# Evolutionary constraints and the distribution of beneficial mutational effects in *Saccharomyces* vineyard adaptation

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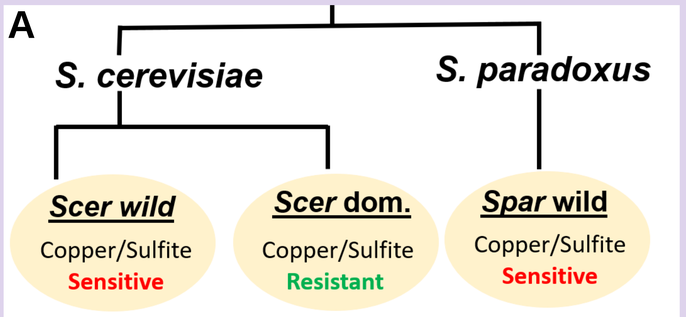
## Introduction

Evolutionary constraints can hinder adaptation by natural selection. The distribution of mutational effects (DME) can impose such constraints if available beneficial mutations are rare, of small effect, or very costly. Differences in adaptability among related species may therefore be due to differences in the DME. *S. cerevisiae*, unlike its sister species, *S. paradoxus,* has adapted to the anthropogenic oenological stressors copper and sulfite[1]. To test whether adaptation to the vineyard environment can be explained by differences in the DME, we mutagenized wild isolates of both species and recovered hundreds of mutants displaying increased resistance to copper. We then subjected them to a high-throughput robotics-based phenotyping assay to precisely measure their effect size and pleiotropic costs. These data allowed us to quantify the mutational target size, mutational effect size, and pleiotropic consequences of mutations conferring copper resistance for both species.



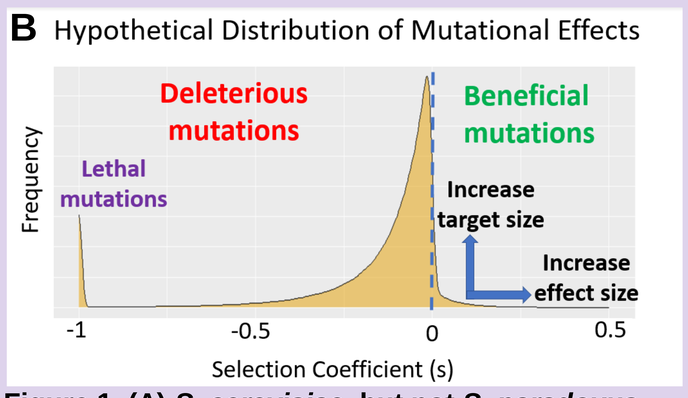




**Figure 1. (A) *S. cerevisiae,* but not *S. paradoxus,* has been domesticated and has adapted to copper and sulfite, stressors found in vineyards. (B) Hypothetical example of the DME and the constraints it can impose. We hypothesized that differences in the DME may underlie the apparent differences in adaptability between these species.**

## Methods

We subjected four copper/sulfite sensitive wild isolates (two *S. cerevisiae* and two *S. paradoxus*) to UV mutagenesis. We then plated mutagenized and control pools of each strain on several concentrations of canavanine and copper. Canavanine served as a control for induced mutation rate. Mutants recovered from the copper plates were then phenotyped on copper and on permissive conditions using a Singer robot to assay the DME for copper resistance mutations in these species.

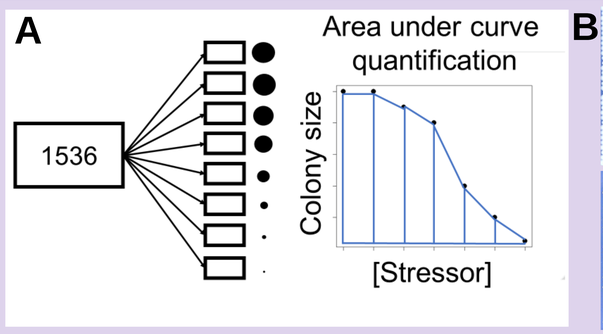
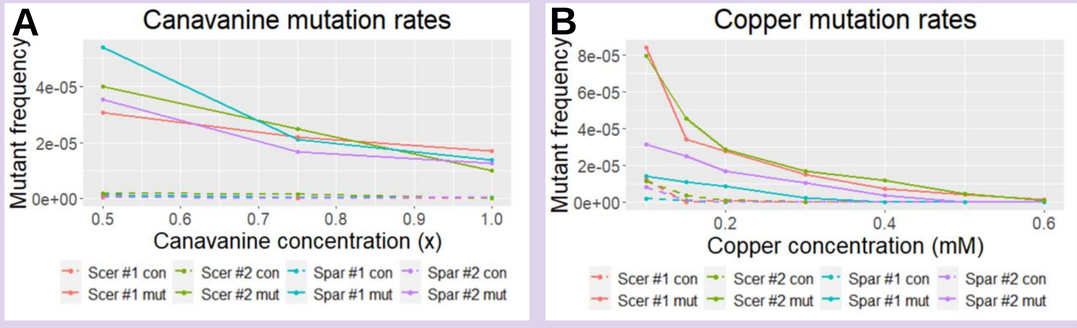



**Figure 2. (A) Schematic of the phenotyping protocol. Resistant isolates are plated en masse on a multitude of concentrations of stressor plates and these colony size measurements are quantified via area under the curve. (B) Example 1536 plates of recovered mutants on various copper concentrations (clockwise beginning in upper left: 0 , 0.07 , 0.2 , and 0.4 mM copper sulfate)**

## Results



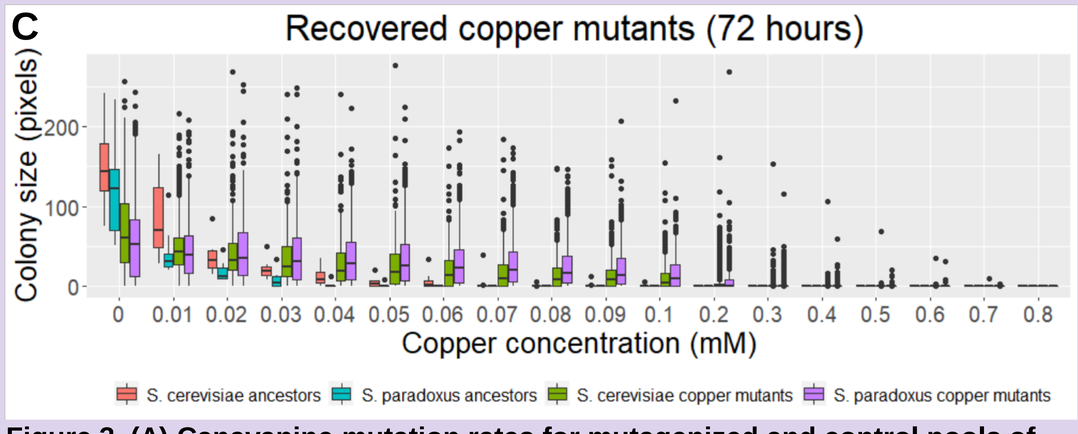




**Figure 3. (A) Canavanine mutation rates for mutagenized and control pools of strains used. (B) Copper resistance mutation rates for mutagenized and control pools of each strain used (C) Raw colony sizes across assayed concentrations of recovered copper mutants and ancestors for both species.**

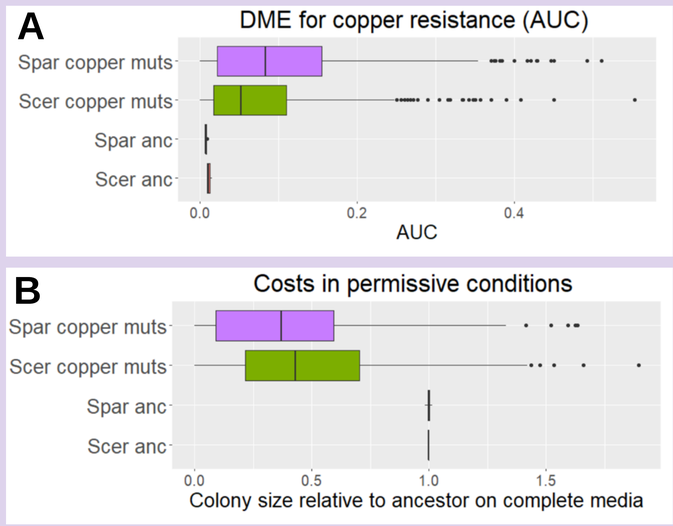



**Figure 4. (A) The DME for both species as quantified by AUC (B) Pleiotropic costs of resistant mutants in permissive conditions as measured by colony size on complete media relative to the ancestor.**

## Conclusions

From the experiments we have performed, it is clear that although *S. cerevisiae* tends to have a higher mutational target and mean effect size from the simple plating assay (fig 3B), the difference in effect size does not stand the scrutiny of follow-up phenotyping. *S. paradoxus* mutants unexpectedly harbor greater average copper resistance than their *S. cerevisiae* counterparts (fig 4A). However, *S. paradoxus* mutants also tend to have a greater growth cost in permissive conditions (fig 4B), suggesting this may be a source of constraints in this system.

## Future Directions

The next steps for this project are to first assess the DME for sulfite resistance mutations across these four strains in a similar manner and then to use WGS and follow-up transgenic experiments to determine the mutational targets for both of these resistance phenotypes in both species. Such work will further our understanding of mechanisms by which the DME can differentially limit adaptation in related taxa.

## References

1. Dashko, Sofia, et al. "Changes in the relative abundance of two Saccharomyces species from oak forests to wine fermentations." Frontiers in microbiology 7 (2016): 215.

## Acknowledgments

- Douda Bensasson, Members of the Fay lab