instar, is much more problematic than aligning amino acid or nucleotide sequences. This is not simply due to the widespread occurrence of evolutionary changes comparable to point mutations, insertions, and deletions, but also depends on more systemic changes that, irrespective of their morphological conspicuousness, complicate the homologization of individual instars. Let us briefly discuss two examples.

In the first case, we try to compare the postembryonic developmental schedules of *Carabus violaceus* (Coleoptera), *Musca domestica* (Diptera), and *Mesovelia furcata* (Heteroptera). These three insects all undergo four postembryonic molts, but what is actually comparable among

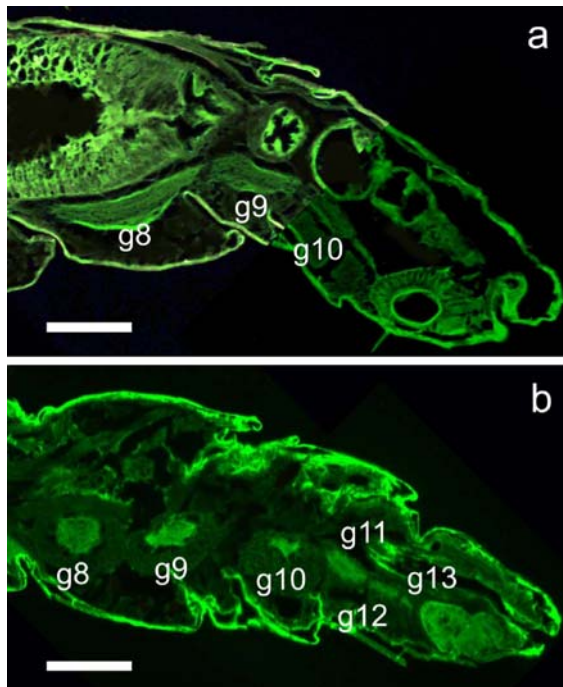


Fig. 2 Differentiation of neuromeres within one larval instar of the lithobiomorph centipede *Lithobius forficatus*. **a** One-day-old larva II; **b** 12-day-old larva II, near to molt to larva III. Mediosagittal sections of the rear of the trunk. Anterior is toward the left, dorsal is toward the top. Immunostaining with an anti-HRP antibody. Labels indicate the neuropile of the leg-bearing trunk ganglia. Bars 0.1 mm

them? That the hatching stage is the same in both endopterygote (*Carabus* and *Musca*) and exopterygote (*Mesovelgia*) insects is far from clear and even explicitly denied by some hypotheses on the origin of holometaboly (Berlese 1913; Truman and Riddiford 1999). However, let us tentatively accept that the final molt to adult, at least, is meaningfully equivalent among the three species under comparison. However, what about of the previous molts? In *Carabus* and *Musca*, there are two larva-to-larva molts followed by a larva-to-pupa molt, whereas in *Mesovelgia*, there are three nymph-to-nymph molts. Besides the molts as such, is there anything left to compare among these three insects? Similarity of molts, stages, and instars is obviously higher between *Carabus* and *Musca*. Indeed, there are good reasons to call in both cases the stage preceding the adult a pupa. However, what about of the individual larval instars? Besides the temporal order in which these instars appear, is there any specific argument in favor (or against) the homology between the first, second, and third larval instars of *Carabus* and the first, second and third larval instars of *Musca*? The significance of this question is perhaps better highlighted by trying a somewhat opposite comparison.

Intraspecific variation in the number of instars is widespread and this produces new problems of homology. Let us consider a butterfly with a variable number of larval instars, e.g., either four or five. Let us call the two alternatives a 4L and a 5L development, respectively. Is there any significance in regarding the last (fourth) larval instar of the 4L development as equivalent to the fourth larval instar of the 5L development, just counting the molts behind them? What if we regard the two last instars as equivalent, irrespective of their previous history? We argue that there is no universally valid answer to this question. In specific cases, a deletion or addition of a particular instar may perhaps be involved, but in the general case, we can expect the difference between 4L and 5L development to be simply one of partitioning the whole span of prepupal development into a different number of instars. If so, no homology would exist between individual instars, as homology only meaningfully exists between the whole larval development of a 4L and the whole larval development of its 5L counterpart (Minelli 2003).

Segmentation schedules in anamorphic arthropods

In anamorphic arthropods, molts punctuate the postembryonic addition of segmental structures, but the number of segments added per molt is not fixed.

In anamorphic centipedes, the quite regular pattern for *Scutigera* can be contrasted with the standard progression of full-formed, leg-bearing segments in *Lithobius*. Their respective schedule up to the attainment of the final number of 15 leg-bearing segments is as follows:

Scutigera $4 + 1 + 2 + 2 + 2 + 2 + 2 = 15$

Lithobius $7 + 0 + 1 + 2 + 2 + 3 = 15$

In millipedes, the patterns of segment addition during postembryonic development are very diverse, varying from the regular addition of (usually) two rings per molt to examples where the number of segments added at each molt varies considerably not only between molts but also, during the same molt, among individuals of the same population and sex (Engelhoff et al. 1993). One of the most extreme examples of intraspecific variation in the schedule of postembryonic segment addition is the platydesmid *Brachycybe* (Fig. 3).

It is generally assumed that most trilobites added one thoracic segment per molt during the so-called meraspid stage, but other patterns of segmentation have been suggested (Chatterton and Speyer 1997; Hughes 2003). It is possible that *Ceraurina typa* and *C. chondra* may have added more than one segment at each molt between two early meraspid instars. Although no case is known in which we can be confident that more than one thoracic segment was added at a single molt, there are several examples in which more than one molt apparently occurred before the addition of a new thoracic segment. For instance, in *Shumardia salopensis*, the number of meraspid instars predicted is almost twice the number of adult thoracic segments: molts that add one thoracic segment seem to almost regularly alternate with molts that do not add a segment. In *Neocobboldia chinlinica*, there is intraspecific variation in the pattern of thoracic segment addition, although all adults had possibly the same number.

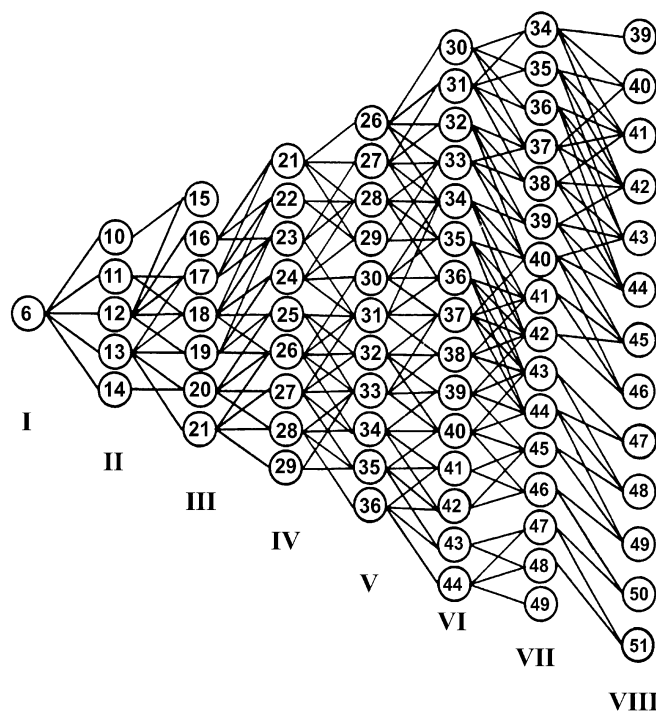


Fig. 3 Alternative segmentation schedules during the postembryonic development of the platydesmid millipede *Brachycybe nodulosa*. Numbers of pleurotergites in successive postembryonic instars (Roman numbers) are connected by observed ontogenetic trajectories (adapted from Murakami 1963)

Contrasting developmental schedules between parts of one arthropod

In isopod crustaceans, ecdysis is a two-step process. The anterior half of the exuvia is released independently of the posterior half, the two events being separated by hours and sometimes by days. This difference between the anterior and posterior half of the body is exaggerated in the parasitic bopyrid isopod *Hemioniscus balani* (Goudeau 1977). This is a protandric hermaphrodite in which the transition from the male to the female condition only affects the posterior half of the body. The anterior half is fixed to the state it achieved during the transient phase of maturity as a male and does not undergo any further molt. The posterior continues to molt, usually five more times, and eventually attains the relatively enormous size and very irregular shape of the female. In this case, molts do not provide a common periodization of the whole animal, which is comprised of two parts of different molt-based age.

Heterochrony—what else?

The basic message behind all matters presented thus far in this paper is that the synchronization (not to say a causal link) between molting and all other kinds of developmental events during an arthropod's ontogeny (hatching and attainment of sexual maturity included) is labile. This can be quickly summarized in terms of heterochrony, something that should hardly be surprising. What is surprising, in our view, is that the conventional periodization of arthropod development has maintained such a firm foothold in the literature even beyond of the level of mere description. Description, however, is one thing, and analysis—be it functional or historical—is another, and requires a sensible choice of structural and process units on which to focus. Minelli and Fusco (2004) and Fusco (2005) argued that the segment of traditional morphology is the product of a typological generalization that seriously narrows our ability to ask meaningful questions about the evolution of development.

This applies also to the vast majority of papers on heterochrony, which deal with growth heterochrony, i.e., with differences between two animals in the time a given developmental process starts or ends, or in the rate at which it proceeds. The main problem with this approach is right one of periodization, i.e., how to establish a temporal frame of reference within which we place the starting point, or the end, of the developmental process we are studying (Smith 2001; Minelli et al. 2006). Another problem is the exclusive focus on just one aspect of development. These problems with growth heterochrony are largely avoidable by adopting the perspective of sequence heterochrony (Smith 2001), where the focus is directly on the relative timing shifts of many developmental events or processes simultaneously.

We argue in this study that a more articulated, factorial approach to the periodization of arthropod development must replace the current framework based on the molting

cycle between hatching and the attainment of sexual maturity.

In the next section, we will show how our perspective on arthropod development may help in understanding some important features of holo- and hypermetaboly in insects.

Terminal addition and the origin of holometaboly

According to our current understanding of insect phylogeny, Holometabola is a monophyletic group whose sister clade is the Paraneoptera (Psocoptera, Phthiraptera, Thysanoptera, and Hemiptera). The morphological and developmental gap between the most plesiomorphic living representatives of the two clades (Holometabola and Paraneoptera) is large, and a direct comparison between them offers very little help to formulate hypotheses about the origin of holometaboly. On this subject, Heming (2003) has recently summarized as many as 11 different hypotheses, and the question is still very open. We will not attempt to offer in this study our 12th solution; instead, we will focus on an aspect that has been largely overlooked until now, probably because of the rigid, typological approach hitherto adopted in framing the periodization of arthropod development.

Let us begin by highlighting two facts related to the origin of holometaboly:

1. The adult stage is not significantly affected by this major evolutionary transition. Indeed, in most keys to adults of the insect orders (cf. Lawrence et al. 1991), hetero- and holometabolous orders do not key out separately from one of the initial couplets but are largely intermingled.
2. The earliest of the two key features of holometabolous development (the larva and the pupa) is first expressed right at the beginning of an insect's active life.

Why is the adult usually so conservative, despite sometimes enormous differences in preceding ontogenetic stages? We believe that the evolutionary stability of the insect adult is strongly conserved by two circumstances. First, there is no AAM. Second, the insect is confined within a usually rigid cuticle. Therefore, during the whole span of its mature life, the gross morphology (a largely cuticular affair) of the adult insect is broadly unchanged. Evolutionary changes utilizing a terminal addition of developmental stages are thus very unlikely, at variance with what would happen to animals whose reproductive life extends over a span of more or less extensive morphological changes. In arthropods, this will be possible when the cuticle is so soft as to allow extensive shape changes during the only adult instar, as among siphonostomatoid copepods. It is also possible that the existence of AAMs has favored terminal addition in lineages such as decapod crustaceans, where larvae are often more conservative than the corresponding adults. The case of the isopod *Hemioniscus balani* cited above may provide an example of the evolutionary potential of the joint occurrence in the same animal (at least in the posterior

part of it) of repeated AAMs together with a very extensible cuticle.

The limited evolvability of the insect adult, by terminal addition at least, brings with it the consequence that any nontrivial deviation of previous ontogenetic stages from the corresponding ancestral condition must be compensated for in a later preimaginal stage. The relationship between increased specialization of immatures and type of postembryonic development is graphically illustrated by Heming (2003) in his Fig. 9.2.

Why does the developmental deviation leading to the occurrence of a holometabolous larva occur at the beginning of the postembryonic life rather than at a later stage? Before providing an explanation, it may be fair to remark that in the Paraneoptera (the sister group to Holometabola) evolutionary novelties may result in a deviation from the original developmental path starting with nymphal instars later than the first: usually, indeed, the second, which is more or less profoundly modified in three out of the four main lineages of sternorrhynch Hemiptera. If we take as a reference point the generally smooth developmental schedule of aphids, we find a radical change from a mobile nymph I to an immobile nymph II in psyllids (jumping lice), aleyrodids (whiteflies), and coccids (scale insects). In these taxa, however, it is easy to imagine a very strong pressure to retain an early exploratory instar, as required for midrange dispersion on the food plant before the animal settles down to a long (psyllids, aleyrodids, and male coccids) or even definitive (many female coccids) sessile life.

That evolutionary novelties in larvae of holometabolans begin with the initiation of postembryonic life becomes possibly easier to understand if we follow the views of Berlese (1913) and Truman and Riddiford (1999). In their perspective (largely accepted by Grimaldi and Engel 2005; but see also Heming 2003 for a different view), the larva would be nothing else than a free-living late embryo or prelarva if compared to developmental stages in hemimetabolous insects. If so, this would be a good example of that decoupling of hatching, molting, and changing from one stage to another that we are stressing as central to the deployment of an arthropod's life cycle, any conventional periodization notwithstanding.

Hypermetaboly and the number of postembryonic molts

The occurrence of further evolutionary novelties at the beginning of postembryonic life is also a feature of hypermetabolous insects. In several families of parasitic hymenopterans, the first instar larva is very different from the subsequent larval instars, which are conservative in respect to the generalized larval type of apocritans. The occurrence of any new type of larvae such as planidia, cyclopoid larvae, mymariform larvae, teleaform larvae, etc. does not seem to necessitate additional adjustment to the life cycle, not even in the total number of larval molts. Things are less clear in hypermetamorphic beetles such as

Rhipiphoridae and Meloidae. In these insects, both the first larva (the very mobile triungulin) and later instars (which are much less mobile) deviate more or less extensively from those of related groups in the superfamily Tenebrionoidea. It must be considered, however, that two diverging selective pressures have evidently shaped larval morphology in Rhipiphoridae and Meloidae. On the one hand, first instar larvae are more active and mobile here than in related families; on the other hand, subsequent larval instars are less mobile than in related families because of the adoption of parasitic habits. What is much less clear is the presence (in Meloidae but not in Rhipiphoridae) of an additional resting stage (the coarctate or pseudopupa), separated from the true pupa by one more active (but nonfeeding) larval instar.

In these two beetle families, the total number of preimaginal instars is high. There are either five or six larval instars, and a pupa in Rhipiphoridae, whereas in Meloidae, the mobile triungulin is usually followed by four active larval instars, followed in turn by a coarctate, a last larva, and a pupa. These numbers are high compared to the three larval instars that probably represent the plesiomorphic condition for beetles, but an increase above this number is common in other nonhypermetabolous families of Tenebrionoidea, where up to 14 larval instars have been recorded (Klausnitzer 2003). Therefore, pending an accurate reconstruction of phylogenetic relationships within the superfamily, we cannot be sure that meloids and rhipiphorids have many preimaginal instars as a result of their hypermetaboly.

Another difficult question is whether the origin of holometaboly was accompanied by an increase in the number of preimaginal instars. Larval instars are quite numerous in the basal orders: there are 10–12 (–15) larval instars in snakeflies (Raphidioptera), 10–12 in alderflies and dobsonflies (Megaloptera), and three to 10 in antlions and lacewings (Neuroptera s.str.). These numbers (except for the lower end of the range in Neuroptera) are higher than the numbers (three to six) found in modern Paraneoptera, six instars being typical of the most plesiotypic group, i.e., the winged forms of Psocoptera. However, current understanding of phylogenetic relationships among holometabolous insects (see Whiting 2004 for an overview) does not compel us to regard the developmental schedule of the neuropteran orders as basal for insects with complete metamorphosis. The question thus remains open.

It is easier to discuss changes in the number of nymphal instars in the Sternorrhyncha. Here, the plesiotypic number of nymphal instars is four (reduced to three in some wingless aphids). With the evolution of a transition from mobile nymph I to sessile nymph II, psyllids have added an extra nymphal instar, but aleyrodids retain four, despite the conspicuous metamorphosis instar IV undergoes. The same is true for male coccids, while female coccids have a truncated development with a fewer number of instars (two or three). Similar to hypermetabolous parasitic hymenopterans, Sternorrhyncha thus do not support the hypothesis

of a link between complexity of postembryonic development and increase (or, indeed, variation) in the number of postembryonic molts.

Conclusions

Many more examples might be added to those discussed in this paper to demonstrate how tenuous is the association between the molting cycle and other important aspects of arthropod development, from late embryo to adult. Of these developmental processes other than molts, some may also be cyclical, but not necessarily linked to molts; others are basically continuous, while still others may occur but once in a life cycle.

We argue in this study that a flexible and critically selected periodization of development is necessary for a deeper understanding of the evolution arthropod life history. One may question whether it is really indispensable to introduce this further level of caution to our comparative analyses. We think it is, both in phylogenetic reconstruction and in the study of evolutionary patterns.

In the same way as complex morphological features like segmentation, body cavities, etc. (cf. Nielsen 2001) are entered with increasing care (not to say suspicion or skepticism) in data matrices to be used in phylogenetic analyses, great attention should be paid in using and coding developmental stages, or stage-related characters. Developmental data based on the standard periodization are currently used in phylogenetic analysis (e.g., Edgecombe and Giribet 2004), but we think these entries hardly contribute to increase the phylogenetic signal of a data matrix.

If we do not bring into the picture the whole complexity of the relative timing of different developmental processes, we do not make a 'reasonable operational simplification'. Rather, we chose to overlook what we know about mechanisms of evolutionary change, and may even introduce a bias in our investigation of evolutionary processes. Because the developmental basis of a temporal module (stage or instar) is interconnected and interdependent with the development of other modules, to trace the evolution of a module could be meaningless, as its identity can be lost (or remolded) continuously with time. Looking for equivalence between stages could thus be meaningless. More subtly, biases may rise in evaluating hypotheses of evolutionary change for developmental traits, which may be (unconsciously) confined to a limited set of alternatives (e.g., Konopová and Zrzavý 2005 vs Truman and Riddiford 1999). Instars are not slots of development that can only fuse, split, and shift forward or backward with respect to other developmental events. Development combines growth, morphogenesis, differentiation, hormonal control, gene expression, etc., and all these different components can evolve with a variable degree of interdependence.

Evolution of robust causal links among different developmental processes should only be expected so far as selection favors their linkage. This perspective mirrors, in the temporal dimension, our view of segmentation (Minelli and Fusco 2004). Different serial features arranged along the main body axis may become integrated to form morphologically identifiable segments. However, this is only a secondary and not necessary consequence of the fact that spatially or temporally periodic features can provide a prepattern for other spatial and temporal features, resulting in something we may erroneously read as a single periodic pattern.

Paying due attention to the time course of different aspects of development without roughly conflating all of them into the usual periodization of stages and instars punctuated by molts offers a good chance for asking interesting questions on the evolution of arthropod development, as shown in our brief discussion about holometaboly and hypermetaboly.

Scholtz (2004) has recently suggested that individual stages along an arthropod's development, far from being elements in a chain of causal necessity, are free to evolve independently from one another. While agreeing with his criticism of developmental determinism, we suggest that the individuality of developmental stages is not intrinsic to them but simply results from the unique composition, at a given time along an arthropod's ontogeny, of specified phases, or conditions, of several developmental processes, each of which may have its own plasticity and evolvability. Different stages, in a sense, have been added, lost, and recreated during some 600 million years of arthropod evolution. A nauplius exists in penaeid decapods but probably does not derive from the same ancestral larva as the nauplius of maxillopodans (Scholtz 2000). Most millipedes, mites, and insects hatch as a six-legged larva, but it is quite unlikely that a six-legged larva was present in the most recent common ancestor of any two of these lineages.

Determining the homology of developmental stages is difficult, but being content with an all-or-nothing assessment of homology, based on the global comparison of stage X of arthropod A with stage Y of arthropod B, will only blur our views of the admittedly complex developmental schedules which, in the end, should nevertheless be accessible to our analytical tools.

We cannot provide a universal recipe to address these questions. We offer a short list of cautionary suggestions. Descriptions must be question dependent. At least, a critical analysis must precede any attempt to recycle available descriptions to address new questions. In comparing ontogenetic schedules, we should carefully select, case by case, those features that best suit the nature of the developmental aspect(s) we choose to investigate.

Acknowledgements Special thanks are due to Jean Deutsch for inviting AM to the Paris meeting on "Development and Phylogeny of Arthropods" (23–24 September 2005), thus stimulating our thoughts on the topic on the present contribution, and to Marta Chiodin for sharing with us her results on *Lithobius* neural development. Authors are also very grateful to Frank Ferrari, Bruce Heming, H. Frederick Nijhout, Peter Weygoldt, and two anonymous referees for their very useful comments on a draft of this paper. Ronald Jenner and Ariel Chipman kindly read our text at the Paris meeting, while the senior author was unable to attend for last-minute health problems. This research was sponsored by the Italian MIUR and the University of Padova.

References

- Andersson G (1979) On the use of larval characters in the classification of lithobiomorph centipedes (Chilopoda Lithobiomorpha). In: Camatini M (ed) Myriapod biology. Academic, London, pp 73–81
- André HM (1988) Age-dependent evolution: from theory to practice. In: Humphries CJ (ed) Ontogeny and systematics. Columbia University Press, New York, pp 137–187
- André HM (1989) The concept of stase. In: André HM, Lions J-C (eds) L'ontogenèse et le concept de stase chez les arthropodes. AGAR Publishers, Warre, pp 3–16
- Bain BA (2003) Larval types and a summary of postembryonic development within the pycnogonids. Invertebr Reprod Dev 43:193–222
- Beck SD (1971) Growth and retrogression in larvae of *Trogoderma glabrum* (Coleoptera: Dermestidae). 1. Characteristics under feeding and starvation conditions. Ann Entomol Soc Am 64:149–155
- Berlese A (1913) Intorno alle metamorfosi degli insetti. Redia 9:121–136
- Budd GE (2001) Why are arthropods segmented? Evol Dev 3: 332–342
- Caussanel C (1966) Étude du développement larvaire de *Labidura riparia* (Derm. Labiduridae). Ann Soc Entomol Fr NS 2: 469–498
- Chatterton BDE, Speyer SE (1997) Ontogeny. In: Kaesler RL (ed) Treatise on invertebrate paleontology, part O, Arthropoda 1, Trilobita, revised. Volume 1: introduction, Order Agnostida, Order Redlichiida. Geol Soc Amer. Boulder, CO; University of Kansas, Lawrence, KS, pp 173–247
- Edgecombe GD, Giribet G (2004) Adding mitochondrial sequence data (16S rRNA and cytochrome *c* oxidase subunit I) to the phylogeny of centipedes (Myriapoda: Chilopoda): an analysis of morphology and four molecular loci. J Zool Syst Evol Res 42:89–134
- Engel H, Dohle W, Blower JG (1993) Anamorphosis in millipedes (Diplopoda). The present state of knowledge and phylogenetic considerations. Zool J Linn Soc 109:103–234
- Ferrari FD, Grygier MJ (2003) Comparative morphology among trunk limbs of *Caenestheriella gifuensis* and of *Leptestheria kawachiensis* (Crustacea: Branchiopoda: Spinicaudata). Zool J Linn Soc 139:547–564
- Fusco G (2005) Trunk segment numbers and sequential segmentation in myriapods. Evol Dev 7:608–617
- Gore RH (1985) Molting and growth in decapod larvae. In: Wenner AM (ed) Larval growth (Crustacean Issues 2). Balkema, Rotterdam Boston, pp 1–65
- Goudeau M (1977) Contribution à la biologie d'un crustacé parasite: *Hemioniscus balani* Buchholz, isopode épicaride. Nutrition, mues et croissance de la femelle et des embryons. Cah Biol Mar 18:201–242
- Grandjean F (1938) Sur l'ontogénie des acariens. CR Acad Sci Paris 206D:146–150
- Grimaldi D, Engel MS (2005) Evolution of the insects. Cambridge University Press, Cambridge, New York
- Hassall M, Grayson FWL (1987) The occurrence of an additional instar in the development of *Chorthippus brunneus* (Orthoptera: Gomphocerinae). J Nat Hist 21:329–337
- Heming BS (2003) Insect development and evolution. Comstock Publ Associates, Ithaca London
- Hobbs HH Jr (1981) The crayfishes of Georgia. Smithsonian Contrib Zool 318:1–549
- Hughes NC (2003) Trilobite body patterning and the evolution of arthropod tagmosis. Bioessays 25:386–395
- Ivanenko VN, Ferrari FD, Smurov AV (2001) Nauplii and copepodids of *Scottomyzongibberum* (Copepoda: Siphonostomatoida: Scottomyzontidae, new family), a symbiont of *Asterias rubens* (Asteroidea). Proc Biol Soc Wash 114:237–261
- Kabata Z (1979) Parasitic copepoda of British fishes. Ray Society, London
- Klausnitzer B (2003) Unterordnung Polyphaga. In: Dathe HH (ed) 5. Teil: Insecta. In: Gruner H-E (ed) Band I. Wirbellose Tiere. In: Kaestner A (founder) Lehrbuch der Speziellen Zoologie. Gustav Fischer, Jena Stuttgart New York, pp 452–526
- Konopová B, Zrzavý J (2005) Ultrastructure, development, and homology of insect embryonic cuticles. J Morphol 264: 339–362
- Krool S, Bauer T (1987) Reproduction, development and pheromone secretion in *Heteromurus nitidus* Templeton, 1835 (Collembola, Entomobryidae). Rec Ecol Biol Sol 24:187–195
- Langton PH, Cranston PS, Armitage P (1988) The parthenogenetic midge of water supply systems, *Paratanytarsus grimmii* (Schneider) (Diptera: Chironomidae). Bull Entomol Res 78:317–328
- Lawrence JF, Nielsen ES, Mackerras IM (1991) Skeletal anatomy and key to orders. In: CSIRO (ed) Insects of Australia. I. Melbourne University Press, Carlton, pp 3–32
- Michalik P, Uhl G (2005) The male genital system of the cellar spider *Pholcus phalangoides* (Fuesslin, 1775) (Pholcidae, Araneae): development of spermatozoa and seminal secretion. Front Zool 2:12
- Minelli A (1996) Segments, body regions and the control of development through time. Mem Calif Acad Sci 20:55–61
- Minelli A (2003) The development of animal form. Cambridge University Press, Cambridge
- Minelli A (2004) Bits and pieces. Science 306:1693–1694
- Minelli A, Fusco G (2004) Evo-devo perspectives on segmentation: model organisms and beyond. Trends Ecol Evol 19:423–429
- Minelli A, Negrisolo E, Fusco G (2006) Reconstructing animal phylogeny in the light of evolutionary developmental biology. In: Hodkinson TR, Parnell JAN, Waldren S (eds) Systematics of species reach taxa: building and using the tree of life. CRC Press, Boca Raton, FL
- Moritz M (1993) Unterstamm Arachnata. In: Gruner EE, Moritz M, Dunger W (eds) 4. Teil: Arthropoda (ohne Insecta). In: Gruner H-E (ed) Band I. Wirbellose Tiere. In: Kaestner A (founder) Lehrbuch der Speziellen Zoologie. Gustav Fischer, Jena Stuttgart New York, pp 64–442
- Murakami Y (1963) Postembryonic development of the common Myriapoda of Japan. XIII. Life history of *Bazillozonium nodulosum* Verhoeff (Colobognatha, Platydesmidae). 3. Zool Mag Tokyo 71:245–249
- Nigon V (1965) Développement et reproduction des Nématodes. In: Grassé PP (ed) Traité de Zoologie, Tome IV, fascicule II. Masson, Paris, pp 218–386
- Nielsen C (2001) Animal evolution: interrelationships of the living phyla, 2nd edn. Oxford University Press, Oxford
- Nijhout F (1994) Insect hormones. Princeton University Press, Princeton, NJ
- Sahli F (1990) On post-adult moults in Julida (Myriapoda, Diplopoda). Why do periodomorphosis and intercalaries occur in males? In: Minelli A (ed) Proceedings of the 7th International Congress of Myriapodology. Brill, Leiden New York København Köln, pp 135–156
- Scholtz G (2000) Evolution of the nauplius stage in malacostracan crustaceans. J Zool Syst Evol Res 38:175–187

- Scholtz G (2004) Baupläne versus ground patterns, phyla versus monophyla: aspects of patterns and processes in evolutionary developmental biology. In: Scholtz G (ed) *Evolutionary developmental biology of Crustacea* (Crustacean Issues 15). Balkema, Lisse, pp 3–16
- Sehnal F (1985) Growth and life cycles. In: Kerkut GA, Gilbert LI (eds) *Comprehensive insect physiology biochemistry and pharmacology*, 2. Postembryonic development. Pergamon, New York, pp 1–86
- Smith KK (2001) Heterochrony revisited: the evolution of developmental sequences. *Biol J Linn Soc* 73:169–186
- Thorens P (1991) Développement et morphologie comparés de *Chorthippus mollis* (Charp.) (Orthoptera, Acrididae). *Mitt Schweiz Entomol Ges* 64:9–25
- Truman JW, Riddiford LM (1999) The origins of insect metamorphosis. *Nature* 401:447–452
- Uvarov B (1966) Grasshoppers and locusts. A handbook of general acridology, vol 1. Anatomy, physiology, development, phase polymorphism, introduction to taxonomy. Cambridge University Press, Cambridge, UK
- Whiting MF (2004) Phylogeny of the holometabolous insects: the most successful group of terrestrial organisms. In: Cracraft J, Donoghue MJ (eds) *Assembling the tree of life*. Oxford University Press, New York, pp 345–361