Commentary

Silicon drives the evolution of complex crystal morphology in calcifying algae

Quote:

"The formation of intricately shaped coccoliths from rudimentary calcite crystals requires the presence of silicon. This novel insight brings us closer to understand the evolution of morphological diversity in algae that have shaped planet Earth"

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Main text

Coccolithophores are oceanic microalgae that have influenced the global climate for millions of years because of their ability to calcify (e.g. Monteiro *et al*. 2016). Their life cycle is haplo-diplontic with significant differences in the structure and morphology of the calcium carbonate plates (coccoliths) between haploid and diploid life-cycle stages (e.g. De Vries *et al.* 2021, Frada *et al*. 2019, de Vargas *et al.* 2007). Whereas coccoliths of haploid life-cycle stages (Holococcoliths – HOLs) are uniform in shape and size, diploid stages are characterized by intricately-shaped coccoliths (Heterococcoliths – HETs) of almost infinite morphology. As HOLs seem to be formed differently and only appear in the fossil record about 30 Mya after the first HETs, it has been suggested that holococcolith-formation represents an independent process of calcification, evolving after the emergence of HETs (e.g. Bown *et al.* 2004, De Vargas *et al.* 2007). Yet, in this issue of New Phytologist, Langer *et al*. (2021; pp. ABC-XYZ) have challenged this view by carefully analysing the process of holococcolith-formation. Combining state-of-the-art microscopy tailored to preserve all subcellular structures, and experiments to reveal the role of silicon in the process of calcification, they show that HOLs are formed in intracellular compartments similar to HETs and that silicon is only required for the formation of intricately shaped coccoliths. These results suggest that HOLs might represent an ancestral form of calcification and that the ability to use silicon in the process of calcification evolved later and is responsible for the synthesis of the elaborately shaped HETs.

Calcification is the most characteristic feature of coccolithophores, which belong to the group of prymnesiophytes and diverged from their non-calcifying ancestors approximately 310 Mya (e.g. Liu *et al*. 2010). There are over 250 known species of coccolithophores in sunlit oceans, contributing up to 10% of annual marine primary production (e.g. Poulton *et al.* 2007). Some species including *Emiliania huxleyi* are so productive that their blooms can be seen from space (Fig. 1). Despite their significance for the global carbon cycle, most studies so far have only focussed on a limited number of diploid coccolithophores with the best studied likely to be *E. huxleyi* (e.g. Gal *et al.* 2018, Read *et al.* 2013). Intricately shaped coccoliths allow easier identification of diploid species, which is possibly why haploid life-cycle stages, many of which do not calcify (e.g. von Dassow *et al*. 2012), have largely been neglected, biasing our current knowledge on coccolithophore biology and evolution.

How and when the life phase transitions occur is not well known for most coccolithophore species although several drivers have been identified for *E. huxleyi* (e.g. Frada *et al*. 2019). For instance, viral infections can cause a switch from the diploid to the haploid life-cycle phase to increase survival rates in response to a virus infection (Frada *et al.* 2008). Typically, though, ploidy and the proliferation of life cycle-stages are decoupled, similar to macroalgae and some plants where gametes develop independent life-cycle stages that reproduce asexually, i.e. gametophytes (e.g. Coelho & Cock 2020, Taylor *et al.* 2005). Thus, both life-cycle stages are exposed to evolutionary forces and therefore might even speciate independently. Generally, it can be assumed that haplo-diplontic life cycles are better at exploring the adaptive landscape of a species because of the larger allelic diversity. Indeed, there is some evidence that different oceanic environments appear to select for different life-cycle stages of coccolithophores (e.g. De Vries et al. 2021), however, our knowledge of the adaptive benefits is still very limited. Nevertheless, it is likely that calcification, which underpins the formation of distinct phenotypes, is under selection and therefore the molecular machinery driving it. Depending on the species, basic calcium carbonate crystals (calcite) can transform into nanopatterned and elaborate coccoliths of seemingly infinite shape and form. HETs are formed inside a specialized Golgi-derived vesicle (e.g. Brownlee *et al.* 2015). Before they are extruded, they are formed by an unknown mechanism that controls crystal morphology and the overall shape and form of HETs. Together they form the coccosphere, which can include various appendages and in which the cell resides. In contrast to well-studied HETs, HOLs have received little attention, but their crystals resemble the typical rhombohedral geometry of inorganic calcite (e.g. Young *et al*. 1999). Furthermore, the morphological diversity of HOLs is much more constrained.

In 2016, the same laboratory at the Marine Biological Association (MBA) in Plymouth, UK, discovered that calcifying coccolithophores have something in common with their silicifying cousins: diatoms (Durak *et al.* 2016). However, diatom shells are made of silica and therefore thought to represent a distinct mechanism of biomineralization. This concept was challenged by the discovery of silicon transporters (SITs) in calcifying diploid coccolithophores (Durak *et al.* 2016). Some of these species even appear to have an obligatory requirement for Si, similar to diatoms. Although the cellular mechanism by which Si contributes to the process of calcification is still unknown, studies in other organisms have suggested that silica might be essential for the formation of ordered calcite crystals as seen in HETs but not in HOLs (e.g. Gal *et al.* 2012).

The study by Langer *et al*. (2021) has tested the hypothesis that HOLs represent an ancestral state of calcification, which is contradictory to the fossil record. As support for their hypothesis, they combined knowledge on the role of silicon for the formation of HETs and applied advanced microscopy to re-assess the calcification processes in HOLs. By using scanning electron microscopy in combination with high pressure freezing and freeze substitutions to preserve both inorganic and organic structures, it was possible for the first time to reveal that HOLs are formed inside the cells in vesicles similar to the synthesis of HETs. Langer *et al.* (2021) argue that this result provides first evidence for the presence of a last common ancestor that was capable of producing both HOLs and HETs as they share not only the same chemical process of calcification but also the same cell biology required to produce calcite crystals. Thus, Langer *et al.* (2021) have provided an evolutionary link between both modes of calcification (Fig. 2). As HOLs are structurally more simplistic, it suggests that they have evolved first, which was already postulated a few years ago by Frada et al. (2019). To identify why the additional complexity observed in HETs evolved later, Langer *et al.* (2021) drew on their insights into the role of silicon for the formation of complex calcite crystal morphology. Remarkably, they found that HOLs do not require Si for crystal formation. They also discovered the presence of rhombohedral HOL crystals in diploid coccolithophores after replacing Si in the growth medium by germanium. These results suggest that Si is required for the synthesis of different crystal shapes as both life-cycle stages develop rudimentary rhombohedral crystals but intricately shaped coccoliths are only formed with the help of Si (Fig. 2).

Although these results seem to have resolved a long-standing paradigm in the evolution of calcification in microalgae (e.g. Bown *et al*. 2004, De Vargas *et al.* 2007), they raise interesting questions. For instance, not all diploid coccolithophores with HETs require Si during formation including the model species *E. huxlei* (Durak *et al*. 2016). Furthermore, although the requirement for Si explains why there is complex calcite crystal morphology, it does not explain the almost infinite morphological diversity of coccospheres. I argue that answers to these questions can be found by applying evolutionary theory to silicon and calcium carbonate metabolism. Although phylogenetics has been applied to reveal relationships between individual genes involved in biomineralization, the field will benefit from revealing how the evolutionary forces of mutation, selection, genetic drift and gene flow shaped the genetic and morphological diversity of biomineralizing microalgae. Langer *et al.*

(2021), speculate that high concentrations of Si in the surface oceans about 250 Mya were driving the evolution of HETs. A subsequent decline of Si due to the rise of diatoms might have caused the loss of an obligate Si requirement at least in some species such as *E. huxlei* and therefore provided a fitness advantage under lower Si concentrations. Thus, they argue that changes in the environment selected for the evolution of complex crystal morphology in calcifying algae.

Combining molecular markers and fossils from the geological record of coccolithophores with demographic inference such as coalescence theory (e.g. Rosenberg & Nordborg 2002), which provides a view backwards in time, will provide evidence as to whether environmental change (e.g. Si concentrations) coincides with the point where gene genealogies (e.g. silicon transporters) come together ('coalesce'). Furthermore, identifying signals of selection will inform biochemical studies because they reveal which genes and functional domains likely contribute to the evolution of morphological diversity, which potentially is the outcome of diversifying selection. As mutational and demographic models are available for coccolithophores (Krasovec *et al.* 2020, Bendif *et al.* 2019), I consider this an exciting avenue for providing further insights into what drives the evolution of complex crystal morphology in calcifying algae. If extended to other biomineralizers, it might even reveal a unifying concept on which the apparently distinctive processes of calcification and silicification coalesce.

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Figure legends

Figure 1. NERC Earth Observation Data Acquisition and Analysis Service (NEODAAS) enhanced-colour view of waters around the British Isles in June 2019 (Median composite between 17th and 23rd of June). Coccolithophores are blue-white and non-calcifying phytoplankton (e.g. diatoms) are from dark to light red. The colour gradients (blue to white; dark to light red) reflect the cell-density gradients of phytoplankton populations in the surface layers with increasing densities from darker to lighter colours. Sediment is yellow.

Figure 2. Conceptual model based on Langer *et al*. (2021) describing the evolution of complex crystal morphology in calcifying algae over the past ca. 350 million years. Anc = non-calcified ancestor of coccolithophores; HOL = coccolithophores with holococcoliths; HET = coccolithophores with heterococcoliths. Si = silicon.