

# Decoding geobiological evolution from microbiomes

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Genomic records of genetic recombination and mutation rates indicate that freshwater ammonia-oxidizing archaea have evolved through paleoclimate and geohydrological history.

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Primary production by phytoplankton, a group of photoautotrophic carbon dioxide-fixing microbes, represents the foundation of aquatic food webs. The availability of nutrients controls phytoplankton activity. This means that increased nutrients, such as nitrogen, will typically stimulate phytoplankton blooms (1), often leading to sizes so large that they can be observed by Earth-orbiting satellites (Fig. 1).

Ammonia-oxidizing archaea (AOA) represent a key group of microorganisms that process the ammonia resulting from the breakdown of organic nitrogen. AOA gain energy by oxidizing ammonia to nitrite, the first step in the nitrification process and a critical component of the aquatic nitrogen cycle (2). The resulting nitrite from AOA activity can be converted to nitrate by nitrite-oxidizing bacteria and then eventually removed from the ecosystem by its conversion to nitrogen gas by denitrifying bacteria (3). In these microbial processes, AOA play a critical role in the first step to removing nitrogen from aquatic ecosystems, helping to keep the balance of nutrients in check that can otherwise lead to harmful algal blooms. AOA reside planktonic and benthic habitats, but the ecology and evolution of freshwater AOA have been historically less studied than marine AOA.

In this issue of *Science Advances*, Ngugi *et al.* (4) show that the genomic evolution of freshwater and brackish AOA species is linked to paleoenvironmental changes, including the extent of glacial ice cover at different periods in Earth's history. The timing of diversification in the freshwater AOA of

European, African, and Asian lakes was shown to correlate with the geological ages of those lakes. Moreover, the molecular dating analyses suggest that evolutionary events of AOA were associated with a major glaciation event, "Snowball Earth," which occurred around 0.7 billion years ago, when glaciers existed at lower latitudes (which today have a tropical climate).

Because the AOA are of global importance for biogeochemical nitrogen cycling, the predicted proteome content of these geographically distinct freshwater AOA likely has important implications for how different freshwater ecosystems process nitrogen. This is particularly important when considering that freshwater habitats are a vital source of drinking water for the world's population. Therefore, the evolution of the freshwater AOA may have implications for how nitrogen is removed from freshwater ecosystems in different geological and hydrological settings.

By applying ClonalFrameML (5) to the environmental metagenome-assembled genomes (MAGs) of archaea, Ngugi *et al.* (4) assessed the contributions of horizontal gene transfer (recombination) and mutation to the evolution of AOA inhabiting the lakes of different continents. This required comparing high-quality gene sets from a relatively large number of AOA genomes collected from numerous lakes in different geographical and climate settings. The resulting analysis from this large phylogenomic dataset allowed Ngugi *et al.* (4) to demonstrate the differential contribution of recombination versus mutation in AOA

associated with the geological history of their habitat.

The phylogenomic analysis showed evidence for quasi-clonal evolution in freshwater AOA. Namely, the genetic diversity within a particular species of AOA, "*Ca. Nitrosopumilus limneticus*" within the phylum Nitrososphaerota, is caused to a larger extent by the accumulation of mutations as opposed to receiving new genetic material from neighbors through recombination and horizontal gene transfer events. Interestingly, in contrast to the European and Asian freshwater lakes, the genomic evolution of AOA from the brackish Caspian Sea showed a higher contribution of horizontal gene transfer from recombination (compared to mutation). This finding indicates that environmental conditions and geo-hydrological settings of the lakes have notably influenced the genome evolution of the AOA residents since the habitat was established.

The ratio of recombination to mutation (defined as  $r/m$ ) in the high-quality MAGs of freshwater AOA reported by Ngugi *et al.* (4) ( $r/m = 0.6$  to  $1.7$ ) can be put into context by comparing against  $r/m$  values reported from other studies. These  $r/m$  values are generally lower than those found for species of the relatively fast-growing cyanobacteria such as *Microcystis* spp., causing harmful algal blooms that tend to have  $r/m$  values over  $2.0$  (6). The  $r/m$  values for the freshwater AOA are also lower than the fast-growing human pathogen *Clostridium difficile* ( $r/m \geq 5.0$ ) (5). This indicates that horizontal gene transfer and recombination in freshwater AOA have made a relatively lower contribution, compared to mutations, in their evolution and therefore an indication of clonal evolution. AOA tend to have relatively slow rates of growth and are adapted to low energy conditions

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**Fig. 1. Phytoplankton blooms on the east coast of the Caspian Sea visualized from space via the Moderate Resolution Imaging Spectroradiometer (MODIS) on the Terra satellite.** Such blooms can be stimulated by increased nutrients such as nitrogen. Photo credit: Jeff Schmaltz, MODIS Rapid Response Team, NASA Goddard Space Flight Center.

(7), which might also help explain their lower  $r/m$  values.

While the  $r/m$  values of the freshwater AOA reported by Ngugi *et al.* (4) are lower than the aforementioned bacteria, they are still an order of magnitude higher compared to subsurface life that persists in sediment under extreme energy limitation over geologic time. For example, the  $r/m$  values from the genomes of subsurface bacteria *Thalassospira* spp. (8) and fungi *Schizophyllum commune* (9), which were isolated from million-year-old sediments from different sites, ranged between 0.05 and 0.07, up to three orders of magnitude lower than that of their terrestrial type strains. The findings of extremely low  $r/m$  values for subsurface life indicate that a lack of homologous recombination in genomic evolution may be a general feature of subsurface microbial

evolution in the deep sedimentary biosphere.

Together, these studies show that novel microbiome techniques can be applied to better understand the role of recombination and mutation in the evolution of microbial populations from diverse environmental samples and taxa. The findings of Ngugi *et al.* (4) open up the possibility for the application of the  $r/m$  and evolutionary rate-based molecular studies to discover the role of dynamic geological processes on microbial evolution and functionality, which may include, but are not limited to, the past dynamic environmental change associated with ocean gateway events at the Drake Passage and the Fram Strait, the massive seawater drought represented by the Messinian Salinity Crisis in the Mediterranean

Sea, and the flooding of the Black Sea ca. 10,000 years ago.

What is important to us today is to understand how microbial life and its ecosystems have responded, adapted, and evolved to tangible climate and environmental changes (and, in turn, shaped the environment) (10). Future studies linking genomic and functional evolution in microbiomes to dynamic geological processes, from the past to the present and the future, have potential to transcend geological and biological disciplines and provide novel insights into the crucial environmental factors driving the coevolution of life and Earth.

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