# Everything is not spinach: A history of evolving carbon affinity and isotopic fractionation in marine algal Rubisco

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The kinetic isotopic fractionaton factor of carbon fixation during photosynthesis, mediated by the enzyme Rubisco, is widely accepted to be ~ 25 ‰, measured on the spinach form 1B of the Rubisco enzyme. This constant lies at the heart of reconstructions of the past carbon cycle. It is employed in both physiological models to derive atmospheric carbon dioxide from the carbon isotopic fractionation by algae, and in isotopically constrained geological models of the partitioning of carbon between the ocean-atmosphere system and burial as inorganic and organic reservoirs. Recent work has suggested that the form 1D Rubisco isotopic fractionation factor could be as low as 18 ‰ in diatoms [1], and 11 ‰ in coccolithophores (*Emiliania huxleyi*, [2]).

We have established a history of positive adaptation within both the Rubisco Large Subunit and Rubisco Small Subunit across the Haptophyta algae suggestive that there have been significant changes in the Rubisco genetic sequence towards the emergence of *E. huxleyi*. Our assays on Rubisco extracted from descendants of these lineages, demonstrate that each genetic change translates to alterations in the enzyme affinity for carbon. Throughout the declining atmospheric carbon dioxide of Earth history, the evolutionary trend is towards a relaxation in Rubisco affinity for carbon due to the increasing employment of a carbon concentrating mechanism to overcome the inate inefficiency of the Rubisco enzyme. We demonstrate that changes in the algal Rubisco affinity for carbon vary linearly with the enzyme carbon isotopic fractionation factor such that over geological history, the carbon isotopic fractionation factor of Haptophyte Rubisco has decreased signficantly. A reduction in Rubisco fractionation factors likely plays a role in the trend of increasingly isotopically heavy marine organic matter since the Cretaceous.

[1] Boller, A. J. *et al*, *GeoBiology*, **13**, 33-43, 2015 [2] Boller, A. J. *et al*, *GCA*, **75**, 7200-7207, 2011