AN AUTONOMOUS SYSTEM FOR EXPERIMENTAL EVOLUTION OF MICROBIAL CULTURES: TEST RESULTS USING ULTRAVIOLET-C RADIATION AND *ESCHERICHIA COLI*. Cynthia Ouandji[1], Jonathan Wang[1], Dillon Arismendi[2], Alonzo Lee[3], Justin Blaich[4], and Diana Gentry[5]. [1]San José State University, San José, CA, USA cynthiaouandji@gmail.com [2]City College of San Francisco, San Francisco, CA, USA [3]University of California, Santa Cruz, Santa Cruz, CA, USA [4]Millennium Engineering & Integration, Sunnyvale, CA, USA [5]NASA Ames Research Center, Moffett Field, CA, USA diana.gentry@nasa.gov.

Introduction: At its core, the field of microbial experimental evolution seeks to elucidate the natural laws governing the history of microbial life by understanding the underlying driving mechanisms, such as adaptation, genetic variation, mutations, and phenotypical changes. However, observing evolution in nature is complex, as environmental conditions are difficult to control. Laboratory-based experiments for observing population evolution provide more controlled environmental conditions, but manually culturing and studying multiple generations of microorganisms can be time consuming, labor intensive, and prone to inconsistencies resulting from different experimenters and accumulation of errors.

We have constructed a prototype, closed system device that automates the process of directed evolution experiments in microorganisms, "AADEC" (automated adaptive directed evolution chamber). It is compatible with any microbial culture, including polycultures and field samples, that survives in liquid culture, conducts flow control and continuous agitation if desired, continuously monitors optical density (OD), and can apply and adjust environmental pressure such as ultraviolet-C radiation (UV-C) and temperature in response to measured conditions. Other environmental pressures such as different chemical agents, and other forms of *in situ* measurement requiring contact sensing, are under development. The results of the AADEC prototype are compared to iterative exposure and survival assays conducted using a traditional hood, a UV-C lamp, and a shutter system.

Materials and Methods: The shutter system acts as a manually-controlled counterpart to the AADEC system. It allows the user to precisely control the UV-C exposure time within an enclosed sterile hood in which open liquid cultures or plated samples can be placed. The shutter system consists of two motor-controlled opaque acrylic blades that are mounted directly beneath a pair of UV-C bulbs. The motors open the blades in response to a user's button push and close after a fixed period of time determined by a user-adjustable potentiometer. The system's 'open' position exposes microorganisms to UV-C radiation at a flux determined by user-swappable aperture plates, and the 'closed' position blocks UV-C light to a limit of 1-6 $\mu W/cm^2$ (reducible to zero through additional shielding). Through careful spatial mapping of the UV-C intensity in the hood, and the shutter system which controls the exposure time, each sample can be consistently exposed to a specified dose.

The shutter hood was used to assay several previously generated experimental samples for UV-C tolerance[1]. These stocks of *Escherichia coli* contained a beta-lactamase pGPSori plasmid, which confers an immunity to kanamycin, encoded with the wild type G238S gene strain[2]. Each stock was created from each of three lineages after each of fifteen iterations of UV-C radiation exposure at one of three regimes: no exposure, 3.2 $J/m^2/s$ for 6 seconds, and 0.5 $J/m^2/s$ for 40 seconds. Each re-inoculated culture was regrown in 5 mL liquid LB Broth with kanamycin (50 µg/mL) to an OD600 of 0.8, washed twice with 0.9% saline, serially diluted over eight orders of magnitude, and plated in 10 µL aliquots onto LB agar and kanamycin (50 µg/mL) plates. One of two duplicate plates were then exposed to UV-C radiation at a dose of 15 J/m^2 using the shutter system; the other was placed in the same hood, but not exposed. The survival ratio, which compares the average number of colonies in an iteration that survive UV-C exposure to their control group that was never exposed, is then calculated to determine the UV-C tolerance of each lineage after each of the iterations.

Results: Preliminary results from 6 iterations (I0, I2, I3, I4, I10, and I11) show that resistance to UV-C radiation increases as the iteration number increases, with the samples previously exposed at 6 and 40 seconds boasting higher survival ratios. The ideal region for exposure within the UV-C hood was found to be from [12.5, 32.5] to [12.5, 48.8] and [20.5, 20] to [20.5, 48], which yielded a consistent UV-C intensity of 2.9-3.1 J/m2/s. The exposure time of the shutter was accordingly adjusted to 5 seconds to achieve the specified exposure dose of 15 J/m^2. Experiments for the remaining iterations, including comparing "by hand" survival assays to assays completed using the shutters, are underway to determine the impact of the shutter system and the effectiveness of the AADEC system.

References:

[1] Moffet, A. (2014) Thesis, UC Santa Cruz.
[2] Moffet, A., *et al.* (2014) *AGU* Abstract #B24A-07