

MACROEVOLUTIONARY PATTERNS AND PROCESSES DURING THE CAMBRIAN RADIATION: INTEGRATING EVIDENCE FROM FOSSILS AND MOLECULES

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ABSTRACT

The Cambrian radiation represents a key episode in the history of life when most of the major animal lineages appeared and diversified in the fossil record. Unravelling the patterns and processes driving the Cambrian radiation has proven challenging. We discuss several lines of evidence that provide additional understanding about the Cambrian radiation including trilobite phylogeny and biogeography, cnidarian fossils and phylogenies, metazoan phylogenies and the molecular clock, genomics and evolutionary development, and palaeoecology. We argue that by integrating these disparate lines of evidence, a more comprehensive view of the Cambrian radiation emerges.

RESUMO

A radiação Câmbrica representa um episódio-chave na história da vida, quando a maior parte das linhagens animais apareceu e se diversificou no registo fóssil. Descobrir os padrões e os processos que conduziram a radiação Câmbrica tem-se mostrado um desafio. Discutimos aqui várias linhas de evidência que proporcionam entendimento adicional sobre a radiação Câmbrica incluindo filogenia e biogeografia das trilobites, fósseis e filogenias de cnidários, filogenias e relógio molecular dos metazoários, genómica e desenvolvimento evolutivo, e paleoecologia. Argumentamos que, integrando essas linhas de evidência variadas, emerge uma visão mais abrangente da radiação Câmbrica.

INTRODUCTION

Macroeolution is the study of the patterns and pro-

cesses relating to the birth, death, and persistence of species. As such, a special aspect of the study of macroevolution

has been a focus on investigating key episodes in the history of life that involve differential proliferation or extinction of species. The fossil record is our one true repository of species' births and deaths. One of the most important episodes in the history of life, at least in terms of its placement in time and phylogenetic space, was the Cambrian radiation. Consideration of the evolutionary significance of this key episode dates back at least to Darwin (1859), and it will be the focus of our contribution. Since there is such a long history of study, scientists' conclusions about the episode and its significance have changed through time (see Lieberman, 1999a, 2003a; Knoll, 2003; Valentine, 2004; Brasier, 2009). Originally this radiation was held to be largely synonymous with the origins and diversification of animals. However, more recently, a nuanced view has emerged, and now it is more typically treated as the initial appearance and proliferation of abundant metazoan remains in the fossil record (Knoll, 2003; Lieberman, 2003a; Valentine, 2004; Brasier, 2009). Part of the transition to this more nuanced view has involved increasing phyloge-

netic precision about the taxa involved. For instance, it is now recognized that several animal phyla proliferated well before the start of the Cambrian including sponges and the Ediacaran biota, which likely contains some stem group cnidarians, or their relatives. However, establishing strong phylogenetic links between elements of the Ediacaran biota and *bilaterian* animal phyla has proven more difficult.

Darwin's (1859) perspective on the Cambrian radiation is worth considering. Notably, Darwin argued that the major groups of taxa that appeared in the fossil record at this time must have evolved well back into the pre-Cambrian. It appears now that Darwin may have been partly inaccurate to the extent that he claimed the roots of Cambrian radiation taxa extended way back into the pre-Cambrian, but he was right to suggest that the Cambrian radiation was not solely an explosive evolutionary event writ large in the fossil record. Instead, the Cambrian radiation had some pre-Cambrian fuse, where the taxa had originated and started evolving (a lit fuse) before the explosive radiation (the bang) appeared on the scene. Because

of this, a key question now is how long before the radiation did the component taxa actually evolve. This is fundamental because it determines whether the Cambrian radiation really is a key episode in the history of life, from an evolutionary perspective, and does indeed represent a dramatic evolutionary proliferation or radiation, or instead marks some set of changing ecological or environmental conditions that allowed already extant organisms to become more visible in the fossil record, either through increases in abundance or size or changes in fossilization potential. Here we consider this issue in greater detail, marshalling various lines of evidence from the fossil record and the extant biota. Then we consider the specific set of changes, genetic and environmental, that may have caused the radiation to happen. We conclude with some discussion on how to forge a synthesis between disparate lines of evidence, from trilobite phylogenies to genetic toolkits, and approaches, from palaeontology to evo-devo, to come up with a broader view of macroevolution both in general and during the Cambrian radiation interval in particular.

Information from palaeontology and development in some respects played an important role in the formulation of what is referred to as the Neo-Darwinian synthesis (e.g., Simpson, 1944; de Beer, 1940) as practitioners from these areas were involved in what is treated as a hallmark event in evolutionary biology. However, by the same token it could be argued that when it came to incorporating actual data or theoretical outlooks, neither of these disciplines was well represented in the body of evolutionary theory that is associated with that synthesis (see Eldredge, 1985; Gould, 2002). Major advances in evolutionary biology have come, and are apt to continue to come, from more fully incorporating information from palaeontology and comparative development.

One important aspect of studying any interval in the history of life is having information about the pattern of evolution during that time period. Thus, phylogenies are a prerequisite for any study that aims to adduce evolutionary processes or mechanisms operating at the grand scale. This is because

“the most important connection between (pattern and process) ...

involves the comparison of both intrinsic and extrinsic features of organisms predicted from theories of process, with those actually found in nature" (Eldredge and Cracraft, 1980, p. 4).

Fortunately, a number of phylogenetic hypotheses for different groups are available that can prove useful in teasing apart the nature of the Cambrian radiation. One set of phylogenies comes from Cambrian organisms themselves, specifically trilobites (e.g., Lieberman, 1998, 1999b, 2001a, 2002); these are in many respects the hallmark Cambrian fossils in terms of the abundance and diversity in Cambrian strata. Another set of phylogenies comes from molecular systematic analysis of an early diverging animal group, the phylum Cnidaria (Collins *et al.*, 2006; Cartwright *et al.*, 2008). These phylogenies, taken in concert with the stratigraphic distribution of various cnidarian fossils, can inform us about the evolutionary nature of the Cambrian radiation (Cartwright & Collins, 2007). Finally, our knowledge of metazoan phylogeny, based on molecular systematic analyses of extant phyla, helps us recognize when and how the different parts of the genetic toolkit evolved

(Putnam *et al.*, 2007; Chapman *et al.*, 2010).

THE TEMPO AND MODE OF EVOLUTION DURING THE CAMBRIAN RADIATION

Trilobite phylogeny, biogeography, and the timing of the Cambrian radiation.

The earliest trilobites appear in the fossil record in the latter part of the Lower Cambrian, roughly 525 Ma (Lieberman & Karim, 2010). When they appear it is effectively simultaneously on several different continents. Moreover, from their earliest appearance they show signs of significant biogeographic differentiation (Fortey *et al.*, 1996; Lieberman, 1999a). This early biogeographic differentiation provides cogent evidence that trilobites may have been evolving for some period of time before they actually appeared in the fossil record. A key question of course is how long were trilobites around before their appearance in the fossil record? Phylogenetic biogeographic analysis provides a means of quantifying the duration of this hidden evolutionary history. In particular, phylogenetic biogeographic analysis can be used to determine if

there were any major tectonic events that may have influenced the early evolution of trilobites, through their effects on patterns of speciation. If there is evidence for congruent biogeographic differentiation that might be related to such tectonic events, and further, if these tectonic events can be dated, it provides a minimum age for the timing of biogeographic differentiation and thus evolutionary origins of the group (Lieberman, 2003a).

Phylogenetic biogeographic analysis on basal trilobite lineages suggests that patterns of early trilobite evolution show an episode of vicariance associated with the breakup of a supercontinent that occurred somewhere in the interval 550-600 Ma (Meert & Lieberman, 2004). (The method of biogeographic analysis employed by Meert & Lieberman, 2004 makes it possible to consider patterns of dispersal and does not simply assume vicariance. In the particular case of these Cambrian trilobites no evidence for dispersal was uncovered. Thus, the biogeographic patterns cannot be explained by a subsequent dispersal event that postdated the breakup of the supercontinent. Of course this does not imply that *no* Cambrian trilobites

ever dispersed before, during, or after 550-600 Ma; the biogeographic history of the majority of Cambrian, and other trilobite groups, has unfortunately not yet been investigated in a phylogenetic framework. Still, based on available evidence it does appear that for the key basal trilobite groups congruent dispersal was absent and their diversification was most prominently influenced by vicariance that occurred sometime between 550-600 Ma. Additional information about the biogeographic method used is provided in Lieberman, 2000). This was a time of major geological changes and for a long time it had been generally recognized that there was some association between these and the major biological changes that were occurring, but phylogenetic biogeographic analysis provides a means of more rigorously demonstrating that correlation. In particular and foremost, consider the fact that biogeographic patterns reveal the prominent stamp of vicariance recorded in congruent biogeographic patterns. This suggests that earth history events exerted a significant control on this key episode in the history of life and influenced the early evolution of

a major group of organisms, the trilobites (Meert & Lieberman, 2004). This provides evidence not only that the radiation is in some ways linked to changes in the abiotic environment but that it was the opportunities for geographic isolation that continental fragmentation afforded that helped spur speciation and the radiation (Lieberman, 2003a, b).

A second key aspect of the biogeographic patterns is that they suggest that the origin of trilobites occurred roughly 20-70 million years before their first appearance in the fossil record. Given that trilobites are at least modestly derived euarthropods, and arthropods are in turn a relatively derived bilaterian phylum, it suggests considerable metazoan, and even bilaterian, divergence must have occurred before the start of the Cambrian. The meaning of these results is clear: the Cambrian radiation had a significant fuse (Lieberman, 2003c; Meert & Lieberman, 2004).

What happened during the Cambrian radiation reflects a more general pattern associated with other evolutionary radiations in the fossil record. In particular, many radiations appear in the fossil record fully formed,

and after the bulk of diversification has occurred (Lieberman *et al.*, 2007; Abe & Lieberman, 2009). For instance, the "Cenozoic" radiation of mammals has roots extending tens of millions of years back into the Cretaceous (Archibald, 1996). (Further, pre-Cenozoic mammals are much rarer than their Cenozoic brethren, typically small, and on the whole morphologically homogeneous.) On the surface this could simply imply an incomplete fossil record, but on closer inspection this pattern could actually be revealing something about the nature of evolutionary radiations in general. (Notably, Simpson, 1944 and Eldredge & Gould, 1972 also argued that the relatively rapid appearance of higher taxa or species in the fossil record told us something important about the nature of the evolutionary process.) The very conditions that encourage evolutionary radiations may also make groups less likely to be commonly retrieved as fossils. This gains special meaning when considered in light of punctuated equilibria (Eldredge & Gould, 1972); this theory posits that the very conditions that encourage speciation, rarity and a restriction to marginal environments,

are likely to conspire to make actual speciation events difficult to recover. Perhaps it should not be surprising then that groups undergoing rapid speciation would do so under conditions that make them less likely to become palaeontologically emergent. Once groups do become commonplace and distributed across a range of environmental settings they are likely to fossilize but the engine of evolutionary radiation will mostly be shut off. Further, it is also worth noting that in the case of the Cambrian trilobites, although we may be missing part of their radiation in the fossil record, the signature of that radiation is still preserved.

Tempo of trilobite evolution during the Cambrian radiation.

Information from trilobite phylogenies can also be used to consider how rapidly speciation was occurring during the radiation. It has been suggested (e.g., Gould, 1989) that evolution at this time was operating unusually rapidly, but results from analyses of stochastic models suggest that, at least in the case of trilobites, rates of speciation cannot be statistically distinguished from rates in other groups and at other time

periods (Lieberman, 2001b, 2003c). The rate of speciation was found to be high during the Cambrian radiation in the trilobite groups considered by Lieberman (2001b), but it was not found to be beyond the pale of evolutionary rates witnessed at other times in the history of life. However, an important aspect of rapid evolution is not just the speed with which speciation transpires but also the amount of change that occurs at each speciation event. Indeed, an important aspect of Gould's (1989) arguments about the pace of Cambrian evolution have centered on the amount of morphological change occurring then and the greater genetic flexibility of Cambrian faunas. At least in the case of basal Early Cambrian trilobites for which phylogenetic information exists, however, there does not seem to be any statistical change in the amount of morphological change occurring at speciation events before and after the Cambrian radiation interval (Smith & Lieberman, 1999). This is not to suggest that greater genetic flexibility plays no role in explaining what was unique about the Cambrian radiation, and we will consider this issue

more fully below, but such processes either did not leave their signature upon basal trilobite speciation or the change in genetic flexibility did not occur until some time after the Cambrian radiation.

Metazoan phylogenies and the molecular clock.

Molecular phylogenies of extant metazoan phyla can provide important information with regard to patterns of evolution between phyla and the relative timing of their origination and diversification. By densely sampling representatives from all major phylum (Paps *et al.*, 2009) and applying phylogenomic techniques to sample DNA sequences from hundreds of genes (Dunn *et al.*, 2008; Hejnol *et al.*, 2009), a consensus is emerging regarding the phylogenetic relationships between major metazoan lineages. We summarize this consensus here. In particular, choanoflagellates are the sister taxon to all Metazoa. Acoelomorpha (acoels + nemertodermatids) is the sister clade to Bilateria. Protostomia comprises two major clades: the Lophotrochozoa, which includes molluscs, annelids, flatworms and bryozoans; and the Ecdysozoa, which includes

arthropods, nematodes, tardigrades and kinorhynchs. Within Deuterostomia, the echinoderms and hemichordates form a clade that is sister to the chordates. Less certainty is the relative placement of several early diverging metazoan lineages. For instance, Porifera is generally thought to be paraphyletic (Borchiellini *et al.*, 2001; Medina *et al.*, 2001), although a recent phylogenomic study has recovered a monophyletic Porifera (Philippe *et al.*, 2009). The pattern of divergence between Ctenophora, Porifera and Cnidaria is also controversial. Most molecular phylogenies support Porifera as the earliest diverging lineage (Glennner *et al.*, 2004; Philippe *et al.*, 2009), whereas other recent phylogenomic studies support Ctenophora as sister to the rest of the Metazoa (Dunn *et al.*, 2008; Hejnol *et al.*, 2009). There is little consensus on the placement of Placozoa, although most evidence indicates they are an early diverging metazoan (Dellaporta *et al.*, 2006; Hejnol *et al.*, 2009; Philippe *et al.*, 2009). There exist two conflicting hypotheses on the placement of the parasitic myxozoans: they could be derived cnidarians or the sister to Bilateria (discussed in Evans *et*

al., 2010). Finally, the enigmatic *Xenoturbella* has been placed as the earliest diverging deuterostome (Philippe *et al.*, 2009) or as sister to Acoelomorpha at the base of Bilateria (Hejnol *et al.*, 2009). Over the next few years, through the application of genomic technologies, there will be dramatic increases in molecular sequence data from a diverse sampling of metazoan taxa. These new data will help to resolve many of the remaining questions in metazoan phylogeny.

Detailed molecular phylogenies, in conjunction with the fossil record, can be useful for estimating dates of the origin of major metazoan lineages. It is well documented, however, that molecules do not actually evolve in a “clock-like” fashion and therefore dates from molecular clocks are highly dependent on the model of molecular evolution used and on the fossil calibrations used to mark minimum and maximum time boundaries at multiple nodes on the tree. Cartwright & Collins (2007) reviewed the literature on some of the earliest fossils representatives of major metazoan lineages and Table 1 summarizes some of the key fossil dates. These fos-

sil dates were used as calibration points on relevant nodes of a molecular phylogeny of Metazoa (Cartwright & Collins, 2007). Specifically, the crown group lineages were used to date the minimum age of the clade that includes that fossil taxon. In addition, sponge biomarker, cnidarian stem fossil and bilaterian trace-fossil evidence were used to assign a maximum-age dates (Table 1). A penalized likelihood model that uses a semi-parametric approach to relax the stringency of a clock was employed (Sanderson, 2002). The results of some of the dates recovered in the molecular clock analysis of Cartwright & Collins (2007) are shown in Table 2. Although the dates of these analyses should be viewed with an appropriate degree of skepticism given that they are highly dependent on both the model of molecular evolution and the accuracy of the fossil calibration, it is interesting to note that although metazoan origins are indicated to extend way back (this is likely a problem related to the available choices to root the tree), most of the major metazoan clades (Cnidaria, Deuterostomia, Ecdysozoa and Lophotrochozoa) are predicted to have originated either towards

TABLE 1. Earliest Fossil Representative of Major Metazoan clades

Earliest Fossil representative	Date (Ma) Formation	Stem/ Crown	Reference
Porifera, Silicea	710 (Biomarkers)	Stem	Love <i>et al.</i> , 2006
Porifera <i>Paleophragmodictya</i>	560	Crown	Gehling & Rigby, 1996
Cnidaria	570	Stem	Xiao <i>et al.</i> , 2000
Scyphozoa, Semaestome	500 Marjum	Crown	Cartwright <i>et al.</i> , 2007
Scyphozoa, Coronate	500 Marjum	Crown	Cartwright <i>et al.</i> , 2007
Hydrozoa, Narcomedusae	500 Marjum	Crown	Cartwright <i>et al.</i> , 2007
Hydrozoa, Filifera	500 Marjum	Crown	Cartwright <i>et al.</i> , 2007
Cubozoa, <i>Tripedalia</i>	500 Marjum	Crown	Cartwright <i>et al.</i> , 2007
Ctenophora <i>Fasciculus</i>	500 Burgess	Stem	Conway Morris & Collins, 1996
Bilateria	560	Stem	Narbonne & Aitken, 1990
Arthropoda <i>Anomalocaris</i>	530	Stem	Collins, 1996
Brachiopoda	525 cosmopolitan	Crown	
Urochordate <i>Shankouclava</i>	525 Chengjaing	Stem	Chen <i>et al.</i> , 2003
Chordate <i>Yunnanozoan Haikouichthys</i>	525 Chenjiang	Stem	Chen <i>et al.</i> , 1995; Shu <i>et al.</i> , 1999
Chordata Agnathan	495 many	Crown	

the very end of the pre-Cambrian (late Neoproterozoic) or even in the Early Cambrian. Thus, the Cambrian radiation, according to the molecular clock analyses,

does appear to approximate the time when major metazoan lineages start to appear and/or diversify in the fossil record. This introduces a bit of a disconnect

TABLE 2. Results of molecular-clock analysis for estimated dates of origin of several metazoan lineages from Cartwright & Collins (2007)

Taxon	Estimated date of origin
Metazoa	1147
Choanoflagellates	837
Ctenophores	390
Silicea	710
Cnidarians	570
Bilaterians	560
Deuterostomes	540
Hemichordates	361
Chordates	495
Protostomes	543
Ecdysozoa	530
Lophotrochozoa	537

relative to the trilobite results already discussed, and we are not sure yet how to square these two disparate data sets. In particular, the patterns from trilobite biogeography suggested that this euarthropod clade had begun to diversify by 550-600 Ma. By contrast, the molecular clock results suggest that Ecdysozoa, which is down the tree relative to Euarthropoda, originated at 530 Ma. This discrepancy illustrates the inherent problems with molecular clock analyses.

While these analyses provide insight into the relative timing of the origin of these lineages, there is likely a fair amount of error in the estimation of actual dates, because of the dependency on a model of molecular evolution and accuracy in fossil calibrations. Ultimately, synthesis in science in general, and evolutionary biology in particular, will come not from deciding which result is right, but explaining how and why there are differences between the two.

MEDUSOZOAN FOSSILS AND PHYLOGENIES AND THEIR BEARING ON THE CAMBRIAN RADIATION

Cnidarians are an important metazoan group because of their exceptional diversity, their prominent role in marine ecosystems and their place as one of the earliest diverging animal lineages. Thus, understanding their evolutionary history, and also their distribution in the fossil record, can provide important clues about the nature of evolutionary patterns and processes, especially during the Cambrian radiation interval. Associated with the Cnidarian Tree of Life project (<http://cnidtol.com/>)

there have been significant advances in our understanding of cnidarian phylogeny (McFadden *et al.*, 2006; Cartwright *et al.*, 2008; Collins *et al.*, 2008; Daly *et al.*, 2008; Evans *et al.*, 2008; Bentlage *et al.*, 2010; Nawrocki *et al.*, 2010). A summary of our current understanding of cnidarian relationships is shown in Figure 1. This information, along with newly discovered cnidarian fossils from the Cambrian, can be put together to provide a picture of evolution at this time, and to add to the perspective from trilobites already presented.

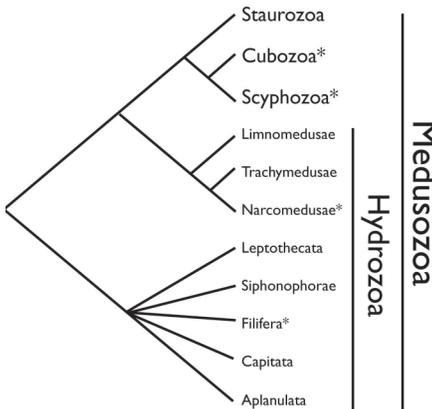


FIGURE 1. Medusozoan phylogeny summarizing our current understanding of cnidarian relationships based on Collins *et al.* (2006), Cartwright *et al.* (2008), Collins *et al.* (2008), and Evans *et al.* (2008). Those taxa with Middle Cambrian fossil representatives are denoted by an “*”.

One difficulty with interpreting early cnidarian fossils, especially those belonging to the Medusozoa, which includes those with a medusae stage (jellyfish) in their life cycle, comprising the classes Cubozoa, Scyphozoa, Staurozoa and Hydrozoa (Daly *et al.*, 2007) is that they are often poorly preserved. Sometimes the “synapomorphy” used to identify a medusozoan basically amounted to “rounded blob”, and often with early putative medusozoan fossils that is all that is visible (Hagadorn *et al.*, 2002). Although such an assignment may in general be valid, it makes it difficult to say much more about these sorts of fossils and especially to determine whether or not they represent stem or crown medusozoans.

Recently, we were fortunate enough to be able to study and describe new and exquisitely detailed Middle Cambrian medusozoan fossils (Cartwright *et al.*, 2007). One of these fossils is shown in Figure 2. These fossils provided enough character data to allow them to be assigned not only to extant medusozoan classes but also to extant orders, families, and in one case a genus. One of these genera, the

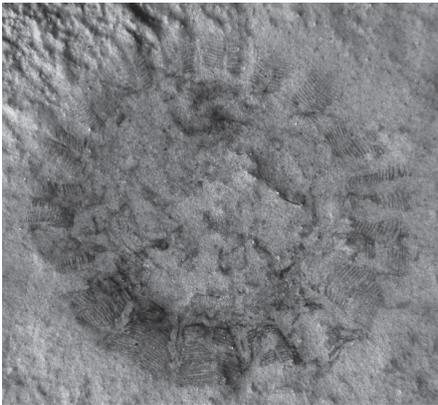


FIGURE 2. Fossil cnidarian identified as a crown group scyphozoan jellyfish that belongs to the extant order Semaestomae. The fossil is from the Middle Cambrium Marjum Formation (approximately 500 million years old) in Utah, U. S. A.; see Cartwright *et al.* (2007) for additional details.

cubozoan *Tripedalia*, today has an advanced visual system and complex reproductive behavior. Although these structures are not visible on the Cambrian fossils, phylogenetic evidence indicates that the character complexes associated with these would have also originated back in the Cambrian.

In Figure 1, the medusozoan taxa that have Middle Cambrian fossil representatives are depicted with an asterisk. As illustrated in this figure, nearly the entire breadth of medusozoan phylogenetic diversity was pre-

sent by the Middle Cambrian. This implies that not only all of the extant medusozoan higher taxa, but even many of the extant orders and perhaps families and genera had begun to diversify by the Middle Cambrian, shortly after the Cambrian radiation. The implications seem clear, and are akin to what was discovered with the trilobites: it is likely that the early Cambrian represents an interval of rapid diversification.

The record from fossils and phylogeny is also informative about the nature of Cambrian ecosystems. For a long time it was thought that these were relatively simple, but the presence of a diverse variety of pelagic organisms provides a cautionary tale. Today jellyfish are dominant predatory forms (and also prey items) in open ocean environments. Their presence and diversity back in the Cambrian suggests that these environments were occupied; furthermore, there must have been prey for the jellyfish to feed on in the pelagic environments. It appears that Cambrian ecosystems were not as simple as once thought and in particular pelagic ecosystems were occupied by a diverse array of taxa.

ANCESTRAL GENETIC TOOLKITS AND EVOLUTION- ARY DEVELOPMENT IN RELATION TO THE CAMBRIAN RADIATION

Recently, entire genomes have been sequenced from a diverse array of metazoan taxa. Most notably for our discussion here, the first complete, assembled genome from the sea anemone *Nematostella vectensis* (Putnam *et al.*, 2007) and the hydrozoan *Hydra magnipapillata* have been published (Chapman *et al.*, 2010). In addition, genome sequencing projects from the demosponge *Amphimedon queenslandica*, the placozoan *Trichoplax*, the ctenophore *Mnemiopsis leidyi* and the coral *Acropora millepora* are expected to be released in the near future. Comparisons of cnidarian genomes with those of bilaterians have revealed unexpected insights into the genetic makeup of early-diverging animals. Prior to the availability of genomic data in non-bilaterian animals, it was thought that many of the complex, signaling pathways were unique to vertebrates, because the model organisms *Drosophila* and *Caenorhabditis elegans* lacked these genes. However, it is now known that

both *Hydra* and *Nematostella* possess a complex genome that contains many developmental regulatory genes/gene families previously thought to be specific to vertebrates (Ball *et al.*, 2004; Kusserow *et al.*, 2005; Technau *et al.*, 2005; Ryan *et al.*, 2006; Chapman *et al.*, 2010) (meaning that these genes were lost in *Drosophila* and *Caenorhabditis*). Prominent signaling pathways shared between bilaterians and cnidarians include Hedgehog, the receptor for tyrosine kinase, Notch, transforming growth factor-B and Wnt (Technau *et al.*, 2005; Chapman *et al.*, 2010). Thus, the ancestor to cnidarians and bilaterians must have been equipped with a diverse genomic toolkit necessary for the specification of complex body plans. It is likely that before the evolution of multicellular animals, many of these genes were performing entirely different functions, and were subsequently co-opted for signaling the development of complex and diverse metazoan body plans. For example, the cellular adhesion gene family cadherins that is important for mediating cell-cell signaling in metazoans, is present in single celled protists such as the choanoflagellates (King *et*

al., 2003; Abedin & King, 2008). The increased availability of genomes from other early-diverging lineages will allow for a more precise reconstruction of the metazoan ancestral genome. Thus far, evidence from genomics indicates that the ancestral metazoan genetic toolkit was complex and enabled the rapid diversification of body plans during the Cambrian radiation.

Although the complex metazoan ancestral genomic toolkit can in part explain rapid diversification of animal body plans, it cannot explain why these body plans appear to become more canalized through time. Peterson *et al.* (2009) proposed that the evolution of microRNAs, because of their key role in transcriptional regulation, may explain the increasing morphological conservatism of body plans through time. Specifically, Peterson *et al.* (2009) note that the evolution of additional microRNA gene families through time means that development becomes more tightly regulated. While this hypothesis is conceivable, it seems a bit premature as very little is known about the role of the diverse microRNA families that exist in metazoans. In addition, although microR-

NA gene families become more diverse through time, the total number of microRNAs does not. That is, there is no correlation between the number of total microRNAs and morphological complexity in extant metazoan taxa. For example, Peterson *et al.* (2009) reports that the sea anemone *Nematostella* has 29 total microRNAs, whereas mouse has only 16. Moreover, pattern and process could be conflated in Peterson *et al.*'s (2009) argument because of the existence of a "left wall" *sensu* Gould (1996). That is to say, the number of microRNA families start out low and through time has to increase because the only direction available for change is for the number to increase (gene families that went extinct and were eliminated could not be sampled). This argument was originally developed by Gould (1996) to explain why apparent biological complexity increases through time. However, any time there is a trend that occurs in a system that involves an originally minimal value that increases through time, one has to be careful not to prematurely invoke a driven trend; the pattern could just involve a random walk, with passive diffusion away from a re-

flecting barrier or minimum value. Moreover, diversification of gene families through time is not unique to microRNAs. Hox genes also show gene duplications and diversification in many separate lineages. (The same “left wall” argument might explain some of these patterns as well.) In summation, it is likely that there is no single explanation for the canalization of body plans in metazoans, but instead it was due to a number of complex changes both in the genomes themselves and in the regulations and interactions amongst the different signaling pathways.

CHANGES IN THE ABIOTIC ENVIRONMENT AND THE TIMING OF THE RADIATION

As we have already described, there is some evidence that changes in the abiotic environment at least partly triggered the Cambrian radiation. In particular, the geological changes at the end of the Proterozoic associated with the fragmentation of a supercontinent expanded the opportunities for vicariant differentiation and allopatric speciation, thereby increased the tempo of evolution at the time (Lieberman, 2003a; Meert

& Lieberman, 2004). These were not, however, the only profound set of environmental changes transpiring at the time. During the very end of the Proterozoic there were also a series of major climatic changes, informally grouped under the rubric of the Snowball Earth (Hoffman *et al.*, 1998). There may have been as many as four episodes when the Earth experienced near total glaciation, being completely encased in ice; the intervening intervals also witnessed extreme environments as the ice melted away only to be followed by episodes of near broiling warmth where global temperatures hovered at close to 50 °C. Given the inhospitable environments, at least for large multicellular organisms, it may be no surprise that it was only after these conditions ameliorated that such organisms evolved (Hoffman *et al.*, 1998; Knoll, 2003). In this case, environmental conditions might well have served as a check on evolution, with environmental moderation creating fodder for evolutionary change.

Another critical aspect in the abiotic environment and evolutionary equation, at least regarding large organisms, are oxygen concentrations. These

seem to have been generally increasing towards the end of the Proterozoic, perhaps then reaching 10% of present atmospheric levels (Fike *et al.*, 2006; Canfield *et al.*, 2007; Li *et al.*, 2010). This might have been an important threshold especially for the generation of key proteins found in many organisms, such as collagen, and also facilitating the building of rigid exoskeletons that make organisms more likely to fossilize (Schopf & Klein, 1992; Bengtson *et al.*, 1994). It also would have facilitated the evolution of large body size because of the issue of surface area to volume constraints (see Bonner, 1988). Again, these environmental changes involve removing a constraint that would have kept a lid on the evolution of animals. The changes do appear to have occurred some time before animals actually are found in the fossil record. Thus, the changes might not have precipitated evolution instantaneously. However, it is worth adding that this difference in timing lessens when one takes into account the evidence from trilobites, which indicates evolution might have significantly preceded first appearance in the fossil record.

In addition, the biotic environment as manifest in ecological interactions in the Proterozoic world would have been impoverished relative to those that prevail today, or even relative to those that prevailed by the late Cambrian. There is some general sense that minimal competition early in the history of animal life in the Proterozoic might have served to facilitate the evolution of animals early on. As competition inevitably increased with increasing diversity in the Phanerozoic, evolution might have later become constrained. However, the evolutionary mechanisms whereby these ecological differences would become translated, specifically from the ecological to the genealogical hierarchies, are not as yet clear and must remain nebulous at this time (Lieberman, 2008).

CONCLUSIONS

Unravelling the patterns and processes involved in the Cambrian radiation is one of the grand challenges in evolution because these events occurred rapidly and in deep time. A comprehensive understanding of the origin and diversification

of major metazoan lineages will likely come from the integration of several fields of inquiry, including a careful study of diversification in the fossil record, detailed paleobiogeographic analyses, paleoecological studies, molecular phylogenetic studies, and studies of genomics and evolutionary development. We predict that from these disparate lines of evidence a macroevolutionary synthesis will emerge where paleontology, phylogenetics and evolutionary development are the key areas of study for understanding this important episode (as well as other important episodes) in the history of life.

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