ADAPTIVE LABORATORY EVOLUTION ELUCIDATES ANCIENT PROTEIN BEHAVIOR THROUGH EXTANT POPULATION LEVEL EVOLUTIONARY DYNAMICS. A. Plesa[1], E. Garmendia[2], B. Kacar[1,3], [1]Harvard University, Cambridge, MA, [2]Uppsala University, Sweden, [3]Blue Marble Space Institute of Science, Seattle, WA

Introduction: Our living world is the result of a complex history spanning billions of years. Scientists have access to artifacts of this history, namely the fossil record and the genomes of many different living organisms, but there are surprisingly limited means with which to infer the exact evolutionary history that resulted in modern biota. It is difficult to reconcile fossil morphology on the one hand, and genetic composition and diversification over time on the other, when so little about the biological behavior of ancestral proteins can be reconstructed to link these data across disparate levels of evolutionary influence. At the molecular level, protein function is influenced by biophysical constraints acting on the protein itself. At the population level, complex cellular interactions influence the fitness effects of mutations on individuals via protein dosage trade-offs and epistatic interactions. At the environmental level, the relationships between population size, structure and nutrient availability impact evolution by influencing entire lineages. Laboratory adaptive evolution experiments exploit the links between genotype and phenotype across all of these levels, connecting molecular level constraints to environment level evolutionary responses. Here we present the results of a new study, where a modern bacteria (E. coli) was genetically engineered to harbor ancestors of an essential ribosomal gene, Elongation Factor (EF-Tu), and then subjected to laboratory evolution through serial propagation of rich media. The impact of the resurrected ancient genes on the competitive fitness, doubling time and gene expression patterns were measured.

Experimental Approach: Genetic Engineering: We investigated the constraints on transfer of an informational protein Elongation Factor-Tu (EF-Tu) encoded by two genes in E. coli, by systematically replacing the endogenous gene with its modern and ancient homologs in the E. coli genome. The modern homologs represented EF-Tu variants obtained from various distant and near homologous organisms. The ancestral homologs represented phylogenetically resurrected EF-Tu sequences dating 0.7 to 3.6 bya. Experimental evolution: Six initially identical E. coli strains harboring five different EF-Tu homologs Evolved in LB culture 10,000x dilution every day for 500 generations. On average, initial population was 1.76E+05 with a final density of growth (after 24 hours) of ~1.76E+0. 9. Mutations at the population level were detected through whole genome sequencing.

Results: All of the foreign tuf genes are transferable to the E. coli genome, provided that an additional copy of the EF-Tu gene remains present in the E. coli genome. When the additional copy was removed, only the variants obtained from the γ-proteobacterial family result in a functional organism, demonstrating the limited functional interchangability of E. coli tuf with its homologs. We found that the amount of EF-Tu produced is directly correlated with organismal fitness and the environmental condition used in the adaptive laboratory evolution experiments impacts population level evolutionary trajectory. Our results permit us to synthesize robust models of adaptation to genetic perturbation, and provide information on how lineages respond to the challenge presented by the restoration of outmoded genetic components. Comparative analysis of evolved populations harboring reconstructed genes of varying age provides a unique opportunity to map historical constraints onto the current genomic state of cell [1-3].

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[3] Kacar, B Rolling the Dice Twice: Evolving Reconstructed Ancient Proteins in Extant Organisms in Chance in Evolution eds. Ramsay and Pence, University of Chicago Press 2016, 265-276