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Review

Cite this article: Wray GA. 2015 Molecular clocks and the early evolution of metazoan nervous systems. *Phil. Trans. R. Soc. B* **370**: 20150046.

<http://dx.doi.org/10.1098/rstb.2015.0046>

Accepted: 29 September 2015

One contribution of 16 to a discussion meeting issue 'Origin and evolution of the nervous system'.

Subject Areas:

evolution, neuroscience, palaeontology, genomics

Keywords:

molecular clock, divergence time, timetree, nervous system evolution, metazoan evolution, Cambrian explosion

Author for correspondence:

Gregory A. Wray
e-mail: gwrays@duke.edu

Molecular clocks and the early evolution of metazoan nervous systems

Gregory A. Wray

Department of Biology and Center for Genomic and Computational Biology, Duke University, Durham, NC 27708, USA

GAW, 0000-0001-5634-5081

The timing of early animal evolution remains poorly resolved, yet remains critical for understanding nervous system evolution. Methods for estimating divergence times from sequence data have improved considerably, providing a more refined understanding of key divergences. The best molecular estimates point to the origin of metazoans and bilaterians tens to hundreds of millions of years earlier than their first appearances in the fossil record. Both the molecular and fossil records are compatible, however, with the possibility of tiny, unskeletonized, low energy budget animals during the Proterozoic that had planktonic, benthic, or meiofaunal lifestyles. Such animals would likely have had relatively simple nervous systems equipped primarily to detect food, avoid inhospitable environments and locate mates. The appearance of the first macropredators during the Cambrian would have changed the selective landscape dramatically, likely driving the evolution of complex sense organs, sophisticated sensory processing systems, and diverse effector systems involved in capturing prey and avoiding predation.

1. Introduction

Knowing when something happened is essential to understanding history. In studying the history of life we have two, and only two, sources of information upon which to estimate the timing of events: the rock record and the genomic record [1,2]. Although both have important limitations, each record presents unique and invaluable information about the history of evolutionary events. Importantly, combining information from the rock and genomic records can lead to insights that might not be clear from either in isolation.

This brief review examines what we know about the timing of very early events in animal evolution and what this understanding implies about the evolution of early nervous systems. The preponderance of molecular evidence, in conjunction with the trace and body fossil records, indicates an extended interval of animal evolution prior to the Ediacaran and perhaps even earlier, followed by an ecologically-driven revolution in animal morphology during the Cambrian. This framework, in turn, suggests two major phases in the early evolution of nervous systems: an initial phase involving relatively simple structure and a later one involving the parallel appearance of more elaborate sensory, processing and effector systems.

2. Using timetrees to understand the history of life

Cladograms provide an indispensable analytical framework for understanding evolutionary history, by allowing one to reconstruct the most likely polarity of character state transformations, patterns of gains and losses and also parallel transformations. Assigning dates to each node converts a cladogram into a timetree, allowing one to address several additional questions of considerable importance [2,3].

The most basic question is when a trait was gained or lost. For instance, when did neurones, ganglia and brains evolve? One can set upper and lower temporal bounds on gains and losses based on the divergence times of the two nodes that bracket it (figure 1a). Another basic question is whether character state transformations in two different lineages happened at about the same

Figure 1. Reconstructing the timing of evolutionary events. Divergence times among extant taxa provide dates for nodes in a cladogram. These, in turn, provide lower and upper bounds on when a trait of interest changed. (a) The arrowheads indicate the earliest and latest time of appearance for the red trait. Even if these two bracketing divergence times were known exactly (which is never the case), the length of the internal branch limits how precisely the time of trait origin can be estimated. (b) The earliest and latest appearance of the red trait and the blue trait do not overlap. This suggests that they evolved at separate times and further that a common extrinsic cause is improbable. (c) The quantitative change of interest took at least as long as the two closely connected arrowheads, but no more time than the widely connected arrowheads. See S2 for details and some important caveats.

time (figure 1b). For instance, did the image-forming eyes of arthropods, molluscs and vertebrates evolve at about the same time, perhaps in response to a common external event? This can be informative when studying parallel or convergent changes in traits of interest. Finally, one can ask how long a complex transformation took (figure 1c). This can be useful when considering the evolution of a quantitative trait or a trait complex composed of individual components.

As an example, how long did it take for the human brain to increase in size from its ancestral state?

For each of these three basic kinds of questions, one can also relate when the character of interest changed relative to other datable events, be they biotic or abiotic. This can be useful when testing hypotheses about ecological factors that may have influenced the origin or loss of a trait. For instance, did the bilaterian ancestor live during a time of particularly

low atmospheric oxygen? Abiotic changes can usually be dated much more precisely than divergence times among lineages, making the evolutionary event often the limiting factor in hypothesis testing.

In all cases, divergence times are used to bound the event or events of interest. This process is analogous to, but generally much less precise than, the process of using overlying and underlying strata that can be dated radiometrically to assign an absolute age to a fossil in sedimentary rock that cannot be dated directly.

Although straightforward in principle, the practical application of these methods of historical inference is subject to several sources of uncertainty. Perhaps, most importantly, each divergence time is associated with uncertainty in estimation, often expressed in terms of confidence intervals or a range of independent estimates. Bounds on the timing of appearance or loss of a particular state can then be expressed in terms of uncertainties in the divergence time estimates. The rather imprecise nature of divergence time estimates means that, in practical terms, only relatively large differences can be confidently assumed to have occurred asynchronously. Another source of uncertainty comes from the topology of the cladogram, which is never known with certainty. Finally, any reconstruction of evolutionary history depends on accurate identification of homologous traits or genomic features among taxa.

3. The problem of the earliest animals

When did animals first appear? The first steps towards answering this question came from the work of geologists in the early nineteenth century, who used fossils to classify rock formations and identified the oldest rocks bearing the remains of animals, which they designated the Cambrian period (for reviews, see [4,5]). One might have naively imagined that these oldest earliest fossiliferous layers would contain the remains that were quite simple in form or clearly ancestral to later groups of animals. But that was not the case. The earliest rocks that bore any recognizable animal fossils contained representatives of several different phyla, including arthropods, molluscs, brachiopods and echinoderms—all of which were rather complex and specialized.

Although what nineteenth century geologists referred to as the Cambrian era has since undergone considerable revision, one important fact has not changed: rocks older than the Cambrian contain very few bilaterian fossils [6,7]. Darwin was famously aware of this seemingly sudden origin of diverse animals in the fossil record and considered it among the most serious objections to his theory of natural selection [8]. He proposed that imperfections of the fossil record were responsible for the abrupt appearance of animal fossils, and that an extended interval of animal evolution remained to be discovered.

The next key event in understanding the origin of metazoans was the discovery of the soft-bodied fauna of the Burgess Shale by Charles Walcott in 1909, a story made famous by Stephen Jay Gould in his book *Wonderful life* [9]. The Burgess Shale provided nearly everything that was known about the soft parts of Cambrian animals for the next half century. Importantly, the exquisite preservation of the Burgess Shale made Darwin's problem even more acute in two ways. First, plausible representatives of several additional, entirely soft-bodied phyla were found to be present, including sponges, cnidarians, annelids, chaetognaths and sipunculids. This more

than doubled the number of phyla that appear abruptly for the first time in the fossil record during the Cambrian [10]. Second, these earliest known soft-bodied animals were still not appreciably less complex than their modern descendants.

So striking and complex were these earliest known metazoans that Walcott formalized Darwin's notion of a missing interval of the rock record, naming it the Lipalian era [11]. This concept was plausible at a time when the only method for dating rocks involved their relative ages based on superposition with a rock formation. Younger strata lay above older strata (with unusual and recognizable exceptions), while index fossils could be used to correlate the age of rocks at different localities. But there was no way to measure the absolute age of rocks, making it impossible to ascertain whether there ever had been a Lipalian era. That all changed when radiometric dating became practical during the 1950s. With the means to assign absolute dates to many strata, it became clear that the Lipalian era did not exist and that rocks from the Ediacaran era, which lack fossils of unequivocal metazoans, transition directly into those of the Cambrian [12].

4. The concept of a Cambrian explosion

If the Lipalian had disappeared, the question of when the first animals evolved had not. A variety of often puzzling fossils were found in pre-Cambrian rocks, and some proposed as early metazoans [13], but even the strongest contenders were not compelling. A turning point came in 1948 with the publication of an influential article by Preston Cloud, where he introduced the term eruptive evolution to describe 'a relatively sudden breaking out of evolutionary diversification' [14]. He argued persuasively that the sudden appearance of animal fossils during the Cambrian recorded a real event. Cloud regarded the term explosion as 'not an altogether felicitous designation' because the Cambrian diversification was 'in process during millions of years and likely did not make a big noise'. Cloud considered several alternative explanations to sudden appearance and one by one dismissed them. These ranged from missing information based on limited rock exposure to biomechanical considerations of the plausibility of entirely soft-bodied versions of arthropods and brachiopods that might have escaped fossilization. Cloud concluded that a prolonged, cryptic evolutionary history of animals was unlikely, and proposed instead that the precursors to the modern phyla diverged rapidly just before and during the Cambrian.

Cloud's conclusions were reinforced by the reanalysis of Walcott's original Burgess Shale material during the 1970s and 1980s by Harry Whittington and his graduate students Derek Briggs and Simon Conway Morris. As detailed by Gould in *Wonderful life* [9], these palaeontologists brought their considerable talents to bear on reconstructing and reinterpreting several rather odd animals that Walcott had misunderstood or largely ignored. Among many of the important results that emerged from these efforts was an even sharper appreciation for the disconnect between the obviously bilaterian animals of the Cambrian and fossils from earlier rocks.

The discovery of additional Lagerstätten (sites of exceptional preservation) from somewhat earlier during the Cambrian than the Burgess Shale [10] bolstered Cloud's view. These sites have yielded many astonishing and beautiful animal fossils. Many show clear affinities to fossils from the

Burgess Shale. Importantly, they are just as morphologically complex and taxonomically diverse despite coming from slightly earlier deposits. As a consequence, these amazing discoveries have not added much towards understanding what the very earliest metazoans looked like.

Cloud's idea that one should read the early animal fossil record literally has proved remarkably resilient. Despite more than 60 years of searching in the most promising locations, very few pre-Cambrian fossils are unambiguously the remains of bilaterians. Strikingly, it is not just body fossils and sclerites that appear abruptly during the Cambrian. The trace fossil record shows a similarly sudden set of transitions, from virtually non-existent to simple traces during the Ediacaran to highly complex traces during the Cambrian [6]. This concordance between body and trace fossil records is an important reason why many palaeontologists half a century later continued to support Cloud's view that animals originated and diversified just before the Cambrian [6,15–18].

5. Challenges to orthodoxy

The orthodox view has always had its sceptics, however. For a start, an argument based on negative evidence is inherently problematic. The exceptional preservation of the Cambrian Lagerstätten is almost unique. Many animal groups appear, for the first time, in the fossil record during the Cambrian and then abruptly disappear for the next 500 million years, even though they survive today (e.g. ctenophores, sipunculids, onychophorans and tardigrades). Clearly, diverse and complex animals can be present and not leave any body fossils for long intervals of time. One wonders what might yet be discovered in Burgess-type preservation in rocks of Ediacaran age.

The absence of trace fossils that can unambiguously be attributed to bilaterians prior to the Cambrian means that the negative evidence needs to be taken seriously [6]. This argument rests, however, on the assumption that early animals were large-bodied and would have burrowed into or ploughed across the sea floor. Some traces from rocks prior to the late Ediacaran have been attributed to metazoans or even bilaterians [19–21], but these are now widely discounted as artefacts [15,22,23]. The only generally accepted bilaterian traces from the pre-Cambrian are associated with *Kimberella*, and show likely feeding marks [24].

The most persuasive evidence would be finding an unambiguous animal fossil from before the Cambrian. For several decades, the strongest contenders came from the Ediacaran fauna of Namibia and Australia. Early proposals that these odd fossils were members of extant metazoan phyla (e.g. [25–29]) met with scepticism, and several other interpretations have been put forward (e.g. [30–33]). The pendulum has since swung in the other direction, and a few pre-Cambrian fossils are now accepted as likely animals by many palaeontologists. Most appear to belong to stem lineages or to basal metazoan phyla [34–37]. However, a few Ediacaran body fossils may be bilaterians, including the lightly skeletonized forms *Cloudina* [38] and *Namacalathus* [39] and a possible ascidian, *Burykhia* [40], all of which make their first appearance during the latest Ediacaran. However, the strongest pre-Cambrian contender for a true bilaterian is *Kimberella*, which has been proposed as a stem or crown group mollusc [24]. Finally, the tiny, soft-bodied organisms in Doushantuo Formation (635–551 Ma) appears to include embryos that may be

metazoan in origin [41–44], although this interpretation remains controversial [22,45,46].

Unlike the fossils of the Cambrian Lagerstätten, which unambiguously contain the remains of more than a dozen different bilaterian phyla, *Kimberella* is the only widely accepted bilaterian present during Ediacaran. The very different mode of preservation as impressions, usually in fairly coarse sediments, makes the Ediacaran material much more difficult to interpret. If, however, we accept that some of the Ediacaran taxa are metazoans, or even bilaterians, the body and trace fossil records indicate that the origin of animals lies at least several tens of millions of years before the base of the Cambrian and more than 50 Myr before the famous Cambrian Lagerstätten (figure 1).

6. Molecular estimates for the origin of animals

Beginning in the 1960s, it became possible to bring another, completely independent, record to bear on the problem of when animals evolved. The concept of a 'molecular clock' originated with Zuckerkandl and Pauling [47] and Margoliash [48], who noted that amino acid substitutions in a particular protein are roughly linear with time.

The earliest application of molecular clocks to date divergence times among major groups of animals were made by palaeontologists who took advantage of some of the first comparative molecular sequence data to become available, that of globins [49,50]. Both of these early studies used a straightforward approach: they calibrated the rates of protein divergence using the fossil record of vertebrates to establish divergence times, then estimated divergence times between phyla based on the assumption of an approximately constant rate of amino acid substitution. The results in both cases implied that divergences among bilaterian phyla took place hundreds of millions of years before the Cambrian. These studies did not attract much attention, in part because they were based on limited data and in part because the surge in interest in the evolution of animal body plans did not begin until the late 1980s.

That increased interest was triggered by the confluence of two events. One was the discovery from the late 1980s–1990s of a common genetic 'toolkit' for patterning the body plans of phylogenetically distant and morphologically disparate animals [51]. This came as a major surprise: at the time there was no reason to expect that the genetic basis for animal body plans would be similar in different phyla [52]. The second event was the publication of Gould's *Wonderful life* [9], which revived interest in the Cambrian explosion and argued persuasively that it constituted an exceptional period of body plan evolution. This immediately raised the question of a causal relationship: Did the evolutionary origin of the 'metazoan toolkit' trigger the diversification of animal body plans?

By the mid-1990s the amount of sequence data available for estimating divergence times had increased considerably. In 1996, along with my colleagues Jeff Levinton and Leo Shapiro, I published an analysis based on several genes [53] that also obtained divergence times long before the Cambrian. While this confirmed, on a qualitative level, earlier molecular clock results [49,50], the estimated data for the protostome–deuterostome split was even older, predating the Cambrian by half a billion years. We concluded that 'The genetic regulatory apparatus that is so strikingly and extensively shared by protostomes and deuterostomes must also have evolved long

Figure 2. Published estimates of the protostome–deuterostome divergence time. The middle of the range of divergence time estimates from several publications are shown. The timeline across the bottom highlights the Cryogenian (Cr), Ediacaran (Ed) and Cambrian (C). The presence of body and trace fossils is indicated directly above: dark colours signify fossils that are unambiguously of metazoan origin and pale colours ones of possible metazoan origin.

before the Cambrian. The antiquity of these genetic regulatory circuits suggests that their appearance was not sufficient to trigger the morphological diversification that occurred during the Cambrian’.

There quickly followed a flurry of publications, some challenging and others supporting the idea of protracted history of animal evolution prior to the Cambrian (figure 2). Using a variety of methods and drawing upon diverse sets of gene sequences, these studies produced a notably broad range of divergence time estimates. Bilaterian divergence time estimates, for instance, ranged from 580 [54] to >1000 Ga [55]. Despite this wide range, mean divergence time estimates in all the studies were consistently older than the base of the Cambrian. Given the uncertainties associated with estimating divergence times, the credible intervals of the shallower estimates overlapped the Cambrian. Nonetheless, the best estimates by all methods were deeper and the credible intervals from many of the studies did not approach the base of the Cambrian.

7. Towards better rate estimates

The lack of a clear consensus about divergence times among animal phyla had an important positive outcome: it prompted the development of better methods for modelling and estimating rates of sequence divergence. With studies producing divergence time estimates that differed by more than two-fold, there was clearly a pressing need for better analytic approaches. Two basic problems were clear from the outset. The first is that the rates of sequence substitution are not in reality clock-like, and can instead vary considerably over time and among lineages [56]. The other problem is that the only calibration points that can be used to understand divergence times among animal phyla come from within phyla. This necessitates extrapolating from shallower, known divergence times in order to estimate the deeper ones of interest.

Efforts to improve the methods for divergence time estimation have focused on three areas: improved sampling of genes and taxa, better use of calibration points and better models of substitution rates. The following brief review of these advances provides a sense of the added rigour that is now possible in dating divergence times with sequence data.

The most basic improvements have come from simply increasing sample size [57]. Basing estimates on many genes helps to reduce the effects of idiosyncratic rate variation [53,58]. A complementary approach is to use sequences from many extant taxa, which can provide mean rather than point rate estimates [58,59]. With the genome sequences of hundreds of relevant species now available, datasets with many genes and broader taxon sampling improvements is increasingly feasible. Even when specific sequences are missing for some species, it is generally better to include more genomic regions [60]. Computational efficiency becomes enormously important with large datasets, and here, too, methods have improved considerably [60,61].

A second important methodological improvement has come from implementing more sophisticated calibration methods as well as best practices in applying them. Because the fossil record is incomplete, calibration points are systematically biased towards underestimating true divergence times. Modelling uncertainties in calibration points is an effective solution [62,63]: the first known appearance is set as a latest possible date for the divergence time, and a range is extended earlier from that date based on the likely missing record, or ‘ghost lineage’. Rock exposure, subsequent gaps in coverage and other empirical evidence can be used to take a principled approach to estimating the missing record [7]. Several methods have been developed to incorporate the resulting soft calibrations [64–67]. Another important improvement has come in best practices for choosing calibration points. Methods that test for consistency among calibration points allow one to identify outliers that can strongly bias divergence time estimates [68,69]. Simulation

studies indicate that deeper calibration points increase precision relative to shallow ones when extrapolating [70].

Third, and perhaps most fundamentally, there are several improvements in modelling the process of sequence divergence. Even early molecular clock studies allowed for multiple substitutions at a site by modelling sequence divergence as a Poisson process [71]. Other significant improvements have involved relaxing the assumption of rate constancy across sites using a gamma distribution and modelling rate heterogeneity across branches using likelihood and Bayesian approaches [66,72,73]. These improvements can reduce systematic biases in divergence time estimates [74]. Using an accurate model for sequence divergence is also important [75,76].

Based on some of these improvements, several studies have re-analysed the results of earlier analyses in an effort to understand why they produced such varying divergence time estimates. It is now clear that some studies arrived at divergence time estimates using flawed assumptions or approaches, providing some important lessons. For instance, allowing rates to vary among branches is clearly important [77], but it is equally important not to impose assumptions about the distribution of rate variation or its direction [56]. Employing many calibration points provides another big improvement, particularly if these include deeper nodes (i.e. as close as possible to the unknown divergence time that is being estimated). Not imposing hard maximum bounds on calibration points is particularly important [63,78], and instead using minimum and soft maximum constraints [79]. Also important is using cross-validation to identify calibration points that are inconsistent with the rest, and which can have a disproportionate impact if they are among the deeper calibration points [80].

These and other lessons have brought improvements in rigour and accuracy to the process of estimating divergence times among metazoan phyla, in much the same way that advances in methods of phylogenetic inference have continued to improve our understanding of phylogenetic relationships among metazoans. The four studies that obtained the shallowest protostome–deuterostome divergence times [54,59,81,82] are all likely to be significant underestimates for technical reasons that are now better understood [56,69,77,78]. Some of the studies that obtained very deep divergence time estimates [53,55] were based on approaches that either do not allow for the rate variation among branches or used unrealistic sequence divergence models, and are consequently likely to be significant overestimates [54,74,83]. Most of the other studies have not been formally re-examined but also used methods that we now understand to be flawed in one way or the other. Even the most recent studies [59,84], however, did not take full advantage of improvements in methodology. Thus, there remains an appreciable scope to produce better estimates of key divergence times in metazoan evolution based on sequences from many genes, inclusion of many taxa, improved knowledge of calibration points, more robust methods for estimation and best practices in data analysis [57].

For the moment, the best we can do is consider what the genomic record can tell us based on published studies, while bearing in mind what we have learned about sources of error in estimating divergence times. Taken together, the current molecular evidence points to the origin of metazoans and bilaterians well before the first appearances of definitive body fossils during the Cambrian. The bulk of the evidence suggests that both of these key divergences occurred during

Figure 3. Divergence time estimates and nervous system evolution. The basic components of eumetazoan nervous systems such as neurones and synapses likely share a single common origin (light blue dots). (The possible exception of ctenophores is not directly relevant here.) The fossil record suggests rapid divergences among bilaterian phyla during or just before the Cambrian (left). This scenario is compatible with, but does not require, a common origin for complex sense organs and central processing systems (purple dot). The molecular record, in contrast, suggests a prolonged interval of pre-Cambrian bilaterians (right). During this time, nervous systems may have been relatively simple (light blue shading) for reasons explained in the text. With the appearance of macropredators during the Cambrian, functional demands would have shifted dramatically, giving rise to parallel origins of complex nervous systems (multiple purple dots). Ch, chordates; Ec, echinoderms; Ar, arthropods; Mo, molluscs; Cn, cnidarians; Po, sponges.

or perhaps even before the Cryogenian, very roughly between 700 and 900 Myr. Indeed, at this point it requires special pleading to propose based on molecular evidence alone that the origin of either metazoans or bilaterians coincides with first appearances in the fossil record (figure 3, left). Instead, the molecular evidence is consistent with a prolonged period of at least tens, and more likely hundreds, of millions of years when metazoans were present but left no unambiguous trace in the fossil record (figure 3, right).

8. Reconstructing early metazoans

What did Cryogenian metazoans look like, and what kind of nervous systems did they possess? Piecing together information from the fossil and genomic records suggests some provisional traits. One can begin by enumerating features shared among all metazoans, or among subclades such as bilaterians, and then drawing conclusions based on biological function (e.g. [85,86]). For instance, the universal presence of collagen and other extracellular matrix proteins in eumetazoans is generally interpreted to mean that multicellularity arose just once in metazoans [87]. The presence of shared proteins involved in specialized cellular junctions in eumetazoans similarly suggests that the eumetazoan ancestor had polarized epithelia and some degree of ionic coupling between cells, and that these features also share a single origin. Of course, proteins can change their detailed functions as seen, for instance, with the co-option of synaptic proteins from unicellular organisms that clearly do not contain neurones and synapses [88,89]. Nonetheless, with appropriate consideration of phylogenetic

and functional context, it is possible to distinguish cases where protein function has been significantly modified from those where it points to a shared trait of interest [85].

Much has been written about a shared ‘toolkit’ of genes encoding the regulatory machinery of bilaterian development (e.g. [90,91]). Some of its components clearly originated outside the Bilateria but acquired unique roles or domains or expanded significantly within metazoans. Other components, some cell signalling systems in particular, appear to be unique to Bilateria. Less clear is what this shared toolkit implies about the traits of the bilaterian ancestor, sometimes called Urbilateria (for a range of perspectives, see [91–95]). A conservative list of features, supported by both genomic and anatomical comparisons, includes a well-defined antero-posterior axis with differentiation into cephalic and trunk regions, a dorsoventral axis, three distinct germ layers, and mesodermally derived muscles. In terms of nervous system organization, the list would also include neurones and neural circuits, including photo- and chemosensory neurones, interneurones, and synapses and neuromuscular junctions. Some authors have gone further, proposing that the bilaterian ancestor possessed a brain with specific circuitry that is reflected in extant protostomes and deuterostomes [93,96,97].

If we accept the divergence times implied by molecular dating, we can add features to the bilaterian ancestor based on the pre-Cambrian fossil and geochemical records. First, and perhaps most obviously, early bilaterians would have lacked a mineralized skeleton. Even microscopic metazoan skeletons would have left a trace, as clearly demonstrated by the extensive pre-Cambrian fossil record of unicellular eukaryotes with skeletons composed of calcium, phosphate and silica. To the extent that skeletons were present, they would likely have been composed of carbohydrates like chitin. For reasons discussed in §§10 & 11, such skeletons would likely have served a role in locomotion rather than defence. Second, early bilaterians probably had very low energy budgets. For most of the Cryogenian and Ediacaran, dissolved oxygen was evidently present at a fraction of its current level in most marine habitats [98,99], likely placing severe constraints on metabolism and feeding modes [100]. Predation, in particular, was not a common feeding mode, consistent with the lack of mineralized skeletons. Third, early bilaterians were probably small, perhaps with body sizes on the scale of one to a several millimetres. Small body size seems likely based on low available oxygen [99] and the paucity of trace fossils that are unambiguously attributable to bilaterians prior to the Cambrian [6].

9. Pre-Cambrian metazoan lifestyles and nervous systems

The characteristics of early metazoans just outlined are compatible with a fairly limited range of ecological and life history possibilities. One possibility is a holoplanktonic life cycle. Many living metazoans from diverse phyla have holoplanktonic life cycles with delicate adults that would not fossilize well (e.g. cnidarians, ctenophores, chaetognaths, salps, appendicularians and pterobranch molluscs). Had tiny, holoplanktonic bilaterians lived during the Cryogenian, we would not necessarily expect to find them in the fossil record. A second possibility is that pre-Cambrian metazoans crept about on the surface of the microbial and algal mats that likely coated the sea floor wherever light penetrated [32], either grazing on them or subsisting on energy

generated by zooxanthellae (symbiotic algae). In either case, these mats would have been thicker than the body size of the early metazoans, and thus would have effectively prevented the formation of trace fossils. A third possibility is that early metazoans were meiofaunal. Twenty phyla contain species that are meiofaunal, and extant meiofaunal communities can contain species from more than a dozen phyla. Adult body sizes in meiofaunal communities are in the range of about 0.05 to 1 mm, which is often smaller than the size of the sediment grains they inhabit. These creatures subsist today on algae, microbes and decaying organic material, a situation not unlike that which would have been present beneath the microbial/algal mats of shallow pre-Cambrian oceans.

These are not incompatible possibilities, and indeed Cryogenian and Ediacaran metazoans may well have included all three lifestyles. But what do these scenarios imply about the nervous systems of early metazoans? The functional demands in all three cases are fairly modest. Certainly sensory systems coupled to effectors would have been important for locating food and mates, and for avoiding unsuitable habitats. The two most important sensory modalities would likely have been physical and chemical: the ability to detect physical contact or flow change and the ability to distinguish diverse chemical cues. Responses to these inputs would generally have been mediated on short timescales through movement and on longer timescales through physiological changes, gamete maturation and production of stress response molecules. Photosensing was likely much less important to Cryogenian than later animals, and was perhaps limited to orientation, establishing circadian rhythms, and locating appropriate habitats. These tasks could have been accomplished with isolated photoreceptor cells embedded in the ectoderm, and would likely not have required image-forming eyes or sophisticated sensory processing systems. Since most bilaterians have directionality of movement, however, some degree of cephalization seems likely, including a higher concentration of mechano-, chemo- and photosensory cells. As such, the central nervous systems of Cryogenian bilaterians probably included a simple brain and perhaps additional ganglia, along with nerve tracts connecting these to muscular and other effector systems.

10. Nervous systems after the Cambrian revolution

Something extraordinary took place during the Cambrian: body fossils from many different bilaterian phyla suddenly appear in the record. Over the past several decades, it has become increasingly clear that the revolution was most likely not the origin of animals or even bilaterians *per se*, but rather the origin of novel trophic modes [101–104]. In particular, macropredators appear for the first time during the Cambrian. Active predation would likely have been limited by the low availability of oxygen during much of the Cryogenian and Ediacaran [99,100]. As oxygen levels began to rise towards the end of the Ediacaran, active feeding modes became possible. The appearance of the first active predators would have imposed new and intense selection pressures. This ecological revolution likely drove the evolution of mineralized skeletons, increases in body size, organs for rapid movement, image-forming eyes and infaunal (burrowing) lifestyles.

The nearly simultaneous appearance of this diverse set of features in several phyla during the Cambrian argues for an

extrinsic trigger; the nature of the traits themselves points to predation as that trigger. The origin of skeletons, for instance, almost certainly took place independently in several phyla rather than in their common ancestor, based on the very different ways that skeletons are produced and on their diverse chemical compositions. It seems implausible that animals as different as brachiopods, echinoderms and trilobites (to name just a few) would have needed mineralized skeletons at the same moment for intrinsic reasons.

Similarly, more sophisticated sensory and central nervous systems would have been needed for both predation and predator avoidance. Image-forming eyes and motion-detecting organs such as sensillae and antennae provide enormous advantages in locating and capturing prey, and these same sense organs are also valuable for predator avoidance. These sophisticated sense organs evolved on many independent occasions during the Cambrian, and at about the same time as skeletons. Image-forming eyes, for instance, incorporated pre-existing photoreceptors but clearly evolved independently in arthropods, cephalopod molluscs and vertebrates. The enormous input of sensory information from image-forming eyes and from sensitive antennae and fields of sensillae would have required the simultaneous expansion of input-processing systems, likely within a brain or multiple ganglia. Effector systems, too, would have needed to change substantially around this time. We know from the fossil record that Cambrian predators had jaws and raptorial appendages, both of which would have required additional control systems. The same is true for prey species, which evolved various systems for avoiding predators, including limbs, fins and the ability to burrow.

11. Two phases of nervous system evolution

The general timing of metazoan origins indicated by molecular clock estimates therefore suggests two distinct phases in the evolution of nervous systems (figure 3, right). The first would have encompassed the metazoan, eumetazoan and bilaterian ancestors, as well as the stem lineages of protostomes and deuterostomes. During this first phase, nervous systems would have included neurones, synapses, and basic effector systems. The likely small size and low energy budgets of early metazoans, combined with the absence of macropredators, would not have required particularly sophisticated sense organs or central processing systems, but certainly could have included cephalization and relatively simple brains.

The second phase of metazoan nervous system evolution took place during the establishment of the crown groups of extant bilaterian phyla. This second phase was likely a response to an ecological revolution precipitated by appearance of the first macropredators, which likely drove the parallel and contemporaneous appearance in multiple groups of mineralized skeletons, energetically expensive capabilities such as rapid movement and burrowing, and increased body size. Macropredators also likely drove the first appearance of complex sense organs, including image-forming eyes, and effector organs requiring complex coordination, such as raptorial appendages and locomotory limbs.

Structural and functional components that evolved during the first phase could have left some degree of shared structural similarity in the organization of central nervous systems in living bilaterians. For instance, several authors have proposed that specific similarities in the organization of protostome and

deuterostome central nervous systems are homologous, rather than convergent [95–97,105,106]. Here the timing of events can perhaps provide insight: nervous system features that evolved during the second phase were independent events since the protostome–deuterostome divergence had already taken place. These later innovations may well have built in parallel ways upon an existing infrastructure of neural components and organization, making it challenging to disentangle the imprint of shared ancestry from convergence. Indeed, similarities in the central nervous systems of extant phyla likely comprise a complex mix of shared and independently derived features. Despite the fact that these two feature sets evolved hundreds of millions of years apart, the intense functional demands of the ecological revolution during the Cambrian makes distinguishing them a challenge.

12. Forecast: cloudy then clearing

Nearly 70 years after the publication of Cloud's influential article [14], the question of when metazoans evolved remains only partially answered. Taken at face value, the variety of age estimates from molecular clock studies might suggest a methodology that is hopelessly inaccurate. This conclusion is not warranted. It is worth bearing in mind the strong parallels to molecular phylogenetic analyses of metazoan relationships. Studies over the past 25 years have produced many, often dramatically different, topologies (reviewed in [86]). A few key relationships have only recently reached consensus, such as the position of cephalochordates outside the urochordate + vertebrate clade. More tellingly, the position of several critical lineages remains unclear today, including ctenophores and acoels, and the monophyly of some phyla remains controversial, most notably sponges.

Yet few would argue for abandoning the practice of phylogenetic analysis based on molecular data. Although methods for estimating divergence times have lagged behind those for estimating phylogenetic relationships, they too have improved steadily. In addition, the sequence data available for analysis has expanded enormously. It would be naive to expect that molecular data will ever produce highly precise divergence time estimates, but there remains considerable scope for extending and refining our understanding of key evolutionary events using this approach. As with phylogenetic analyses of metazoan history, we can expect consensus to emerge as methods continue to be refined.

Another important point to bear in mind is that even inaccurate estimates of divergence times can be enormously informative if they help to rule out specific hypotheses. In particular, hypotheses that hinge on divergence times among metazoan phyla occurring during or just before the early Cambrian can be falsified by independent divergence time estimates whose credible intervals are much deeper. For this particular application, the essential point is not exactly how much earlier those divergences took place, but that they are clearly earlier than the Cambrian or latest Ediacaran.

Competing interests. I have no competing interests.

Funding. I received no funding for this study.

Acknowledgements. Thanks to Frank Hirth and Nick Strausfeld for organizing the symposium on the Origin and Evolution of the Nervous System where these ideas were presented. Thanks also to Jennifer Israel and Sasha Makohon-Moore and two anonymous referees for perceptive and constructive comments on the manuscript.

- Smith AB, Peterson KJ. 2002 Dating the time of origin of major clades: molecular clocks and the fossil record. *Annu. Rev. Earth Planet. Sci.* **30**, 65–88. (doi:10.1146/annurev.earth.30.091201.140057)
- Donoghue PC, Benton MJ. 2007 Rocks and clocks: calibrating the Tree of Life using fossils and molecules. *Trends Ecol. Evol.* **22**, 424–431. (doi:10.1016/j.tree.2007.05.005)
- Avise J. 2009 Timetrees: beyond cladograms, phenograms, and phylograms. In *The timetree of life* (eds S Hedges, S Kumar), pp. 19–25. Oxford, UK: Oxford University Press.
- Thackray JC. 1976 The Murchison–Sedgwick controversy. *J. Geol. Soc. Lond.* **132**, 367–372. (doi:10.1144/gsjgs.132.4.0367)
- Rudwick M. 1988 *The great Devonian controversy*. Chicago, IL: University of Chicago Press.
- Budd GE. 2008 The earliest fossil record of the animals and its significance. *Phil. Trans. R. Soc. B* **363**, 1425–1434. (doi:10.1098/rstb.2007.2232)
- Marshall CR. 2008 A simple method for bracketing absolute divergence times on molecular phylogenies using multiple fossil calibration points. *Am. Nat.* **171**, 726–742. (doi:10.1086/587523)
- Darwin C. 1859 *On the origin of species by means of natural selection*. London, UK: John Murray.
- Gould S. 1988 *Wonderful life*. New York, NY: W. W. Norton.
- Shu D, Isozaki Y, Zhang X, Han J, Maruyama S. 2014 Birth and early evolution of metazoans. *Gondwana Res.* **25**, 884–895. (doi:10.1016/j.gr.2013.09.001)
- Walcott C. 1914 Cambrian geology and paleontology. *Smithson. Misc. Collect.* **57**, 14.
- Knoll A, Walter M, Narbonne G, Christie-Blick N. 2006 The Ediacaran period: a new addition to the geologic time scale. *Lethaia* **39**, 13–30. (doi:10.1080/00241160500409223)
- Raymond PE. 1935 Pre-Cambrian life. *Geol. Soc. Am. Bull.* **46**, 375–392. (doi:10.1130/GSAB-46-375)
- Cloud PJ. 1948 Some problems and patterns of evolution exemplified by fossil invertebrates. *Evolution* **2**, 322–350. (doi:10.2307/2405523)
- Budd GE, Jensen S. 2000 A critical reappraisal of the fossil record of the bilaterian phyla. *Biol. Rev. Camb. Philos. Soc.* **75**, 253–295. (doi:10.1017/S000632310000548X)
- Briggs DEG, Fortey RA. 2005 Wonderful strife: systematics, stem groups, and the phylogenetic signal of the Cambrian radiation. *Paleobiology* **31**, 94–112. (doi:10.1666/0094-8373(2005)031[0094:WSSSGA]2.0.CO;2)
- Conway Morris S. 2006 Darwin's dilemma: the realities of the Cambrian 'explosion'. *Phil. Trans. R. Soc. B* **361**, 1069–1083. (doi:10.1098/rstb.2006.1846)
- Valentine J. 2006 *On the origin of phyla*. Chicago, IL: University of Chicago Press.
- Seilacher A, Bose PK, Pflüger F. 1998 Triploblastic animals more than 1 billion years ago: trace fossil evidence from India. *Science* **282**, 80–83. (doi:10.1126/science.282.5386.80)
- Rasmussen B, Bengtson S, Fletcher IR, McNaughton NJ. 2002 Discoidal impressions and trace-like fossils more than 1200 million years old. *Science* **296**, 1112–1115. (doi:10.1126/science.1070166)
- Pecoits E, Konhauser KO, Aubert NR, Heaman LM, Veroslavsky G, Stern RA, Gingras MK. 2012 Bilaterian burrows and grazing behavior at >585 million years ago. *Science* **336**, 1693–1696. (doi:10.1126/science.1216295)
- Huldtgren T, Cunningham JA, Yin C, Stampanoni M, Marone F, Donoghue PCJ, Bengtson S. 2011 Fossilized nuclei and germination structures identify Ediacaran 'animal embryos' as encysting protists. *Science* **334**, 1696–1699. (doi:10.1126/science.1209537)
- Gaucher C, Poire DG, Bossi J, Bettucci LS, Beri A. 2013 Comment on 'Bilaterian burrows and grazing behavior at >585 million years ago'. *Science* **339**, 906. (doi:10.1126/science.1230339)
- Fedonkin MA, Simonetta A, Ivantsov AY. 2007 New data on *Kimberella*, the Vendian mollusc-like organism (White Sea region, Russia): palaeoecological and evolutionary implications. *Geol. Soc. Lond.* **286**, 157–179. (doi:10.1144/SP286.12)
- Sprigg R. 1947 Early Cambrian (?) jellyfishes from the Flinders Ranges, South Australia. *Trans. R. Soc. South Aust.* **71**, 212–224.
- Glaessner M. 1959 The geology and Late Precambrian fauna of the Ediacara fossil reserve. *Rec. South Aust. Mus.* **13**, 369–401.
- Glaessner M. 1984 *The dawn of animal life*. Cambridge, UK: Cambridge University Press.
- Gehling JG. 1987 Earliest known echinoderm: a new Ediacaran fossil from the pound subgroup of South Australia. *Alcheringa* **11**, 337–345. (doi:10.1080/03115518708619143)
- Fedonkin MA. 1990 Systematic description of Vendian Metazoa. In *The Vendian system: paleontology*, vol. 1 (eds BS Sokolov, AB Iwanowski), pp. 71–120. Heidelberg, Germany: Springer.
- Seilacher A. 1989 Vendozoa: organismic construction in the Proterozoic biosphere. *Lethaia* **22**, 229–239. (doi:10.1111/j.1502-3931.1989.tb01332.x)
- Retallack GJ. 1994 Were the Ediacaran fossils lichens? *Paleobiology* **20**, 523–544.
- Narbonne G. 1998 The Ediacara biota: a terminal Neoproterozoic experiment in the evolution of life. *GSA Today* **8**, 1–6.
- Seilacher A, Grazhdankin D, Legouta A. 2003 Ediacaran biota: the dawn on animal life in the shadow of giant protists. *Paleontol. Res.* **7**, 43–54. (doi:10.2517/prpsj.7.43)
- Li C, Chen J, Hua T. 1998 Precambrian sponges with cellular structures. *Science* **279**, 879–882. (doi:10.1126/science.279.5352.879)
- Maloof AC *et al.* 2010 Possible animal-body fossils in pre-Marinoan limestones from South Australia. *Nat. Geosci.* **3**, 653–659. (doi:10.1038/ngeo934)
- Tang F, Bengtson S, Wang Y, Wang XL, Yin CY. 2011 *Eoandromeda* and the origin of Ctenophora. *Evol. Dev.* **13**, 408–414. (doi:10.1111/j.1525-142X.2011.00499.x)
- Yin Z *et al.* 2015 Sponge grade body fossil with cellular resolution dating 60 Myr before the Cambrian. *Proc. Natl Acad. Sci. USA* **112**, E1453–E1460. (doi:10.1073/pnas.1414577112)
- Grant SWF. 1990 Shell structure and distribution of Cloudina, a potential index fossil for the terminal Proterozoic. *Am. J. Sci.* **290A**, 261–294.
- Grotzinger JP, Watters WA, Knoll AH. 2000 Calcified metazoans in thrombolite-stromatolite reefs of the terminal Proterozoic Nama Group, Namibia. *Paleobiology* **26**, 334–359. (doi:10.1666/0094-8373(2000)026<0334:CMITSR>2.0.CO;2)
- Fedonkin MA, Vickers-Rich P, Swalla BJ, Trusler P, Hall M. 2012 A new metazoan from the Vendian of the White Sea, Russia, with possible affinities to the ascidians. *Paleontol. J.* **46**, 1–11. (doi:10.1134/S0031030112010042)
- Xiao S, Zhang Y, Knoll AH. 1998 Three-dimensional preservation of algae and animal embryos in a Neoproterozoic phosphorite. *Nature* **391**, 553–558. (doi:10.1038/35318)
- Chen J-Y, Oliveri P, Li CW, Zhou GQ, Gao F, Hagadorn JW, Peterson KJ, Davidson EH. 2000 Precambrian animal diversity: putative phosphorized embryos from the Doushantuo formation of China. *Proc. Natl. Acad. Sci. USA* **97**, 4457–4462. (doi:10.1073/pnas.97.9.4457)
- Hagadorn JW *et al.* 2006 Cellular and subcellular structure of neoproterozoic animal embryos. *Science* **314**, 291–294. (doi:10.1126/science.1133129)
- Chen L, Xiao S, Pang K, Zhou C, Yuan X. 2014 Cell differentiation and germ-soma separation in Ediacaran animal embryo-like fossils. *Nature* **516**, 238–241. (doi:10.1038/nature13766)
- Bengtson S, Budd GE. 2000 Comment on 'Small bilaterian fossils from 40 to 55 million years before the Cambrian'. *Science* **306**, 1291. (doi:10.1126/science.1101338)
- Cunningham JA *et al.* 2012 Experimental taphonomy of giant sulphur bacteria: implications for the interpretation of the embryo-like Ediacaran Doushantuo fossils. *Proc. R. Soc. B* **279**, 1857–1864. (doi:10.1098/rspb.2011.2064)
- Zuckerkandl E, Pauling L. 1962 Molecular disease, evolution, and genic heterogeneity. In *Horizons in biochemistry* (eds M Kasha, B Pullman). New York, NY: Academic Press.
- Margoliash E. 1963 Primary structure and evolution of cytochrome c. *Proc. Natl Acad. Sci. USA* **50**, 672–679. (doi:10.1073/pnas.50.4.672)
- Brown RH, Richardson M, Boulter D, Ramshaw JA, Jefferies RP. 1972 The amino acid sequence of cytochrome c from *Helix aspersa* Muller (garden snail). *Biochem. J.* **128**, 971–974. (doi:10.1042/bj1280971)
- Runnegar B. 1982 A molecular-clock date for the origin of the animal phyla. *Lethaia* **15**, 199–205. (doi:10.1111/j.1502-3931.1982.tb00645.x)

51. Knoll AH, Carroll SB. 1999 Early animal evolution: emerging views from comparative biology and geology. *Science* **284**, 2129–2137. (doi:10.1126/science.284.5423.2129)
52. Raff RA, Kauffman TC. 1983 *Embryos, genes, and evolution*. New York, NY: Macmillan.
53. Wray GA, Levinton JS, Shapiro LH. 1996 Molecular evidence for deep Precambrian divergences among animal phyla. *Science* **274**, 568–573. (doi:10.1126/science.274.5287.568)
54. Aris-Brosou S, Yang Z. 2003 Bayesian models of episodic evolution support a late precambrian explosive diversification of the Metazoa. *Mol. Biol. Evol.* **20**, 1947–1954. (doi:10.1093/molbev/msg226)
55. Bromham L, Rambaut A, Fortey R, Cooper A, Penny D. 1998 Testing the Cambrian explosion hypothesis by using a molecular dating technique. *Proc. Natl Acad. Sci. USA* **95**, 12 386–12 389. (doi:10.1073/pnas.95.21.12386)
56. Welch JJ, Fontanillas E, Bromham L. 2005 Molecular dates for the 'Cambrian explosion': the influence of prior assumptions. *Syst. Biol.* **54**, 672–678. (doi:10.1080/1063515050947212)
57. Wheat CW, Wahlberg N. 2013 Critiquing blind dating: the dangers of over-confident date estimates in comparative genomics. *Trends Ecol. Evol.* **28**, 636–642. (doi:10.1016/j.tree.2013.07.007)
58. Schulte II JA. 2013 Undersampling taxa will underestimate molecular divergence dates: an example from the South American lizard clade Liolaemini. *Int. J. Evol. Biol.* **2013**, 628467. (doi:10.1155/2013/628467)
59. Erwin DH, Laflamme M, Tweedt SM, Sperling EA, Pisani D, Peterson KJ. 2011 The Cambrian conundrum: early divergence and later ecological success in the early history of animals. *Science* **334**, 1091–1097. (doi:10.1126/science.1206375)
60. Filipiński A, Murillo O, Freydenzon A, Tamura K, Kumar S. 2014 Prospects for building large timetrees using molecular data with incomplete gene coverage among species. *Mol. Biol. Evol.* **31**, 2542–2550. (doi:10.1093/molbev/msu200)
61. Tamura K, Battistuzzi FU, Billings-Ross P, Murillo O, Filipiński A, Kumar S. 2012 Estimating divergence times in large molecular phylogenies. *Proc. Natl Acad. Sci. USA* **109**, 19 333–19 338. (doi:10.1073/pnas.1213199109)
62. Hug LA, Roger AJ. 2007 The impact of fossils and taxon sampling on ancient molecular dating analyses. *Mol. Biol. Evol.* **24**, 1889–1897. (doi:10.1093/molbev/msm115)
63. Ho SY, Phillips MJ. 2009 Accounting for calibration uncertainty in phylogenetic estimation of evolutionary divergence times. *Syst. Biol.* **58**, 367–380. (doi:10.1093/sysbio/syp035)
64. Sanderson MJ. 1997 A nonparametric approach to estimating divergence times in the absence of rate constancy. *Mol. Biol. Evol.* **14**, 1218–1231. (doi:10.1093/oxfordjournals.molbev.a025731)
65. Thorne JL, Kishino H, Painter IS. 1998 Estimating the rate of evolution of the rate of molecular evolution. *Mol. Biol. Evol.* **15**, 1647–1657. (doi:10.1093/oxfordjournals.molbev.a025892)
66. Sanderson MJ. 2002 Estimating absolute rates of molecular evolution and divergence times: a penalized likelihood approach. *Mol. Biol. Evol.* **19**, 101–109. (doi:10.1093/oxfordjournals.molbev.a003974)
67. Britton T, Anderson CL, Jacquet D, Lundqvist S, Bremer K. 2007 Estimating divergence times in large phylogenetic trees. *Syst. Biol.* **56**, 741–752. (doi:10.1080/10635150701613783)
68. Shaul S, Graur D. 2002 Playing chicken (*Gallus gallus*): methodological inconsistencies of molecular divergence date estimates due to secondary calibration points. *Gene* **300**, 59–61. (doi:10.1016/S0378-1119(02)00851-X)
69. Battistuzzi FU, Billings-Ross P, Murillo O, Filipiński A, Kumar S. 2015 Resolving the conundrum of Cambrian times for the divergence of animal phyla from molecular data. *Mol. Biol. Evol.* **32**, 1907–1912. (doi:10.1093/molbev/msv075)
70. Mello B, Schrago CG. 2014 Assignment of calibration information to deeper phylogenetic nodes is more effective in obtaining precise and accurate divergence time estimates. *Evol. Bioinform. Online* **10**, 79–85.
71. Kimura M. 1983 *The neutral allele theory of molecular evolution*. Cambridge, UK: Cambridge University Press.
72. Rambaut A, Bromham L. 1998 Estimating divergence dates from molecular sequences. *Mol. Biol. Evol.* **15**, 442–448. (doi:10.1093/oxfordjournals.molbev.a025940)
73. Kishino H, Thorne JL, Bruno WJ. 2001 Performance of a divergence time estimation method under a probabilistic model of rate evolution. *Mol. Biol. Evol.* **18**, 352–361. (doi:10.1093/oxfordjournals.molbev.a003811)
74. Douzery EJ, Snell EA, Baptiste E, Delsuc F, Philippe H. 2004 The timing of eukaryotic evolution: does a relaxed molecular clock reconcile proteins and fossils? *Proc. Natl Acad. Sci. USA* **101**, 15 386–15 391. (doi:10.1073/pnas.0403984101)
75. Battistuzzi FU, Filipiński A, Hedges SB, Kumar S. 2010 Performance of relaxed-clock methods in estimating evolutionary divergence times and their credibility intervals. *Mol. Biol. Evol.* **27**, 1289–1300. (doi:10.1093/molbev/msq014)
76. Schwartz RS, Mueller RL. 2010 Branch length estimation and divergence dating: estimates of error in Bayesian and maximum likelihood frameworks. *BMC Evol. Biol.* **10**, 5. (doi:10.1186/1471-2148-10-5)
77. Ho SY, Phillips MJ, Drummond AJ, Cooper A. 2005 Accuracy of rate estimation using relaxed-clock models with a critical focus on the early metazoan radiation. *Mol. Biol. Evol.* **22**, 1355–1363. (doi:10.1093/molbev/msi125)
78. Blair JE, Hedges SB. 2005 Molecular clocks do not support the Cambrian explosion. *Mol. Biol. Evol.* **22**, 387–390. (doi:10.1093/molbev/msi039)
79. Warnock RC, Yang Z, Donoghue PC. 2012 Exploring uncertainty in the calibration of the molecular clock. *Biol. Lett.* **8**, 156–159. (doi:10.1098/rsbl.2011.0710)
80. Battistuzzi FU, Billings-Ross P, Murillo O, Filipiński A, Kumar S. 2015 A protocol for diagnosing the effect of calibration priors on posterior time estimates: a case study for the Cambrian explosion of animal phyla. *Mol. Biol. Evol.* **32**, 1907–1912. (doi:10.1093/molbev/msv075)
81. Ayala FJ, Rzhetsky A, Ayala FJ. 1998 Origin of the metazoan phyla: molecular clocks confirm paleontological estimates. *Proc. Natl Acad. Sci. USA* **95**, 606–611. (doi:10.1073/pnas.95.2.606)
82. Peterson KJ, Lyons JB, Nowak KS, Takacs CM, Wargo MJ, McPeck MA. 2004 Estimating metazoan divergence times with a molecular clock. *Proc. Natl Acad. Sci. USA* **101**, 6536–6541. (doi:10.1073/pnas.0401670101)
83. Cutler DJ. 2000 Estimating divergence times in the presence of an overdispersed molecular clock. *Mol. Biol. Evol.* **17**, 1647–1660. (doi:10.1093/oxfordjournals.molbev.a026264)
84. Chernikova D, Motamedi S, Csuros M, Koonin EV, Rogozin IB. 2011 A late origin of the extant eukaryotic diversity: divergence time estimates using rare genomic changes. *Biol. Direct.* **6**, 26. (doi:10.1186/1745-6150-6-26)
85. Richter DJ, King N. 2013 The genomic and cellular foundations of animal origins. *Annu. Rev. Genet.* **47**, 509–537. (doi:10.1146/annurev-genet-111212-133456)
86. Dunn CW, Giribet G, Edgecombe GD, Hejnol A. 2014 Animal phylogeny and its evolutionary implications. *Annu. Rev. Ecol. Evol. System.* **45**, 371–395. (doi:10.1146/annurev-ecolsys-120213-091627)
87. Nielsen C. 2012 *Animal evolution: interrelationships of the living phyla*. Oxford, UK: Oxford University Press.
88. Emes RD, Grant SG. 2012 Evolution of synapse complexity and diversity. *Annu. Rev. Neurosci.* **35**, 111–131. (doi:10.1146/annurev-neuro-062111-150433)
89. Burkhardt P. 2015 The origin and evolution of synaptic proteins: choanoflagellates lead the way. *J. Exp. Biol.* **218**, 506–514. (doi:10.1242/jeb.110247)
90. Carroll SB, Grenier JK, Weatherbee SD. 2005 *From DNA to diversity*. Malden, MA: Blackwell.
91. Hejnol A, Martindale MQ. 2008 Acoel development supports a simple planula-like urbilaterian. *Phil. Trans. R. Soc. B* **363**, 1493–1501. (doi:10.1098/rstb.2007.2239)
92. Balavoine G, Adoutte A. 2003 The segmented *Urbilateria*: a testable scenario. *Integr. Comp. Biol.* **43**, 137–147. (doi:10.1093/icb/43.1.137)
93. Hirth F. 2003 An urbilaterian origin of the tripartite brain: developmental genetic insights from *Drosophila*. *Development* **130**, 2365–2373. (doi:10.1242/dev.00438)
94. Miller DJ, Ball EE. 2009 The gene complement of the ancestral bilaterian—was *Urbilateria* a monster? *J. Biol.* **8**, 89. (doi:10.1186/jbiol192)
95. Northcutt RG. 2010 Cladistic analysis reveals brainless *Urbilateria*. *Brain Behav. Evol.* **76**, 1–2. (doi:10.1159/000316443)
96. Strausfeld NJ, Hirth F. 2013 Deep homology of arthropod central complex and vertebrate basal ganglia. *Science* **340**, 157–161. (doi:10.1126/science.1231828)

97. Tosches MA, Arendt D. 2013 The bilaterian forebrain: an evolutionary chimaera. *Curr. Opin. Neurobiol.* **23**, 1080–1089. (doi:10.1016/j.conb.2013.09.005)
98. Lyons TW, Reinhard CT, Planavsky NJ. 2014 The rise of oxygen in Earth's early ocean and atmosphere. *Nature* **506**, 307–315. (doi:10.1038/nature13068)
99. Planavsky NJ *et al.* 2014 Earth history. Low mid-Proterozoic atmospheric oxygen levels and the delayed rise of animals. *Science* **346**, 635–638. (doi:10.1126/science.1258410)
100. Sperling EA, Frieder CA, Raman AV, Girguis PR, Levin LA, Knoll AH. 2013 Oxygen, ecology, and the Cambrian radiation of animals. *Proc. Natl Acad. Sci. USA* **110**, 13 446–13 451. (doi:10.1073/pnas.1312778110)
101. Stanley SM. 1973 An ecological theory for the sudden origin of multicellular life in the late Precambrian. *Proc. Natl Acad. Sci. USA* **70**, 1486–1489. (doi:10.1073/pnas.70.5.1486)
102. McMenamin MA, McMenamin DLS. 1990 *The emergence of animals*. New York, NY: Columbia University Press.
103. Peterson KJ, Cotton JA, Gehling JG, Pisani D. 2008 The Ediacaran emergence of bilaterians: congruence between the genetic and the geological fossil records. *Phil. Trans. R. Soc. B* **363**, 1435–1443. (doi:10.1098/rstb.2007.2233)
104. Butterfield NJ. 2011 Animals and the invention of the Phanerozoic Earth system. *Trends Ecol. Evol.* **26**, 81–87. (doi:10.1016/j.tree.2010.11.012)
105. Benito-Gutierrez E, Arendt D. 2009 CNS evolution: new insight from the mud. *Curr. Biol.* **19**, R640–R642. (doi:10.1016/j.cub.2009.06.020)
106. Holland LZ, Carvalho JE, Escrava H, Laudet V, Schubert M, Shimeld SM, Yu J-K. 2013 Evolution of bilaterian central nervous systems: a single origin? *Evodevo* **4**, 27. (doi:10.1186/2041-9139-4-27)
107. Turner SL, Young JPW. 2000 The glutamine synthases of Rhizobia: phylogenetics and evolutionary implications. *Mol. Biol. Evol.* **17**, 309–319. (doi:10.1093/oxfordjournals.molbev.a026311)
108. Pisani D, Poling LL, Lyons-Weiler M, Hedges SB. 2004 The colonization of land by animals: molecular phylogeny and divergence times among arthropods. *BMC Biol.* **2**, 1. (doi:10.1186/1741-7007-2-1)
109. Otsuka J, Sugaya N. 2003 Advanced formulation of base pair changes in the stem regions of ribosomal RNAs: its application to mitochondrial rRNAs for resolving the phylogeny of animals. *J. Theor. Biol.* **222**, 447–460. (doi:10.1016/S0022-5193(03)00057-2)
110. Hedges SB, Blair JE, Venturi ML, Shree JL. 2004 A molecular timescale of eukaryote evolution and the rise of complex multicellular life. *BMC Evol. Biol.* **4**, 2. (doi:10.1186/1471-2148-4-2)
111. Gu X. 1998 Early metazoan divergence was about 830 million years ago. *J. Mol. Evol.* **47**, 369–371. (doi:10.1007/PL00013150)