EVOLUTIONARY INSTABILITY OF GENOMIC MUTATION RATE IN RAPIDLY ADAPTING ASEXUAL MUTATOR *ESCHERICHIA COLI* POPULATIONS: AN EXPERIMENTAL STUDY UNDER BOTH HARD SELECTION AND SOFT SELECTION.

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Introduction: Mutations that influence the phenotype are more likely to be deleterious, rather than beneficial – however, mutations are also the ultimate source of all evolutionary novelty. Mutators are mutant alleles that confer elevated genomic mutation rates. It is known that in an asexual evolving wild-type population, a mutator can spontaneously arise and 'hitchhike' to fixation, because there is no recombination to break the genetic linkage between the mutator and the beneficial mutations that it inflicts. The evolution of the mutation rate is highly relevant to the study of early life: it has been proposed that many early life forms had extremely high mutation rates, due to the lack of mechanisms for DNA proofreading, repair, and recombination. Furthermore, theoretical models suggest that intolerably high mutation rates may culminate in a population's extinction. We are studying the effects of hard selection and soft selection on the mutation rates in evolving asexual mutator microbial populations.

Mutation rate evolution under hard selection: In the hard selection project, we investigated whether populations of asexual mutator *E. coli*, when exposed to multiple rounds of hard selection (via lethal antibiotic plates), can be enriched for hypermutator individuals. Here we define "hypermutators" as possessing two or more mutator mutations with a cumulative effect on the mutation rate. The single-mutator ancestor (with a mutation rate 100-fold higher than wild-type, due to a mutation in the mismatch repair gene *mutL*) was sequentially exposed to a series of three different antibiotics in increasing order of lethal selectivity (henceforth the "canonical order"). Clones were randomly isolated from each stage of the selection series. Their mutation rates were measured by fluctuation tests. The ancestral mutator was also exposed to the antibiotics in decreasing order of selectivity (henceforth the "reverse order"). Hypermutator enrichment was found to be minimal after single-exposure, double-exposure, and reverse order exposure. However, the canonical triple-exposure order resulted in strong enrichment for hypermutators. To determine what mutations were responsible for the changes in mutation rate, we sequenced two clones from the end stage of the canonical series. Both clones retained the ancestral *mutL*- allele, but also had a frameshift mutation in the well-known mutator gene *mutT*. When the two clones were transformed with a *mutT*+ plasmid, the single-mutator ancestral mutation rate was restored. This is the first time that a mutator allele has been shown to spontaneously arise and fix in an asexual evolving population with a preexisting high mutation rate. Our findings support theoretical work predicting that adapting asexual populations may evolve progressively higher mutation rates.

Mutation rate evolution under soft selection: In the soft selection project, 30 near-isogenic asexual single-mutator populations (with mutation rates 100-fold higher than wild-type, due to a mutation in the mismatch repair gene *mutS*) were propagated by daily batch transfer in a liquid environment with limited nutrients. After ~900 generations, five clones were randomly isolated from each evolved population. Of the 150 clones, 9% had mutation rates significantly higher than that of the mutator ancestor, 23% had mutation rates significantly lower than that of the mutator ancestor, and 68% had mutations rates similar to that of the mutator ancestor. 22 out of the 30 populations displayed some degree of change in the mutation rate: 20 populations were polymorphic for the mutation rate, one population had potential fixation of a mutator, and one population had potential fixation of an antimutator. All 30 evolved populations had increased fitness relative to the single-mutator ancestor.

We sequenced the genomes of 20 evolved clones, representing a wide range of mutation rates. The ancestral *mutS*- allele was retained in all 20 sequenced clones. Thus, the observed changes in mutation rate in the sequenced clones are due to mutations in other loci. In light of the extensive polymorphism across the evolved populations, we hypothesize that the increases in mutation rate are most likely due to hitchhiking of new mutator alleles, and that the decreases in mutation rate are most likely due to direct pleiotropic fitness effects of the new antimutator alleles.