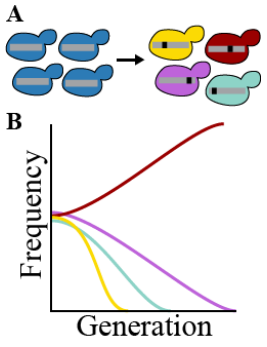
The effects of loss of function mutations over adaptive trajectories in *S. cerevisiae*

In order to understand evolution, we need to understand the complex relationships between biological systems and fitness. This is a difficult task, because there are an enormous number of possible genetic states, and the mutations underlying these states interact non-additively to produce fitness. We can frame this problem by thinking of evolution as a process occurring on a high-dimensional map between this space of genetic possibilities and the fitness of each possibility, a function often referred to as the "fitness landscape." As a population adapts to a particular environment, it moves between neighboring genotypes, constrained by the force of selection to follow paths of increasing fitness. By understanding the general properties of the fitness landscape, we can answer questions about the functional nature of a biological system - *If a mutation knocks out this gene, what effect will that have on fitness?* - and ask broad theoretical questions - *Is evolution predictable, or does it depend on chance events?*

The growing field of experimental evolution provides an avenue for addressing these questions by empirically testing important features of the fitness landscapes of microbes. We now know that in the budding yeast *Saccharomyces cerevisiae*, the effect of a beneficial mutation depends on the fitness of the genetic background where it arises [1], but whether a similar pattern holds for deleterious mutations is an open question. Deleterious mutations may be common in populations due to environmental changes or population bottlenecks, and they provide a novel way to study adaptation and to test the role of contingency in evolution. **In my PhD research, I will study the fitness landscape of *S. cerevisiae* by investigating the interplay between deleterious mutations and adaptation.** I will complete my PhD research in Dr. Michael Desai's lab at Harvard University, where I am uniquely situated to conduct work that combines genetics, experimental evolution, and deep sequencing.

Aim 1. Changes in the fitness effects of loss of function mutations over adaptive trajectories

Given that most loss of function (LOF) mutations are deleterious, competing models make different predictions about how their effects should change with increasing population fitness. The Desai lab recently found that in *S. cerevisiae*, the fitness effect of a beneficial mutation in a particular genetic background is primarily predicted by the fitness of the background, creating a pattern of "diminishing returns" during adaptation [1]. If this "global epistasis" model holds for all mutations, deleterious mutations should also become less deleterious as the population becomes more fit. In contrast, Fisher's geometric model predicts that the fitness effect of some mutations will change from negative to positive at different levels of adaptation [2]. I will use transposon mutagenesis and sequencing fitness assays (Tn-seq) to measure the fitness effects of a large set of loss of function mutations in populations with different initial fitness backgrounds. *Hypothesis:* **I predict that, in accordance with the global epistasis model [1], LOF mutations will be less deleterious in populations with higher fitness.** *Methods:* First, I will evolve 24 *S. cerevisiae* populations in standard liquid media for 1000 generations (100 days), following a similar protocol to [3]. I will freeze samples every 250 generations to create a "frozen fossil record" of each population as it adapts and gains fitness. At each of the five timepoints in this record, I will unfreeze my populations and use Tn-seq to systematically probe the fitness effects of a large number of LOF mutations. As shown in the figure at right, Tn-seq consists of two steps. In A, I transform a gene disruption library into the population, causing a diverse set of single insertion mutations. In B, I track the frequency of each mutation over 30 generations using deep sequencing of a barcode region in the insertion



[4]. Using this method, I can determine the fitness effect of every mutation in parallel by analyzing the change in its frequency [3,4]. I will create my DNA-barcoded transposon (Tn) gene disruption library using genomic DNA from *S. cerevisiae* [3], and by sequencing this library, I will associate a unique barcode with each gene disruption. These associations will allow me to connect my data to specific genes, yielding additional biologically relevant information about how *S. cerevisiae* adapts to laboratory conditions.

Aim 2. Adaptation after disruption of the genetic system

Evolution often involves transient environmental changes that alter selection pressure or population size, both of which can lead to the fixation of mutations that are not beneficial in the organism's primary environment. Do these events affect long-term outcomes of evolution? The dynamics of adaptation after a population has been "bumped" off of its adaptive trajectory are not well understood, but they have the potential to distinguish between models of adaptation. For fitness landscape models in which mutations interact only additively, any deleterious mutation simply slows adaptation. However, in "rugged" fitness landscape models where mutations interact non-additively, it is possible that deleterious steps can lead to exploration of a previously inaccessible part of genotype space, potentially allowing a population to ultimately reach higher fitness. I will capitalize on the Tn-seq method to distinguish between these models by evolving "disrupted" populations alongside "undisrupted" populations and comparing their fitness trajectories. *Hypothesis:* **I hypothesize that deleterious mutations will be more likely to improve evolutionary outcomes in poorly adapted populations, as predicted by [5]. Therefore, I predict that disruption due to Tn insertions will lead to higher final population fitness relative to the undisrupted populations only when the original disruption occurs at early time points from the frozen fossil record.** An alternate prediction is that disruption will slow adaptation in all cases, which would support additive landscape models. *Methods:* I will propagate "Tn-disrupted" populations from Aim 1 for 500 generations. I will measure mean population fitness every 100 generations in these populations and at the corresponding timepoints in the "undisrupted" populations using standard fluorescence-based competitions [1].

Intellectual Merit

My project aims to connect ideas about the dynamics of adaptation on fitness landscapes to a functional understanding of how a model organism changes as it adapts. Using massively parallel, sequencing-based fitness assays, this project will provide unprecedented resolution of the functional changes a population experiences during adaptation, and through evolution of Tn-disrupted clones, this study will test basic questions about the fitness landscape of evolving *S. cerevisiae*.

Broader Impacts

We now know that large asexual populations, in the form of pathogens or cancer cells, are involved in over a quarter of deaths worldwide [4]. While my research is centered on basic science questions, these basic principles of asexual adaptation are an important part of building models of how these diseases progress. I will publish my work in peer-reviewed journals aimed at a scientific audience, but I will also use the power of animations and interactivity to make my evolution research come alive on my web site, where it can be shared with the general public. As detailed in my personal statement, I will also use science communication and video to empower young people to pursue STEM careers by showing them the human side of research.

[1] Kryazhimskiy S *et al.* 2014. *Science* 344: 1519-1522. [2] Fisher RA. 1930. Clarendon Press, Oxford, U.K.
[3] Van Opijnen T *et al.* 2009. *Nature Methods* 6: 767-772. [4] Levy SF *et al.* 2015. *Nature* 519:181–186.
[5] Nahum JR, *et al.* 2015. *Proc Natl Acad Sci USA* 112:7530–7535.