

Research Plan

Identifying Mutations that Promote Microbial Evolvability

Evolvability describes an organism's capacity for producing descendants that are better adapted to a given environment. Evolvability is a complex trait that depends not only on how developmental and regulatory processes render underlying genetic changes into phenotypic variation, but also on the dynamics of how beneficial mutations arise and compete within a population. Laboratory evolution experiments and theoretical models have demonstrated that mutator strains of microorganisms, which have elevated genomic mutation rates due to defects in DNA proofreading and repair pathways, can take over chronic pathogenic populations because they promote evolvability. Little is currently known about how mutations affecting other cellular processes impact microbial evolvability or about how evolvability varies during adaptive evolution.

In large populations of asexual microorganisms adapting to a novel environment, competition among contending beneficial mutations can slow down the fixation of any one adaptive genetic change long enough that secondary mutations begin to accumulate. In this situation, more evolvable single-step mutations may be favored, even if the immediate fitness advantage that they confer is not the greatest among the contending single-step mutations, because they are able to generate two-step variants with higher fitnesses. Thus, natural selection may avoid dead-end peaks in a fitness landscape by selecting for mutations that least restrict further evolvability. The categories of mutations that are important for modulating evolvability during step-wise adaptation can be determined by examining laboratory populations of microbes with long evolutionary histories. Conditions where more evolvable genotypes have a chance to outcompete more fit individuals in a population can also be exploited to discover genetic changes that increase evolvability in functional genetic screens of mixtures of signature-tagged strains. When comparing two strains differing by a single mutation, it is important to correct relative evolvability measurements for the relative fitnesses of the test strains, because genotypes of higher fitness generally have fewer available beneficial mutations of large effect.

We will use an integrated approach that relies on evolution experiments with laboratory *Escherichia coli* populations coupled with population genetic models and simulations to design and interpret experiments that systematically search for new categories of mutations that increase microbial evolvability.

Specific Aim 1: Use marker divergence experiments to establish the extent to which *E. coli* evolvability increases in mutation accumulation lines with arbitrary series of deleterious mutations.

Specific Aim 2: Reconstruct the complete histories of mutations in independent lineages from the *E. coli* long-term evolution experiment. Identify and characterize mutations that were ultimately successful because they increased evolvability rather than due to their immediate fitness effects.

Specific Aim 3: Search for genetic changes that increase evolvability with respect to many environments with high-throughput screens of *E. coli* gene deletion and overexpression strain libraries.

Limiting the evolution of drug resistant microorganisms and slowing the progression of chronic infections are among the greatest challenges of modern medicine. Understanding what categories of genetic changes generally promote microbial evolvability would be useful for anticipating and perhaps even frustrating pathogen adaptation. The full promise of many green chemistry, renewable bioenergy, and biotechnology applications hinges on rapidly and efficiently creating domesticated bacterial strains with a desired property. Knowledge of targeted genetic changes that could increase evolvability and serve as tools to accelerate this development process would have many practical applications relevant to medical research and industry.