EXPERIMENTAL EVOLUTION OF UV-C RESISTANCE: MUTATIONS IN FITNESS-POSITIVE AND FITNESS-NEUTRAL GENES. A. Moffet[1], N. Wong[2], A. Okasinski[3], C. Sloan[4], J. M. Grace[5], M. Camps[6], D. M. Gentry[7]. [1]Bioengineering Department, University of California, Santa Cruz, [2]Electrical Engineering Department, San Jose State University, [3]College of San Mateo, [4]Cañada College, [5]Economics Department, University of California, Santa Cruz, [6]Microbiology and Environmental Toxicology Department, University of California, Santa Cruz, [7]Biospheric Science Branch, NASA Ames Research Center

Introduction: Experimental evolution, a process in which artificial selection pressures are applied to microbes to study their responses and adaptations, is a key tool in the study of the history of life on Earth. We conducted a series of experimental evolution procedures using UV-C radiation, one of the major challenges to life in an early Earth environment. The evolved microbes' changes in UV-C tolerance, as well as the changes in an unrelated trait (antibiotic resistance), were measured and correlated with comparative sequencing data.

Current methods in experimental evolution typically require the experimenter to expose the cultures to a selection pressure, perform an assay to observe the degree of the cultures' adaptation, and potentially iterate on this process dozens of times [1]. A single iteration can take hours or days to perform, potentially losing information from intermediate microbial generations. We have been developing a system, Dynamically Controlled Directed Evolution (DCDE), which takes advantage of automation and machine learning to generate time-resolved intermediate culture samples throughout experimental evolution protocols [2]. The goal is to generate and characterize synthetic extremophiles under user-defined environmental conditions.

To establish a baseline characterization for DCDE, we performed a basic implementation of directed evolution to UV-C radiation tolerance. In these experiments, we measured the change in UV-C survival rate in cells exposed to multiple cycles of either a 6-second or a 40-second exposures, with fluxes adjusted to yield the same fluence (total dose). The exposed cells carried a plasmid bearing a TEM beta-lactamase gene, which in the absence of antibiotic treatment is a neutral reporter for mutagenesis.

Determining the relationship between the intensity and duration of the UV-C exposure, individual mutations in the beta-lactamase gene, changes in beta-lactamase-mediated antibiotic resistance, and emergence of UV-C resistance has informed our understanding of both the emergence of radiation-related extremophile in natural environments and strategies for generating artificial extremophiles in the laboratory.

Results: UV-C radiation exposure acted as both a selection pressure and a mutagen, leading to a high rate of change in both UV-C-tolerance-related and non-UV-C-tolerance-related genes. (Adaptation dynamics may also include changes in linkages between traits.)

We found no significant difference in UV-C resistance between the two fluxes, with each leading to approximately a one-million-fold in UV-C tolerance over seven iterations. Using beta-lactamase as a mutation rate marker, we empirically found that this gene gained functionality (change to broad-spectrum resistance) before losing functionality (loss of narrow-spectrum resistance) under cycles of UV-C exposure, demonstrating that increased mutation rates can be beneficial. Sequencing of beta-lactamase allowed us to determine the baseline mutation frequency for each flux of UV-C radiation tested.

Expanding on these results, immediate future work will focus on identifying the genomic changes responsible for the change in UV-C tolerance; determining the mechanisms of the emerged UV-C tolerance; and performing competition experiments between the iteration strains to quantify fitness tradeoffs resulting from UV-C adaptation. Other future work will be expanding DCDE to work with other selection pressures, incorporating different mutagens, working with full microbial communities, and detecting not only the microbes but the materials they produce.

References:

[1] Moffet, A. (2014) *UCSC Bioengineering Senior Thesis.*

[2] Liang, J., Wong, N., Zhou, J. (2014) *SJSU Electrical Engineering Senior Thesis.*