Introduction

Atmospheric carbon dioxide: a driver of photosynthetic eukaryote evolution for over a billion years?

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Exciting evidence from diverse fields, including physiology, evolutionary biology, palaeontology, geosciences and molecular genetics, is providing an increasingly secure basis for robustly formulating and evaluating hypotheses concerning the role of atmospheric carbon dioxide (CO₂) in the evolution of photosynthetic eukaryotes. Such studies span over a billion years of evolutionary change, from the origins of eukaryotic algae through to the evolution of our present-day terrestrial floras, and have relevance for plant and ecosystem responses to future global CO₂ increases. The papers in this issue reflect the breadth and depth of approaches being adopted to address this issue. They reveal new discoveries pointing to deep evidence for the role of CO₂ in shaping evolutionary changes in plants and ecosystems, and establish an exciting cross-disciplinary research agenda for uncovering new insights into feedbacks between biology and the Earth system.

Keywords: atmospheric carbon dioxide; evolution; fossils; geochemistry; molecular genetics; photosynthesis

1. INTRODUCTION

The papers in this thematic issue arose from presentations given during a two-day scientific meeting held at the Royal Society’s Kavli International Centre. Speakers were invited to consider the evidence for (or against) the proposition that a changing atmospheric carbon dioxide (CO₂) concentration might represent a driver of the evolution of photosynthetic organisms. The premise for this proposition arises from three fundamental considerations. First, the central role the enzyme Rubisco (ribulose-1,5-bisphosphate carboxylase–oxygenase) plays in the photosynthetic productivity of the biosphere. Second, the substantial changes in CO₂ and oxygen (O₂) that characterize the evolution of Earth’s atmosphere over the past 2.4 Gyr since the advent of oxygenic photosynthesis. Third, the co-dependency between vascular land plant evolution and the organic and inorganic carbon cycles through the production of organic matter for burial in sediments, and acceleration of the chemical weathering of silicate rocks, respectively [1–4]. Biological enhancement of silicate weathering may be especially important because, as several contributions point out, it influences the concentration of atmospheric CO₂ over evolutionary timescales. The overall process can be expressed by the following simplified equation [2,5,6] representative of all calcium silicates:

$$\text{CO}_2\text{[atm]} + \text{CaSiO}_3\text{[continent]} \rightarrow \text{SiO}_2\text{[continent + ocean]} + \text{CaCO}_3\text{[ocean]}. \quad (1.1)$$

where [atm] refers to atmosphere, [continent] refers to continental crust and soil, and [ocean] refers to sedimentary deposits on the ocean floor. Silica (SiO₂) is incorporated in weathering products such as clay minerals on land, or deposited as siliceous oozes and diatomaceous sediments at sea. A similar, but more complicated, expression prevails for magnesium silicates.

Fossil evidence indicates the appearance of eukaryotic marine algae by around 1.2 Ga [7–9], with the possible colonization of freshwater lake margins by 1.1 Ga [10]. These organisms, and the majority of photoautotrophs that have evolved since that time, use the enzyme Rubisco to catalyse the carbon-reduction step in the photosynthetic carbon-reduction pathway [11]. Therefore, Rubisco is the primary carboxylase powering photosynthetic life forms and linking the organic and inorganic carbon cycles. However, variations in the Earth’s atmospheric composition are likely to have major effects on its efficiency, with feedbacks on CO₂ via the operation of these cycles. The inefficiency arises because the active site of Rubisco can bind with either CO₂ or O₂, leading to catalysis of two competitive reactions: photosynthetic CO₂ assimilation (carboxylation) or photorespiratory CO₂ loss (oxygenation), respectively [12]. The oxygenation...
reaction also generates toxic intermediates that are metabolized via photorespiration to render them harmless [13]. Variations in the Earth’s atmospheric composition additionally change the bioavailability of key elements, including nitrogen, phosphorus, iron, zinc and magnesium, required by organisms to produce and operate Rubisco [14]. Such considerations create the potential for feedbacks between biology and geology on evolutionary timescales. While consideration has been given to the interplay between the evolution of oxygenic photosynthesis and the early redox history of the ocean and the atmosphere [15], the potential for atmospheric CO\textsubscript{2} change (or atmospheric CO\textsubscript{2} and O\textsubscript{2} combined) to influence the evolutionary history of eukaryotic photosynthetic organisms has generally received far less attention.

Recent advances now indicate it is an opportune time to redress the balance. Over the last decade, an expanding wealth of information from diverse fields—including physiology, evolutionary biology, palaeontology, geochemistry and molecular genetics—is increasingly providing evidence to robustly formulate and evaluate hypotheses concerning atmospheric CO\textsubscript{2} as a driver of the evolution of photosynthetic eukaryotes. The papers in this issue reflect the breadth and depth to which novel and exciting approaches are being used to address this topic, and reveal new insights into the coevolution of the terrestrial and marine carbon cycles and the Earth’s atmosphere.

### 2. CARBON DIOXIDE AND THE EVOLUTION OF ALGAE

The first two contributions consider algal evolution and adaptation in relation to atmospheric CO\textsubscript{2}. Young et al. [16] report new analyses of the kinetic properties of Rubisco with the aim of defining its history of adaptation in red and chromistan algae that possess a red chloroplast during atmospheric CO\textsubscript{2} and O\textsubscript{2} change over the past 1.5 Gyr. They detect a signature of positive selection in the rbcL gene sequence encoding for the large subunit of Rubisco linked to physiological adaptations to falling CO\textsubscript{2}. Focusing on the geochemically and ecologically important groups, the Haptophyta (including coccolithophores) and the Bacillariophyta (diatoms), they find evidence for strong positive selection of Rubisco during periods of falling CO\textsubscript{2} one around 1.1 Ga and the other at approximately 0.41 Ga. The latter event implies an evolutionary response of algal Rubisco to significant reductions in CO\textsubscript{2} during the formation of the Neoproterozoic ‘snowball’ Earth.

Carbon-concentrating mechanisms (CCMs) induced with biochemical and/or biochemical mechanisms that suppress the oxygenation reaction of Rubisco are an important evolutionary response to falling CO\textsubscript{2}. Indeed, the 1.1 Ga burst of Rubisco evolution detected by Young et al. appears linked to an endosymbiotic event that produced a CCM and relaxed constraints on the need for high CO\textsubscript{2} specificity. The origin and function of CCMs in photosynthetic marine algae is reviewed by Raven et al. [14]. CCMs are widely distributed among algae and very likely evolved as an adaptation to low CO\textsubscript{2} by effectively accumulating CO\textsubscript{2} in the compartment containing Rubisco to a higher steady-state concentration than in the growth medium, thereby increasing its efficiency. As Raven et al. [14] emphasize, there are numerous documented episodes of low CO\textsubscript{2} in the past when such adaptations could confer obvious advantages, including the Permoo–Carboniferous glaciation (300–270 Ma) and repeated glacial intervals of the Pleistocene (2.1 Ma).

Less obvious, however, are answers to the fascinating question of why CCMs have been retained by algae during subsequent warm, high CO\textsubscript{2} ‘greenhouse’ intervals of the Earth's history, given their operation has an energetic cost to the organisms concerned [14,17]. The answer appears to be that CCMs confer an advantage under a combination of other environmental factors besides low CO\textsubscript{2} [14]. Under a high CO\textsubscript{2} environment, such as might be expected in the future, CO\textsubscript{2}, HCO\textsubscript{3} and pH all increase in surface sea water, while the depth of the upper mixed layer of the ocean decreases. Taken together, these effects increase the availability of inorganic carbon, photosynthetically active radiation and ultraviolet-B radiation and decrease the supply of nutrients, especially N and P, which evidence from short-term experiments reveals can alter algal CCM expression [14]. This evidence points to the need to move away from the existing paradigm viewing algal CCMs solely as an adaptation to a low CO\textsubscript{2} atmosphere [14].

### 3. CARBON DIOXIDE AND THE EVOLUTION OF TERRESTRIAL PLANTS

The evolutionary appearance of land plants marked emergence of the terrestrial biosphere, and Pires & Dolan [18] synthesize recent advances in evolutionary developmental biology that shed light on how plant body plans and morphology diversified during their successful colonization of terrestrial environments. They conclude that the core genetic toolkits of distantly related plants are remarkably similar, and that the evolution of form derives from altered expression patterns of ancient lineages of regulatory genes throughout land plant evolution. A similar situation also exists for animals, suggesting general principles of evolution are emerging from evolutionary developmental biology [18]. An important outcome from the recent sequencing of plant genomes has been the realization that many transcription factor (TF) gene families regulating gene expression are highly conserved in land plants, but with the size of the gene families increasing substantially over time, mostly as a result of gene duplication events [18]. This implies that an expansion of TF gene families over time may lie behind the dramatic diversification of land plants, and is reminiscent of what has been discovered regarding the mechanisms contributing to the rise in complexity of metazoans [18].

The origin of land plants and early terrestrial animals linked to the evolution of genes and developmental mechanisms marks the early development of the organic carbon cycle on land. Fossil land plant remains provide direct evidence for the emergence of the terrestrial biosphere, and the assembly of early terrestrial ecosystems with herbivorous arthropods consuming organic detritus [19]. Kenrick et al. [20] argue for the possibility...
that we are being deceived by the nature of the fossil evidence because of major facies changes in the rock record. Taking analyses of western Europe as an example, they point out that there are very few unequivocal terrestrial deposits known before the latest Silurian, which only become widespread later during the Early Devonian [20]. This raises the intriguing possibility that plant evolution feeds back to influence its own preservation as fossils in sediments by changing the nature of the sedimentary environments, including fluvial systems [21,22]. Constraining when early land plants originated is important for understanding when in the Early Palaeozoic they began exerting an influence on organic and inorganic carbon cycles. This challenge requires a more robust approach than relying solely on fossils. Kenrick et al. [20] suggest an approach combining evidence from the fossil record with time-trees derived from molecular calibrations of relevant living species of early land plants and arthropods. Their analyses support the idea that facies changes in the rock record mask an earlier origin of life on land than is evident from certain classes of fossil data.

Stomata are widely regarded as critical structures contributing to the success of land plants, with a relatively rich fossil record extending back over 400 Ma, and far-reaching effects on the global environment [23]. Analyses of changes in the size (S) and density (D) of fossil stomata of land plants over the past 400 Myr indicate both variables are sensitive changes in the concentration of atmospheric CO₂ [24]. In particular, high densities of small stomata appear to be an adaption for allowing high maximum leaf CO₂ diffusive conductances (cmax) and countering CO₂ starvation during Permo-Carboniferous and Cenozoic glaciations [24]. Franks et al. [25] report new experimental observations for a range of plant taxa, from clubmosses through to late-branching angiosperms, which provide supporting evidence for the causal action of CO₂ on coordinated changes in S and D. These results suggest that S- and D-controlled adaptation of cmax to atmospheric CO₂ is widespread in vascular plants, possibly with a conserved underlying genetic basis.

Clues to that genetic basis emerge from findings reported by Doheny-Adams et al. [26]. These authors take a novel approach to investigate the relationship between S and D using genetic mutants and transgenic lines of Arabidopsis with downregulation or over-expression of genes controlling a family of secreted peptide signals known as the epidermal patterning factors (or EPFs) that play a role in stomatal development [27,28]. By altering EPF family expression levels to increase or decrease D, Doheny-Adams et al. [26] show an opposite effect in S, thereby confirming the inverse relationship between these variables originally observed in fossils holds in Arabidopsis. This work suggests the genetic pathways controlling both S and D appear to be linked. Moreover, plants lacking the EPF1 and EPF2 inhibitors of stomatal development failed to show the usual increases or decreases in D when grown at reduced or elevated CO₂ concentrations, respectively. If supported by further investigations, then it suggests the involvement of an intact EPF signalling pathway in an exceedingly ancient stomatal CO₂ response. Interestingly, phylogenetic analyses of the EPF-like gene family reveal several members are present in diverse lineages of land plants, including the moss Physcomitrella patens and the lycophyte Selaginella moellendorfii, suggesting they originated in the common ancestor to all land plants [29].

Allied research reported by Franks et al. [30] reveals a strong positive correlation between nuclear genome size (1C DNA amount), nucleus volume and a simple volumetric measure of guard cell size. They exploit this finding to reconstruct genome sizes throughout the past 400 Myr of land plant evolutionary history using evolutionary comparative analyses and the fossil record of stomatal S. It emerges that genome size appears to correlate with changes in atmospheric CO₂ over this long timescale, possibly as a result of selection toward optimal leaf gas exchange, via guard cell size. Experimental evidence provides some support for this possibility [30] by revealing correlations between increases in S, guard cell nucleus size and average 1C DNA amount in epidermal cell nuclei in response to increases in growth CO₂ concentration. The mechanistic details of these observations are unclear. Nevertheless, this work opens up a new area of investigation concerning the possible role of changing CO₂ in driving adaptations of stomata and leaves that may be linked to changes in genome size.

Until recently, incorporating the effects of the evolutionary rise of terrestrial ecosystems facilitated by genetic networks and stomata on silicate rock weathering into models of the geochemical carbon cycle have largely relied upon scaling observations from field studies with empirical functions [1,2]. Taylor et al. [31] make a significant contribution in this area by reporting a process-based approach to estimate weathering by the terrestrial biosphere, focusing on the effect of nutrient uptake by fine roots and mycorrhizal fungi on soil chemistry and the biological proton cycle [32,33]. The biological proton cycle is controlled by the stoichiometry of reactions during the uptake of mineral nutrients by plants and symbiotic root fungi during growth, and the subsequent return of these nutrients to the soil during decomposition of organic matter [32,33]. Overall, these reactions determine the net flux of protons into and out of the sub-surface environment, and therefore the acid–base balance and its influence on the pH of the mineral weathering environment [32,33]. The approach of Taylor et al. [31] should eventually prove amenable to investigating the effects of critical transitions in early land plant evolution on silicate weathering, like the evolution of trees and deep-rooting systems. In the meantime, global simulations for different time slices over the past 200 Myr highlight the possibility that vegetation exerts substantially larger effects (by up to a factor of 4) than predicted by empirical functions [31]. If borne out by further analyses, these findings suggest that we have been significantly underestimating the effect of terrestrial ecosystems on silicate rock weathering for several decades.

4. CARBON DIOXIDE AND THE EVOLUTION OF ECOSYSTEMS

Two contributions deal with the role of CO₂ as a driver of the evolution of terrestrial ecosystems in which
grasses with the C₄ photosynthetic pathway play an ecologically important role. In plants with the C₄ photosynthetic pathway, inorganic carbon from the atmosphere is initially fixed into four-carbon organic acids via the enzyme phosphoenolpyruvate carboxylase [34]. C₄ acids are then shuttled into a specialized compartment of the cell or leaf and the CO₂ is liberated to feed the Rubisco-driven C₃ photosynthetic pathway [35]. A major adaptation of C₄ plants is the physical isolation from the atmosphere of the compartment where C₃ photosynthesis occurs, typically in bundle sheaths, to enrich CO₂ at the active site of Rubisco and suppress the oxygenation reaction. However, the C₄ CCM has an energetic cost and C₄ grasses are typically only more photosynthetically efficient, in terms of productivity and nitrogen-use efficiency, than C₃ plants under high-light, warm temperature environments that promote high rates of photorespiration in C₃ plants. These considerations generally confine C₄ grasses to open habitats in the warm tropics and subtropics where they form important components of flammable biomes, including savannahs.

Phylogenetic analyses suggest C₄ photosynthesis evolved in more than 60 plant lineages [36], possibly because the pathway uses enzymes that are already present in C₃ leaves [37] and is constructed from pre-existing gene networks [38]. Current evidence supports the contention that C₄ photosynthesis evolved in response to the selection pressure of a low or falling CO₂ atmosphere [38–41]. Recent time-calibrated molecular phylogenies, for example, indicate that C₄ photosynthesis originated in various clades over the past 30 Myr (reviewed in Osborne & Sack [42]), after a dramatic decline in atmospheric CO₂ levels during the Oligocene (23–34 Ma) and the onset of Antarctic glaciation [43].

Osborne & Sack [42] provide a new twist on this low CO₂ debate by introducing whole-plant water relations into the picture. Drawing on physiological information and creative model simulations, they identify C₄ plants as having markedly improved water relations over C₃ plants in open habitats typical of those into which their ancestral forms migrated. The advantage derives from the fact that excessive demand for water transport associated with low CO₂ and highlight and temperature environments would have selected for C₄ photosynthesis as a water-conserving mechanism as well as being beneficial for photosynthetic productivity. In particular, the low stomatal conductance (and transpiration) characteristic of C₄ plants, even at low atmospheric CO₂ concentrations, could protect the hydraulic system from failure under desiccating environmental conditions and, suggested by Osborne & Sack [42], may have contributed to their ecological and evolutionary success.

These detailed physiological considerations of factors underlying the evolutionary success of C₄ grasses in low latitudes over the past 30 Myr are complemented by the contribution of Bond & Midgley [44], who consider the central role of CO₂ in controlling the balance between trees and grasses in savannahs. In these fire-prone ecosystems, fires help maintain grassy vegetation, even though the climate is suitable for woodlands or forests. The global atmospheric CO₂ concentration is thought to act by influencing the growth rate of tree saplings. Large saplings more easily escape fire mortality, and are therefore more readily recruited, accelerating the conversion of open savannahs to woodlands. Against this background, Bond & Midgley [44] highlight documented cases for large increases in the numbers of trees in the mesic savannahs of South and North America, Australia and Africa. These increases cannot easily be explained by historical changes in land use. In the Kruger National Park, for example, they report dramatic increases in tree densities between the 1950s and the present-day, with the dominant tree species (Terminalia sericea) showing a strong response to CO₂ enrichment in glasshouse experiments [44]. These observations, and evidence from experiments and vegetation modelling studies, highlight the fact that increases in the tree cover of tropical and subtropical savannahs around the world appear to be consistent with a global driver—the rapid rise in atmospheric CO₂ concentration over the past 70 years [44].

5. CARBON DIOXIDE AND THE FUTURE

A common recurring theme emerging from the several contributions to this issue is that low or falling atmospheric CO₂ concentrations, rather than a high CO₂ or rising CO₂ concentrations, appear to be a potent selective force driving evolutionary adaptations in plants and ecosystems. This raises the scientific challenge of understanding the responses of photosynthetic organisms to current (and future) increases in atmospheric CO₂, driven to a large extent by increasing rates of emissions from anthropogenic combustion of fossil fuels. Leakey & Lau [45] critically address this issue by considering how an evolutionary perspective might illuminate efforts to understand and manage vascular land plant responses to rising atmospheric CO₂ concentrations. The range of fossil studies reviewed confirms the impression that low or falling CO₂ is a potent agent shaping key moments in the evolutionary history of land plants. Experiment-based multi-generation selection studies in vascular plants and green algae show little evidence for either large or consistent evolutionary responses to elevated CO₂ concentrations, albeit granted the caveat that most are conducted under conditions where abiotic stress is minimal. Leakey & Lau [45] also find no evidence for incidental breeding of crop varieties to achieve enhanced yield under future elevated CO₂. Not surprisingly, therefore, efforts to improve crop productivity in the current CO₂ atmosphere centre on the improving the performance of Rubisco or mimicking the evolutionary success of CCMs in C₄ grasses by engineering a version of it into rice, an important C₄ food crop [46].

Feedbacks between terrestrial plants and the Earth’s geochemical carbon cycle, such as those modelled by Taylor et al. [31,33], operate on long timescales (hundreds of millions of years) and are not therefore obviously relevant to the current CO₂ crisis. However, mitigating the damaging effects of rising CO₂ on future climates and ecosystems over the next century will require the drastic reductions in CO₂ emissions from fossil fuel combustion, especially coal-fired power stations, together with some form of CO₂
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This might involve mandatory carbon capture and sequestration from point sources such as power stations or removing it directly from the atmosphere. One proposition is the injection of CO2 dissolved in water into reactive basaltic rock formations to form carbonate minerals that provide permanent and safe storage for CO2 [48]. It essentially artificially mimics the action of terrestrial plants on silicate rocks with carbonate minerals [49].

More recovered water samples suggesting a significant reduction in Ca and Mg ions in Magmatic Province in eastern North America has demonstrated major reductions in in situ reaction of fossilized CO2 with silicate minerals [49]. More recently, a joint industrial–academic project in Iceland (CarbFix) has been developing and trialling promising technology to exploit the acceleration of weathering as a feasible means of capturing CO2 from a power station by pumping captured CO2 into basaltic formations to generate in situ carbon mineralization [50].

More detailed mechanistic understanding of the actions of plants and associated root-symbionts on silicate rocks from nanometric to ecosystem scales may yet gain increasing relevance to these technologies.

6. CONCLUSION

Analyses of the evolutionary, physiological and genetic traits of extant marine and terrestrial photosynthetic organisms reveal deep underlying evidence supporting the proposition that variations in the Earth’s atmospheric CO2 (and O2) composition have shaped their evolutionary history. As evidence emerges from diverse fields, it provides insights into our understanding of the coevolution of the Earth’s atmosphere, geosphere and biology. At the same time, as the papers in this issue demonstrate, it identifies exciting future directions for investigating the feedbacks between biology and the Earth system.

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