THE EVOLUTION OF MICROBIAL STEROL SYNTHESIS AS IT PERTAINS TO THE SPONGE BIOMARKER HYPOTHESIS. D.A. Gold[1] and R.E. Summons[2]
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Organic geochemistry provides a rich source of molecular "fossils" that can fill in gaps left by traditional forms of paleontology. One celebrated molecular fossil is 24-isopropylcholestane, which is found in abundance in certain Neoproterozoic rocks ~640 Ma [1], [2]. This sterane is interpreted as the diagenetic product of 24-isopropylcholesterol (24-IPC), a sterol that is produced in abundance today by a subset of sea sponges from the clade Demospongia. This sterol has thus been widely accepted as a "sponge biomarker", predating the first unambiguous sponge fossils by approximately 100 million years, and suggesting that animals evolved in a unique environmental setting that lay between two global "snowball" events, the older Sturtian and younger Marinoan glaciations [3].

However, several recent papers have challenged the sponge biomarker hypothesis based on three major arguments [4], [5]. Firstly, a recent genome-sequencing project from a bacterial symbiont of demosponges recovered several genes involved in sterol synthesis, suggesting that these bacteria might be responsible for 24-IPC [6]. Secondly, 24-IPC is also produced as a trace product during 24-n-propylcholesterol synthesis in some pelagophyte algae, meaning they could also be responsible for the biomarker. Thirdly, few of the lineages closely related to sponges have been assayed for their sterol repertoires [7], so it remains unclear when or how often 24-IPC evolved during eukaryote evolution.

Addressing these challenges requires an understanding of how the underlying sterol synthesis pathway has evolved, as well as the distribution of sterols in several critical, understudied lineages. To this end, we combined gas chromatography-mass spectrometry with comparative genomics to reconstruct the history of sterol synthesis across evolutionary time. We first demonstrate that putative sterol synthesis genes from sponge-associated bacteria were the result of genome assembly errors, and that the synthesis of 24-IPC can be safely constrained to the eukaryotes. We next analyzed the sterol repertoires of four eukaryotic microbes, which collectively represent the closest living relatives of the sponges (the choanoflagellate *Salpingoeca rosetta*, the filasterean *Capsaspora owczarzaki*, and the icthyosporeans *Sphaeroforma arctica* and *Creolimax fragrantissima*). We did not find 24-IPC in any of these taxa, suggesting that among the opisthokonts (animals, fungi, and their relatives), 24-IPC is restricted to demosponges. We did however find a close correlation between the complexity of sterols produced by each species and the number of *C-24 sterol methyltransferase* (*SMT*) genes in their respective genome. This correlation appears widely applicable across eukaryotes—including pelagophyte algae—with the major exception of demosponges, who have a single *SMT* copy despite producing the widest range of sterols. This suggests that the exotic sterols of demosponges resulted from the evolution of a more promiscuous *SMT* enzyme, as opposed to a series of gene duplication events.

Our results suggest that sponges and pelagophyte algae convergently evolved the ability to synthesize complex sterols, using divergent sets of *SMT* genes. When the *SMT* gene tree is mapped onto a time-calibrated evolutionary tree, we find that the genes necessary for pelagophyte 24-n-propylcholesterol synthesis did not evolve until the middle Paleozoic, and therefore cannot be responsible for the steranes reported in [1]. Ultimately, our results strongly support the "sponge biomarker" interpretation of 24-isopropylcholestane, and offer a case study in combining genomics and organic chemistry to improve our understanding of the geological record.

References:

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