

# Breathing room for early animals

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If life has been present on our planet for much of its history—more than three and a half billion years—why did it take so long for animals to appear? This question emerged long ago from the incipient fabric of the fossil record, which embodies the sudden appearance of metazoans in sedimentary strata deposited near the end of Precambrian time—and scientists have wrestled with this issue now for well over a hundred years (1). For the past 50 or so years, the most popular and pertinacious hypotheses have concerned atmospheric dioxygen (2–5). Because of their aerobic physiology, the idea was that O<sub>2</sub> levels were perhaps too low to have supported animals until sometime near the end of Precambrian time, and that rising O<sub>2</sub> levels thereafter provided a quantum evolutionary leap of sorts for aerobic biology culminating in the “late” evolution of animals (3). A recent study of middle Proterozoic sedimentary rocks in China, however, favors a different view—suggesting that O<sub>2</sub> levels capable of supporting animal physiology were present more than 500 million years before the appearance of animals (6).

Animals evolved in an aerobic world; that much is clear. They need large O<sub>2</sub> fluxes to support the metabolic demands of their multicellular body plans (7), and O<sub>2</sub> is used copiously during the biosynthesis of their tissues (8). Exactly how much dioxygen did early animals require? Phylogenetic relationships near the base of the Metazoa remain unsettled, but based on the degree to which Porifera (sponges) provide a useful measure of the aerobic requirements of early animals, experimental observations illustrate that the O<sub>2</sub> concentrations needed appear to be exceptionally low, perhaps 0.5–4% present atmospheric levels (PAL) (Fig. 1) (9). A similar view was garnered from environmental observations of oxygen minimum zones (OMZs)—subsurface zones in marine basins typically a couple hundred meters in water depth where O<sub>2</sub> levels are at their lowest due to high respiration demands (10). These environmental data also showed that although small animals may be present at very low O<sub>2</sub> concentrations, the ecological richness associated with the Cambrian biota (particularly carnivory) requires O<sub>2</sub> concentrations perhaps closer to 10% PAL (Fig. 1) (11, 12). Under the hypothesis that animal

evolution and O<sub>2</sub> are inexorably linked, these physiological and ecological constraints make a set of predictions for the geological record—specifically, that stable atmospheric O<sub>2</sub> concentrations did not rise above 1% PAL until relatively late in the Proterozoic Eon.

Reconstructing ancient atmospheric O<sub>2</sub> levels from geological observations has been notoriously tricky (Fig. 1). To date, there is no simple paleobarometer for O<sub>2</sub>. The best historical observations come from direct measurements of atmospheric O<sub>2</sub> from bubbles of ancient air trapped in ice, now as old as 1 Ma (13). All older estimates for atmospheric oxygen come from subtle geological and geochemical measurements of sedimentary rocks, quantified in the context of mass balance models. It is worth noting that the geochemical measurements, in particular, are typically highly precise; rather, it is the models and mechanistic frameworks that take these data and invert for O<sub>2</sub> concentrations that introduce substantial estimate uncertainty. Walking back from the ice measurements, constraints on atmospheric O<sub>2</sub> are provided by fire in terrestrial ecosystems. The record of fossil charcoal shows that as long as sufficient plant biomass existed on land that could burn, it did (14), requiring O<sub>2</sub> levels greater than ~70% PAL (15). Before the appearance of land plants, reconstructions—particularly of Proterozoic O<sub>2</sub> levels—become murky. Ironically, in a number of ways, it has been easier to constrain Archean atmospheric O<sub>2</sub>, because a number of geological and geochemical proxies are sensitive to tiny amounts of O<sub>2</sub>, and constrain Archean dioxygen to levels  $\ll 10^{-3\%}$  PAL. The loss of redox-sensitive detrital grains, appearance of fluvial and near-shore marine red beds, and other sedimentary features indicative of oxidative weathering during continental denudation mark a major rise in atmospheric O<sub>2</sub> concentrations beginning at ~2.35 Ga; estimates suggest this corresponded to O<sub>2</sub> levels of 1% PAL or greater (Fig. 1) (16, 17). Empirically, the atmosphere was forever thereafter oxygenated. However, at what point did O<sub>2</sub> reach concentrations sufficient to support animals?

Classical reconstructions of Proterozoic O<sub>2</sub> concentrations were drawn near 1% PAL to honor both the

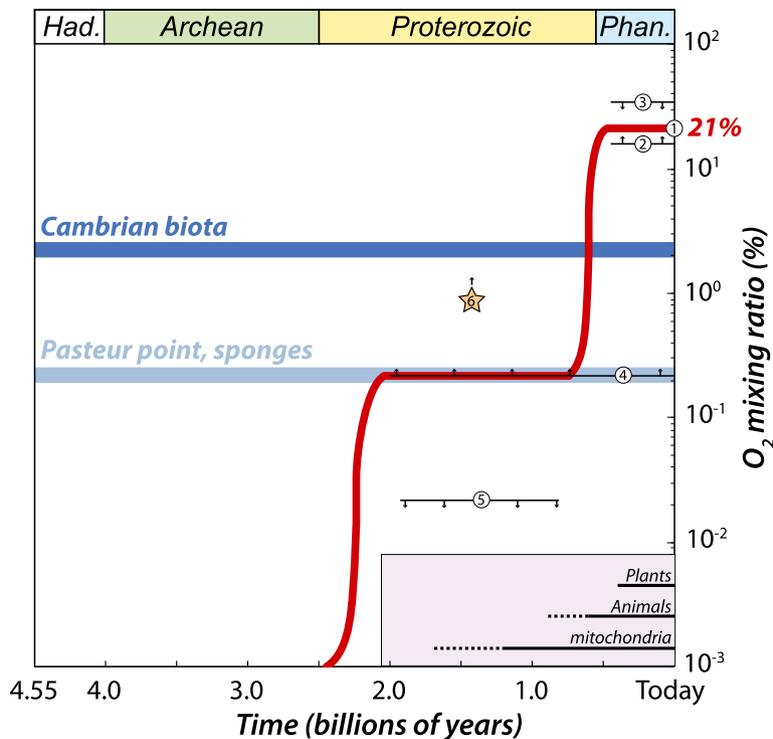
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**Fig. 1.** Reconstructing ancient atmospheric  $O_2$ . For point 1, direct observations can be made back to 1 Ma via atmospheric bubbles trapped in Antarctic ice (13). All other  $O_2$  constraints are the results of models. In point 2, observations of charcoal in terrestrial sedimentary rocks provide a record of combustion nearly as old as plants themselves (14). Point 3 is a limit set by persistent wildfire (24). Point 4 takes into account oxidative weathering and loss of redox-sensitive detrital grains from sedimentary basins (16, 17). Point 5 relates to a general lack of Cr isotope fractionation observed in sedimentary rocks (21). The starred point 6 incorporates the new constraint from sedimentary strata of the middle Proterozoic Xiamaling Formation (6). The estimates from this study are remarkable because they are higher than anticipated and exceed  $O_2$  concentrations required for animals respiration. The red curve highlights a classical view of Earth's  $O_2$  history (e.g., ref. 16)—one that respects oxidative weathering, but remains near the lower bound to explain a perceived higher frequency of Proterozoic ocean anoxia. In light blue, the Pasteur point generally defines the point at which many organisms forgo aerobic respiration for fermentation, and/or perish. In dark blue, a likely threshold  $O_2$  level required for Cambrian biota is shown (11, 12). Also shown for reference are the age ranges of animals, plants, and mitochondria. Although eukaryotes marked by a ribosomal lineage may be quite old, mitochondria appear to be younger on the basis of fossils and molecular clocks. Their evolutionary origin and persistence in Proterozoic surface environments also provide some general constraints on  $O_2$ .

oxidative weathering constraints and the widely held—but hard to quantify—view that anoxia occurred with greater frequency in ocean basins than in Phanerozoic counterparts (Fig. 1) (16, 18). In general, these reconstructions have received support from datasets of the abundance of redox-sensitive trace metals in fine-grained sedimentary rocks that tend to show low Proterozoic values contrasting with high Phanerozoic values (19, 20). More recently, data from the emerging Cr isotope system pushed Proterozoic estimates even lower. Measurements of Cr isotope ratios in iron-rich sedimentary rocks displayed a general lack of the isotope fractionation expected at high  $O_2$  concentrations, and, viewed through the lens of an (admittedly imperfect) isotope mass balance model, it was argued that these data constrain  $O_2$  levels to  $<0.1\%$  PAL (21). If  $1\%$  PAL might be viewed as marginal for animal respiration,  $0.1\%$  PAL certainly would have been (Fig. 1). Additionally, the Cr isotope data showed a large secular trend with “modern” levels of fractionation beginning  $\sim 800$  Ma, and this result breathed fresh air into the idea that rising amounts of  $O_2$  in the atmosphere (above a respiration threshold) and the origin of animals were correlated in time. This is where the new study from Zhang et al. (6) comes in.

Working in fine-grained marine sedimentary rocks of the Xiamaling Formation on the North China Platform, Zhang et al. (6) observed patterns of enrichments of trace elements V, U, and Mo similar to those seen in Phanerozoic strata that imply at least

episodic bottom water oxygenation. Additionally, these rocks are exceptionally well preserved for their age, and, because they have not been substantially buried and heated, the organic-rich rocks still contain hydrocarbon biomarkers. The authors observed stratigraphic trends in the abundances of aryl isoprenoids—molecules formed predominantly as breakdown products from the aromatic  $C_{40}$  carotenoid isorenieratene. Iso-renieratene is a valuable paleoenvironmental indicator molecule because it is predominantly (although not uniquely) synthesized by members of the bacterial phylum Chlorobi capable of anoxygenic photosynthesis using sulfide as an electron donor. These biomarkers are important because they highlight the presence of photic zone euxinia—free sulfide (and thus the lack of dioxygen) in parts of the water column that still contain useful light for photosynthesis.

Together with the trace metal enrichments, these stratigraphic datasets argue convincingly that these rocks were deposited in a marine basin with an overlying OMZ—perhaps similar to what one would find today in the productive waters along the western continental margins of South America or Namibia. Indeed, one would be forgiven for confusing the depositional characteristics of middle Proterozoic Xiamaling Formation with those of Cretaceous-age petroleum source rocks in the Middle East, were it not for the paleontological attributes to tell them apart. Armed with their paleoenvironmental and biogeochemical data, Zhang et al. (6)

then did a range of calculations using a simple model of the marine carbon cycle to reconstruct the O<sub>2</sub> levels that best explained the geochemical data. They found that O<sub>2</sub> levels of ~4% PAL or higher were required to explain the observations (Fig. 1). This estimate pushes O<sub>2</sub> concentrations higher than classical reconstructions and is much greater than was estimated from Cr isotopes; if correct, it highlights a location and time when dioxygen was assuredly high enough for animal respiration, far before any animals prowled the seas.

With the new results from Zhang et al. (6), are we ready to toss aside the hypotheses that connect O<sub>2</sub> and animals? Probably not yet. Proterozoic proxy estimates for atmospheric O<sub>2</sub> concentrations still manifest conflict, and O<sub>2</sub> curves will remain unsatisfying as long as we don't have a good understanding of why. It's also possible that Proterozoic O<sub>2</sub> levels did occasionally exceed thresholds for animal respiration but were not sufficiently stable (because O<sub>2</sub> has a geologically short characteristic residence time of <1,000 y) to enable the evolution of animals, a prerequisite noted by Nursall (2). Additional studies of Proterozoic rocks will be needed to test this possibility. It might also be the case that atmospheric O<sub>2</sub> concentrations were long permissive of animal origins (~4% PAL), but that they didn't rise to above a threshold (near ~10% PAL) to fuel the ecological complexity associated with the Cambrian biota until near the end of the Proterozoic Eon (Fig. 1) (12). However, other hypotheses would still seek to link the histories of O<sub>2</sub> and animals but reverse the causality (22).

One of the enduring challenges to understanding the history of Earth's O<sub>2</sub> stems from the current requirements that we view atmospheric O<sub>2</sub> through the lens of complex (typically marine) biogeochemical processes that have evolved with the biota over Earth's history. It is becoming more apparent that the sedimentary record can make the world look more anaerobic than it ever really was. For example, a new comprehensive dataset of aromatic carotenoids—biomarkers for photic zone euxinia like those observed in the Zhang et al. (6) study—illustrates that these compounds are much more pervasive in sedimentary strata of all ages (Proterozoic and Phanerozoic) than previously thought (23). Strategies are needed that can accurately parse this aliasing to arrive at more accurate measures of ancient atmospheric O<sub>2</sub>. Perhaps on the horizon remains a more direct and reproducible O<sub>2</sub> proxy that will allow us to definitively evaluate the hypotheses that connect animals and O<sub>2</sub>. Indeed, new approaches to evaluating Earth's redox history are a burgeoning pursuit in the Earth sciences. However, a profitable path forward might require taking a step back to better understand the mechanics of O<sub>2</sub> in the more recent geological past. Earth has done a number of geological (e.g., climate change and Northern Hemisphere glaciation) and biological (C<sub>4</sub> photosynthesis) experiments over just the past 10 million years that should have—if we can understand them properly—impacted atmospheric O<sub>2</sub> in predictable and measurable ways. For now, though, the study of Zhang et al. (6) leaves us with some important questions, and suggests that we keep an open mind about why animals appear so late in Earth history.

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