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## Animal Evolution: Trilobites on Speed

**A new study quantifies rates of morphological and molecular evolution for arthropods during the critical Cambrian explosion. Both morphological and molecular evolution are accelerated — but not so much to break any speed limits.**

**Graham E. Budd**

The sudden appearance of animals in the fossil record has exercised minds as far back as Cuvier in 1812 [1], and ever since around the time of Steven Stanley's 'cropping' hypothesis [2], it has become customary to call this event the 'Cambrian explosion'. We now date the first definite animal fossils in the record to around 540 million years ago (mya), and by about 515 mya exceptionally preserved biota such as that from Chengjiang followed by the slightly younger Burgess Shale reveal that a wide range of animal taxa with different life-styles had evolved. The implication of the 'Cambrian explosion' tag is thus that the fossil record is telling us something real about the speed and nature of the evolutionary events that we can dimly perceive behind it. However, there has always been an alternative view, namely that the oldest fossil record of animals should not be read literally, and instead is the product of a long period of cryptic evolution — in other words, that the first animal fossils post-dated the first animals by some considerable time. The most famous exponent of this view was of course Darwin, and the problematic nature of the event has therefore become known as 'Darwin's

Dilemma'. Darwin's view, that there must have been an extensive but hidden Precambrian history of animals, became largely discredited by the work of skeptics such as Preston Cloud who showed that most putative Precambrian animals fossils could easily be refuted [3]. Nevertheless, this view was revived during the 1990s when some molecular clock studies (e.g. [4]) that use rates of change of molecules such as DNA to assess times of divergences of different lineages suggested that animal lineages in fact had deep roots perhaps hundreds of millions of years older than their appearance in the fossil record. Such views became fashionable partly because of worries that if animal evolution really took place within the Cambrian, it implied very fast rates of evolution that might not be easily reconcilable with Darwinian modes of gradualistic evolution [5]. Now, Lee *et al.* [6] present in this issue of *Current Biology* a groundbreaking study of arthropod evolution in the Cambrian and later. They find firstly that Cambrian rates of evolution for both morphology and molecules really were fast compared to average later ones, and secondly that even so they do not appear to break any supposed speed limits — similar rates

are known from later evolutionary radiations.

It is rather remarkable, perhaps even embarrassing, that the basic question above about the early animal fossil record has yet to be fully resolved. One of the problems has been that the molecular clock method of assessing times of origin has been controversial because of its demonstrably uneven rate through time and in different organisms. Furthermore, molecular clocks need to be calibrated, eventually against the fossil record. Recalibration of the invertebrate molecular clock [7] has in recent years tended to push opinion back towards the explosion option for animal origins, although molecular clocks still currently date the origin of at least sponges to some 200 million years before the Cambrian [8], despite not being convincingly recorded by the fossil deposits [9].

Of course, it has long been known that at least morphological rates of evolution must be highly uneven through time, a pattern categorised as 'bradytely' (slow), 'tachytely' (fast) and 'horotely' (normal) rates of evolution for particular groups. [10]. There is even an official unit of measurement, inevitably called the Darwin, based on proportional change in (for example) the size of a particular feature per unit time [11]. One pattern that has been suggested is that morphological evolutionary rates during the early stages of evolution of a group appear to be fastest, before settling down to more staid middle and old age (e.g. [12]). So much for morphology, but what of the enticing question of the

molecular background to this? In other words, given that we know that rates of morphological change vary through time, what about the correlated changes in the DNA that must ultimately lie behind such change? An intriguing study [13] based on Bayesian analysis suggested some years ago that basal animal evolution only *appeared* to have taken place well before the Cambrian explosion because of an increase in molecular rates around the base of the Cambrian itself. However, the conclusions of this study have not been universally accepted because of methodological problems centred on how sensitive such analyses are to prior assumptions [14]. Lee and colleagues [6] have shown that at least for arthropods, this pattern seems real: just like in morphology, which they also quantify, the molecular evolution of animals appeared to be unusually fast during the Cambrian (at least, relative to post-Cambrian rates). The work is based on a relaxed-clock study of a previously-published set of 62 protein-coding genes and a broad-based phylogenetic study of living arthropods that allows the calculated molecular clock rate to vary somewhat through time, all time-calibrated with the (generally) excellent arthropod fossil record. What is particularly satisfying about this study is that the authors take the issue of possible deep Precambrian roots to animal evolution head on, and show that even if one does assign such early dates to animal origins, this only has marginal effects on the elevated rates deduced for the deep branches.

Putting dates of a “this group must have evolved by this time” type on taxa is relatively straightforward. For example, given the presence of branchiopods, an important group of living crustaceans, by the end of the Lower Cambrian some 510 mya, which are quickly followed by other modern crustacean groups [15], we know that crustaceans of modern aspect must have evolved at *least* by then (Figure 1). But fixing lower limits is a much more delicate task, as it must partly rely on negative evidence: allowing absence of fossils to imply absence of organisms. Here, the complex problem of fossil preservation arises: if early animals were very small, or preservational conditions were notably different to later, then early fossils might simply be much harder to find than later ones. The oldest known arthropod body fossils

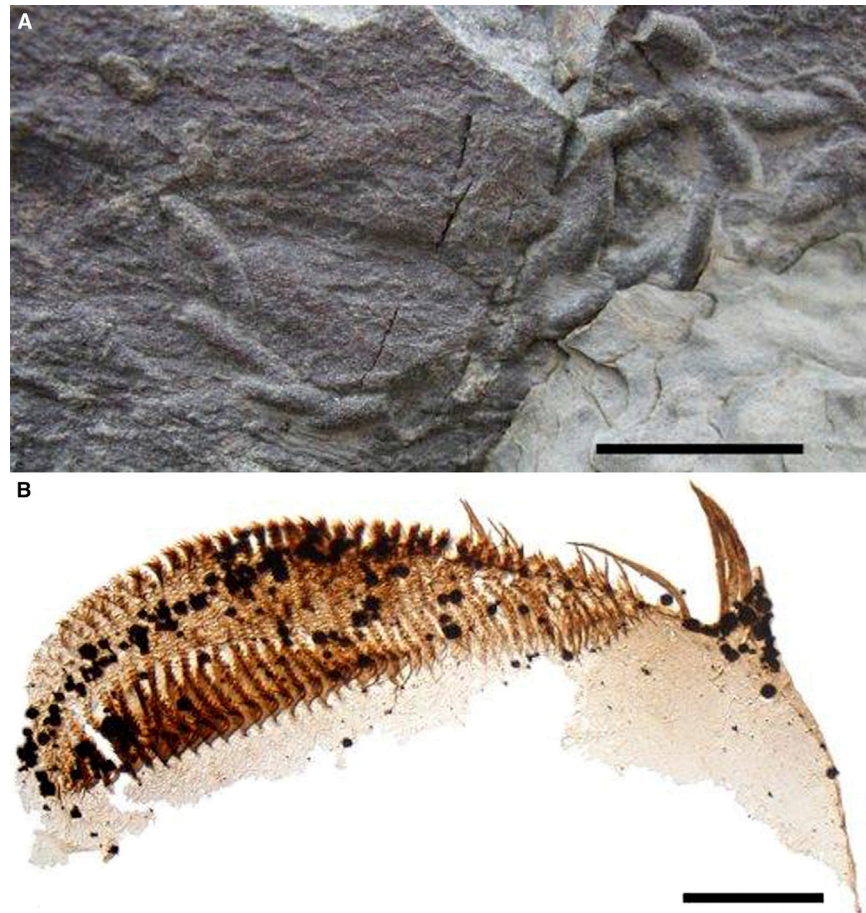


Figure 1. Constraining the basal arthropod radiation with fossils.

(A) *Treptichnus pedum*, a large and complex but non-arthropodan trace fossil from the base of the Cambrian of north Norway, around 540 mya (scale bar: 2 cm; image courtesy of Søren Jensen). (B) A mandible of a crown-group branchiopod crustacean from the Middle-Late Cambrian of Canada, approx. 505 mya [15]. The presence of complex trace fossils from the base of the Cambrian from rocks that should, but do not, preserve any arthropod traces suggests that trace-making arthropods were yet to evolve. Conversely, by the time of the Middle-Late Cambrian, definitive crown-group crustaceans had evolved, suggesting that a major arthropod radiation took place between these dates. (Scale bar: 50  $\mu$ m; image courtesy of Tom Harvey and Nick Butterfield.)

are probably trilobites from around 520 mya, but trace fossils that can be really confidently attributed to large complex arthropods of some type are known from quite close to the base of the Cambrian (perhaps at around 535 mya). From the end of the Ediacaran at around 545 mya onwards are relatively large trace fossils that do *not* appear to be arthropods (Figure 1), although they are preserved in similar environments as later arthropod traces, and from this a reasonable assumption is that large complex arthropods had not evolved by then. Depending on what exactly is the phylogenetic significance of ‘large, complex arthropods’, one reasonable interpretation is that a large proportion of arthropod evolution took place

between the end of the Ediacaran at about 545 mya and the end of the lower Cambrian at about 510 mya when modern crustaceans appear, i.e. during a period of about 35 million years at the most, and possibly in considerably *less* time. What the trace and body fossil records taken together tell us, though, is that in principle it should be possible to find arthropod body fossils that are at least 15 million years older than those presently known — but they almost certainly lacked hard parts, and preservational conditions do seem to have been annoyingly poor this early in the Cambrian [16].

Whether or not 35 million years or less is enough time to evolve an entire phylum of animals from a potentially

very simple ancestor is a matter of taste. Presumably there is an as-yet not understood upper “speed limit” for such rates, but Lee and colleagues [6] point out that even the most elevated rates recovered do not seem to go over the limit for known rapid periods of either morphological or molecular evolution. As a result, one can indeed have one’s evolutionary cake and eat it — it is possible to squeeze the radiation of the animals into a short period of time just before and during the Cambrian without having to invoke any peculiar non-Darwinian mechanisms to explain it.

Of course, this raises a further and potentially even more interesting question about how the elevated rates of gene and morphological evolution are related to each other. Lee *et al.* [6], perhaps wisely, steer clear of addressing this fraught issue directly, and indeed previous attempts at examining it have come to conflicting conclusions [17,18]. However, the mere observation that both rates are elevated is unlikely to be coincidental, and suggests that, contrary to various developmental scenarios where large-scale morphological change in the Cambrian explosion is driven by a few changes in some key developmental genes, there must be an ecological basis to it that would naturally link the two rates. The discovery that some molecular evolutionary rates are elevated during a particular interval of time will naturally

re-awaken some of the vexatious issues around selection versus neutrality in molecular evolution [19]. If there is a true correlation between the two rates, this could come about by a variety of means [20], not all of which involve direct causality. Exploring the relationship between the two will in future work surely add valued ammunition to fire at the many problems presented by early animal evolution.

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## Rapamycin Resistance: mTORC1 Substrates Hold Some of the Answers

The mechanism of action of the mTOR inhibitor rapamycin is poorly understood and why certain mTORC1 phosphorylation sites are rapamycin insensitive remains elusive. Site-specific analysis of mTORC1 substrates now suggests that the sequence composition of a phosphorylation site determines whether it is sensitive to rapamycin and starvation.

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Cells respond and actively adapt to a variety of environmental and intracellular stimuli, such as nutrients, energy, oxygen and growth factors. The mammalian target of rapamycin complex 1 (mTORC1) integrates these

cues to regulate key anabolic and catabolic processes, including protein, lipid and nucleotide synthesis, as well as autophagy [1,2]. Not surprisingly, mTORC1 signaling is commonly deregulated in human diseases, including cancer and diabetes, making mTOR an attractive therapeutic target with numerous clinical

applications. Extensive efforts to develop improved analogs of the mTOR inhibitor rapamycin — so-called rapalogs — have resulted into two types of FDA-approved molecules, including temsirolimus (Torisel) and everolimus (Afinitor). While these drugs were found to be effective against certain neoplasms, including advanced kidney cancer and mantle cell lymphoma, many types of cancer respond poorly to rapalogs [3]. Given the crucial role played by mTORC1 in cell growth and proliferation, these intriguing findings have fueled interest in better understanding the elusive mechanism of action of rapamycin.

Extensive studies have revealed that mTORC1 phosphorylation substrates are not equally impacted by rapamycin