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Improving scientific understanding of the origin and rise of complex life

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Abstract

Understanding of the triggers and timing of the rise of complex life ca. 720-520 million years ago has expanded dramatically in recent years. This volume brings together diverse and novel geochemical and palaeontological data presented as part of the Royal Society "*The origin and rise of complex life: integrating models, geochemical and palaeontological data*" discussion meeting held in September 2019. The individual manuscripts offer prescient insights from multiple disciplines. Here we summarise their contribution towards the goal of the meeting; to create testable hypotheses for the differing roles of changing climate, oceanic redox, nutrient availability, and ecosystem feedbacks across this profound, but enigmatic, transitional period.

Introduction

Scientific interest in the origin and rise of complex life is centuries old, and since Darwin's 'Origin of Species' [1] it has been seen as one of the central puzzles of evolution. 'Darwin's dilemma' - that fossil evidence of complex life appears abruptly in Cambrian strata, seemingly at odds with the gradual nature of evolution by natural selection - has been at least partly resolved by the discovery of an earlier soft-bodied and skeletal record of complex life (as well as an even older microfossil record). Nevertheless, why complex life appeared when it did remains unresolved, and encapsulates a multitude of scientific questions from diverse disciplines.

The contemporary literature is replete with hypotheses on how and why key evolutionary transitions occurred, or even why they might have occurred much later than seems reasonable [e.g. 2]. The contributions to this thematic issue of *Interface Focus* address several key episodes in the history of life, and highlight the challenges faced in establishing the causes of their evolutionary origin and the consequences of their subsequent diversification. This has been an active area of research for several decades, involving diverse disciplines, but the time has come for the integration of the diverse perspectives that these disciplines and their data afford.

Research into the origin and rise of complex life has benefitted hugely from this interdisciplinary perspective, e.g. in providing parallel genomic, anatomical, ethological, sedimentological, stratigraphic, geochemical, biomolecular, palaeoenvironmental and palaeogeographic insights into the same evolutionary events. However, these insights have not generally been integrated, to the extent that geochemical records from the same stratigraphic sections do not always use the same geologic age model, precluding even a basic combined understanding of how the same biogeochemical cycles varied over time and space. This is unfortunate, since attempts to move from hypothesis formulation to hypothesis testing frequently require that the attendant data, characterising both biological evolution and the physical environment, are calibrated to the same temporal framework, such that the difference between local and global signals can be constrained. The recurrence of conferences drawing together researchers to address the origin and rise of complex life is evidence, if any were needed, that we are some way from achieving this ideal.

One of the challenges to obtaining a holistic understanding of the major evolutionary transitions concerns the insufficiently specific nature of many hypotheses, which results in them being much more difficult to test than to formulate. Our thematic set of papers encompasses the origin and rise of complex life, but what does that even mean? It is clear from the contributions that we restrict our scope to eukaryotes, including their origin and diversification, but also the origin of major clades and grades that have had a formative impact on the evolution of the modern biosphere. This includes the rise of eukaryotic algae, which have a complex polyphyletic origin that has generated recent interest, following reports of biomarkers evidencing their surprisingly late rise to ecological prominence in the Cryogenian Period [3]. This is surprising because fossils of multicellular algae are known from at least the Mesoproterozoic. There is also considerable interest in the emergence of eukaryotic predation, which significantly predates Metazoa.

However, it is the origin and diversification of animals that continues to engender most interest (Figure 1). This is doubtless a consequence of our own animal origins, and because the compositions of our genomes and the nature of our embryology, are intimately related to the events that occurred at the origin of animals and in their subsequent diversification. Indeed, many congenital human diseases are rooted in the failure of molecular processes that were established early in animal evolution [4]. However, animals have co-evolved with the planet, both shaped by environmental and physical constraints as well as shaping the nature of their environment and the biogeochemical processes that operate within it. Even at the broadest of scales, the origin of animal-grade multicellularity (with apoptosis; programmed cell death) ranks as a rare, major transition in evolution [5] where a new level of selection arose and was stabilised against disruptive lower-level selection (cancer excepted), becoming irreversible. Naturally, then, many of the contributions to this volume focus on the origin and diversification of animals.

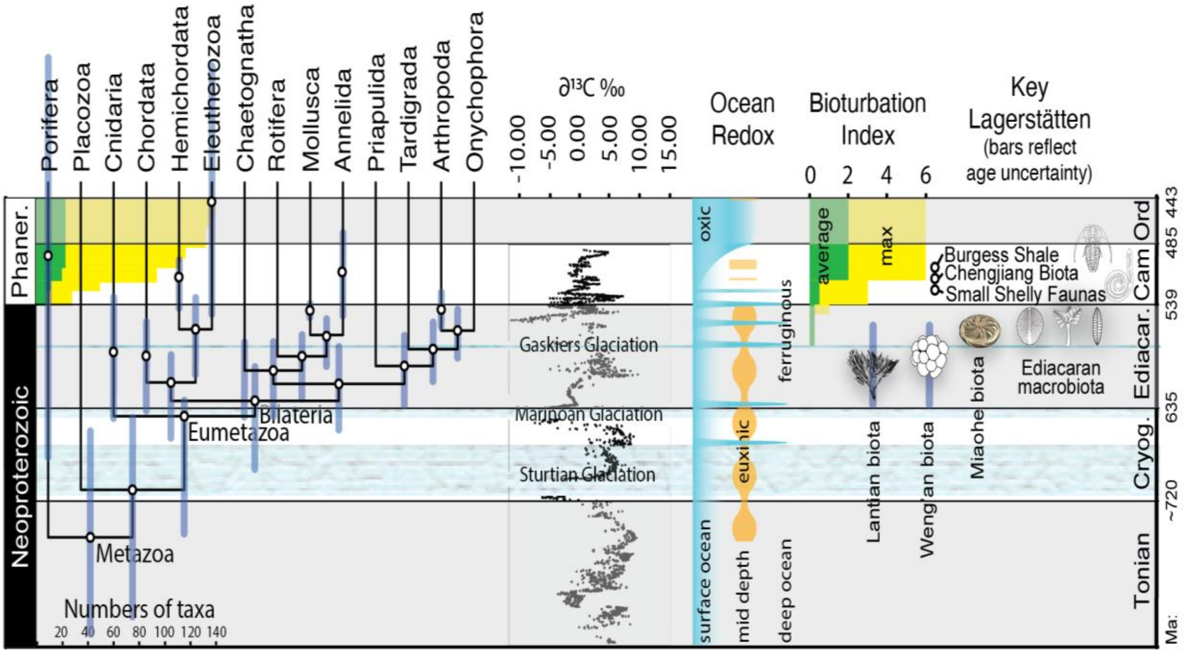


Figure 1: *Uncertainty in the timing of the evolution of animals and their environments across the Proterozoic-Phanerozoic transition. From left to right: Appearance of animal phyla (green) and classes (yellow) in the fossil record; age uncertainty in the molecular timescale of animal evolution (purple lines); δ^{13}C and redox variation - local or global signals?; bioturbation extent, which varies temporally and spatially; Neoproterozoic Lagerstätten, the precise ages of which are extremely uncertain, yielding in turn uncertainty that is reflected in molecular clock estimates of clade age. Data from refs. [6-11].*

A holistic understanding of the major evolutionary episodes that led to the origin and rise of complex life requires that explanatory hypotheses are formulated sufficiently precisely that they are practically testable. This is a comparatively high bar for most contemporary hypotheses that seek to explain the origin or diversification of major clades. For example, attempts to explain the origin and diversification of animals have invoked diverse causal mechanisms: the emergence of new gene families [12]; the evolution of coding [13] or non-coding [14] gene regulation; the origin of new life history stages [15]; escalatory ecological interactions [16]; salinity [17, 18]; trace metal nutrient availability [19]; global cooling into snowball Earth [20]; the challenges of increasing oxygenation of the biosphere [21] or its effects in releasing constraints on body size [2, 22]; vicariance biogeography driven by continental fragmentation [23]; release from the ecological and physiological constraints of global glaciation [24, 25]; polar wander [26]; and the mutational effects of pulses of cosmic radiation that result from our galaxy passing through nebulae [27]. These hypotheses are not (all) mutually exclusive. Some may be rejected because they are untestable and, therefore, unscientific. Otherwise, most of these causal hypotheses may have contributed to the shaping of animal diversity and, doubtless, some are at least in part a consequence of animal evolution, while others are likely to be entirely irrelevant to the question at hand. How can we even begin to discriminate among them?

At the very least, it would be helpful if these hypotheses were formulated with respect to a specific clade. However, to some researchers, the key phenomenon to be explained is not the origin of Metazoa, but the more complex Eumetazoa, Bilateria, or more usually, the emergence of bilaterian diversity i.e. the origin of the bilaterian animal phyla themselves. One thing is for sure, no single causal hypothesis can explain the gamut of early animal evolution since there cannot have been any progressive deterministic drive towards the origin of, for example, a bilaterian acme of animal evolution. Sponges do not exhibit eumetazoan-envy and, with the possible exception of *Buddenbrockia*, it is not clear that cnidarians have bilaterian-ambition. Thus, whatever the ultimate cause of eumetazoan and bilaterian innovation, its effects were not pervasive, leading to competitive displacement of what some researchers seem to perceive as inferior evolutionary grades. The origin of Metazoa, Eumetazoa, Bilateria, and the modern diversity of bilaterian phyla, are all genuinely interesting and important historical evolutionary episodes, but to render explanatory hypotheses testable, these specific taxonomic phenomena need to be identified explicitly, ideally with material justification in terms of the key anatomical, ecological or physiological innovations that link them to a hypothetical causal mechanism. Only then can we render such hypotheses testable and achieve the beginning of the end to debate over the origin and rise of complex life.

Oxygen and animals

Perhaps the most seductive, and certainly the most widely popularised, hypotheses (of late) concern possible causal links between oxygen levels and the origin and/or rise of animals, which began as early as 1959 [28]. There are at least three distinct hypotheses entertained in the recent literature: The first is that insufficient oxygen prevented the origin of animals (i.e. metazoan crown). Second is that insufficient oxygen constrained particular ecophysiological traits or modes of animal life (e.g. eyesight, carnivory). Third is that pulses of increased oxygenation (and/or productivity) promoted diversification, i.e. episodic early metazoan radiation events. (Though there is also a competing view that rising O_2 was a problem for metazoan evolution [21]).

The first two hypotheses are often taken in their inverse sense - namely that when oxygen levels rose, (i) animals originated, or (ii) new ecophysiological traits or modes of animal life arose. This assumes increased oxygen levels were both a *necessary* and a *sufficient* condition in each case. Conceivably, some other necessary condition could have precluded the origin of animals in each case - but then that last necessary condition to be fulfilled tends to be viewed as the true (and sole) cause (rather than oxygen).

These examples serve to exemplify the challenges of testing any hypothesis concerning a causal effect of an environmental variable on a phylogenetic (or ecological) ‘event’. Several things must be established, notably: the effect of the environmental variable on the lineage(s) in question - usually through extant representatives; the variation over time and space of the environmental variable - using indirect proxies and models; and the timing of the biological event itself - from molecular clocks informed by the fossil record. These all carry considerable challenges and uncertainties.

Starting with the first hypothesis, that insufficient oxygen prevented the origin of animals, proposed tests have involved: establishing the O_2 requirement of extant non-bilaterian animals; establishing environmental O_2 levels over geological time; establishing the timing of origin of animals; and then comparing the timings. The O_2 requirement of some extant sponges has been established and found to be very low [29, 30] (but of course one can question whether the O_2 requirements of extant animals reflect those of their earlier ancestors). Establishing environmental O_2 levels over geological time has triggered much ongoing research and debate - addressed in this volume [11, 31]. Currently it is unclear whether O_2 levels in the atmosphere and surface/shallow ocean (equilibrated with the atmosphere) were low enough to prevent sponges existing at any point after Earth's Great Oxidation at ~2.3 Ga [29]. Sponges tend to live at depths below the influence of regular mixing (by wind or tides) as turbid sediment impairs their function. In seasonally-stratified shelf seas $[O_2]$ can drop below the equilibrated level with the atmosphere thanks to respiration of sinking or dissolved organic material [32]. However, 'oxygen oases' would have occurred locally near primary producers, including benthic microbial mats (at least in the daytime) [32, 33]. If chromium isotopes do indicate there was a rise in atmospheric O_2 levels from limiting low levels [34], then it happened several times in the Mesoproterozoic and Neoproterozoic [35], and it is unclear when it became persistent [35]. Inevitably, there is debate over the timing of the origin of animals linked to the veracity of both molecular clock estimates and the fossil record on which they rely for calibration [9, 36, 37]. Integrating over this uncertainty is challenging since the breadth of estimates from this range of approaches and perspectives spans the late Tonian – middle Ediacaran. Inevitably, such imprecision is not incompatible with evidence of oxygenation of the environments within which some of the earliest undoubted fossil animals lived [38], however temporally and spatially heterogeneous. However, this is not a very stringent test [9].

For the second hypothesis, that insufficient oxygen constrained particular eco-physiological traits or modes of animal life, the first challenge is establishing the corresponding O_2 requirements. This has been approached by theoretical estimates [39], noting organism size, geometry, and making assumptions about the means of oxygen transport – by diffusion or circulation. Alternatively, extant benthic animal community composition and modes of life – such as carnivory – have been related to dissolved $[O_2]$ levels [40, 41]. For specific traits such as eyesight, more advanced forms of eye and means of supplying them with oxygen have subsequently evolved, making it difficult to infer the oxygen requirement of ancestral eyes. When it comes to establishing environmental $[O_2]$ levels over time, at the ocean-sediment (benthic) interface, these are predicted to be spatially and temporally variable. For water columns deeper than ~50 m that are not perennially mixed by the tides, $[O_2]$ can depend on: nutrients; ocean circulation; the nature of the biological pump, the presence of microbial mats, and atmospheric pO_2 [32]. Whether one should take the 'most habitable' benthic $[O_2]$ conditions as the potentially constraining ones is rarely considered. If so, for a given atmospheric pO_2, the highest $[O_2]$ would be expected in relatively shallow, permanently (tidally) well-mixed shelf seas, at cooler higher latitudes [32]. Once again, uncertainties in the interpretation of the fossil record and the evolutionary timescales that we can ultimately derive from it [9, 36, 37], currently preclude effective tests [9].

For the third hypothesis, that pulses of increased oxygenation triggered episodic early metazoan radiation events, which we presume reflects the origin of the bilaterian animal phyla, the challenge is to demonstrate a coincidence of increased oxygenation with increased rates of origination. Given environmental heterogeneity, the best prospects for testing involve integrating local redox proxies and biotic records in the same sections, with the help of a mechanistic model (rather than a global approach). However, it is not at all clear how oxygenation events would have promoted innovation and diversification when the principal effect of environmental oxygenation is to release constraints on tissue thickness [42] and facilitate the emergence of differentiated bodyplans – a eumetazoan, rather than a derived crown-bilaterian phenomenon.

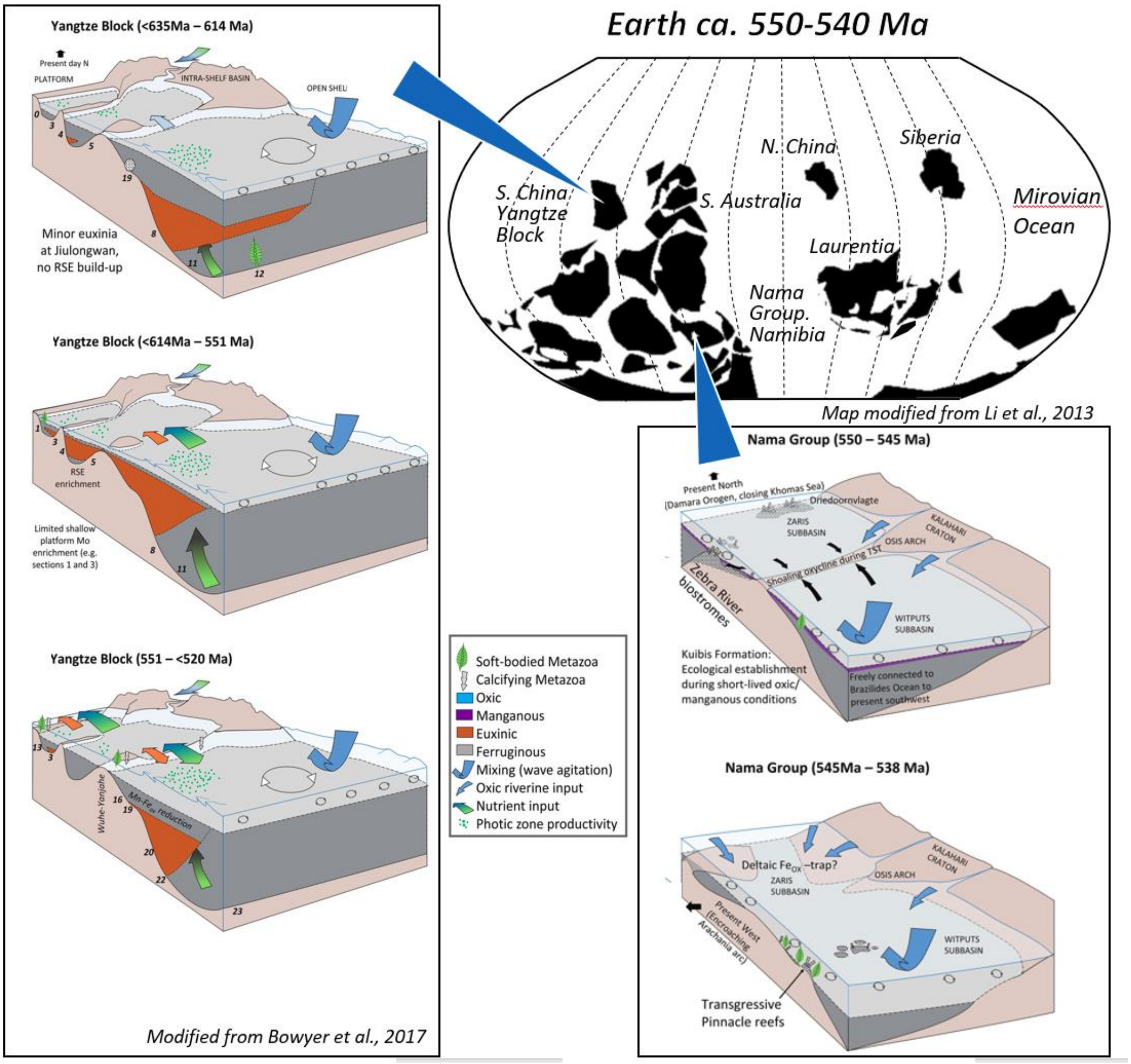
Clearly, testing even the best-formulated causal hypotheses on the origin and diversification of animals is challenging. We remain far from making most causal hypotheses experimentally tractable, and that is before we consider challenges in discriminating local from global patterns of evolution of the environments in which early animals originated, evolved and diversified, which is the subject to which we turn next.

Environmental heterogeneity across space and time

Often we consider the record of changes of seawater oxygenation and chemistry as a single evolving temporal narrative, which can be reduced to an eye catching and frequently reproduced synthetic image (e.g. Figure 1). Not only does this often ignore many transient extrinsic changes, which even though geologically fleeting nonetheless have a significant evolutionary impact (such as mass extinctions), but also such simplicity of message belies considerable heterogeneity at a basin, or even local scale. For example, we know that the coeval records of redox dynamics over the Ediacaran-Cambrian interval were very different on the Yangtze Platform of South China compared to the Nama Group of Namibia; such records formed at very different palaeolatitudes driven by their own regional climates, oceanography, and tectonics (Figure 2).

This begs the question, what is representative of any interval of time? The Yangtze Platform developed shallow marine euxinia during the Ediacaran-Cambrian and supported a notably different metazoan biota from the Nama Group, which rather showed an initially dynamic ferruginous OMZ that deepened towards the Ediacaran-Cambrian boundary [38, 43]. This may result from differences in physical coupling to the open ocean and associated nutrient inputs and/or the evolution of weathering regimes in these regions, but this is far from understood. Indeed, many other fossiliferous sections from this time interval have yet to be fully described in any integrated palaeontological, geochemical, and tectonic-sedimentological way. Such multidisciplinary, regional studies are vital to understand the driving processes that control the Ediacaran-Cambrian radiation of animals.

Hence even for an environmental variable like oxygen, which has been well-mixed in the atmosphere at least since the Great Oxidation Event, its concentration in marine environments where early animals - which may themselves have been diverse - lived, is and was spatially and temporally variable thanks to a varying balance of oxygen supply (via local air-sea exchange, dissolution, diffusion and advective mixing, as well as localised biological oxygen sources) and oxygen demand (mostly from biological oxygen consumption). Given such spatial-temporal variability it is hard to relate hypotheses framed in terms of ‘single’ oxygen levels (often defined for the atmosphere) to the reality of where early animals and their ancestors lived.



Modified from Bowyer et al., 2017

Map modified from Li et al., 2013

Figure 2: *The importance of understanding heterogeneity, both at a regional spatial scale and on a short-term (Myr) temporal scale. Compare the short-term redox dynamics and metazoan distributions from two disparate palaeocontinents - the Yangtze Platform, S. China, from ca. 635-520 Ma, and the Nama Group, Namibia from ca. 550 - 540 Ma (modified from refs. [38, 44]).*

Moving forward

The contributions in this volume do much to advance our knowledge of the origin and rise of morphological complexity in eukaryotes, metazoans, bilaterians, and entire ecosystems. They provide new hypotheses for the biological pathways by which eukaryotic cells were constructed, and the timing of when this took place. They improve our understanding of the mechanisms by which fossil evidence for these events is preserved within the rock record, and highlight the fact that we require a comprehensive understanding of all of the potential influences on a record, be they biological, geochemical, diagenetic or sedimentological, if we are to accurately interpret that record and incorporate it within a theoretical framework.

To effectively test hypotheses regarding the interplay between organisms and their environments across key evolutionary events, several ambitions need to be realised: (1) improve underlying data and embrace all the uncertainties inherent in their interpretation; (2) integrate disparate datasets more effectively; and (3) improve models to incarnate and test causal hypotheses against the collated data. Many research groups are attempting to do these things. We view it as the framework via which advances can be made. To elaborate:

1) While more data are always welcome, we need to improve our understanding of the data we have. Making data available is the responsibility of us all, and large compendia of data clearly have value for teasing out general patterns and testing hypotheses on a global scale. However, the data within those databases must be reliable, and its context fully understood, if it is to be utilised effectively and appropriately. Basic scientific research on individual sections forms the foundational building blocks of all of our datasets, and the value of detailed, integrated, geological studies on a local or regional scale cannot be overstated. Such detailed studies will be invaluable going forward not only to fill gaps in our existing knowledge, but to refine our understanding of classic sections or localities to provide essential context for the data they provide. Uncertainties in individual datasets also need to be acknowledged and reported when those datasets are combined into integrated studies. Appreciation of the differences between local, regional or global spatial scales, or ecological, micro- or macro-evolutionary timescales, is also a necessity. The tendency to over-interpret must be reined in.

2) Accurate chronological frameworks are required, onto which data can be tied. We concur with suggestions (e.g. [45]) that the field would benefit from 4-dimensional (space-and-time) databases bringing together available evidence. Such frameworks have significant potential to permit precise correlation of biological and environmental/geological events, thus enabling testing of causal relationships between them. To achieve these outcomes, we need more geochronological data, obtained via dating of key reference sections and the correlation of data between them. Age models provide a potential way to approach this (e.g. [26]), and are beginning to come online for this time interval (e.g. [46]).

3) We need to improve models so that they can test causal hypotheses. Modelling, when done appropriately, provides a powerful tool with which to combine disparate datasets and either formulate predictions that can be tested in the rock record, or to interrogate the viability of proposed hypothetical multivariate mechanisms. Models can be readily adapted and developed to incorporate new data, and their ability to incorporate an increasing array of feedbacks and uncertainties will only improve as advances are made in computing power. In order to test hypotheses about causality we need models that can causally relate variables hypothesised to be important. This means models that can: (i) capture spatial and temporal environmental heterogeneity, where it is reasonable to think it is causally important, and (ii)

accurately relate proxy variables to environmental variables proposed to be causally important. There also needs to be an appropriate statistical framework for hypothesis retention or rejection.

These challenges require a concerted and co-ordinated international effort to overcome, but they are not insurmountable. As the studies in this volume attest, our knowledge of the origin and rise of complex life is being iteratively accumulated at a phenomenal rate. The suggestions above, which stem from our discussions with colleagues both at the September 2019 meeting and elsewhere over the past few years, seek to encourage a step-change in approach to tackling these interdisciplinary questions, promoting a truly international collaborative ethos, intelligent integration of disparate datasets, and a commitment to the scientific process of developing, and robustly interrogating, testable hypotheses. Our Royal Society meeting was successful in galvanizing discussion between disciplines on the fundamental outstanding questions in our field. Our hope is that this momentum can be carried through to inspire real breakthroughs in deciphering the origin and rise of biological complexity through time.

Overview of the published contributions

We construct a narrative for the contributions to this volume, which explore issues of uncertainty and test the hypotheses that surround key evolutionary events as well as specific topics. These first concern the origin of eukaryotes, and the facilitating power as well as the biases in interpretation of the fossil record created by the vagaries of taphonomy and preservation. How we might interrogate and quantify the ecological dynamics of the oldest metazoan communities, and how oxygen might in fact have presented a physiological challenge rather than a facilitator for early animals, is then considered. The next set of contributions concerns the influence of secondary alteration on geochemical signals, and how choice of appropriate principles and conceptual frameworks should underpin all model building and testing. The volume concludes with exploration of the possible long-term relationship between the evolution of complex life and the apparent stabilisation of the Earth system through geological time.

The origin of eukaryotes

Porter [47] presents a barn-burning reinterpretation of the timing and tempo of eukaryogenesis, the process by which the key characteristics of modern eukaryotic cells were assembled over time, from their archaeal and alpha-proteobacterial antecedents, through to the last common ancestor of all living eukaryotes. The contemporary view is of stem and crown eukaryotes emerging early in the Proterozoic, existing at low diversity and perhaps in restricted environments or ecologies until a late Mesoproterozoic diversification of crown-eukaryotes. This view has been supported by molecular phylogenetics, the fossil record and their integration in molecular clock analyses. However, the wheels have begun to loosen on this bandwagon with evidence that eukaryotic sterols, presumed to have been present in the eukaryotic crown-ancestor, do not occur in the rock record until the early Neoproterozoic. Many molecular clock analyses may also have been compromised by the way in which they have used *Bangiomorpha pubescens*, the oldest widely accepted fossil record of a crown eukaryote, in calibration. *Bangiomorpha* has recently been re-dated 150 Myrs younger than previously assumed and its affinity as a derived crown rhodophyte has been questioned, with many reclassifying it as a total-group rhodophyte. Porter also observes that eukaryote fossils are not consistently associated with aerobic environments until the early Neoproterozoic. Together, this evidence can be interpreted to reflect a deep Palaeoproterozoic origin of eukaryotes, but a latest Mesoproterozoic origin of the crown group, with the key eukaryote characters such as encystment evolving early in the stem lineage, but sterol biosynthesis and aerobic respiration evolving very late, evidencing the late origin of crown-eukaryotes.

D. Mills [48] goes even further and argues that phagocytosis - the ability of eukaryotic cells to enfold their cell membrane around other objects and engulf them - evolved late and multiple times. This is contrary to the textbook view that phagocytosis is a fundamental, ancestral trait of stem group eukaryotes that was associated with the founding fusion event in which a mitochondrion became the engine room of the first eukaryote cell. It is true that prokaryotes are occasionally found inside other prokaryotes - and no one is quite sure how they got there. Mills appeals to such uncertain “alternative mechanisms” for the fusion of an ‘Asgard’ archaea and alphaproteobacteria mitochondrion to form the first eukaryote cell. The appeal of the ‘phagocytosis late’ hypothesis is that it could help reconcile the late ecological expansion of algae seen in the biomarker record - as some form of predation was necessary to create size-structured marine food webs. But that just adds the puzzle of how algae were created in the first place. The origins of the ancestral alga and subsequent secondary endosymbiotic events forming different algal lineages are all conventionally thought to involve phagocytosis. Given that a multicellular red alga *Bangiomorpha pubescens* was already present in the earliest Neoproterozoic, it would seem hard to push the origin of phagocytosis this late, let alone later. Mills suggests that the diversity of proteins associated with phagocytosis across the eukaryotic tree of life is supportive of multiple late origins, but it could be equally supportive of a long time interval of diversification since an early ancestral form of phagocytosis. It is always good to entertain bold hypotheses that challenge dominant paradigms - and we look forward to seeing how this one fares.

The fossil record and its preservation

In order to recognise past interactions between biological evolution and the global environment, we need confidence both in our biological and environmental records, and in our interpretation of them. From a biological perspective, the fossil record provides the only direct insight into evolutionary history, but disentangling true biological features from artefacts generated by taphonomic pathways/processes is critical to our ability to accurately interpret the likelihood that a given preserved feature is phylogenetically informative. This volume includes several papers that provide timely overviews of key aspects of the available fossil record.

Interpreting the fossil record of early eukaryotes is challenging because their key distinguishing characteristics, such as organelles and nuclei, are not commonly preserved. Even when candidate structures are recognised, their interpretation remains highly contentious. ***Sun et al.*** [49] seek to reduce uncertainty in the timing of the appearance of eukaryotes by testing the hypothesis that presumed fossil nuclei can indeed be preserved.

They compare the results from experimental degradation and mineralisation of nuclei in living eukaryotes to putative nuclei in embryo-like fossils from the early Ediacaran Weng'an Biota. Presumed fossil nuclei, although consistent in size and position within the cell, show a diversity of shapes, internal morphologies and mineralogies, and are often preserved as external moulds. Experimental taphonomy of modern cells demonstrates that nuclei are more decay resistant than their host cells, and often maintain their original physical dimensions on timescales compatible with rapid fossilization. Hence, both the characteristics of fossil preservation and experimental evidence can be explained by the same taphonomic pathway, involving selective mineralization of the cell cytoplasm and inner body at different stages of decay. The authors conclude that the presumed nuclei found in the Ediacaran Weng'an Biota are indeed just that, albeit preserved as external casts. This then enables more faith to be placed in our fossil record to potentially reveal the timing of the evolutionary assembly of eukaryote-grade cells.

Much of our early record of organic eukaryotic microfossils comes from fine-grained, clay-rich, rocks (mudstones), which begs the question, why are such settings conducive to exceptional preservation? And are such permissive environments spatially or temporally restricted? Using high-resolution in-situ geochemical techniques, ***Anderson et al.*** [50] test the hypothesis that certain minerals promote the fossilisation of organic material. They find that exceptionally preserved Proterozoic (ca. 1000 and 800 Ma) organic microfossils from mudstones are surrounded by a halo of aluminium-rich clay, probably kaolinite. Kaolinite is therefore implicated as promoting preservation, supporting previous work that has also suggested that kaolinite aids the spectacular preservation of non-skeletal metazoans of the Cambrian Burgess Shale and similar deposits [51]. Kaolinite is known to slow the growth of degrading bacteria, and may attach to or precipitate upon soft tissues early in diagenesis, so conserving them by providing a protective coating [52]. The incipient fossils themselves may also have facilitated organic molecule polymerisation and kaolinite enrichment [53]. Evoking a role for particular clays to enable exceptional preservation leads the authors to propose that the post-Proterozoic record of microfossils may be biased towards settings conducive to kaolinite formation, which are dominantly those formed under tropical weathering regimes. The authors therefore argue that the lack of evidence for metazoan life before ~800 Ma may reflect a genuine absence, since the fossilisation conditions that preserve non-mineralising metazoans in younger deposits are expected to have been present through the entire Neoproterozoic.

Hall et al. [54] apply a similar analytical approach to 3-dimensionally preserved macrofossils of soft-bodied organisms from clay-bearing sandstones of the late Ediacaran Wood Canyon Formation of Nevada. Using petrography, Raman spectroscopy, X-ray fluorescence microprobe and X-ray diffraction to assess candidate Erniettomorph specimens, the authors note an absence of evidence for either sulphide mineralization, or silica cements: minerals that might be expected by some taphonomic models for the preservation of soft-bodied organisms in the late Ediacaran [55, 56]. This raises questions about the preservation process here. The authors identify a carbon- and aluminium-rich thin layer delineating the fossil surface within the sediment, and illite and kaolinite on the exposed fossil surfaces, which, as with the Anderson et al. [50] study, they interpret to implicate a role for clay minerals in the preservation process. Does this mean that clay minerals are integral to Ediacaran macrofossil preservation? It is too early to say, and further work on clay-poor facies is required to test such a suggestion. However, pyritised tubular macrofossils in other lithofacies within the Wood Canyon Formation evidence alternative taphonomic pathways even within this same unit, and the authors explore hypotheses including differences in tissue composition, and the preservation of internal versus external membranes, in attempting to explain these observations.

The trace fossil record documents the behaviour of organisms rather than their actual physical remains. ***Mángano & Buatois*** [57] provide a timely overview of bilaterian traces across the Ediacaran-Cambrian transition. They review not only the ichnofossil record, but also the methods currently employed to study it, discussing the pros and cons of a variety of measures and indices, and advocating integration of multiple tools when assessing broad-scale changes in ichnological records. Emphasising the now widely recognised principle that the activity of organisms is capable of substantially changing (engineering) the properties of the ecosystems in which they reside [58, 59], the authors argue that the early trace fossil record of bilaterians is not a simple record of gradually increasing complexity across the Ediacaran-Cambrian boundary, but a complex interplay between organisms and their environments. A four-phase model that encapsulates the changes in bilaterian locomotory behaviour from the late Ediacaran through to Cambrian Age 4 is presented, which in addition to illuminating the trajectory of bilaterian evolution, clarifies the temporal disconnect between the popular concepts of the Agronomic Revolution (the seemingly protracted replacement of matgrounds by mixgrounds; [60]), and the Cambrian Substrate Revolution [61].

Ecology of early complex communities

The rules that govern the biodiversity and structure of the earliest presumed animal communities of the benthic Ediacaran Macrobiota remain of fundamental interest. Do the same rules that control modern ecosystem dynamics apply? And how did the environment influence ecology? The incredible natural laboratory of the fossil-bearing Ediacara Member at Nilpena offers opportunities to address the specific impact of biostratinomy, and particularly current-mediated hydrodynamic conditions, on bedding plane assemblages of Ediacaran macrofossils. ***Droser et al.*** [62] utilise the knowledge they have gained during excavation of multiple extensive bedding plane surfaces [63] to provide a palaeoecological baseline against which surfaces suspected of having been influenced by environmental conditions can be compared. Using the taxa *Dickinsonia* and *Arborea* as examples, they are able to tease apart the competing ecological, environmental and biostratinomic signals that have contributed to assemblage composition on specific surfaces. The authors show that currents can not only distort the morphology of individual specimens, sometimes creating distinct taphomorphs, but also alter assemblage ecology and composition, for example by size-selectively removing certain specimens from individual palaeocommunities, or transporting individuals into an assemblage from elsewhere. Identifying bedding plane assemblages that have been affected by these processes facilitates more accurate interpretation of the palaeoecology and biology of their component organisms.

Once a clear understanding of how much taphonomic bias can affect fossil communities has been obtained, hypotheses as to the ‘rules of assembly’ can be tested. ***Mitchell et al.*** [64] use spatial point process analyses (SPPA) to test the hypothesis that environmental heterogeneity may control community structure by examining seven late Ediacaran (558–550 Ma) macrofossil assemblages. Taxa in different settings show significant differences in their habitat associations, interactions, and response to small-scale habitat heterogeneities on the seafloor, with shallow, nearshore, palaeocommunities being more heavily influenced by local habitat heterogeneities than contemporaneous deeper-water assemblages. Shallow-water communities were also more patchy in their organisation, perhaps further promoted by the presence of grazers and detritivores. It is suggested that such extrinsic and intrinsic sources of heterogeneity may promote higher biodiversity in shallow settings, although it is difficult to isolate these factors from other controls such as elevated nutrient resources in the water column or intermediate disturbance levels. The relationship between environmental spatial heterogeneities and biodiversity of modern communities is well established, with habitat variability enabling the coexistence of species by creation of different niches. The authors posit a potential mechanism to link early animal diversification and benthic habitat patchiness prior to the evolution of metazoan predators and wide-spread pelagic organisms.

Budd and Mann [37] build on these early records of animals and their absence from older strata to challenge molecular timescales for Cryogenian or Tonian estimates of animal antiquity. They reason that the fossil record exhibits a stratigraphically consistent phylogenetic structure, with the earliest possible animal fossils first occurring in the middle Ediacaran, followed by the earliest possible eumetazoans in close succession, with trace and body fossil evidence indicating an origin of crown-bilaterians at or around the Proterozoic-Phanerozoic boundary. They rationalise unreasonably ancient molecular estimates for the timing of origin of these same clades as a consequence of biases in the birth-death models on which molecular clock analyses rely, along with calibrations based on unduly conservative interpretations of the fossil record. Their bottom line is that the fossil record can perhaps be read more or less literally.

Oxygen as a problem for early animals

Hammarlund [65] presents a new take on the role of oxygen in early animal evolution. Rather than viewing increased oxicity of Neoproterozoic environments as having released a constraint on size and complexity in animal evolution, she presents this same biogeochemical transition as a hurdle that early animals and their forebears had to overcome. This challenge to the contemporary narrative is rooted in the observation that cells that drive tissue formation lose their function on exposure to oxygen, and the maintenance of cell stemness - the ability of cells to differentiate and transdifferentiate - requires hypoxia. Hammarlund views the early deep-water habitats of rangeomorphs, among the earliest credible records of animals, as reflecting this sensitivity to oxygen, living in stable extrinsic environments where oxygenation can be controlled by the gradients within the organisms' tissues. Animals were able to release themselves from sessility in such environments by adopting fast cell fate turnover, as seen in modern sponges and cnidarians, but this necessarily limited the diversity of cell types and bodyplan complexity that they could achieve. The emergence of genetic factors in bilaterians that could induce hypoxia within cells, released their evolutionary potential for living in diverse habitats and achieving more complex body plans.

Geochemistry and modelling

Geochemistry provides one of the principal platforms by which environmental drivers and feedbacks relating to early animal evolution can be interrogated. Over the last two decades, spurred on by the development of new techniques and innovative new hypotheses, there has been an explosion in studies aimed at utilising geochemical and isotopic techniques to reconstruct environmental conditions at the dawn of animal life. Inherent in all of these studies, is the premise that the signal we measure in rocks today is that which was initially trapped in the rocks, either at the time of deposition or after diagenesis. However, with the huge number of studies being produced, this aspect is difficult to police, particularly with regard to the potential for post-depositional oxidation of redox sensitive elements.

Planavsky et al. [66] bring this issue firmly to the fore, with a detailed study of the secondary alteration of rare earth elements in a major global data-set spanning the entirety of Earth history. The authors note significant differences in cerium (Ce) anomalies, a common paleoredox proxy, between outcrop and drill-core samples, implying that geochemical data from altered samples have frequently been published in the peer-reviewed literature. Is this factor responsible for the high degree of spatial heterogeneity observed in environmental conditions across the late Precambrian to early Phanerozoic? The answer to this is that we simply do not know, but it appears likely that secondary alteration may have amplified this apparent heterogeneity, against a natural background of inherent variability. While an official community-wide approach for addressing this issue is fraught with practical difficulties, this study provides a timely focal point for all researchers who strive to use the geological archive to reconstruct ancient environmental conditions.

Tostevin & B. Mills [11] present a review of proxy records of atmospheric oxygen and marine redox state through the Neoproterozoic-Paleozoic. Alongside this they update the ‘COPSE’ model of long-term biogeochemical cycling, bringing together multiple updates to the model and providing updated predictions of atmospheric oxygen through the Neoproterozoic-Paleozoic. This suggests that atmospheric oxygen fluctuated by about an order of magnitude between 0.1 and 1 PAL (present atmospheric level) during the Ediacaran-Cambrian periods. The cause of this in the model is that, building on recent work [67], the ‘Shuram’ negative carbon isotope excursion is assumed to be driven by an input of oxygen from the weathering of sulphate minerals. This generates a late Ediacaran peak in atmospheric oxygen that is predicted to have temporarily oxygenated the ocean. In fact the proxy data suggest multiple ‘ocean oxygenation events’ through the Cryogenian-Ediacaran-Cambrian. This might hint at multiple peaks in oxygen, not least because the COPSE model doesn’t yet capture other negative carbon isotope excursions that may have had a similar cause. The model also needs to account for the times when sulphate evaporite deposition exceeded weathering, which would be expected to cause oxygen to decline. Overall this will serve to reinforce Tostevin and Mills’ conclusion that there was no single ‘Neoproterozoic oxygenation event’ but rather there was what they call a ‘Neoproterozoic oxygenation window’ (NOW) in which oxygen fluctuated up and down. NOW that is something for early animals to contend with!

As demonstrated by Tostevin and Mills [11], combining geochemical proxy data with biogeochemical modelling constraints opens up tremendous possibilities for testing hypotheses and feedbacks in relation to potential links between the environment and the evolution of complex life, in all its forms. However, for a great majority of the community, including those who greatly value this approach, modelling can appear intangible. For non-modellers, it can be difficult to assess the limitations of individual models, or the applicability of different modelling approaches to successfully address different problems. In a scholarly review of key existing models and their limitations and future possibilities, ***Lenton*** [31] opens a window into the world of modelling for the non-specialist. The approach is firmly centred on implications for reconstructing oxygen and nutrient levels as complex life evolved and diversified, but a particularly valuable aspect comprises an overview of the principles that should underpin all biogeochemical models, combined with a critical evaluation of key existing models. It is argued that some of the disagreements between existing models and the reconstruction of atmospheric oxygen and ocean nutrient levels associated with the rise of complex life stem from the application of conceptual frameworks designed for inappropriate timescales, as well as variable model representations of different processes, and flaws in process representation. The upshot is that given current uncertainties in atmospheric oxygen levels and the minimum oxygen requirements of early complex life, it remains unclear whether the evolution of stem group animals, sponges or simple bilaterian animals was prohibited by a lack of oxygen. But Lenton takes us beyond this, and in addition to outlining how current biogeochemical models can be improved to better address the main issues at hand, links to other modelling approaches are advocated, including consideration of how trait-based physiological and ecological models may capture environmental constraints on particular types of complex life, which would then allow subsequent feedbacks to be interrogated by biogeochemical modelling.

Payne et al. [68] consider the relation between the evolution of complex life and the apparent stabilisation of the Earth system in the Phanerozoic. It is well known that background extinction rates decline through the Phanerozoic, but why? Payne et al. discount the options that interactions between species have decreased over time (they haven't) or that geologic or extraterrestrial perturbations have decreased over time (they haven't). Rather, they argue that animals have evolved to gain more physiological resilience to environmental changes. But something much larger in scale also appears to be going on. For example, the 'Snowball Earth' events have never been repeated in the Phanerozoic, and it is well established that a key stabilising feedback in the long-term carbon cycle from silicate rock weathering has become more effective with the evolution of land plants. Furthermore, the evolution of larger, mobile animals mixing sediments [69] and water columns is argued to have stabilised biogeochemical cycles. Overall, Payne et al. agree with and augment previous analyses [70] that "the evolution of complex life has, on the whole, strengthened stabilising feedbacks in the climate system" [68]. If so, this is a remarkable observation - consistent with Lovelock and Margulis' Gaia hypothesis [71] - that the evolution of life has been making the Earth system more stable and resilient over time.

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