

Origin and Dynamics of Adaptation in *Schizosaccharomyces pombe*: Standing Genetic Variation vs. De Novo Mutation

Alexandre Rego, Utah State University | Dr. Zach Gompert, Utah State University



Introduction

When populations encounter a novel environment, adaptation can occur via *de novo* mutations or standing genetic variation (Hermisson, 2005). Theory predicts that adaptation should occur more rapidly when standing variation exists, but this has rarely been tested. For example, experimental evolution studies rarely consider both *de novo* mutation and standing variation because the generation time for sexually reproductive organisms is often too long for *de novo* mutations to contribute, while experiments with quickly reproducing asexual organisms typically start with genetically homogeneous clines.

Here we test the hypothesis that standing variation facilitates adaptation to high temperatures in fission yeast (*S. pombe*) (Fig. 1). To this end, survival data were generated and analyzed from genetically homogeneous and variable colonies of *S. pombe*, a sexually reproducing yeast, under the stressful growth condition of 35°C.

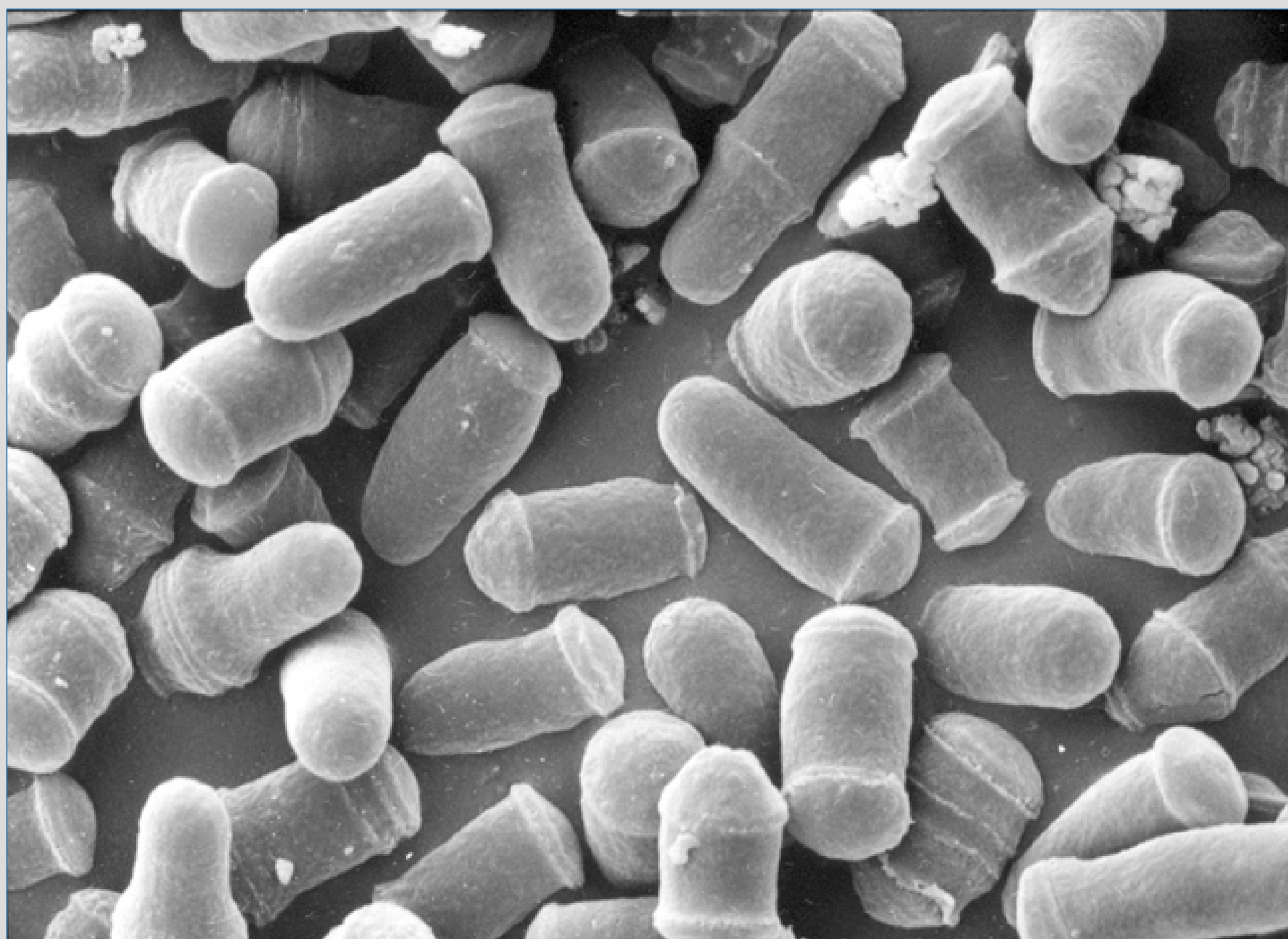


Fig 1: Electron microscopy of the fission yeast, *S. pombe*. Image captured by Mary Parker, Institute of Food Research, Norwich.

Methods

I. Induction of Genetic Variation

A clonal colony of *S. pombe* was split into two separate colonies grown on malt-extract agar plates and incubated at 25°C. 5M Hydrogen peroxide (H_2O_2) was applied to one colony to induce oxidative damage to *S. pombe* and generate genetic variation (Fig 2). DNA point mutations and deletions have been observed following the addition of high molarity hydrogen peroxide to yeast (Thacker, 1976).

II. Colony Replication and Propagation

Once the oxidatively damaged colony was allowed to recover, each colony was split into 10 replicates. 5 replicates of each treatment were placed into 25°C and 35°C incubation. Each replicate was allowed to grow for 1 week before inoculation on a new plate. Inoculations onto new plates were repeated once a week.

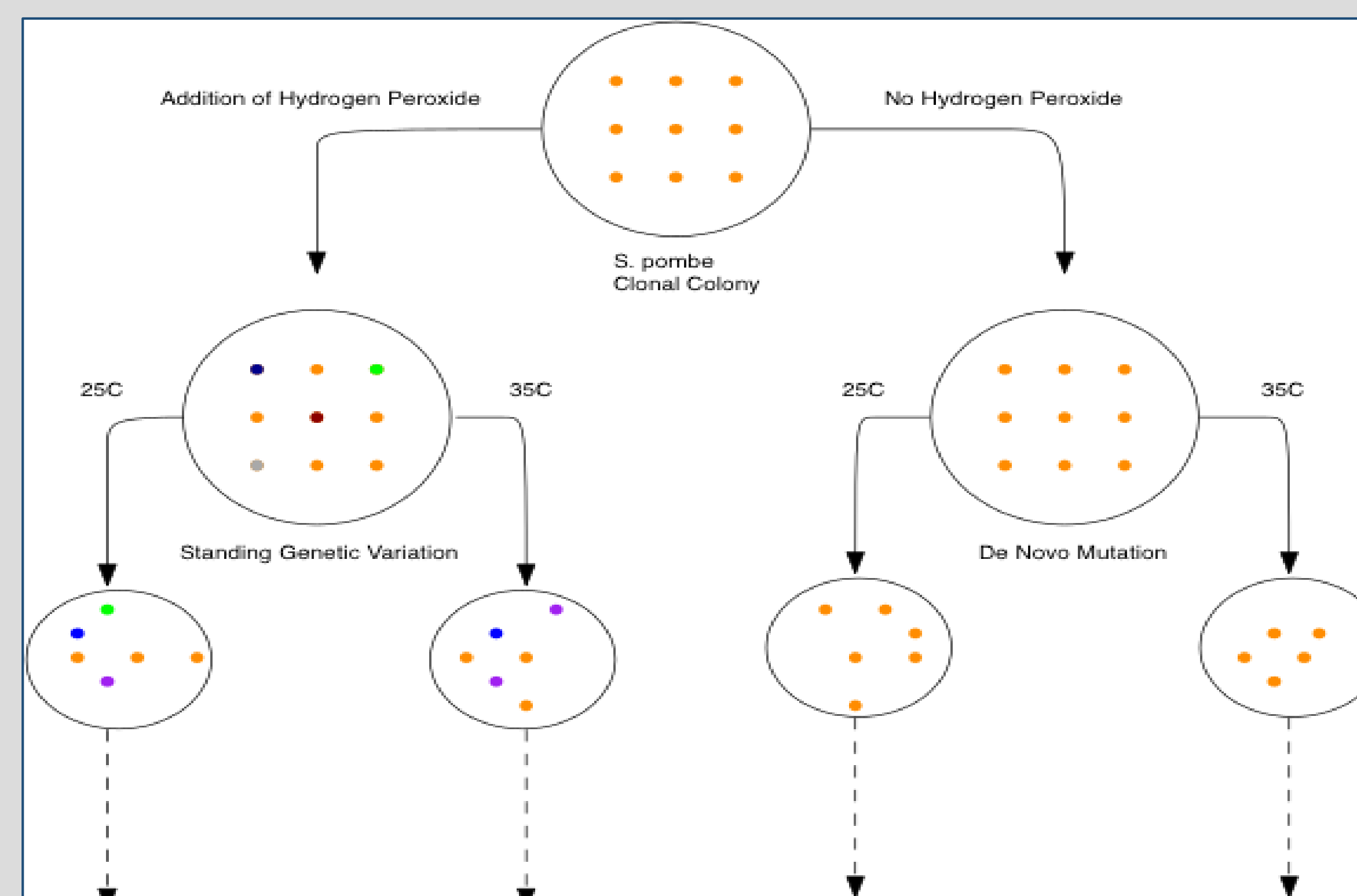


Fig 2: Summary of experimental set-up. Replicates for standing genetic variation and de novo mutation colonies in 25°C and 35°C are not shown.

III. Phenotypic Fitness Spot Assay

Before each transfer, approximately 100µL of yeast growth from each replicate was added to 1mL of ddH₂O. Serial dilutions were produced of each sample, and subsequently 10µL were pipetted onto malt-extract agar plates in grid fashion (Fig 3). Two assays were completed, both at 25°C and 35°C. Yeasts were allowed to grow for 72 hours before presence of growth in the highest dilution was measured.



Fig 3: Spot assay of *S. pombe*. Each column represents a 10⁻¹ dilution of the previous sample. Rows represent individual replicates.

Results

I. 35°C Assays

No colonies are observed initially for yeast that contain standing genetic variation (SGV). After the second assay, the proportion of yeast successfully grown increases quickly until all colonies are observed to have grown successfully. Clonal yeast are seen to have moderate fitness in 35°C which stays somewhat constant until achieving high fitness in later assays.

II. 25°C Assays

SVG yeast initially showed decreased fitness in 25°C, which is expected due to a high probability of incurring deleterious mutations from oxidative damage. De novo yeast initially do well, but dip in fitness during assays 3 and 4 before returning to high fitness levels.

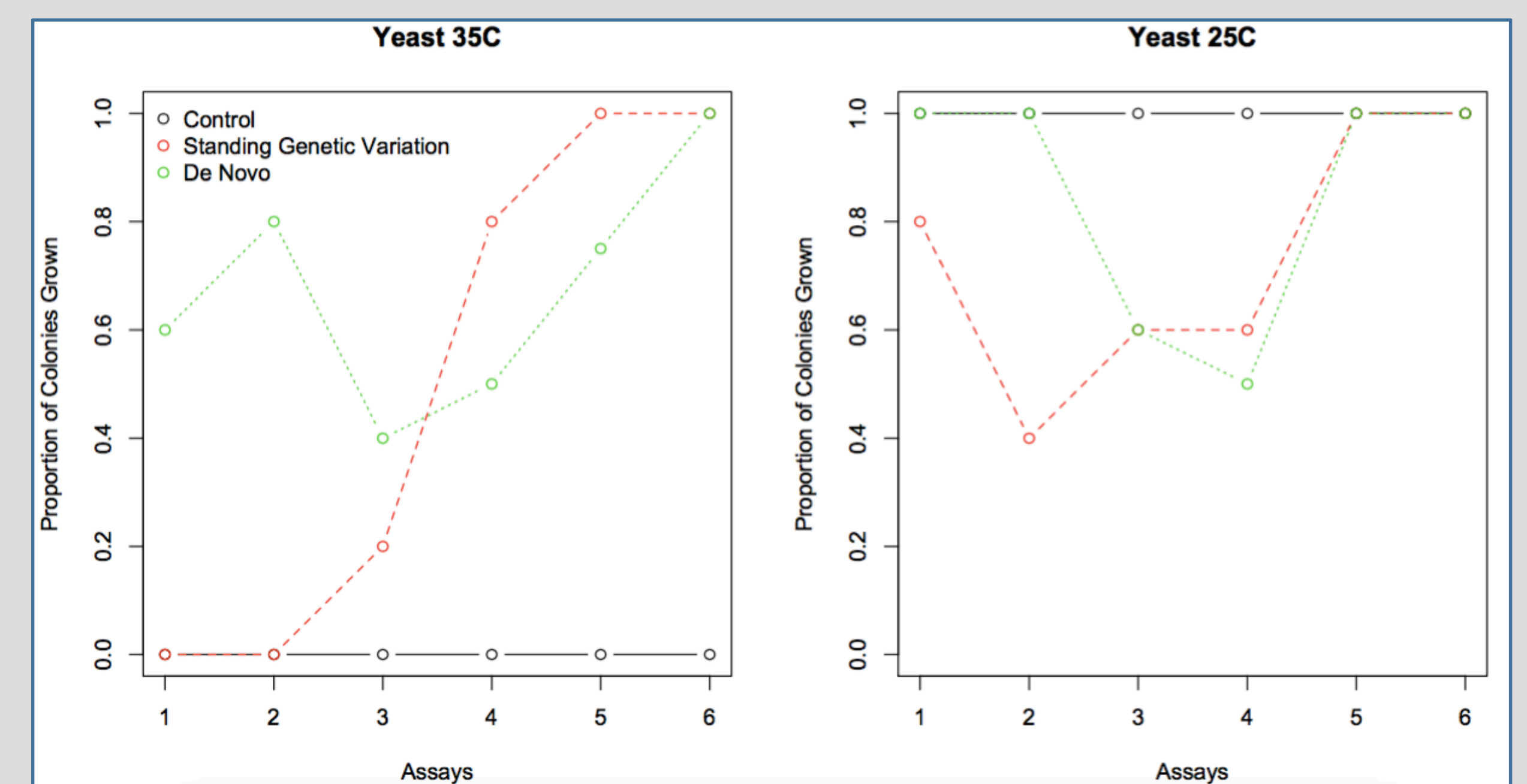


Fig 4: Results of yeast spot assays. Proportions of colonies grown was measured from dilutions of 10⁻³.

Conclusions

The survival assays summarized in Fig. 4 suggest that standing genetic variation allowed the yeast to adapt more quickly despite initial deleterious effects. This is consistent with theory, as genetic variation is necessary for evolution by natural selection and some small subset of the mutations introduced should be beneficial (Hermisson, 2005). With that said, because of limited replication and variability among replicates, a larger-scale study is warranted to follow up on these results. DNA sequencing of these experimental colonies is underway to identify the specific genetic variants responsible for adaptation to high temperature, and to compare this between colonies with and without standing variation.

References

- Hermisson J, Pennings PS. (2005) *Soft sweeps: molecular population genetics of adaptation from standing genetic variation*. *Genetics* 169:2335–2352. 10.1534/genetics.
- J. Thacker, W.F. Parker (1976) *The induction of mutation in yeast by hydrogen peroxide*, *Mutation Research/Environmental Mutagenesis and Related Subjects*, Volume 38, Issue 1, Pages 43-52, ISSN 0165-1161.