A metabolic network plasticity approach to the evolution of C4 photosynthesis

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Short Abstract — Many groups of plants have independently developed a common mechanism, C4 photosynthesis, for increasing the efficiency of carbon dioxide assimilation. Understanding possible paths to the C4 state and the high evolvability of the system could guide attempts to introduce it artificially to other plants. We apply a nonlinear optimization method, incorporating key relationships between CO_2 levels and reaction rates, to find feasible C3, C4, and intermediate flux distributions in a genome-scale plant metabolic network model, allowing us to explore the plasticity of the network and characterize the fitness landscape of the transition from C3 to C4 photosynthesis.

Keywords — C4 photosynthesis, constraint-based modeling, metabolic networks.

I. INTRODUCTION

In a remarkable example of convergent evolution, over 50 groups of plants have independently developed C4 photosynthesis, a mechanism which increases the efficiency of carbon dioxide assimilation [1]. In these plants, the carbon-fixing enzyme rubisco is restricted to compartments in the leaf where a high CO₂ concentration is maintained, suppressing the competing process of photorespiration by favoring CO₂ over O₂ in competition for the enzyme's active sites. Efforts are underway to engineer the system into non-C4, or C3, plants to increase crop yield [2].

A broad sequence of steps which may be common to most evolutionary transitions between C3 and C4 photosynthesis has been proposed [3], but the level of flexibility in this sequence and the details of the recruitment of nonphotosynthetic enzymes to new C4 roles are not known, and it is not clear what makes the system so readily evolvable.

As the enzymes necessary for the C4 cycle are also present in C3 plants, these questions could be approached by describing C3 and C4 systems and all intermediate stages of transitions between them as different flux states of a single metabolic network model, extended to include two differentiated tissue types. However, flux balance analysis, the standard tool for predicting fluxes in such models, relies on linear programming, which cannot capture the nonlinear relationships among CO_2 levels, diffusion rates, and rubisco carboxylation and oxygenation rates [4].

II. METHODS

We developed a tool to generate nonlinear programming problems from metabolic network models, incorporating both the standard linear constraints of flux balance analysis and nonlinear constraints on reaction rates derived from kinetic laws or thermodynamics, and solve them with the IPOPT package [5] to obtain consistent flux predictions. We applied this approach to a two-compartment model of generic plant metabolism, based on an experimentally validated model for *Arabidopsis thaliana* [6].

We parameterized the model to describe C3 or C4 plants, verified that each parameter set led to a realistic nonlinear dependence of carbon dioxide assimilation on atmospheric CO_2 levels, and determined the maximum CO_2 assimilation rate, a proxy for overall fitness, along many paths between the C3 and C4 endpoints in parameter space.

III. RESULTS

At moderate atmospheric CO_2 levels, for several different transition path models, intermediate states between C3 and C4 photosynthesis show reduced carbon assimilation rates, suggesting that a fitness barrier separates the two states. As the CO_2 level decreases, this barrier becomes lower or disappears. This result is consistent with the historical record, which shows that low CO_2 conditions promote C4 evolution [7]. Further application of the technique may allow more detailed predictions of likely sequences of evolutionary changes at the enzymatic level.

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