Fossil and Transcriptomic Perspectives on the Origins and Success of Metazoan Multicellularity

James W. Valentine and Charles R. Marshall

Abstract Multicellularity independently evolved numbers of times—many estimates are in the mid to high twenties—but it is within only two clades, Embryophyta and especially Metazoa, that the multicellular condition led to the evolution of richly diverse and morphologically disparate taxa that have so transformed the biosphere over the last half billion years. Here we first examine the fossil record of metazoans for clues to this morphological profligacy. Part of the reason for their success appears to lie in their early macroevolutionary pattern of rapid invasions of newly accessible adaptive zones followed by exploitation of the morphological possibilities inherent in their new adaptive capacities—their bodyplans–which led to the hierarchical pattern exploited by Linnaean taxonomy. The recent ability to investigate genomically the initial morphological radiation of the phyla through the comparison of ontogenetically dissected transcriptomes has revealed a genomic signature of the phylotypic stage. This suggests, in combination with the paleontologic pattern, that the phylum-level radiation of the metazoans involved the radiation of the phylotypic stages of the phyla. Further transcriptome data offer the possibility of testing evolutionary hypotheses, such as proposed heterochronies, which may be associated with the origin of major morphological novelties; for example, the possibility that the eumetazoan phylotypes descended from sponge larval developmental modules, rather than from those of adult sponges. Finally, we suggest that the morphological disparity of the metazoans (and embryophytes) may be due to their developmental architecture, which includes a mid-embryonic morphological conservatism and transcriptional complexity, and the ability to modify the transcriptome at any developmental stage.

Keywords Cambrian explosion · Metazoa · Phyla · Phylotypic stage · Transcriptome modules · Paedomorphosis · Heterochrony · Disparity · Macroevolution

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Introduction

Among the major clades that have composed Earth's biosphere, Metazoa stand out for the richness of their forms, habits and behaviors, and for the breadth of their disparity and diversity. These attributes are clearly derived from their multicellular construction. Although unicellular clades continue to dominate the major biogeochemical processes of our planet, metazoans exemplify the vast morphological distances that organic evolution is capable of traversing. Here, we use the metazoan fossil record to ask how metazoans have managed to accomplish those feats, and why their particular approach to exploiting the multicellular condition has been so transformative of the biosphere. As paleontologists we have naturally turned to morphologies in the early fossil record for clues, but it is in concert with the exploration of the genome and developmental processes that answers to these questions may eventually be found.

The fossil record has been largely silent on the origins of multicellularity, and although molecular evidence has tentatively identified the clades most closely related to the metazoans (and to the embryophytes and many algal groups; see Butterfield 2009), these groups lack or have very poor fossil records during the periods when their multicellular sister groups arose. Details of earliest metazoan morphologies are also not recorded, although they can be inferred from a morphological comparison of the most basal metazoans (the sponges or possibly the ctenophores (Dunn et al. 2008; Hejnol et al. 2009), although these unusual trees seem to be the result of insufficient taxon sampling and long branch attraction (Pick et al. 2010)) with their putative sister group (the choanoflagellates). Clues to the earliest metazoans can also be garnered from non-morphological fossils such as traces left by their activities, by chemical fossils (biomarkers), and by paleontological dating of branching events in molecular phylogenies, which suggest that stem metazoans arose about 780 Ma (million years ago) (Erwin et al. 2011; also see Chapter "Timing the Origins of Multicellular Eukaryotes Through Phylogenomics and Relaxed Molecular Clock Analyses"). However, nodes on molecular phylogenies simply record the divergences between clades, and not the origin of the significant morphological novelties that those clades may come to possess (Marshall and Valentine 2010). Thus, the metazoan stem lineage may have consisted of species that were morphologically at a choanoflagellate grade of organization for tens of millions of years after the origin of the last common ancestor (LCA) of metazoans and crown choanoflagellates (Fig. 1)—although it might sound counter-intuitive, the first stem group metazoans were not sponges nor were they multicellular (Fig. 1). It is thus useful to supplement the molecular clock estimates for the time of origin of morphologically defined taxa, such as phyla and classes, with morphologies mapped onto phylogenies (rather than cladograms— see Fig. 1), as well as examine the fossil record of the early appearances of those morphologies, and evaluate the patterns of disparity that are found over time.
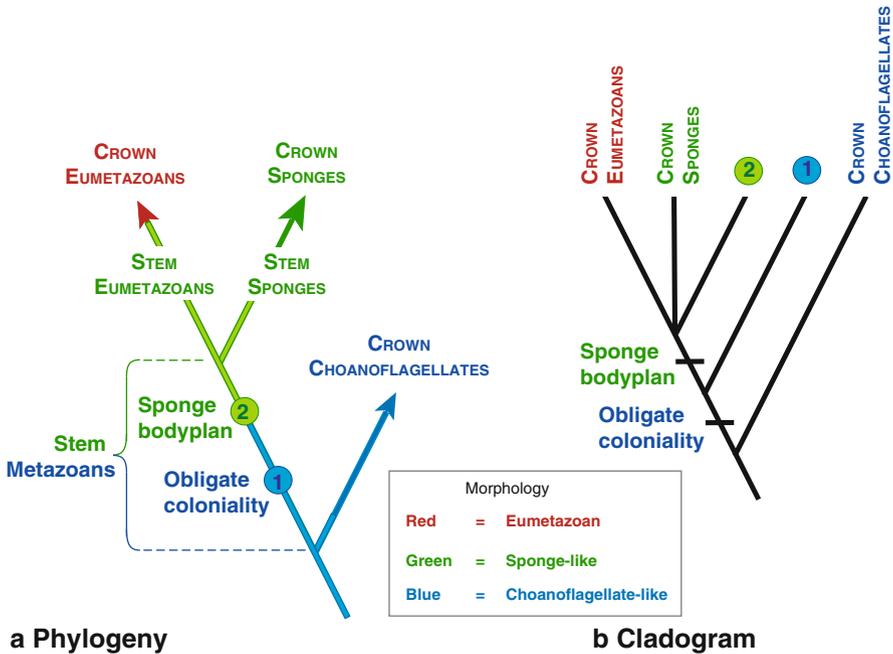
Fig. 1 Morphologies associated with the emergence of the metazoans. **a** Morphologies (*colors*) and morphological changes within a phylogeny depicting the emergence of the metazoans. Lineages with choanoflagellate-like morphologies (*blue*) split to produce crown choanoflagellates and the lineage that gave rise to living sponges and metazoans. At some point the ancestors of metazoans, which were biologically choanoflagellates, evolved obligate coloniality (*1*), which in turn gave rise to a differentiated multicellular organism with a sponge-like bodyplan (*green*, at *2*). Later, a lineage of these sponges, which cladistically were stem metazoans, split, with one lineage giving rise to the eumetazoans, the other to living sponges. If living sponges are paraphyletic (Sperling et al. 2009, 2010) then there will have been more than one branch leading to living sponges. Thus, the earliest eumetazoans (*red*) had a sponge-like bodyplan. **b** The same events captured in a cladogram. Note that the cladogram, in lacking the explicit temporal perspective, does not adequately capture the sequence of morphological changes associated with the emergence of the metazoans

Metazoa Before the Cambrian Explosion

Choanoflagellates to Sponges

The ancestry of sponges in Choanoflagellata (Chapter "Choanoflagellates: Perspective on the Origin of Animal Multicellularity") is suggested by morphology, i.e., the similarity of sponge choanocytes to choanoflagellates, e.g. Laval (1971), although there are differences in the cell structure and cytoskeleton between the two (Karpov and Leadbeater 1998). Choanoflagellate ancestry of sponges is also suggested by molecular evidence (e.g. Lang et al. 2002; Medina et al. 2003; King 2004), but we note that there has been important gene loss in choanoflagellates with respect to the metazoan developmental toolkit (Sebé-Pedrós et al. 2010, 2011; and Chapter

"Transcription Factors and the Origin of Animal Multicellularity"), making the continuity between choanoflagellates and metazoans less clear. That the last common ancestor (LCA) of choanoflagellates and sponges was colonial is possible but not certain, although obligate coloniality was almost certainly present in the metazoan stem lineage as an early step in the evolution of multicellularity (Fig. 1). The exact mechanism by which the colonial form evolved is uncertain, but recent evidence from ichthyosporeans suggests it may have been via cellularization of a multinucleate syncytium (Suga and Ruiz-Trillo 2013; Chapter "Filastereans and Ichthyosporeans: Models to Understand the Origin of Metazoan Multicellularity"). Assuming a choanoflagellate ancestry, feeding chambers were produced as sponges evolved by clustering choanocytes within a scaffold of supporting cells to provide for a more powerful feeding stream, clearing a larger volume of water and permitting the rise of larger individuals. New specialized cell types also appeared as the sponge bodyplan was elaborated, although this almost certainly predated the divergence between the lineage that led to living sponges and the eumetazoans (Fig. 1). As the sponges use food items, largely bacteria, that are quite similar to those of choanoflagellates, they would have fitted easily into the benthic trophic web of the time; no novel conditions of trophic ecology seem required for their emergence. The adaptive advantages of becoming a sponge probably involved feeding and reproductive efficiencies associated with larger body sizes.

The earliest fossils that most likely represent metazoans are indeed possible sponges, that are found in rocks deposited earlier than the Ediacaran Period (c. 630–542 Ma), perhaps significantly before 635 Ma (Maloof et al. 2010), while biomarkers that characterize demosponges today also appear earlier than 635 Ma (Love et al. 2009). Hexactinellids seem to be closely allied with and perhaps basal to demosponges, but most early records of their spicules are suspect, and their crown groups may have diversified as late as the early Cambrian (Dohrmann et al. 2013). It has been postulated that the appearance of large sponge populations filtering the water column influenced the oxygenation of the oceans (Laflamme et al. 2009) and could have played an important role in the eventual appearance of larger, active eumetazoans.

The morphological bridge between sponge and eumetazoan bodyplans is unknown. Many early phylogenies postulated that sponges branched from a protistan lineage while eumetazoans arose independently from another (probably colonial) protistan (reviews in Clark 1964; Willmer 1990). The alternative, a route to eumetazoans through the sponge body plan (see Sperling et al. 2009, 2010 for the case based on the paraphyly of the living sponges) seems more difficult, because the sponge bodyplan (although not the genome) must then be lost. In the past, a common solution has been to suggest that eumetazoans descended from sponge larvae via paedomorphosis (e.g. Salvini-Plawen 1978), shifting reproduction into a worm-like larva (Fig. 2). Crown sponge larvae include somewhat elongate forms with anteroposterior axes that might suggest how bilaterality was foreshadowed (e.g. the trichimella of hexactinellidans and the cinctoblastula of homoscleromorphs; Boury-Esnault et al. 2003; Leys and Ereskovsky 2006), although the fact that adult calcareans and hexactinellids have polarized bodyplans leaves the door open to the possibility that
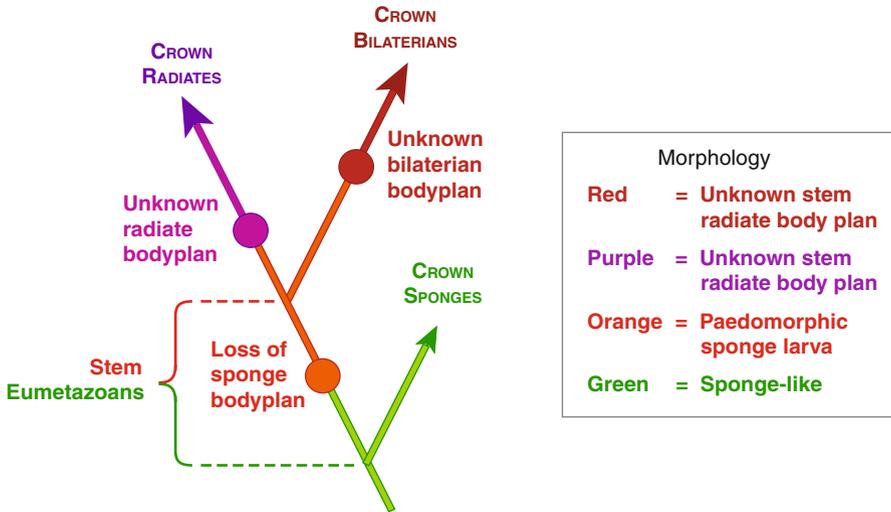
Fig. 2 Morphologies associated with the emergence of the eumetazoans. Morphologies (*colors*) within a phylogeny depicting the emergence of the eumetazoans. Lineages with sponge morphologies (*green*) split to produce crown sponges (or more than one crown group if they are paraphyletic) and the lineage that gave rise to eumetazoans. Under the paedomorphic theory for the origin of eumetazoans, their ancestors, which were biologically sponges, lost the sponge bodyplan with the transfer of reproduction to the larval stage (*orange*). Later, a split occurred with one lineage (or perhaps more than one) that went on to become the radiates, while the other gave rise to the bilaterians. At present, we do not know what the associated bodyplans looked like before these superphyla differentiated

eumetazoans passed through a sponge-like organism that had a limited adult morphology in comparison to the adult morphologies seen in the living sponges. At any rate, the fact that some cell lineages in sponges may readily be transformed from one cell type to another (T. L. Simpson 1984)—for example, archaeocytes into gametes—suggests that paedomorphosis might be more accessible to sponges than to most metazoans. Nevertheless, the issue of whether eumetazoans descended from sponge larvae remains unsettled.

The Ediacarans

A group of large-bodied taxa that are commonly interpreted as early metazoans appeared near 578 Ma. These are the "ediacarans", which seem to have been multicellular and appeared later than the first sponges but a bit earlier than the first convincing evidence for bilaterians (see Xiao and Laflamme 2009). They persisted at least to about the Ediacaran/Cambrian boundary interval (c. 542 Ma). They have been allied with a wide variety of taxa, including various unicellular forms, lichens, or fungi, or as multicellular forms that arose independently of metazoans, or, commonly, as early branches of epithelial metazoans themselves. When they

first appear their bodies are highly patterned in frond-like architectures, many of which are fractal, indicating well-regulated developmental processes. If these first ediacarans were metazoans they must have branched along the eumetazoan stem. All lack organs and appear to have been sessile. It has been suggested that they fed by osmotrophy (Laflamme et al. 2009).

Early work on the ediacaran faunas (e.g. Seilacher 1992) tended to lump the early occurring frondose forms with a fauna of more mobile forms that appeared somewhat later and lived on and under microbial mats, likely feeding on those mats (and perhaps on biofilms) in the fashion of Placozoans (Sperling and Vinther 2010), or by scraping the mats or films in the fashion of some algal-feeding mollusks. Most of the mobile forms also have highly patterned bodies and appear to include more than one major group, and may include bilaterians. Only recently has a practical taxonomy been set up for these ediacaran groups (see Laflamme et al. 2012).

The Cambrian Explosion

Patterns of Preservation

The fossil record improves dramatically during the Cambrian (c. 542–489 Ma), especially with the appearance of the rich, chiefly benthic assemblages of the Chengjiang fauna of south China (c. 520 Ma) and the later Burgess-shale type faunas from British Columbia, Canada (c. 510 Ma; see Zhu et al. 2006). These fossils are so exceptionally well preserved that they include many details of their soft-part anatomies, even though some of them appear to lack stiffened organic or mineralized integuments. These assemblages provide a window into an important fraction of Cambrian faunas that would otherwise be lost. Another mode of preservation that appears in the early Cambrian is represented by "small shelly fossils", largely phosphatized skeletons, chiefly under 2 mm in their largest dimension, commonly of sclerites (individual plates, spines, etc., typically components of multi-element skeletons or scleritomes). And the most common Phanerozoic mode of benthic marine fossil preservation, as mineralized or otherwise stiffened organic skeletons, is well represented in the Cambrian as well. Thus, there are three important modes of preservation represented in Cambrian faunas, two of which are unusual.

The highest taxonomic levels, for example the Linnaean phyla and classes, are well represented in the Cambrian fossil faunas, while the faunas seem underrepresented by taxa at lower levels, such as species and genera, at least by modern standards. For example, there are 20 Phanerozoic echinoderm taxa that are deemed distinctive enough to be assigned to class level by Linnaean criteria (Sprinkle and Kier 1987), but many of the first appearing ones are extinct, have very short durations compared with living classes, and are represented by relatively few lower taxa; eight of them are known from fewer than 10 genera each. It is possible to interpret the low generic (and specific) diversities recorded for these classes as owing to a fossil record so spotty that it fails to sample most lower-level taxa, while still leaving a relatively complete record at the higher taxonomic levels (given that it takes

only one record of one genus or species to establish the presence of its class). And the spottier the record, the fewer lower taxa will be captured, and the shorter the apparent temporal range of the phylum or class will be. Thus one can ask, is the Cambrian pattern of high rates of appearance of often short-lived higher taxa, and low rates of appearance of lower taxa simply owing to a record biased in this way? Or does the fossil record portray the true underlying macroevolutionary dynamics of the Cambrian explosion—high rates of major morphological innovation with low rates of lower taxonomic innovation? The latter possibility is supported by the fact that the Cambrian is characterized by the three major modes of high quality preservation as described above, and by the observation that peak rates of origination shift towards lower taxonomic levels through time (Valentine 1969; Erwin et al. 1987), as if evolution was running out of novel gambits. That is, the higher the taxon, the earlier its peak rate of diversification: phylum-level diversification peaks during the early Cambrian, class-level diversification peaks during the late Cambrian to early Ordovician, orders in the mid Ordovician, while families peaked later still.

Building a Macroevolutionary Case for Early Metazoan Diversification Patterns

Morphometric studies can, in principle, shed light on the relationship between the observed patterns of Cambrian diversification and the processes that produced them. However, there are methodological problems that make such studies difficult. Two are particularly discouraging: the lack of reasonably large numbers of lower-level taxa, required for statistical significance; and, the lack of common morphological landmarks among the very disparate Cambrian higher taxa, many of which are stem groups at the level of phyla or superphyla (Budd and Jensen 2000). Trilobites are a major exception, for they are represented by more species than all other major invertebrate clades combined during the Cambrian, although at its appearance this clade is already distinctive and its branching position among arthropods is difficult to establish. But extensive studies of trilobite lineages find nothing in their developmental pattern that is unique among arthropods (review by Hughes 2005)—the emergence and initial radiation of trilobites does not seem to be the result of an unusual mode of development or macroevolution, although their success may owe in part to their earlier acquisition of more easily preserved (chiefly mineralized) skeletons than other contemporaneous clades.

Perhaps a better place to test for a similar macroevolutionary patterns is among Ordovician faunas (c. 488–444 Ma); they are better preserved and much more diverse than those of the Cambrian thanks to the "great Ordovician biodiversity event" (see Webby et al. 2004). That diversification raised overall family diversity by a factor of about 2.5 (Sepkoski 1981) to a standing diversity that held reasonably steady until the end-Permian extinctions, despite some extinction spikes. The numbers of lower taxa available within many of the more common phyla and classes became large enough to support morphometric treatments, which were successfully pursued among Echinodermata in a series of important studies by Foote (especially 1992, 1994,

1995). These studies are particularly interesting because the Cambrian appearance of echinoderms is emblematic of the low-diversity, high-disparity pattern described above, and some classes continued that pattern during the Ordovician. Foote's studies included Paleozoic blastozoans (Foote 1992) and crinoids from the Ordovician to Devonian (Foote 1994) and also from the Carboniferous to Permian (Foote 1995).

The morphological pattern that Foote found to be the most common in his strato-morphometric studies was that of the early radiation of higher taxa relative to lower ones. The earliest members of the diversifying taxon were highly disparate, more or less defining the full morphological space that was to become more densely occupied as the clade reached its peak diversity—the later appearing members tend to fill in the morphospace between the founders. Thus, the founders were more distinctive from each other (and usually assigned to a higher taxonomic level) than were the later appearing members from each other. Within more inclusive clades, their sub-clades typically repeat this pattern, even if late evolving—*their* early branches tending to outline the morphological region that their descendants would come to occupy, and so on. The early morphospace regions staked out by early-appearing novelties became more densely occupied over time as later branches originated. Such a history of the filling of morphospace produces a hierarchy of disparities, which lend themselves easily to classification in the Linnaean fashion. Thus, the dissection of diversity patterns at different levels of the Linnaean hierarchy is highly informative (despite arguments to the contrary, e.g. Smith (1994), and see Foote (1996) for a well-reasoned counter-argument).

This pattern of early rise of disparity is by no means universal, but it occurs in other phyla that were important in early metazoan history for which data are available. Other major clades in which disparities among groups appear abruptly before many lower-level taxa appear include: arthropods, the most dominant fossil group during the Cambrian explosion, which reach a level of disparity in the Cambrian that approximates that of the modern marine arthropod fauna (Briggs et al. 1992; Wills et al. 1994); brachiopods, of which 12 orders appear in the Cambrian (Curry and Brunton 2007); and, also at the ordinal level, early bryozoans (appearing in the early Ordovician—Anstey and Pachut 1995). The generality that emerges is that for taxa in the Cambrian as in the Ordovician, the higher the taxonomic level the earlier they diversified. This pattern holds from the level of phylum down to the family level (see Valentine 1969; Sepkoski 1981; Erwin et al. 1987; Campbell and Marshall 1986 for echinoderms).

Subsequent expansion of morphospace regions certainly does occur within some higher taxa, especially after major extinctions that presumably released spatial and trophic resources utilized by the extinct groups that were then taken up by a surviving group; this may be the case with the origins of some orders following extinctions (Erwin et al. 1987). Especially clear cases of morphospace expansion of established clades involve the invasion of large but previously unoccupied regions of adaptive space, most spectacularly by the invasion of the land, as by insects and by tetrapods. However a group arises, the size of its morphospace is most strongly affected by the presence of large peripheral subgroups (Foote 1993).

As the reality of this "top-down" filling of morphospace has been confirmed for Ordovician taxa, and as a similar (but spottier) pattern can be seen in the much less

diverse Cambrian faunas, it seems likely that similar macroevolutionary processes were responsible for the similarities in the record of higher taxa origination during both Periods. This raises two questions: (1) Is it reasonable to assume that the Ediacaran pattern was also similar, even though we may not have a record of a much larger proportion of the taxa of the time; and (2) What caused or permitted the relatively rapid origin of the novel bodyplans that founded the Cambrian explosion, and of the sub-plans that begat the awesome morphological diversity of the Metazoa. We tentatively answer the first question affirmatively since there is no obvious reason that the best-known macroevolutionary pattern among major metazoan morphologies was not inherited from that of earlier times. We cannot answer the second question, but recent advances in the analysis of change in gene expression during development offers an opportunity to better understand the evolutionary processes responsible for the "top-down" filling of morphospace, which we now explore.

The Phylotype, Transcriptomes, and the "Top-Down" Filling of Morphospace

The Phylotypic Stage

A long-standing observation is that metazoan morphological lability during ontogeny is shaped like an hourglass, the neck of which corresponds to a conserved "phylotypic stage" (Slack et al. 1993) with more divergent morphologies developed both earlier and later (Duboule 1994; Raff 1996). Descriptively, morphological lability is said to be constrained at that stage because morphological similarities in the developing embryos among species belonging to the various subgroups within a phylum (or other taxon) resemble each other most closely then. Common features are seen across the subgroups, and these features can be referred to at the phylum level as constituting its underlying bodyplan.

The "top-down" filling of morphospace implies that the phylum-level morphological differences were among the first stages in the evolution of the metazoans, and thus that, in essence, the beginnings of the radiation of metazoans was the radiation of the morphologies that retrospectively we identify as the phylotypes. Thus, given that the genome captures a great deal of historical information in its sequences and its functions, we here posit that the genomic analysis of the phylotypic stages of living taxa should offer insight into the first steps in the diversification of the phyla.

The Search for the Genomic Signatures of the Phylotypic Stage

Recently, phylostratigraphic analyses (Domazet-Loso et al. 2007) of ontogenetically dissected transcriptome data from model organisms, fruitflies (Kalinka et al. 2010) and zebrafish (Domazet-Loso and Tautz 2010), have been used to examine transcriptional lability during development. Kalinka et al. (2010) studied six species of

Drosophila and found that a reduction in temporal lability of transcription occurs in mid-embryogenesis and that this developmental stage is characterized by the relatively enhanced transcription of the regulatory genes that are chiefly responsible for the bodyplan of the phylum—the arthropod phylotypic stage. Furthermore, tests for the strength of stabilizing and directional selection among the species were consistent with the presence of significant stabilizing selection during mid-embryogenesis.

Domazet-Loso and Tautz (2010) used a different type of analysis, asking what is the average phylogenetic age of the genes expressed during each ontogenetic stage of the developing zebrafish. They found that mid-embryonic phylotypic stages were both preceded and followed in ontogeny by the transcription of, on average, phylogenetically younger genes—their data suggested that the hourglass shaped lability of morphology seen in development is mirrored by an hourglass-shaped average age of the genes expressed during ontogeny.

However, re-analysis of Domazet-Loso and Tautz's (2010) data by Piasecka et al. (2013) suggest that rather than the average age of the genes expressed at each developmental stage exhibiting an hourglass shape, the average age of the genes expressed simply gets younger through ontogeny. In that case the phylotype is not reflected in the average age of the genes expressed during ontogeny. Nonetheless, Piasecka et al. (2013) show a significant enrichment in the number of transcription factors expressed during the phylotypic stage, and that those transcription factors have significant concentrations of highly conserved non-coding elements and transposon-free regions. Thus, consistent with Kalinka et al.'s (2010) observations and the ideas of Duboule (1994) and Raff (1996), the zebrafish phylotypic stage is at least characterized by a peak in evolutionary stability.

Further, and perhaps most significantly, Piasecka et al. (2013) showed that there is a significant over-representation of genes common to all bilaterians expressed during the vertebrate phylotypic stage. Unfortunately, they were only able to employ a few model systems in their analysis, so it is unclear how many of those genes have origins that lie deeper in the tree, for example at the origin of the Eumetazoa or Metazoa or even deeper. Nonetheless, Piasecka et al.'s (2013) analysis of Domazet-Loso and Tautz's (2010) data lends considerable weight to the hypothesis that the first stages of the metazoan radiation were characterized by the divergence of the developmental stages that were later to become entrenched in the phylotypic stages of the differentiated phyla (see de Mendoza et al. 2013).

Using Transcriptome Profiles Help to Solve Evolutionary Problems in the Deep Past?

The increasing sophistication of the approaches used to understand the transcriptional history of genes in the early history of the metazoans offers hope that further work will yield solutions to some of the most vexing problems associated with the nature of the morphological innovation among early metazoans. The most obvious major problem for which help may arrive involves the identification of transcriptional shifts