

SULFUR ISOTOPE FRACTIONATION IS A PHENOTYPIC TRAIT THAT RESPONDS TO EVOLUTIONARY ADAPTATION.

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Motivation: Dissimilatory sulfate reduction is a respiratory process used by some bacteria and archaea to generate energy under anaerobic conditions. Because of the preferential consumption of ³²S-substituted sulfate during this process, sulfate (SO₄²⁻) in sedimentary environments is ³⁴S enriched, while the sulfide (H₂S) produced during this process is ³⁴S depleted, providing a phenotypic trait for the underlying metabolism that can be preserved in sedimentary minerals. This trait is quantified by

$$^{34}\epsilon = \left[\frac{^{34}\text{S}/^{32}\text{S}_{\text{sulfate}} - ^{34}\text{S}/^{32}\text{S}_{\text{sulfide}}}{^{34}\text{S}/^{32}\text{S}_{\text{sulfide}}} \right]$$

and expressed in parts per thousand (‰). Isotopic measurements from sedimentary rocks show that ³⁴ε has varied dramatically over the last 3.5 billion years. Using the isotope phenotype of modern microbes, this record is typically interpreted solely in terms of environmental change. Our underlying goal is to evaluate whether this hypothesis of microbial metabolic uniformitarianism is valid. As a first step, we present here an investigation of the response of ³⁴ε to changes during evolution experiments with sulfate-reducing microbes

Experimental microbial evolution: We performed microbial evolution experiments with a pair of strains of sulfate-reducing bacteria (Desulfovibrio vulgaris Hildenborough - DvH; and Desulfomicrobium baculatum - DBac). In these experiments, genetically pure ancestral populations of DvH and DBac were continually serially transferred for about a year apiece in the same chemically-defined growth media. At regular intervals during the experiment, samples of all populations were preserved in a -80°C freezer, producing a “fossil record” of approaching 1000 generations for both strains. We used molecular and optical techniques for tracking the relative size of ancestral and descendent populations during head-to-head competition, quantifying how the growth rates for the evolved and the ancestral lines. The ratio of these two values is the ‘Malthusian’ fitness, which continuously and monotonically improved during the selection experiments. In 6 different repeat lines, the descendent DvH population was always more fit than its ancestor, with an average improvement in growth rate of 25% after 1000 generations. Similarly, the 3 distinct descendent DBac populations were always more fit than their ancestors, with an average improvement in growth rate of improve-

ment in growth rate of a little over 200% after ≈750 generations.

The cell-specific sulfate reduction rate increased weakly over the course of the DvH experiment, while ³⁴ε did not change significantly, leading to a near-horizontonal pattern of fractionation with rate. A single metabolic model can reproduce this trend, suggesting that adaptive changes in fractionation can recapitulate physiological ones [1]. One possible mechanism for this would be loss of an unneeded cellular function enabling more resources to be devoted to the production of respiratory enzymes. Over the first 300 generations of selection, all of the DBac populations exhibit a common trajectory that is similar to that seen in the DvH experiments. RNASeq results show higher expression for the components of the sulfate reduction pathway (*Sat*, *Apr*, *Dsr*) in the evolved strains at 300 generations relative to the ancestral population, potentially supporting our hypothesized adaptive mechanism. Later generations of DBac, however, fall off the predictions of a single metabolic model calibrated to earlier generations, and seem to be starting on somewhat divergent trajectories. In light of recent bioisotopic models for dissimilatory sulfate reduction [2], these trajectories suggest that fewer respiratory enzymes are needed rates in later generations of the DBac experiment to maintain similar cell-specific sulfate reduction rates as in earlier generations.

Coda: Although we have yet to falsify our inferences about the molecular mechanisms behind the adaptive trajectories in these experiments, the results demonstrate that sulfur isotope fractionation is a phenotypic trait that responds to evolutionary adaptation. Accordingly, the ³⁴ε record in sedimentary rocks is unlikely to be a simple dipstick for environmental change over the last 3.5 billion years. The likely presence of environmental and evolutionary signals in these records releases our interpretations from the constraints of microbial metabolic uniformitarianism.

References: [1] Pellerin A. P. et al. (2015) *AEM*, 81, 2676–2689. [2] Wing B. A. and Halevy I (2014) *PNAS*, doi:10.1073/pnas.1407502111.