Macroevolution of Animal Body plans
Jenner, R.A.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 4

Evolution of Animal Body Plans: the Role of Metazoan Phylogeny at the Interface between Pattern and Process

Ronald A. Jenner

Published in Evolution & Development 2: 208-221 (2000)
Evolution of animal body plans: the role of metazoan phylogeny at the interface between pattern and process

Ronald A. Jenner
Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O. Box 94766, 1090 GT Amsterdam, The Netherlands
Correspondence (email: jenner@bio.uva.nl)

SUMMARY Comprehensive integrative studies are the hallmark of evolutionary developmental biology. A properly defined phylogenetic framework takes a central place in such analyses as the meeting ground for observation and inference. Molecular phylogenies take this place in many current studies on animal body plan evolution. In particular, 18S rRNA/DNA sequence analyses have yielded a new view of animal evolution that is often contrasted with a presumed traditional or classical view. First, I expose this traditional view to be a simplified historical abstraction that became textbook dogma. Second, I discuss how two recent important studies of animal body plan evolution, examining the evolution of the platyhelminth body plan and the evolutionary significance of indirect development and set-aside cells, have actively incorporated two problematic aspects of the newly emerging molecular view of animal evolution: incomplete and unresolved phylogenies.

EVOLUTIONARY DEVELOPMENTAL BIOLOGY AND THE CENTRALITY OF PHYLOGENETIC INFORMATION

George Gaylord Simpson (1951, p. 51) wrote “Over and over again in the study of the history of life it appears that what can happen does happen.” Particularly studies of animal development and phylogeny have recently played key roles in elucidating the potentialities and actualities of the evolutionary history of the animal kingdom, and it is in the field of evolutionary developmental biology (popularly called “evo-devo”) where such studies of development and phylogeny meet. Evo-devo seeks to explain the evolution of morphology and development and the underlying developmental and genetic mechanisms by comparing different organisms. A properly defined phylogeny is the only meaningful framework for comparison if the goal is the reconstruction of the nature and direction of evolutionary change, and the discrimination between convergent similarity and homology (Raft 1996; Arthur 1997; Hall 1999). Consequently, it is instructive to consider in detail the basis of some of the recent advances in our understanding of the evolution of animal body plans. The use of 18S rRNA/DNA phylogenies is rapidly gaining prominence as the basis for building evolutionary scenarios. At present there is no generally accepted morphology-based phylogeny of the Metazoa to compete with it, even with the widespread adoption of cladistic methods (Jenner and Schram 1999). Often, a traditional textbook phylogeny based on morphology is contrasted with the emerging view from molecular systematics.

In this paper I address the following issues from a phylogenetic perspective. First, I consider the traditional textbook phylogeny and explore its historical roots. Second, I critically discuss the newly emerging view of animal evolution based on 18S rRNA/DNA sequence data, which is sketched in the latest generation of review papers as a basis for advancing scenarios of body plan evolution. The new view of animal evolution is often presented in phylogenies characterized by two striking features: lack of resolution and incompleteness. These data limitations have subsequently been actively incorporated as essential ingredients into various important new hypotheses of animal body plan evolution. Third, I will re-evaluate two of these hypotheses that are beginning to be considered major advances in our understanding of animal evolution: platyhelminthes as derived coelomates, and the evolutionary significance of set-aside cells and their role in the Cambrian explosion.

METAZOAN MORPHOLOGY, LIBBIE HYMAN, AND THE "TRADITIONAL" VIEW OF ANIMAL EVOLUTION

Since Ernst Haeckel codified our profession by coining its current name and by establishing a coordinated research program for the elucidation of evolutionary relationships be-
between organisms, the study of higher level animal phylogeny has yielded an expansive literature but relatively little detailed consensus. It is therefore not surprising often to find merely the shortest of possible summaries of morphological phylogenetics in current papers on the subject. Winnepennickx et al. (1998a, p. 888) epitomize this approach in the first sentence of their paper: “Despite several decades of morphological and anatomic research, numerous aspects of metazoan relationships have remained uncertain.” Their discussion then turns to the revisionary views from molecular systematics. Aguinaldo and Lake (1998), Holland (1998), Balavoine (1998), and Erwin (1999) adopt a similar approach by invariably presenting the Anglo-Saxon “traditional textbook view,” the acelomate-pseudocelomate-coelomate series, as an icon for metazoan phylogeny based on morphology. Balavoine (1998), Balavoine and Adoutte (1998), Adoutte et al. (1999), and Valentine et al. (1999) in fact claim that such a phylogenetic series represents “... a glorious saga of progressive increase in complexity ...” (Adoutte et al. 1999, p. 105), arising from “... the intellectual bias for increasing complexity in evolution ...” (Balavoine and Adoutte 1998, p. 397). It would be a serious problem indeed if our efforts to reconstruct animal phylogeny would produce nothing more than manifestations of our intellectual disposition for increasing complexity in evolution. However, I will show that these assessments are distorted and misleading.

In point of fact, there exists no such thing as “the traditional textbook phylogeny.” A diversity of different schemes can be found, influenced by factors such as nationality, depth of historical treatment, and a whole range of personal perspectives of the textbook authors (e.g., Willmer 1990; Willmer and Holland 1991). The most frequently mentioned source in Anglo-Saxon works is Hyman’s influential series The Invertebrates (1940–1967) (e.g., Field et al. 1988; Raff et al. 1989; Schram 1991; Willmer and Holland 1991; Ruppert and Barnes 1994; Balavoine 1998; Garey and Schmidt-Rhaesa 1998; Adoutte et al. 1999). Hyman is often cited in support of the traditional textbook phylogeny (e.g., Barth and Brosnhears 1982; Willmer 1990; Schram 1991; Willmer and Holland 1991; Ruppert and Barnes 1994; Willmer 1995; Garey and Schmidt-Rhaesa 1998; Adoutte et al. 1999), which can be summarized as follows: after the divergence of the Radiata (Cnidaria and Ctenophora), the bilaterians are arranged in the acelomate-pseudocelomate-coelomate (protostomes and deuterostomes) series, which reflects successive branchings of these three clades. How well is this phylogenetic scheme corroborated in Hyman’s work?

It is not easy to represent accurately Hyman’s opinion on metazoan relationships for several reasons. First, she regarded classification and phylogeny as two logically distinct devices to represent the “relationships” between organisms (1940, chapter II). Consequently, when Hyman described “affinities” it may be difficult to determine whether this implies structural resemblance or evolutionary propinquity. Second, Hyman published The Invertebrates over a time span of 27 years, creating enough room for change of opinion. She considered views on animal relationships as ephemeral products, necessarily changing with the accretion of new data (Hyman 1940, p. 27; 1959, p. 697). Nevertheless, her distilled views have been widely accepted as “traditional” consensus by many biologists, and Hyman’s viewpoint has been considered as so well established and typical that it is no longer necessary to refer to any particular of her works (see Barth and Brosnhears 1982; Ruppert and Barnes 1994), and that her view can stand as an epitome for the Anglo-Saxon view of animal relationships (Willmer and Holland 1991). I will provide an alternative perspective on Hyman’s views on animal phylogeny, which I regard as better supported by her own statements than the traditional textbook tree for which her support is commonly claimed.

Hyman indeed divided the Metazoa into diploblasts and triploblasts, although her difficulties with the germ layer theory led her to regard the distinction between Radiata and Bilateria as more meaningful (Hyman 1940). Within the Bilateria, Hyman did indeed distinguish between acelomates, pseudocelomates, and coelomates stating: “Such a division stands firmly on a realistic anatomic basis and eschews all theoretical vaporizings . . .” (Hyman 1940, p. 35). She stated that “These groupings [acelomates, pseudocelomates, coelomates] do not, however, entirely correspond to taxonomic relationships” (Hyman 1951a, p. 23). Hyman interpreted these groups as not clades or phylogenetic groups, but as organizational grades (Hyman 1940, chapter II). Her consideration of priapulids strikingly illustrates this distinction: priapulids are classified as schizocoel coelomates based on structural grade (Hyman 1940, p. 34) but phylogenetically united with the pseudocelomate aschelminths (Hyman 1951b, pp. 54, 56, 196, 197). Hyman stated that schizocoel coelomates are related to the acelomates and pseudocelomates, together comprising the Protostomia (Hyman 1940, p. 36; 1951a, p. 5). She envisioned the immediate splitting of the Bilateria from the last, acelomate, common ancestor into two lineages (Hyman 1951a, p. 4): one leading to the Deuterostomia (enterocoel coelomates) and one leading to the Protostomia, which includes the extant acelomates, pseudocelomates, and coelomate protostomes. She considered the Protostomia as “... evidently related to each other” (Hyman 1951a, p. 5). The only metazoan phylogeny from The Invertebrates (Hyman 1940, Fig. 5) reflects this grouping.

Her provisional acceptance of this diphylectic theory of bilaterian relationships therefore contradicts the monophyletic division of the Metazoa, comprising both coelomate protostomes and deuterostomes, which is at the heart of the traditional textbook phylogeny for which Hyman’s support is often claimed (Barth and Brosnhears 1982; Field et al. 1988; Raff et al. 1989; Willmer 1990; Schram 1991; Willmer and Holland 1991; Ruppert and Barnes 1994; Willmer 1995; Garey and Schmidt-Rhaesa 1998;
Deducing support for Eucelomata as a phylogenetic unit from Hyman's discussions of body cavities is based on a confusion of grades and clades. As mentioned above, Hyman regarded the division of bilaterians into acoelomates, pseudocelomates, and eucelomates not as phylogenetic units, but as organizational grades. Only by misinterpreting these grades as clades, by denying the argued phylogenetic unity of the Protostomia, and by judiciously connecting the branches in ascending order from acoelomate to eucelomate can one defend Hyman's discussion of body cavities to support the traditional textbook tree. This reasoning may explain the appeal to Hyman in support of the traditional phylogeny in various papers (e.g., Schram 1991; Willmer 1995; Carranza et al. 1997; Garey and Schmidt-Rhaesa 1998; Adoutte et al. 1999).

A second potential source of support for the traditional interpretation of Hyman may be her discussion of the lophophorates (which she considered protostomes) as intermediates between celomate protostomes and deuterostomes. The most convincing statement is that "... deuterostomes show a further development of characters beginning in an unclear way in lophophorates, and thus branch off from protostomes by way of the latter" (1959, p. 605), although Hyman does not supply any information as to how this link may be conceived. This may be the only real support from Hyman for the traditional textbook phylogeny. Strikingly, in the same volume Hyman argued that chaetognaths may have diverged very early from the common ancestor of the Bilateria at the time that the diploelura ancestor of the deuterostomes became differentiated (1959, p. 66). This again points to the independence of the protostome and deuterostome lineages since their last common acoelomate ancestor, and is in obvious contrast to the traditionally presented "Hymanian" phylogeny.

How well does Hyman's work support the traditional acoelomate-pseudocelomate-celomate series as a story of linear progressive increase in complexity (Balavoine and Adoutte 1998; Adoutte et al. 1999; Valentine et al. 1999)? Hyman was convinced of the branching nature of evolution, and quite in contrast to our current penchant for relying on dichotomously branching diagrams, she conceived of many groups of bilaterians as arising at the same level, thus creating polytomies (1940, p. 39, Fig. 5). She emphasized the bush-like branching of the animal evolutionary tree, arguing against a linear phylogenetic arrangement of organisms according to their structural complexity (1940, p. 39). Thus, in Hyman's exegesis we cannot trace a conscious intellectual bias to impose notions of necessary evolutionary progress in complexity on her views of animal phylogeny. When Hyman describes "higher" and "lower" groups, and specifies the order of treatment of the phyla in The Invertebrates according to "... the general order of their structural complexity" (1940, p. 38), she refers to a series of organizational grades, not a phylogenetic series (e.g., see Willmer 1990, p. 5 for an illustration of this confusion). The only potential genuine phylogenetic series according to body cavity construction in Hyman's work is the hypothesized series of ancestors leading to the protostomes (1951a, p. 17): planuloid ancestor—acoeloid ancestor (acoelomate grade)—trochozoan (pseudocelomate grade). But this is distinct from the progressive series leading to a monophyletic Eucelomata depicted in most recent papers quoting Hyman's work.

In conclusion, citing Hyman as the principal source for the "traditional textbook tree" is either based on a confusion of grades and clades or is at best a simplified abstraction of her views. Nevertheless, the Hymanian phylogeny has become dogma in subsequent works, and an icon for the Anglo-Saxon view on animal phylogeny. In contrast, I would argue that her most cogently argued opinion on animal phylogeny is outlined in the first volume of The Invertebrates (mainly chapter II) and is reinforced at various places in later volumes, and paints a different picture of animal phylogeny summarized in Fig. 5 of Hyman (1940). It emphasized the unity of the Protostomia, including acoelomates, pseudocelomates, and celomates (including lophophorates), and did not support a monophyletic Eucelomata and a linear, progressive phylogenetic series according to body cavity organization. Nevertheless, the 27 year time span between the first and the last volume of The Invertebrates obviously provided enough room for a creative thinker such as Hyman to change her views and explore different alternatives. It may be difficult to interpret Hyman's phylogenetic views precisely since the theory and practice of phylogenetics have changed so profoundly since her time. Representing Hyman's view in a cladogram (e.g., Eernisse et al. 1992; Adoutte et al. 1999) can therefore never encompass the full subtlety of her perspective because cladistics cannot easily accommodate the central position for direct ancestors such as the planuloid and acoeloid ancestors, which would be needed for an accurate representation of historical information.

Accepting the traditional textbook tree as an impoverished historical abstraction, and given the absence of a detailed consensus of animal phylogeny on the basis of morphology, many biologists have turned to molecular systematics in search of a phylogenetic framework.

**MOLECULAR PHYLOGENY AND ANIMAL EVOLUTION: THE "NEW VIEW"**

Molecular phylogenetics is currently dominated by the study of 18S rRNA/DNA sequences. This research has yielded a
consensus phylogeny, albeit of limited resolution (e.g., Aguinaldo et al. 1997; Aguinaldo and Lake 1998; Balavoine 1998; Balavoine and Adoutte 1998; Maley and Marshall 1998; McHugh 1998; Carey and Schmidt-Rhaesa 1998; Halanych 1998; Winnepenninckx et al. 1998a; Adoutte et al. 1999; Knoll and Carroll 1999). This consensus proposes three major bilaterian clades. The first split divides the deuterostomes and protostomes, with protostomes consisting of the sister clades Lophotrochozoa and Ecdysozoa. The deuterostomes are the echinoderms, hemichordates, and chordates. Lophotrochozoa comprises groups such as molluscs, annelids, platyhelminths, rotifers, phoronids, bryozoans, and brachiopods; Ecdysozoa unites molting animals such as arthropods, tardigrades, and nematodes. The Acoela may be basal bilaterians (Ruiz-Trillo et al. 1999), although data from elongation factor 1-α suggest they are lophotrochozoans (Berney et al. 1999). The relationships within the three main clades of the Bilateria are especially unresolved. Causes for the lack of resolution are discussed elsewhere (e.g., Lecointre et al. 1993; Phillipe et al. 1994; Abouheif et al. 1998; McHugh 1998; Foster and Hickey 1999). Not all analyses agree in all aspects. A few analyses yield a paraphyletic Protostomia uniting deuterostomes either with ecdysozoans or lophotrochozoans (see Ernisse 1997; Winnepenninckx et al. 1998a; Zrzavy et al. 1998 for recent examples).

The icon of the new view of animal phylogeny depicts the three bilateral clades (Lophotrochozoa, Ecdysozoa, Deuterostomia) as polytomies of very limited membership, virtually never depicting all the relevant taxa (Aguinaldo and Lake 1998; Balavoine 1998; Balavoine and Adoutte 1998; Adoutte et al. 1999; Knoll and Carroll 1999). Of course, this mode of representation should be regarded as a convenient shorthand, illustrating the uncertainty of the phylogenetic relationships in a cladogram of manageable size, whereas in older studies these incomplete trees reflect limited availability of sequence data. However, I will show that these incomplete and unresolved trees are being taken too literally as representations of our new understanding of animal evolution in a significant proportion of these papers. Strikingly, these limitations now form integral parts of recently proposed scenarios of animal body plan evolution, two of which will be discussed in detail: the evolution of the platyhelminth body plan, and the evolution of primary larvae and set-aside cells. A properly expanded molecular phylogeny including all pertinent taxa forms the basis of the following re-evaluation.

**PLATYHELMINTHS AS DERIVED COELOMATES**

Balavoine (1997, 1998) investigated the phylogenetic position of the phylum Platyhelminthes on the basis of limited 18S rRNA and *Hox* gene data. He proposed that these data favor the grouping of platyhelminths with the coelomate spri-
A variety of molecular phylogenetic studies published before Balavoine’s papers indicated that various aschelminth or pseudocoelomate phyla are likely to be closely associated with the lophotrochozoans and ecdysozoans (e.g., Winnepenninckx et al. 1995; Garey et al. 1996a, 1996b; Hanelt et al. 1996; Aguinaldo et al. 1997; Eernisse 1997). Recent studies confirm this picture (e.g., Aleshin et al. 1998; Garey and Schmidt-Rhaesa 1998; Littlewood et al. 1998, 1999; Schmidt-
We refer the reader to recent reviews by Balavoine (Balavoine 1996; Eernisse 1997) for an overview of the current status of the concept of a coelom in the Bilateria.

In the cladistic view, the presence of a coelom in the ground pattern (the set of characters primitively present in a clade) of the Bilateria is a synapomorphic character of a monophyletic clade that includes at least the Ecdysozoa and Lophotrochozoa. This view is based on the following evidence:

1. The presence of a defined body cavity, the coelom, which is lined with mesothelium.
2. The presence of a definitive mesoderm, which is the source of the coelom peritoneum.
3. The presence of a definitive segmentation, which is the basis for the delineation of mesoderm and cavity.
4. The presence of a definitive pattern of development, which is the basis for the delineation of mesoderm and cavity.

However, recent molecular and developmental studies suggest that the concept of a coelom is complex and may not be applicable to all phyla. The following arguments support this view:

1. The presence of a coelom in the ground pattern does not necessarily imply the presence of a definitive mesoderm or segmentation. In some cases, a coelom may be derived from an existing cavity or from a primitive mesodermal derivative.
2. The presence of a coelom in the ground pattern does not necessarily imply the presence of a definitive pattern of development. In some cases, a coelom may be derived from an existing pattern or from a primitive pattern of development.
3. The presence of a coelom in the ground pattern does not necessarily imply the presence of a definitive pattern of evolution. In some cases, a coelom may be derived from an existing pattern of evolution or from a primitive pattern of evolution.

In conclusion, the concept of a coelom in the ground pattern is complex and may not be applicable to all phyla. The presence of a coelom in the ground pattern does not necessarily imply the presence of a definitive mesoderm or segmentation, the presence of a definitive pattern of development, or the presence of a definitive pattern of evolution.
ously limited, especially in distantly related taxa (e.g., Davidson 1997; Scholtz et al. 1998; Holland and Holland 1999; Janies and DeSalle 1999).

In summary, Balavoine's conclusions that platyhelminths are derived coelomates that lost the coelom and segmentation cannot be upheld. His pruned and unresolved phylogeny bears at best ambiguous testimony, while a proper consideration of morphology in the context of molecular phylogeny argues against his hypothesis. Instead, multiple convergences of coelom and segmentation are indicated.

Finally, Balavoine's interesting heterochronic scenario for the origin of platyhelminths is effectively proposing a process where pattern is lacking. Balavoine (1997) stated that "... the Müller larva of polyclads is, in some traits, reminiscent of the trophophore of annelids and molluscs, which may indicate that the possession of a trophophore-like larva is ancestral in (polyclad + euchrocochoans) clad" (p. 92). He also suggested homology of nemertean pilidium and polyclad Müller's or Götte's larva, following the argument of Nielsen (1995). Balavoine (1998) then proposed a progenetic series beginning with an annelid-like ancestor with a trophophore larva, through a nemertean intermediate with a pillidium larva, and culminating in a platyhelmint with a Müller's or Götte's larva. Balavoine (1998) stated that the progenetic origin of platyhelminths "... always seemed to be a 'nice story'... but it is not readily testable" (p. 856). Indeed, testing progenesis as a causal evolutionary process is difficult, but the first step can already be taken by rigorously applying available phylogenetic information, which will necessarily necessitate a reinterpretation on two levels.

First, we need to ascertain that Müller's or Götte's larva and pillidium larvae are homologous, and primitive for the platyhelminths and nemerteans, respectively. Conceding sufficient special morphological similarities to warrant initial homology of polyclad and pillidium larva (but see Salvini-Plawen 1980; Rouse 1999), can we consider these larval types primitive for these taxa? There is general consensus that the polyclad Müller's and Götte's larva, and the nemertean pillidium larva, are not present in the ground pattern of the two phyla (Haszprunar et al. 1995; Nielsen 1995, 1998). Morphological and molecular phylogenetic studies support this conclusion in the platyhelminths (e.g., Carranza et al. 1997; Campos et al. 1998; Littlewood et al. 1999; Ruiz-Trillo et al. 1999), whereas a phylogeny for the Nemertea is not yet available, allowing only a tentative conclusion (Henry and Martin Dale 1997). However, pillidium larvae are restricted to some anoplon species only. Paradoxically, Nielsen (1995) united platyhelminths and nemerteans as Parenchymia on the basis of proposed similarities in polyclad and pillidium larvae, while realizing that these larval types are not likely to be ancestral for these phyla, and clearly his adherence to the trochaean theory is responsible for this conclusion. Support from Nielsen (1995) for Balavoine's hypothesis is therefore suspect.

Second, the evolutionary branching order of the annelids, nemerteans, and platyhelminths needs to be consistent with Balavoine's progenetic series if annelids and nemerteans are to exemplify precursors in a series of evolutionary transitions. It is only by virtue of the lack of resolution in his cladogram that Balavoine can propose his speculative scenario. None of the current molecular and morphological studies clearly indicate a phylogenetic position for annelids and nemerteans as steps in an evolutionary series culminating in platyhelminths (see Figs. 1b and 1d). It is more probable that the trophophore larva evolved in a more restricted set of taxa after platyhelminths split off, a hypothesis supported by Rouse (1999), while pillidium and polyclad larvae are derived within Nemertea and Platyhelminthes, respectively.

Some 22 years ago, Gould warned about the speculative invocation of heterochronic mechanisms to explain the origin of higher taxa: "It is unfortunate that most literature on paedomorphosis is cast in the same mold that bolstered recapitulation during the previous half century—speculative phylogeny of higher taxa" (p. 277) (Gould 1977). Heterochrony is a powerful concept to understand evolutionary changes in animal form (e.g., McNamara 1995). Although heterochronous processes have been proposed to account for the origin of many higher level animal taxa, from mystacocarid crustaceans to larvacea unechordates, a properly specified phylogenetic framework is essential to determine the ancestral and descendant states, and therefore a properly resolved phylogeny can serve as an efficient test for a heterochronic scenario.  

SET-ASIDE CELLS AS A MECHANISTIC EXPLANATION OF THE CAMBRIAN EXPLOSION

The set-aside cell hypothesis developed by Davidson and colleagues (Davidson 1991; Davidson et al. 1995; Peterson et al. 1997; Cameron et al. 1998; Arenas-Mena et al. 1998) has been regarded as an important advance in our current understanding of animal body plan evolution (Hall 1999; Knoll and Carroll 1999; Olsson and Hall 1999). It addresses the controversial subject of the origin and evolution of animal life cycles (e.g., Strathmann 1993; Strathmann and Emerie 1994; Haszprunar et al. 1995; Hart et al. 1997; Nielsen 1998; Pechenik 1999). The core argument of this elegant scenario is an ingenious synthesis of descriptive and molecular embryology, metazoan phylogeny, and the fossil record. The hypothesis can be regarded as an explanation for the absence of a pre-Ediacaran metazoan fossil record, although it is rooted in negative evidence, and it may be a viable hypothesis explaining the origin and diversification of large bilaterian body plans (Vermeij 1996; Wray et al. 1996; Conway Morris 1997; Erwin 1999; Lieberman 1999; Smith 1999). The central points of the scenario are: a biphasic life cycle.
with primary larvae and maximal indirect development (i.e., embryonic [zygote to embryo/larva] and postembryonic [larva to adult]) stages of the life cycle are completely separable both in terms of morphology [larval cells vs. set-aside cells] and developmental process [Type I embryogenesis vs. pattern formation]) is primitive for Bilateria; the bilaterian ancestor is similar to a ciliated primary larva with a population of set-aside cells; and the rapid divergence of adult body plans (Cambrian explosion) from homologous larvae with homologous set-aside cells.

To put the following analysis into perspective, I will briefly summarize pertinent discussions of the set-aside cell hypothesis in the literature. Critiques of the set-aside cell hypothesis can be arranged along four lines dependent on the chosen perspective: convergence of adult body plans; adaptationist reasoning; correlation of reproductive traits; and distribution of developmental types within the Metazoa.

Lieberman (1999) and Smith (1999) considered the unlikelihood of the synchronous, independent evolution of many macroscopic, adult body plans during the Cambrian explosion as the biggest stumbling block of the hypothesis. This appears to be an important caveat, but at present we have no established criteria for judging the amount of expected convergence in animal evolution, although convergent evolution may be prominent in invertebrates (Moore and Willmer 1997).

Lacalli (1997) and Wolpert (1999) used adaptationist reasoning as the basis for their critiques. Lacalli (1997) argued that evolution should favor rapid development over slow development, for example, to decrease predator pressure. The need to maximize the rate of development should favor the evolution of rigid modes of development from more flexible antecedents, making it likely that the developmental processes forming the larva (Type I embryogenesis) have been secondarily imposed upon pre-existing, more flexible pattern formation processes (forming the adult), although this does not explain why these primitive and supposedly slower developmental processes evolved in the first place. Wolpert (1999) argued that every evolutionary transition must be gradual and adaptive. The evolutionary origin of set-aside cells would then be insurmountable, because what could have constituted the selective advantage of these cells before the adult stage that they would eventually give rise to had evolved? Although these criticisms may appear logical, they are difficult to test. I think Lacalli (1997) only shifts the problem, while Wolpert (1999) does not make the distinction between historical origin of a feature and its current utility, a distinction that holds the key to some evolutionary riddles and that cannot be discounted in principle (Gould and Lewontin 1979; Gould and Vrba 1982). Although it may be difficult to reconstruct the evolutionary origin of set-aside cells with respect to their current function, set-aside cells might turn out to be the most significant exaptation in the history of the Metazoa.

Conway Morris (1998a, 1998c) argued against the primacy of maximal indirect development on the basis of known correlations between reproductive traits in marine invertebrates. Indirect development is virtually restricted to large-bodied invertebrates (Olive 1985), and this would argue against the likelihood of a small indirectly developing bilaterian ancestor. I do not consider this to be an effective argument since the set-aside cell hypothesis proposes a bilaterian ancestor reminiscent of a larva that itself represents the adult stage, thus exhibiting direct development (see discussion below). The subsequent evolution of a biphasic life cycle could be linked with the origin of a large-bodied, new, adult stage.

Conway Morris (1998a, 1998b, 1998c) and Wolpert (1999) argued that direct development may be primitive for metazoans based on the study of living taxa, in large measure deriving support from the work of Haszprunar et al. (1995). However, the principal message in Haszprunar et al. (1995) is not that direct development is likely to be primitive for the Bilateria, but rather that a biphasic life cycle with planktotrophic larvae is not likely to be plesiomorphic for the Bilateria. Indirect development with lecithotrophic larvae remains a viable option for the original bilaterian life cycle. Moreover, since Haszprunar et al. (1995) do not provide a phylogenetic framework for their comparison of animal life cycles, I do not regard this study to be effective as an argument against the presence of indirect development in the primitive bilaterian life cycle. Similarly, Conway Morris' (1998a, 1998b, 1998c) and Wolpert's (1999) claim for support for an original metazoan life cycle with direct development deduced from the proposed presence of various directly developing fossil metazoans in the Cambrian (Bengtson and Yoe 1997) cannot be convincing since alternative modes of development, including direct and indirect development, are widespread within the living phyla (e.g., Nielsen 1998). A more effective argument would have to rest on comparisons within a phylogenetic framework of the Metazoa, allowing a robust assessment of plesiomorphic and apomorphic character states.

The hypothesis claims that a biphasic life cycle with maximal indirect development is primitive for Bilateria. This necessitates the evolution from essential direct development (for the bilaterian ancestor resembled a reproductively mature larva-like organism) to maximal indirect development with a separate larva and adult bearing no morphological resemblance at the base of the Bilateria. This hypothesis is only testable by a phylogenetic study when the out-groups suggest direct development as primitive at the out-group node, and the in-group suggests indirect development as primitive for the in-group node, thereby enabling an evolutionary change at the internode basal to the Bilateria (Fig. 2). Note that we are then dealing with two ancestors. This would better accommodate the evolution of set-aside cells, because we can circumvent contradictory characters in one ancestor (at the same
time the larva is the adult and there is maximal indirect development with a maximally different larva and adult).

Let us examine the central points of this hypothesis in detail. First, we must determine the phylogenetic level at which the set-aside cell scenario is said to be effective. Davidson et al. (1995) and Peterson et al. (1997) proposed to explain the diversification of the Bilateria, although their cladograms exclude most of the non-coelomate directly developing aschelminths (Figs. 3a and 3b). It should be noted that without sufficient attention to non-coelomate bilaterians there is a problem to pinpoint the exact clade under discussion. (For instance, Conway Morris [1998a, 1998b], Hall [1999], and Olsson and Hall [1999] regard the hypothesis as explaining the origin of the Metazoa.)

The scenario hinges on the assumption that "maximal indirect development" is primitive for Bilateria, and monophasic life cycles with direct development (absence of a primary larva) are thought to be derived within the Bilateria. To buttress this conclusion, Davidson et al. (1995) and Peterson et al. (1997) provided two phylogenies, Figs. 3a and 3b, with a mapping of maximal indirect development. Neither of these cladograms allow the primitive developmental mode of the Bilateria to be deduced with certainty. Moreover, the likelihood for maximal indirect development in the bilaterian ground pattern decreases when the previously discussed caveats for indirect development in platyhelminths and nemertians is considered. In addition, the provided phylogenies are heavily pruned, leaving out all direct developing "minor" bilaterian phyla, except Nematoda (Fig. 3a), or Rotifera (Fig. 3b). Understanding the phylogenetic positions of these excluded taxa is crucial if the goal is the reconstruction of the ancestral mode of bilaterian development. Rather than giving a detailed justification for their premise, Davidson et al. (1995, p. 1320) state: "We are convinced that the direction of the transition is from indirect to direct development and not the reverse," because this direction of evolution is frequently observed within phyla. So they write about "... the premise that [maximal indirect development] represents the ancestral mode by which adult body plans are ontogenetically produced." Cameron et al. (1998, p. 615) conclude that, "The important point is that the process of indirect development from a ciliated larva, which itself bears little or no relation in structure to the adult body plan to which it will give rise, is a character shared by the majority of animal phyla. Therefore, we postulate that the latest common ancestor of bilaterians minimally had a larval stage similar to what is found in modern indirectly developing marine organisms."

If higher level phylogenetic research is to have any independent status as a potential source of insight into evolutionary processes, we have to be rigorous in analyzing pattern. In this context, a consideration of the acelomate and pseudocoelomate bilaterians is necessary. Aschelminths, for example, have direct development, completely lacking primary ciliated larvae and set-aside cells that give rise to the adult stage. As discussed above, 18S rDNA data indicate basal positions in the bilaterian clade of various directly developing groups (Fig. 3c). Therefore, it would appear that primary larvae are not homologous in the Bilateria, and the evolution of set-aside cells is convergent. It should be noted that the phylogenetic positions of the directly developing Acoela and the problematic echinophores and placozoans will be crucial for a proper resolution.

Davidson et al. (1995) and Peterson et al. (1997) seal their argument by discussing the arthropods and chordates as directly developing exceptions to the general dominance of maximal indirect development. Both groups are characterized by the loss of larval characters present in closely related taxa. Molecular systematics, however, suggests an interesting alternative. According to 18S rDNA information the Chordata (Urochordata, Cephalochordata, and Vertebrata) most likely are the sister group to the Enteropneusta + Pterobranchia + Echinodermata (Turberville et al. 1994; Halanych 1995; Ermis 1997; Littlewood et al. 1998; Wada 1998; Winnepennickx et al. 1998a; Zrzavy et al. 1998; Bromham and Degnan 1999). A diploseudula type larva then is an autapomorphy of the hemichordate/echinoderm clade, indicating that chordates and deuterostomes may in fact be primordially direct developers. When confirmed, this may have important consequences for recent hypotheses deriving the chordate body plan with reference to enteropneust anatomy (e.g., Nübler-Jung and Arendt 1999; Ruppert et al. 1999) or larval morphology (Lucalli 1994; Salvini-Plawen 1998; Nielsen 1999).

Arthropods fall within the echinoderm clad. All ecdysozoans lack classic primary larvae and indirect development. Studying the distribution of indirect development on the consensus 18S rDNA phylogeny (Fig. 3c) reveals the possibility that lack of larvae in chordates and arthropods (as well as the
basal ecdysozoans and lophotrochozoans) is primitive. In this scheme indirect development and set-aside cells would have evolved multiple times in parallel, e.g., in various lophotrochozoans such as cyclophorans, platyhelminths, nemerteans, and the coelomate lophotrochozoans, and in hemichordates and echinoderms. Presence of set-aside cells in the directly developing arthropods (e.g., imaginal discs, teloblasts, and derivatives) indicates decoupled evolution of set-aside cells and maximal indirect development, and the independent evolution of set-aside cells in arthropods. These conclusions corroborate and extend the suggestion by Valentine et al. (1999) that set-aside cells may not be homologous across the Bilateria. Application, therefore, of set-aside cells as a mechanism to explain the Cambrian explosion should be regarded with caution. The basal position of directly developing taxa without set-aside cells in the Ecdysozoa and Lophotrochozoa indicates that in-

Fig. 3. Cladogram from Davidson et al. (1995) (a); Peterson et al. (1997) (b); 18S rDNA consensus cladogram (c), with mapping of maximal indirect development.
dependently evolved set-aside cells could have contributed to the radiation of distinct clades of macroscopic metazoans. The radiation of the various small-bodied basal taxa such as gastrotrichs, rotifers, loriciferans, and kinorhynchs probably occurred independently of set-aside cells.

Do primary larvae actually exist? Historically, the definition of primary larva was developed as a phylogenetic rather than morphological concept. Diagnosing a primary larva then is inferential, not direct observation. Francis Maitland Balfour was perhaps the most devoted British recapitulationist of the 19th century, who sought to explain everything about the nature of larval forms by exclusive use of evolutionary arguments. Accordingly, Balfour (1880, p. 383) defined primary larvae as "... more or less modified ancestral forms, which have continued uninterruptedly to develop as free larvae from the time when they constituted the adult form of the species."

More recently, Gösta Jägersten (1972) in his influential book, *Evolution of the Metazoan Life Cycle: A Comprehensive Theory*, similarly defined primary larva as a phylogenetic concept, although not in the same recapitulationist mold as Balfour. Jägersten (1972, p. 4) states that, "The qualification 'primary' thus neither refers to any special characters in the morphology of the larva...nor to the degree of complication of structure, but to the fact that the pelagic larval type has persisted in ontogeny without interruption since its first appearance."

Therefore not surprisingly, Jägersten designates both crustacean nauplii and sponge larvae as primary larvae despite very different morphologies. Without explicit morphological justification, these definitions are linked with a particular view of metazoan phylogeny, and thus represent phylogenetic concepts. Primary larvae can then only be recognized with reference to a particular phylogeny. The specter of these phylogenetic definitions is still present in the literature. In discussing the phylogenetic position of the most recently discovered animal phylum, the Ciliophora, Funch and Kristensen (1997) cite Jägersten (1972) to support their notion that the ciliophoran chordoid larva is a primary larva. Cameron et al. (1998) cite Jägersten (1972) for arguing a priori that direct development is secondary, failing to recognize the nature of Jägersten’s phylogenetic concepts. Zrzavy et al. (1998) use the presence of a primary larva to sort animal relationships without providing the necessary morphology-based definition, thus entering a phylogenetic concept as primary data into the reconstruction of a phylogenetic concept. It is therefore noteworthy that Cameron et al. (1998, p. 617) conclude in the Balfourian mode that, “The larval forms of modern indirect developing marine species indicates to us the probable nature of these ancestral organisms [the ancestral bilaterians], except that they then constituted the terminal or adult stage of development...”

In summary, the bilaterian ancestor did probably not resemble a ciliated primary larva. More likely, this ancestor (at least the protostome ancestor) was at the organizational grade of a noncoelomate as suggested by the basal ecdysozoans and lophotrochozoans. However, with current phylogenetic information we cannot conclusively decide whether a biphasic life cycle is primitive for the Bilateria. At least independent evolution of set-aside cells and larvae in deuterostomes and protostomes is indicated (Fig. 3c). The combination of Type I embryogenesis and later pattern-formation processes were probably already in place in the bilaterian ancestor. The modern basal noncoelomate bilaterians did not lose maximal indirect development and set-aside cells. Notwithstanding, the set-aside cell hypothesis has great heuristic value for evolutionary developmental biology when placed in a proper phylogenetic framework. The study of the development of larvae and adults in terms of molecular developmental processes in various indirect developing deuterostomes has already yielded very important insights into body plan formation (e.g., Arenas-Mena et al. 1998; Peterson et al. 1999a, 1999b). Nevertheless, detailed study of indirectly developing protostomes and the relatively unknown directly developing noncoelomates is much needed if our goal is to understand the evolutionary origin and significance of set-aside cells across the Bilateria.

These two studies are not unique in evolutionary developmental biology in that they can benefit from greater attention to the phylogenetic basis of their conclusions. Problematic hypotheses of animal body plan evolution can be identified in a range of other recent studies, suffering from biased taxon sampling and/or equivocal phylogenetic deductions. For example, we can diagnose ambiguous inferences of ground pattern attributes in the bilaterian ancestor such as presence of a coelom (e.g., in Balavoine and Adoutte 1998; Martindale and Henry 1998; Adoutte et al. 1999; Knoll and Carroll 1999), the presence of enterocoely based on misrepresenting the conclusions of Valentine (1997) (e.g., in Martindale and Henry 1998; Knoll and Carroll 1999), the presence of “anterior tentacular appendages formed by coelomic outpouching” (Knoll and Carroll 1999), the presence of morphological serialisation or metamersism (e.g., in Balavoine 1997, 1998; Adoutte et al. 1999; Holland and Holland 1999; Knoll and Carroll 1999), and the presence of antenniform appendages in the protostome ancestor (e.g., in Pangianian et al. 1997; Tabin et al. 1999). Rigorous application of phylogenetic information has yielded remarkable insights into animal evolution at lower taxonomic levels for diverse taxa and characters (e.g., Haeg 1995; Sturmbauer et al. 1996; Lee and Shine 1998; McHugh and Roux 1998; Werdelin and Nilsson 1999). If studies on animal body plan evolution aim at a similar effectiveness, phylogenetic information of higher level animal taxa should be approached with appropriate care.

**CONCLUSIONS**

Evolutionary developmental biology attempts to understand the genesis and evolution of organismal design by compara-
tive research, integrating information from disparate fields of biological and paleontological research. A proper phylogenetic framework is necessary because the interpretive framework in many studies of evolutionary developmental biology. The emergence of a consensus on higher level metazoan relationships on the basis of 18S rRNA/DNA sequence data has provided new framework for studying animal body plan evolution. Frequently, papers contrast this new molecular view of animal evolution with a traditional, morphology based perspective. I explored the nature and historical basis of this widely cited traditional textbook tree, and provided an amended reconstruction that better reflects historical information.

Next I discussed the newly emerging molecular view on higher level animal relationships as a basis for studies of animal body plan evolution. Two recent studies, on the evolution of the platyhelminth body plan and on the evolutionary significance of indirect development and set-aside cells, are then used to illustrate the danger of overlooking two aspects of the newly emerging molecular metazoan phylogeny depicted in many recent papers: incompleteness and lack of resolution. In addition, the need for rigorous phylogenetic reasoning is stressed. Both case studies rely on phylogenies that exhibit a strong taxon selection bias for coelomate, indirectly developing bilaterians. In both instances, by ignoring pseudocoelomates, the probability of recovering "linear" transformation series and homology is maximized by maintaining a maximum of connections between features in different taxa. Pseudocoelomates have been the classical locus of some of the most enduring disputes in comparative zoology, namely the origin and evolution of body cavities and larvae. The properly expanded new molecular view of animal evolution forcefully draws attention to the necessity to better incorporate the poorly understood bilaterians into our hypotheses of animal body plan evolution.

Acknowledgments
I thank Professor Frederick Schram for his many valuable comments on the manuscript. My work is supported by grant 805-33.431-P from the Earth and Life Sciences Foundation (A. L. W.), of the Netherlands Organization for Scientific Research (N. W. O.).

Note added in proof: While this article was in press, a paper (Peterson et al. 2000) was published that contains new relevant information for the discussion on the evolutionary significance of set-aside cells. Exhibiting a striking series of parallel lines of thought, Peterson et al. (2000) independently resolved some of the specific criticisms aired in this paper. Their adjusted perspective now also includes a more balanced sampling of bilaterian taxa; a new phylogenetic framework chiefly based on 18S rRNA/DNA sequence data, a consideration of a sequence of succeeding bilaterian ancestors rather than a single ancestor to better accommodate the evolution from direct to indirect development at the base of the Bilateria; and the interpretation of the directly developing bilaterian ancestor as representing the organizational grade of modern primary larvae, rather than possessing specific larval structures. However, our different methodologies of "telling the tree" remain standing, as illustrated by the continued reference to their earlier papers for support of their phylogenetic interpretations.

REFERENCES


