Defining phyla: evolutionary pathways to metazoan body plans

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SUMMARY   Phyla are defined by two sets of criteria, one morphological and the other historical. Molecular evidence permits the grouping of animals into clades and suggests that some groups widely recognized as phyla are paraphyletic, while some may be polyphyletic; the phyletic status of crown phyla is tabulated. Four recent evolutionary scenarios for the origins of metazoan phyla and of supraphyletic clades are assessed in the light of a molecular phylogeny: the trochaea hypothesis of Nielsen; the clonal hypothesis of Dewel; the set-aside cell hypothesis of Davidson et al.; and a benthic hypothesis suggested by the fossil record. It is concluded that a benthic radiation of animals could have supplied the ancestral lineages of all but a few phyla, is consistent with molecular evidence, accords well with fossil evidence, and accounts for some of the difficulties in phylogenetic analyses of phyla based on morphological criteria.

INTRODUCTION

Concepts of animal phyla have changed importantly from their origins in the six Linnaean classis and four Cuvieran embranchements. As knowledge of the comparative development and morphology of metazoans has accumulated over the years, the number of taxa generally recognized as living phyla and believed to represent uniquely derived body plans has grown to about 35. Phyla, if defined as groups of animals that share a body plan due to common history, must be monophyletic in the original sense of the term (Haeckel 1866) but may be either paraphyletic or holophyletic. Morphologically intermediate forms that link phyla to common ancestors have not come to light. Lacking such linkage, precladistic, morphology-based phylogenetic analyses of phyla had to rely upon hypothetical evolutionary events to explain transitions from assumed ancestral forms to the descendant body plans that we see. In some cases, speculations about these events were woven into overarching scenarios that later served as assumptions on which to base interpretations of the entire tree of metazoan life. However, data from comparative morphological studies, at all life stages, have been insufficient to establish one generally accepted metazoan phylogeny, chiefly because they are not powerful enough to falsify alternative hypotheses. And cladistic techniques, though providing a fresh approach to investigating phylogenies, have proven difficult to apply to the body plans of phyla because clearly identifiable, informative homologs are rare. Therefore, numbers of competing scenarios have remained viable, some for over a century (reviews given by Clark 1964; Willmer 1990).

Molecules have provided an important operational advance to addressing questions about the origins of animal phyla. Molecular developmental and comparative genomic evidence offer insights into the genetic bases of body plan evolution. Molecular phylogenetic evidence of branching topologies is not related to developmental or morphological data as such, providing welcome independent tests of previous hypotheses. The affinities indicated by molecular trees are subject to interpretation, and many questions of relationships remain, but molecular phylogenies have been able significantly to further our knowledge of the branching patterns of descent among many of the phyla. However, molecules can only be sampled from extant members of phyla. Molecular phylogenies indicate divergences between the last common ancestors of extant phyla members but do not indicate their morphologies. Fossils therefore are important sources of data for studies of early animal evolution. In contrast with molecules, interpretation of the early fossil record is dependent upon morphology. Fossil studies have revealed numbers of important but extinct taxa that can either be allied with living phyla on morphological grounds or that are so distinctive that they cannot be associated with a living group. What fossils have not revealed are chains of morphological intermediates that link living phyla to common ancestors.

Since the reviews cited above, there has been a new round of evolutionary scenarios that attempt to account for patterns of the origin and radiation of metazoan phyla. Three seem particularly pertinent: those of Nielsen (1995), Davidson et al. (1995), and Dewel (2000). Here we add a fourth, arguing for the predominantly benthic origins of major metazoan

taxa (Valentine et al. 1999; Valentine and Collins 2000). Elements of these new scenarios bear eerie resemblances to those found in the overarching theories of schools of precladistic, morphology-based phylogenetics. Our purpose here is to examine the nature of metazoan phyla and to review critically these four evolutionary scenarios for early metazoan radiations with evidence not only from molecular sequence comparisons but from conventional comparative approaches, from molecular developmental findings, and from the fossil record of the early history of metazoans.

ANCESTRAL BODY PLANS AND PHYLA

Molecular phylogenies indicate patterns of branching, but not the origins of the morphological features that characterize body plans (Valentine 1996; Runnegar 1996; Fortey et al. 1996). Since morphologically based phyla do not originate until characteristic features of their body plans have been evolved, combining molecular and fossil data introduces semantic problems into discussions of body plans (Valentine and Hamilton 1997). The fossil record has shown that many higher taxa, such as phyla, undergo important radiations early in their histories, producing morphologically disparate branches. In many cases some of those branches are more basal than those of any living members of the phylum. The taxa composing those branches are defined as stem groups (Jefferies 1979). The stem ancestor of a morphologically based phylum is the last common ancestor that possessed the characteristic body plan of the phylum, while the crown ancestor of such a phylum is the last common ancestor of its extant members. Neither of these sorts of ancestors is certainly known for any phylum. Interpretations of the body plan of the last common ancestor of any two higher taxa such as phyla are dependent upon hypotheses about the phyletic status of the taxa. For instance, if an ancestor gave rise to two phyla that are thought to be holophyletic clades, then the common ancestor of the two phyla cannot be assumed to have had a body plan like that of either of the derived phyla. A considerably more detailed inference can be made if it is determined that one phylum is paraphyletic with respect to a second because the body plan of the last common ancestor of the two phyla very likely had a body plan characteristic of the former phylum rather than the latter. Determining the phyletic status of animal phyla is therefore of considerable value.

In a few cases, molecular evidence suggests that the extant members of a phylum are in fact probably paraphyletic with respect to other phyla or supraphyletic groups. Such taxa can be termed paraclades. Examples of probable cases of paraclades among the animal phyla are Porifera, Annelida, and Rotifera. Multiple lines of molecular evidence suggest that Porifera is paraphyletic with respect to metazoans possessing tissues (Cavalier-Smith et al. 1996; Collins 1998;

Kruse et al. 1998; Peterson and Eernisse 2001; Medina et al. 2001). Nonsponge animals therefore are very likely derived from an ancestor with a sponge body plan. Similarly, molecular sequences for two genes suggest that the phylum Rotifera may have given rise to the phylum Acanthocephala (Garey et al. 1996; Mark Welch 2000). If Acanthocephala is retained as a phylum, then the body plan of the earliest acanthocephalan is directly derived from the body plan of a rotifer. We do not especially recommend retention of phylum Acanthocephala, but use this case to present a clear example of our approach to the definition of phyla and the usefulness of identifying the phyletic status of phyla. Recognizing a phylum as a paraclade provides information about events subsequent to the origin of its body plan, namely, that one (or more) of its branches became so morphologically distinctive as to constitute a new phylum.

Budd and Jensen (2000) suggest that the body plans of phyla should be restricted to those features that are encompassed by crown taxa. However, there is no reason to believe that the synapomorphies associated with extinct branches of a phylum, whether they branched before or after crown branches, are any less interesting or important than those associated with surviving taxa. Removing stem groups reduces the morphological disparity of phyla and delays their appearance in the fossil record. Furthermore, restriction of body plans to crown taxa confuses factors associated with the origin of body plan features—our interest here—with those that are associated with their extinction and that thus create stem and crown groups. The problems of defining body plans cannot be avoided by this definitional expedient.

MOLECULAR PHYLOGENIES OF PHYLA

Although the use of molecular sequence comparisons seems to have established the general architecture of the metazoan tree of life, many uncertainties remain as to the placement of a number of phyla and of the branching orders of others within major clades. The gene most widely sampled to investigate the phylogeny of phyla codes for the small subunit RNA of the ribosome gene (*SSU* rRNA). In some cases, *SSU* rRNA gene sequences are known from one or very few species in an entire phylum. Further, the use of different exemplars, of different mixes of phyla, or of different algorithms for inferring trees from the sequence data produce different branching patterns. In some animals, the *SSU* rRNA gene has experienced a relatively elevated rate of evolution, undergoing so many changes that its sequence similarity to the homologous genes in its nearest relatives is obscured. Such "long branches" tend to place phyla in branches lower on the tree than their actual branches—closer to phyla that have branched much earlier than to close relatives. Long branch taxa also erode support for alliances that otherwise appear

robust. In cases in which numbers of phyla have branched in a relatively short space of geologic time, the slowly evolving *SSU* rRNA gene may not have recorded their branching order or retained changes that can be recognized after over half a billion years of subsequent evolution. Still other problems plague the establishment of molecular phylogenies (see Huelsenbeck 1995; Hillis et al. 1996).

With these caveats in mind, an hypothesis of the phylogeny of the groups usually recognized as metazoan phyla, based on conservative interpretations of *SSU* rRNA studies, is presented in Fig. 1. For each putative phylum, we also provide our best evaluation of the phyletic status of its extant members, along with an estimate of the extent of sampling of the SSU rRNA molecule (Table 1). At present, hypotheses of monophyly (either paraphyly or holophyly) for metazoan phyla are not contradicted by *SSU* rRNA data, with the possible exception of two cases. *SSU* rRNA evidence suggests that Platyhelminthes (Ruiz-Trillo et al. 1999; Peterson and Eernisse 2001) and Bryozoa (Mackey et al. 1996) may be polyphyletic. It should be stressed, however, that taxon sampling is poor for many phyla, and the phyletic status for most phyla is not clearly worked out. We have tried not to overinterpret the molecular data. When the phylogenetic placement of a phylum is rather unstable with respect to different methods of forming trees, we have remained reasonably agnostic

about its relationships, other things being equal. Even so, it must be said that even the best-supported clades represent hypotheses that should be subject to further extensive testing. In particular, much denser taxon sampling is sorely needed; not only does such sampling lead to more stable topologies, but it tests the monophyly of distinctive clades within phyla and identifies the more basal groups.

There are four major alliances of phyla that are relatively stable under different methods of *SSU* rRNA sequence comparisons and that are supported by or are consistent with other data (for example *Hox* gene assemblages [de Rosa et al. 1999]; mitochondrial gene sequences and arrangements [Boore 1999]; some protein homologies [Manuel et al. 2000]; and *LSU* rRNA sequences [Medina et al. 2001]). These are nonbilaterians (the paraclade Porifera through Placozoa in Fig. 1), deuterostomes (Echinodermata through Urochordata in Fig. 1), ecdysozoans (Kinorhyncha through Tardigrada in Fig. 1), and lophotrochozoans (other "Bryozoa" through Xenoturbella in Fig. 1). A few points, indicated by question marks in Fig. 1, require comment. Myxozoa, Orthonectida, and Rhombozoa are wholly parasitic long-branched groups that are not positively allied to other phyla on *SSU* rRNA evidence; their eventual placement is certainly of interest but is not likely to affect general scenarios for the origin of the other metazoan body plans. Acoela, on the other hand, ap-
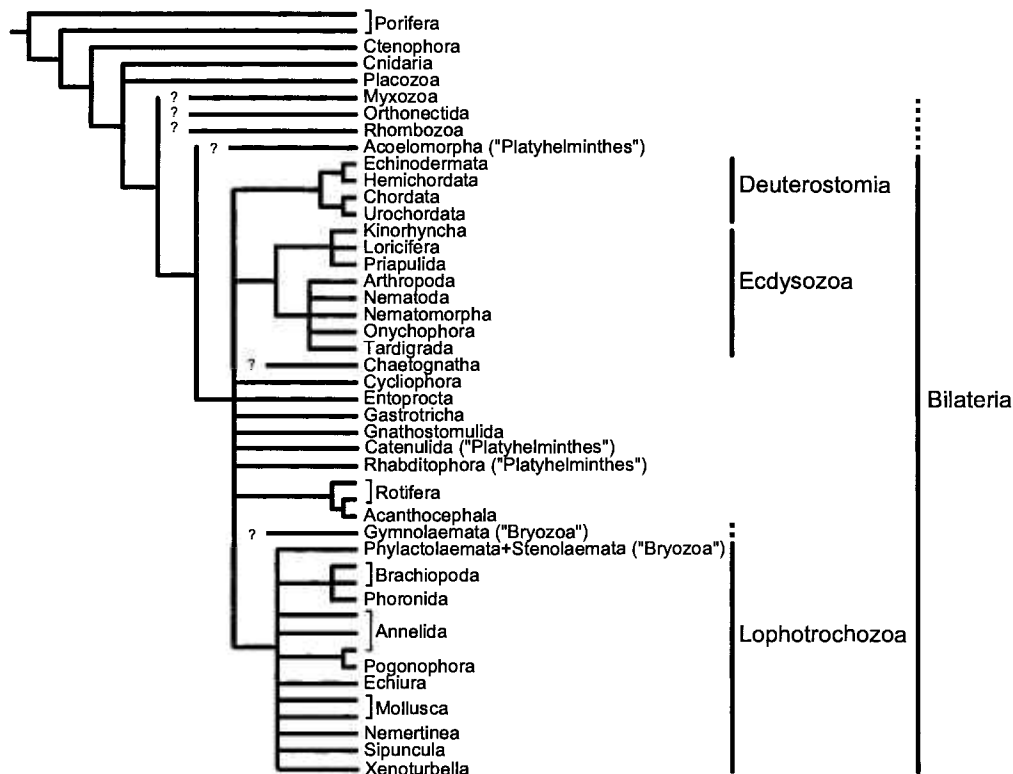

Fig. 1. A conservative hypothesis of the phylogeny of groups usually recognized as phyla, based on various *SSU* rRNA studies.

Table 1. Phyletic status of animal phyla,[1] as indicated by *SSU* rRNA sequences

#	Phylum	Species Known[2]	*SSU* rRNA Samples[3]	Phyletic Status and Notes
1	Acanthocephala	1,150	25	Likely holophyletic
2	Annelida	11,600	119	Possibly polyphyletic; likely paraphyletic with respect to Pogonophora
3	Anthropoda	1,000,000	990	Likely holophyletic
4	Brachiopoda	325	48	Possibly holophyletic; possibly paraphyletic with respect to Phoronida
5	Bryozoa	5,000	6	Possibly polyphyletic with Gymnolaemata separate
6	Chaetognatha	70	3	Possibly holophyletic
7	Chordata	45,000	105	Possibly holophyletic
8	Cnidaria	9,000	107	Likely holophyletic
9	Ctenophora	100	3	Likely holophyletic
10	Cycliophora	1	1	Uncertain
11	Echinodermata	6,000	55	Likely holophyletic
12	Echiura	140	2	Possibly holophyletic
13	Entoprocta	150	3	Possibly holophyletic
14	Gastrotricha	450	3	Possibly holophyletic
15	Gnathostomulida	80	3	Possibly holophyletic
16	Hemichordata	90	9	Likely holophyletic
17	Kinorhyncha	150	1	Uncertain
18	Loricifera	50	0	Unstudied
19	Mollusca	50,000	128	Possibly polyphyletic
20	Myxozoa	1,200	18	Likely holophyletic
21	Nematoda	12,000	89	Likely holophyletic
22	Nematomorpha	320	5	Possibly holophyletic
23	Nemertinea	900	3	Possibly holophyletic
24	Onychophora	70	2	Possibly holophyletic
25	Orthonectida	10	2	Uncertain, one species sampled
26	Phoronida	14	8	Likely holophyletic
27	Placozoa	2	2	Possibly holophyletic
28	"Platyhelminthes"	3,000	331	Possibly polyphyletic with Acoela separate
29	Pogonophora	80	2	Possibly holophyletic
30	Porifera	5,000	24	Likely paraphyletic with respect to Eumetazoa
31	Priapulida	16	5	Possibly holophyletic
32	Rhombozoa	70	1	Possibly holophyletic
33	Rotifera	2,000	7	Likely paraphyletic with respect to Acanthocephala
34	Sipuncula	320	3	Possibly holophyletic
35	Tardigrada	600	7	Likely holophyletic
36	Urochordata	1,250	16	Likely holophyletic
37	Xenoturbella	1	1	Uncertain

[1]Extant members only.
[2]Rough estimate of known species; actual species richness is probably much greater for some phyla.
[3]Approximate number of complete or near complete (>1700 bp) *SSU* rRNA sequences in GenBank.

pears on some *SSU* rRNA evidence to be the most basal living bilaterian group known, rather than part of a monophyletic Platyhelminthes (Ruiz-Trillo et al. 1999; Peterson and Eernisse 2001; and see Littlewood et al. 2001). Many acoels are quite long-branched and this basal position is not yet beyond doubt, but if it is supported by future work its implications for bilaterian ancestry are important. Chaetognatha and Gymnolaemata (bryozoans) are both long-branched and their

positions in *SSU* rRNA trees are unstable and uncertain. It is with respect to the topology indicated in Fig. 1 that we evaluate proposals as to the evolutionary pathways leading to metazoan body plans, which necessarily involve developmental and morphological considerations.

FOUR SCENARIOS OF EARLY METAZOAN EVOLUTION

The Trochaea hypothesis

This scenario derives major metazoan clades from adult planktonic forms (Fig. 2) (Nielsen and Nørrevang 1985; Nielsen 1995), modeled in part after embryonic and larval stages in metazoan development. An actual adult blastula stage, Blastaea, evolves through Gastraea, Trochaea, and Protornaea body plans to a Tornaea. These forms are all visualized as having been adult holopelagic organisms, feeding and reproducing as plankters. Organisms at the gastraean grade produced a branch, a clade ancestor, that colonized the benthic environment, evolving into Cnidaria, while a branch that remained holopelagic

evolved into Trochaea. A branch of trochaeans colonized the benthos as the bilaterian clade ancestor, and within that clade the first stem bilaterian body plan evolved, with an antero-posterior axis as appropriate to a creeping form. As the adult stage evolved, the trochaean body plan was retained as a planktonic larval stage, producing a biphasic bilaterian life cycle. Further evolution within each phase resulted in the ancestral protostomian stem body plan, termed Gastroneuron, with a trochophore-like planktotrophic larval phase.

In the plankton, a branch of trochaeans continued to evolve as holopelagic organisms, passing through the body plan of Prototornaea to Tornaea. A group of tornaeans then also colonized the seafloor. Among this group, evolution of the adult phase produced the stem ancestor of Deuterostomia. This ancestor is termed Notoneuron, which it is postulated had either a tornaria-like (i.e., enteropneust larva-like) or a dipleurula-like planktotrophic larval phase (Fig. 2). The evolution of features within the holoplanktonic adults is worked out so that Gastroneuron and Notoneuron displayed different organizations that lead to the distinctive protostome and deuterostome characters (see Nielsen 1995).
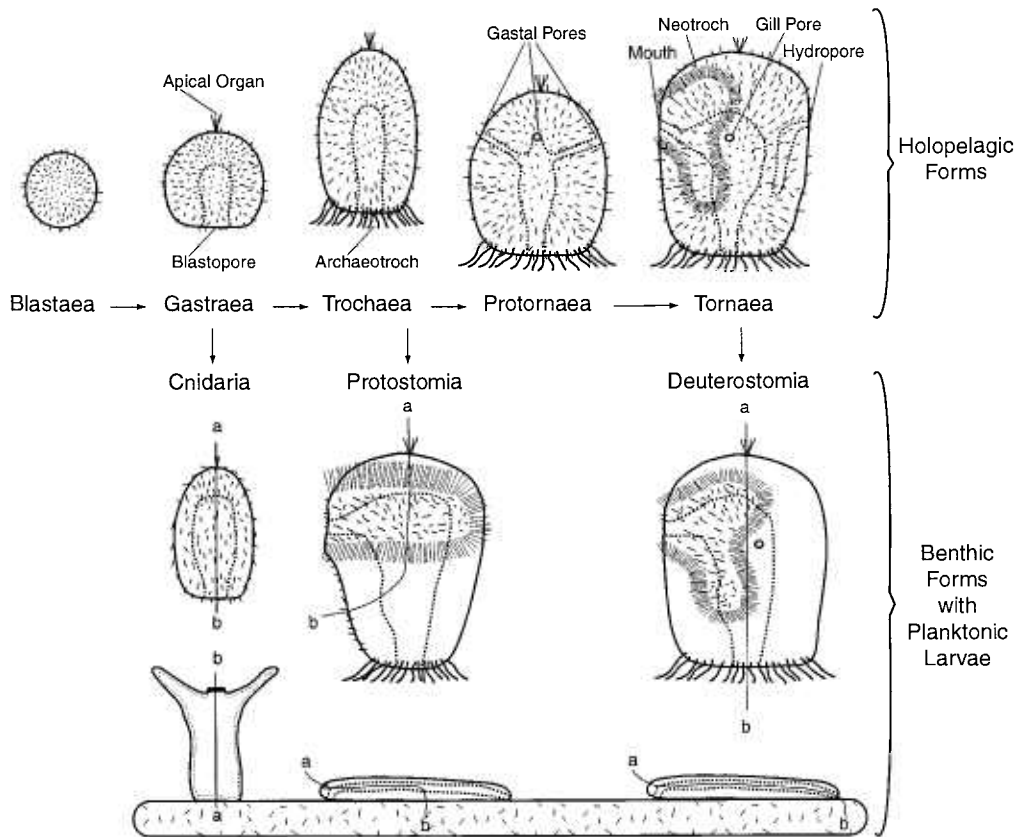

Fig. 2. Elements of the Trochaea hypothesis, after Nielsen (1995). In later versions, Nielsen suggests that the ancestral benthic deuterostome, Notoneuron, possessed a dipleurula-like larva rather than a tornaria-like larva; a–b = apical-blastoporal axis.

This hypothesis is at odds with subsequent molecular phylogenetic findings. For example, both the Spiralia and Trochozoa of Nielsen contain mixtures of phyla that are separated into Ecdysozoa and Lophotrochozoa on *SSU* rRNA evidence, and his Deuterostomia contains forms now considered as lophotrochozoans. However, the scheme could be modified to take into account new findings and to retain the overriding notion that differences in organization between major metazoan clades occurred in ancestral holopelagic forms. But there is no independent evidence that those putative holopelagic forms ever existed, once one rejects a theory of recapitulation (e.g., Haeckel 1866), which evidently does not serve to order the phyla correctly into natural associations. Indeed, lecithotrophy or direct development is most likely plesiomorphic for a wide variety of bilaterian phyla, suggesting that one of these states is most likely for the crown ancestor of Bilateria (Hazsprunar et al. 1995; Jenner 2000; Rouse 2000).

The set-aside hypothesis

Another hypothesis that invokes larval-style body plans as ancestral to complex metazoans was proposed by Davidson et al. (1995; see also Peterson et al. 1997, 2000; Arenas-Mena et al. 1998). In some metazoans, many adult organs are not derived from cells within larval organs, but rather from pleuripotent cells sequestered during larval life, set aside as primordia from which adult structures form, such as the imaginal discs of insects. The distribution of set-aside systems in any plausible phylogeny certainly suggests that this developmental tactic is very ancient. Davidson et al. (1995) suggested that such a set-aside system is homologous across Bilateria and evolved as a response to the growing complexity of adult body plans. The body plans that are now represented by the planktotrophic larvae of many deuterostomes and lophotrochozoans are envisioned as characteristic of adult body plans of early branches of Bilateria. As more complex body plans were evolved, the cells that were used for adult body plans were not employed in the larval phase but were set aside then, and their fates specified during or after a metamorphosis. Like the Trochaea hypothesis, this scenario implies a highly recapitulatory bilaterian history.

Peterson et al. (1997) suggested that the rotifers, which are direct developers and lack set-aside cells, may broadly represent the less complex, ancient, adult body type from which complex bilaterians evolved (an idea dating to Hatschek 1878). These simpler forms are considered to have given rise to an indirect-developing clade that began to employ set-aside cells before the crown ancestor of Bilateria; the set-aside strategy was thus plesiomorphic with respect to protostomes and deuterostomes. This suggestion was supported by a phylogenetic tree that is at odds with some *SSU* rRNA data (see Peterson and Eernisse 2001). Many trees derived from *SSU* rRNA data suggest that the body plans of rotifers and their living aschelminth-like allies arose, not only after the protostome-deuterostome split, but after the split that separated ecdysozoans and lophotrochozoans. Homology of the lophotrochozoan set-aside cells with those in ecdysozoans would thus be essentially precluded since indirect-developing bilaterians probably evolved later, suggesting that set-aside cells were evolved independently in several lineages (Valentine and Collins 2000).

The set-aside hypothesis was subsequently modified to accord with molecular phylogenetic and clock data (Peterson et al. 2000). In this formulation, metazoans are assumed to have originated over 1500 Ma, and the origin of set-aside cells is placed at some later date, but prior to the origin of Bilateria (Fig. 3). The *Hox* cluster is inferred to have been assembled later still, after the origin of adult development via set-aside cells but before the evolution of the crown bilaterian ancestor. Bilaterian stem groups that predated the crown ancestor are presumably extinct. The early dating of metazoan origins is highly unlikely, being unsupported by the fossil record (their ancestral protistan taxa had probably not appeared by 1500 Ma) (Knoll 1996; Butterfield 2000) or by any well-corroborated molecular dating methods (Bromham et al. 2000). Furthermore, *Hox* and *ParaHox* genes are now known to have originated before the evolution of crown cnidarians (Finnerty and Martindale 1999) and are inferred to have been present in clusters, implying that the "modern" patterning apparatus was in place long before the origin of the early bilaterians in which set-aside cells are inferred to have arisen. The early *Hox*-type genes were clearly not used to pattern the sorts of body plans found in adults of complex crown Bilateria. The crown ancestor of Bilateria had at least seven *Hox* genes (de Rosa et al. 1999) and three *ParaHox* genes (Ferrier and Holland 2001). These were without doubt used in patterning the adult body plan, and their activities must have long predated the evolution of set-aside cells in complex bilaterians. Some of the more derived bilaterian phyla have larger *Hox*-type clusters (cephalochordates have a cluster of 14 *Hox* genes) (Ferrier et al. 2000), some smaller (nematodes have a cluster of six *Hox* genes [see de Rosa et al. 1999], and only a single *ParaHox* gene is known [see Ruvkun and Hobert 1998]). However, so far as is known, planktotrophic larvae of bilaterians employ few *Hox* genes in their developmental repertoires (e.g., Arenas-Mena et al. 1998). Evidently the larval sequestration of classic *Hox* activities found in higher Bilateria is owing to the evolution of larval features that are intercalated between gastrulation and the adult stages and that employ other patterning systems (Wolpert 1999; Valentine and Collins 2000); in indirect developers, adult patterning is usually delayed until metamorphosis.

Perhaps the employment of set-aside-like systems can be more generally related to the development of a second body
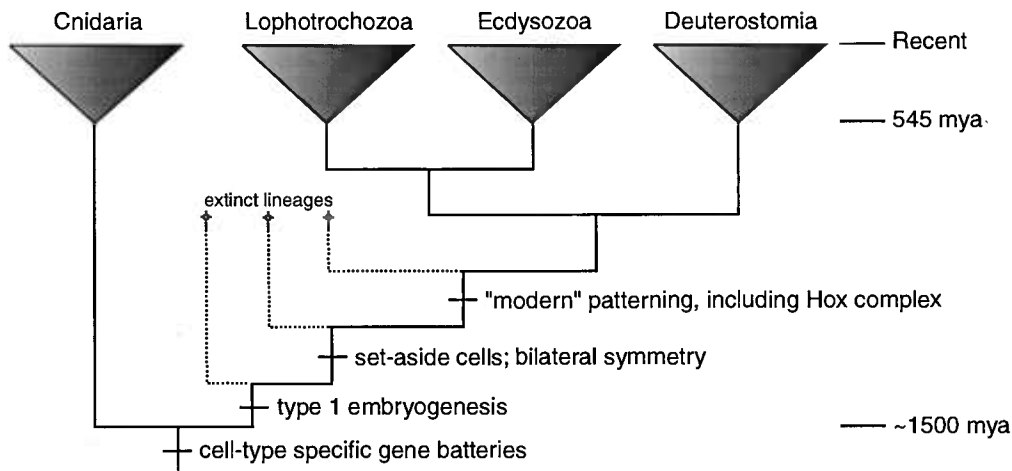
Fig. 3. Elements of the set-aside hypothesis, after Peterson et al. (2000). mya; million years ago.

type within an ontogeny. In relatively simple forms such as hydrozoans, the medusoid subumbrellar structures develop in many cases from the entocodon, a small ectodermally derived cell mass intercalated between germ layers of the polyp. In complex insects, the imaginal disks containing set-aside cells produce adult structures at metamorphosis. These structures are not homologous with the set-aside regions in ciliated feeding larvae. Within echinoderms there is fossil evidence of evolution toward reduction in direct larval contribution to adult bodies and therefore of increasing use of a set-aside system in metamorphosis (Mooi and David 1998). In stem echinoderms, in which metamorphosis appears to have been relatively inconspicuous, larval body walls contribute cell lines to much of the adult. Moreover, even within crown echinoderms, crinoids lack set-aside cells and develop directly (Hyman 1955). Set-aside systems appear to be convergent adaptations to situations where there is a significant disjunction between the selective regimes facing larva and adult.

The benthic colonial hypothesis

Dewel (2000) has suggested that the ancestral Bilaterian body plan arose by the evolution of a colonial diploblastic body plan into a modular triploblastic one. She has hypothesized that the sort of complexity that arose from the association of choanocyte cells to form colonies, which then became individuated as multicellular sponges, was continued in an association of functional sponge modules, which became individuated to form pennatulacean-like Cnidaria on one hand and ancestral Bilateria on the other. The ancestral bilaterian modules were serially arranged, producing a segmented body plan. The early bilaterians were thus not particularly small organisms, and as they evolved they became quite complex, so that the crown ancestor of Bilateria was at a rather

advanced coelomate grade. This ancestor was regionated into a head, segmented trunk, and tail, and had gill slits. Blocks of innervated mesoderm occurred within the segments, as did gonads with associated gonocoels, gonoducts, other serially repeated coeloms, nephridial organs, and elements of a circulatory system. Still other unsegmented coelomic compartments, a brain, and a contractile "heart," were also present.

In hypothesizing such a complex crown bilaterian ancestor, it is also necessary to postulate the simplification of many metazoan body plans, a requirement that is reminiscent of archicoelomate hypotheses that derive segmented coelomates directly from Cnidara (e.g., Sedgwick 1884; Remane 1954). Dewel has listed phyla that may have been simplified; they include 20 (or 20 1/2]) of the 29 bilaterian phyla she recognizes. As she points out, the simpler phyla are generally either meiofaunal, and therefore small-bodied, or are parasitic. However, only small-bodied forms are indicated by Neoproterozoic trace fossils. As Budd and Jensen (2000) argue persuasively, benthic forms with complex larger body plans would not go undetected in the Neoproterozoic fossil record, for such organisms produce trails, burrows, and bioturbations with significant preservation potentials.

Much of Dewel's scheme rests on the assumption that similar morphological features found in complex bilaterians are homologous. For example, coelomic cavities and segmentation are considered by Dewel to be synapomorphies of protostomes and deuterostomes, and therefore properties of the crown bilaterian ancestor. However, the homology of those features seems unlikely; they are solutions to particular biomechanical problems in elongate organisms that pursue certain modes of life, and their details are consistent with independent evolutionary origins. Dewel argues the homology of complex morphological features from the standpoint of their

developmental genetics as well, pointing out that similar and often homologous developmental genes control similar features (such as limbs, eyes, nerve cords, etc.) in both protostome and deuterostome taxa. While these homologs indicate the presence of the genes (or of some paralogs) in a common ancestor, they do not certify the homology of the features whose development they mediate. In these cases, it is more likely that the genes were involved with the development of features in the last common ancestors that were precursors to the complex organs in the descendants: eyespots instead of eyes (e.g., Tomarev et al. 1997), body wall protuberances instead of limbs (e.g., Panganiban et al. 1997), diffuse nerve nets instead of condensed nerve cords (Gerhart 2000). Finally, Dewel believes that the large number of developmental genes inferred to be present in early bilaterians (see Gerhart and Kirschner 1997; Knoll and Carroll 1999) indicates that complex organs and organ systems were present. However, many of the genes cited by Dewel as indicating homologies of bilaterian organs are found in prebilaterians that lack those organs (Müller et al. 1999; Gröger et al. 2000; Spring et al. 2000), as are signaling elements of bilaterian patterning systems (e.g., Samuel et al. 2001). Present evidence favors the idea that the genomic architecture that characterizes metazoan development was in place before the last common ancestor of living Eumetazoa appeared, in conjunction with the evolution of cell differentiation and body plan patterning in relatively simple multicellular organisms.

The benthic individual hypothesis

A fourth scenario is that the origin and early evolution of animals occurred among lineages of solitary individuals and was mainly a benthic phenomenon (Valentine 1994; Budd and Jensen 2000) with relatively few invasions of the pelagic realm that produced stem ancestors of living phyla. The majority of metazoan adult body plans contain basic features that are adaptations to a benthic life habit (Valentine et al. 1999). The origin of Metazoa itself is likely to have occurred in the benthos. The body plan that probably founded multicellular Metazoa is that of the phylum Porifera. The poriferan body plan is plausibly hypothesized to have evolved from some choanoflagellate-like ancestor, with which sponge choanocytes share considerable cytoarchitectural similarity (Laval 1971; Salvini-Plawen 1978). Benthic forms abound within the choanoflagellates, and there is no reason to believe that the entirely benthic sponges are derived from an ancestor that was not also benthic. A scenario that derives sponges from a colonial benthic form with cell types that have phenotypic phases for feeding (protochoanocytes) and reproduction (protoarchaeocytes) fits the facts well. The evolution of the sponge grade involved many important innovations, as multicellularity, with extracellular matrix to provide cohesion and support, and cellular differentiation.

Such novelties imply the evolution of regulative genes in signaling cascades controlling a pattern of gene expression, which reliably provided an assemblage of specific cell types. Many sponge cell types are not terminally differentiated, but nevertheless they are not simply phases in a pathway of cell differentiation, but rather are parts of a multicellular body plan that is mediated by key developmental genes (including numbers of *homeobox* genes) (Manuel and Le Parco 2000). At least three simple larval types have evolved in sponges; none of them feed.

Evolution of tissue-grade organisms has often been hypothesized to occur from a small, solid, flagellated organism—the "planuloid" hypothesis, originally suggested to explain the origin of the diploblastic grade (Lankester 1877) but later generalized to account for the origin of Bilateria as well (see Hyman 1951; Salvini-Plawen 1978). As sponges stand in the direct ancestry of diploblastic organisms, an ancestral planuloid would not likely have been at a tissue level of organization; epithelial tissue sheets had yet to appear. Furthermore, planuloids would lack the principal adult feeding structures of sponges, so that a new trophic style must have been acquired. It seems plausible that these problems have a common solution in the evolution of digestive tissue. The evolution of digestive cells from archaeocyte-type cells is not hard to imagine, but this must have occurred before dispensing with choanocytes.

That sponges can replace their choanocyte-based feeding system through the evolution of other feeding methods is confirmed by the discovery of carnivory in Cladorhizidae, a group whose demosponge affinities are indicated by their siliceous spicule types (Vacelet and Boury-Esnault 1995). These sponges trap minute (<1mm) crustaceans on hook-shaped spicules that coat filamentous extensions of the epithelium; the crustaceans are then overgrown by migrating cells and digested. There are no traces of choanocytes or of intake pores or oscula. In effect, these forms have abandoned the entire pumping system that is the basis for the sponge body plan. Vacelet and Boury-Esnault (1995) suggest that this trophic shift is adaptive to environments that are poor in the usual sponge food items. Thus, there is some basis to hypothesize that the Neoproterozoic sponges that gave rise to diploblastic animals shifted from feeding on minute suspended food items to feeding on captured aggregates of unicellular organisms or detritus, and therefore that pumping systems with choanocytes became obsolete in those forms. Organisms of diploblastic grade might evolve from such sponge derivatives. Among the body fossils of the Neoproterozoic are benthic forms that seem to be constructed of modular elements, the Vendobionta of Seilacher (1992). These forms differ in constructional details from Cnidarians, but appear to be at a similar, diploblastic grade. Whether there are crown cnidarians among the benthic Neoproterozoic assemblages is uncertain, though there are

likely candidates (as the so-called "medusoid" taxa, some of which may be stem anthozoans). At any rate, the earliest fossil diploblasts would seem to be benthic, and *SSU* rRNA evidence indicates that the earliest diverging crown cnidarians are the benthic anthozoans (Bridge et al. 1995; Collins 2000; Medina et al. 2001). Pelagic cnidarians were presumably derived from benthic ancestors. The pelagic ctenophores also appear to be independently adapted to pelagic life.

If early bilaterians were benthic, they may well be represented among Neoproterozoic trace fossils. Among the more common metazoan fossils of the late Neoproterozoic are small surface trails and horizontal burrows. These trace fossils are chiefly about 1 mm in width or less; a few are larger, ranging to 5 mm (e.g., Droser et al. 1999). There is little bioturbation in Neoproterozoic sediments (Droser et al. 1999; McIlroy and Logan 1999); most infaunal biological activity was quite shallow. Although similar surface trails may be made by cnidarians (Collins et al. 2000), it is quite likely that most of the Neoproterozoic traces were produced by bilaterians. Thus our earliest fossil clues as to the nature of Neoproterozoic bilaterians suggest that they were small, vagile, and benthic, creeping or burrowing horizontally on or just below the seafloor. The route from the ancestral sponge body plan to such small bilaterians could either have been through a series of adults or through a demersal, ciliated planuloid; there seems to be no way to distinguish these evolutionary paths at present. For a small ciliated form with differentiated endoderm, evolution of increasing anteroposterior differentiation and of mesodermal body wall muscles would make good adaptive sense. Such a benthic worm is essentially an aschelminth-like paracoelomate (a triploblastic architecture of acoelomate or pseudocoelomate construction) (Inglis 1985).

A common evolutionary pattern is for stem groups to radiate and then to be sorted by subsequent events. We speculate that stem bilaterians of paracoelomate grade radiated into varied ecological roles primarily within benthic habitats, with distinctive morphologies appropriate to their assorted modes of life. Some lineage within this fauna became the crown ancestor of Bilateria and continued to diversify to produce the stem ancestors of Deuterostomia, Ecdysozoa, and Lophotrochozoa. The branching sequence among such various ancestral nodes would not necessarily be indicative of the relative timing of the origins of the body plans of stem or crown groups of phyla, and morphological diversifications within those paracoelomate clades need not have produced nested synapomorphies that have been retained in crown phyla. We suggest that it is a history of this sort that has created the difficulties that are encountered in phylogenetic analyses at the level of phyla, particularly in contrasting or combining molecular and morphological evidence.

CONCLUSIONS

Important new findings from molecular studies that bear on the evolutionary paths of metazoan phyla include support for general aspects of the molecular phylogenetic tree and the discovery of many key features of triploblastic developmental systems among diploblastic forms. These findings permit assessment of scenarios of the early evolution of Metazoa and the origin of animal body plans. Although the phylogenetic hypotheses involved in the Trochaea hypothesis are not supported by molecular evidence, the possibility that pelagic body plans played a part in early metazoan evolution is not falsified. However, with the possible exception of Ctenophora, Chordata, and perhaps Urochordata, there is nothing to be found in the early fossil history of metazoan phyla, in developmental and morphological evidence, in metazoan genomes, or in molecular phylogenies that requires pelagic ancestors. Indeed, there is a long Neoproterozic and Early Cambrian fossil record of metazoans that is replete with positive evidence of benthic inhabitation, which is amenable to interpretation as suggesting that nearly all metazoan body plans arose as adaptations to benthic environments.

The finding that genes that play essential roles in bilaterian body patterning are present in diploblastic forms contradicts scenarios that suggest a gradual assembly of these genomic elements as metazoan body plans became increasingly complex (e.g., Valentine et al. 1996). It appears that the genetic tool kit of the Cambrian was essentially the tool kit of the late Neoproterozoic. Scenarios that implicate the evolution of these genes to permit the rise of higher bilaterian body plans, as in the set-aside hypothesis, and scenarios that require genetic homologs to correlate with morphological ones, as in Dewel's colonial hypothesis, need revision. The notion that early metazoan evolution occurred essentially in the benthic realm is not contradicted by the available evidence. We submit that the most parsimonious scenario of early metazoan diversification is that it occurred among lineages of individuals rather than of colonies, and essentially in the benthos rather than within environments where it conveniently escapes our attention.

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