Summary & Critique of “An adaptive interaction between cell type and metabolism drives ploidy evolution in a wild yeast” presented by John Crandall.

Introduction

The long-term effects of ploidy variation in eukaryotic evolution have long been recognized. In *Saccharomyces cerevisiae*, short-term evolutionary consequences have also been described (Gerstein & Sharp 2021). However, little is known about how organismal fitness and ploidy variation interact. The precise molecular basis in particular has remained obscure. Further complicating the interpretation and prediction of fitness differences between yeast ploidy states is the intricate link between ploidy and cell type across species. In wild-type *Saccharomyces* ploidy controls cell type through the presence or absence of alleles at the *MAT*ing type locus. Two nonhomologous alleles, MATa and MATα, which code for regulators of the two distinct haploid mating types and of the diploids produced by combining both, define the mating type (Haber 2012). Selection on cell type has been increasingly researched in pathogenic fungi species (Magditch et al. 2012). When microbial traits and their underlying genotypes have a direct impact on human health, are crucial for biotechnological processes, or act as models for eukaryotic evolution, they are of significant interest. In this study, the developing model yeast *Saccharomyces eubayanus* was examined to investigate how maltose consumption could be restored after secondary loss utilizing adaptive laboratory evolution (ALE). Surprisingly, haploids emerged and rose to high frequency in replicate ALE populations founded with a diploid strain, which is a highly unusual ploidy transition for *Saccharomyces*. Further experiments were performed on these haploid clones to unravel the molecular underpinnings of environment-cell type fitness interactions. Additionally, these experiments

demonstrated how selection on traits unexpectedly linked to ploidy states or cell types can drive karyotypic evolution in fungi, a phenomenon that may be more significant in fungal life cycles than is currently understood (Naranjo-Ortiz & Gabaldon 2020).

Summary

In order to elucidate the molecular pathways for maltose metabolism, extensive RNA sequencing experiments were carried out on maltose-grown cultures to examine the differential expression of the *S. eubayanus MALT* genes (Brickwedde et al. 2018). Aiming to identify mechanisms by which maltose utilization might be regained after secondary loss, diploid *S. eubayanus* strains were subjected to ALE under selection for improved growth on maltose. Evolution of maltotriose utilization by *Saccharomyces* species is all but a straightforward process. Many factors play an important role, such as the presence or absence of maltotriose transporters as well as potential chimeric proteins carrying out novel functions, making it challenging to describe this adaptive evolution (Baker & Hittinger 2019). By mapping the genetic basis of the adaptation in the clones, Crandall discovered that evolved *S. eubayanus* isolates harbored mutations incongruous with ancestral ploidy. Growth on maltose paired with WGS was used to identify and assess the phenotypic and karyotypic evolution of the isolates. The identified cryptic single nucleotide polymorphisms (SNPs) and shard aneuploidy suggested a common target for selection. Both isolates had gained the ChrXV, which encompasses the glucoside transporter encoded by *AGT1*. The expression of *AGT1*, encoding a general α-glucoside transporter, has long been known to be maltose inducible (Han et al. 1995). Previous studies have proposed that the presence of several copies of the AGT1 gene on the *S. eubayanus* ChrXV region functions as a buffer to prevent loss of maltotriose utilization

(Brouwers et al. 2019). Using flow cytometry, the ploidy states of the evolved isolates and the ancestral strain were determined to be haploid and diploid respectively. Haploids became more prevalent in the experimental populations during the ALE, which led researchers to wonder whether they might have an advantage over other types of organisms in terms of fitness.

Crandall posited two different hypotheses to account for his findings. The evolved clones may have a conditional fitness advantage in the ALE environment, or there may be a direct ploidy advantage. The latter hypothesis was evaluated in this study, which is consistent with previous research that showed the fitness benefits of heterozygosity reduction in *Saccharomyces* hybrids (Lancaster et al. 2019). A sensitive competition analysis was carried out in both the rich medium and the ALE conditions to gauge the fitness of isogenic diploids and haploids from fully tetrads. Although the haploids were less fit under typical culture settings, they did have a considerable direct advantage over the diploids under the conditions of the initial evolution experiment. A notable differential fitness between haploids of opposing mating types was also observed from the experiments in both scenarios. How absolute ploidy and cell type impact fitness is one of the primary issues covered in this study. Changes in ploidy had previously been shown to be harmful to fungi, leading to decreased fitness, but they can also boost fitness under stress as they fundamentally alter how a cell perceives and reacts to its environment (Todd et al. 2017).

To distinguish between ploidy dependent physiology and ploidy-linked cell type, engineered genotypes that can decouple ploidy from cell type were utilized. The effects of ploidy, mating-type specification, and cell type-specific gene expression patterns on fitness were measured in the ALE conditions. These experiments suggested that cell-type selection is the

main factor contributing to adaptive fitness. Therefore, in the ALE conditions, the cell type specified by the MAT locus, rather than absolute ploidy per se, has the greatest impact on fitness.

As transcriptomes may reveal expression patterns driving haploid advantage, mRNA-seq was employed to ascertain how different cell types interact with central metabolism. Crandall reasoned that the parallelism between independent isolates should be attributable to a shared genotype. In both haploids, an increased expression of an α-glucoside transporter gene was observed. The *AGT1* transporter on the ChrXV had been amplified in both evolved haploids and displayed an expression higher than the twofold increase expected commensurate with its relative copy number. However, increased *AGT1* expression was not reflected globally. This suggests that the gene is likely to encode a transporter with broad substrate affinity. Adding another copy of the *AGT1* further increased fitness in maltose growth conditions showing that increased expression of *AGT1* is in fact adaptive.

Crandall examined why *AGT1* might be more highly expressed in haploids by analyzing regulatory networks. The *AGT1* promoter was shown to integrate cell-type as well as regular-responsive networks. Both canonical and non-canonical regulatory modules were observed, with transcription factors for carbon metabolism and growth mode. The results suggest that, through selection, the generalist transporter encoded by *AGT1* was subjected to a larger transcriptional response to famine or glucose depletion as part of a scavenging strategy, thus decoupling maltose metabolism genes from its stringent canonical regulation.

The experiments also identified a Tec1 binding site, a motif for haploid-specific transcription factors, in the promoter of *AGT1*. Crandall et al. performed a fluorescence-based

expression analysis comparing the native *AGT1* promoter and a variant in which the Tec1p consensus site was altered by point mutations. Decreased expression was observed in Tec 1 mutants, but did not abolish expression completely, showing that *AGT1* expression is partially dependent on Tec1. Integration of cell-type and carbon-responsive regulatory networks was thus identified as an adaptive target that drives ploidy evolution. The expression and regulatory pattern of *AGT1* in haploids and its dependence on a haploid-specific transcription factor described in the seminar might model adaptive loci in other fungi.

Critique

Crandall spent a considerable amount of time on explaining what ploidy is and the variation in shows on different scales. Using graphs to demonstrate how ploidy varies across nuclear cycles, and images to show how it varies within individuals, Crandall truly emphasized our current knowledge. He indicated how ploidy variation manifests on macroevolutionary as well as on short timescales, generating intraspecific variation. Outlining the phenomenon of ploidy drive, he showed that evolution towards ancestral ploidy state is common using *S. cerevisiae* as an example. This organism was further used to demonstrate how ploidy variation influences evolutionary processes, with accelerated evolution indicated in one of the chromosomal copies of the yeast.

Crandall strongly used these examples and background information to introduce some of the topics of his research and seminar. What are the immediate causes and consequences of ploidy change? Is there selection on ploidy variation? Does ploidy affect fitness in yeasts, and if so, what is the basis for this altered fitness? To show how ploidy is linked to cell type in yeasts, Crandall used the elucidating example of the mating type locus in *Saccharomyces*, encoding for

three different cell-type specific genes. This example was strongly presented to the audience, in an understandable and clear way.

Before presenting his work, Crandall introduced the organism he used. *S. eubayanus* was described as an emerging model yeast, with only 20% nucleotide divergence with *S. cerevisiae*. This importance of this yeast for biotechnological processes was strategically mentioned to capture the attention of the audience. Although many *eubayanus* are phenotypically similar, the ability to grow on maltose is an exception. To ensure the audience could follow along, Crandall summarily introduced the three linked genes required for maltose metabolism. The research question "Can maltose growth be regained under strong selection?" resulted in evolved clones with an improved maltose metabolism. Crandall's main research question followed: "What is the genetic basis of this adaptation?". This rather long introduction was crucial for the audience to understand the research presented in the rest of the seminar.

Both orally and visually, the findings were introduced and described to the audience. She also articulated and supported the expected results of her experiment, encouraging the audience to consider the science behind the information being provided. The predicted and experimental outcomes were presented in an understandable way, giving sufficient consideration to previous research. Crandall correctly indicated it is important to bear in mind the possible bias in some of the results. For instance, the possibility that cells experienced modest off-target fitness defects as a result of their extensive genetic engineering in the stringent ALE condition was mentioned to possibly result in slight underestimation of the fitness benefit attributable to haploid-like cell type in the ALE condition.

When probing new methods, such as the sensitive competition assay, Crandall reiterated the procedure to ensure the audience understood why he had chosen to include this experiment in his research as well as how precisely his original research questions had changed and new ones had emerged during the course of the experiments. To elucidate his reasoning and make an effort to connect with the audience, he maneuvered strong illustrative slides. Crandall, exuded the energy and body language necessary to captivate the audience when presenting. He adopted a confident, steady pace which was beneficial for the audience. Additionally, his nonverbal communication reflected confidence and passion for his own research.

Graduate and undergraduate students with a wide range of prior knowledge were present in the audience for Crandall's seminar, in addition to career researchers with PhDs and other degrees. Notwithstanding, the data and study were presented in a way that was understandable for diverse academic levels, particularly when techniques like *MAT* locus genotyping and flow cytometry were introduced and repeatedly explained. Crandall was able to respond with clarity and precision to queries on methodology and perspective, both simple and complex questions.

Conclusion

John Crandall's exciting findings as to how an adaptive interaction between cell type and metabolism drives ploidy evolution in a wild yeast have an important contribution to resolving the genotype-to-phenotype map in yeasts, which remains a key objective in genetics and evolutionary biology. I was ecstatic to get a mechanistic insight into the molecular underpinnings of environment-cell type fitness interactions, and learn more about how

selection on traits unexpectedly linked to ploidy states or cell types can drive karyotypic evolution in fungi. As a genetics student who has a keen interest in biotechnology, these findings are of particular interest to me. The presentation's slides and organizational structure were excellent and encouraged audience participation and active thinking. This lecture piqued my interest in fungal karyotypic evolution and its potential applications as well as the potential implications of this finding for fungal (evolutionary) biology more broadly.

Work Cited:

Baker, E., and Hittinger, C.T., (2019). Evolution of a novel chimeric maltotriose transporter in *Saccharomyces eubayanus* from parent proteins unable to perform this function. PLOS Genetics, 15(4).

Brickwedde, A., Brouwers, N. Van Den Broek, M., Gallego Murillo, J.S., Fraiture, J.L., Pronk, J.T., Daran, J.G., (2018). Structural, Physiological and Regulatory Analysis of Maltose Transporter Genes in *Saccharomyces eubayanus* CBS 12357T. Frontiers in Microbiology, 9.

Brouwers, N., Brickwedde, A., Gorter de Vries, A., van den Broek, M., Weening, S., van den Eijnden, L. Diderich, J. Bai, F., Pronk, J. Daran, J. (2019). Himalayan *Saccharomyces eubayanus* Genome Sequences Reveal Genetic Markers Explaining Heterotic Maltotriose Consumption by *Saccharomyces pastorianus* Hybrids. Applied and Environmental Microbiology, 85(22).

Gerstein, A., and Sharp, N., (2021). The population genetics of ploidy change in unicellular fungi. FEMS Microbiology Reviews, 45(5).

Haber, J., (2012). Mating-type genes and MAT switching in *Saccharomyces cerevisiae*. Genetics, 191(1), 33-64.

Han, E., Cotty, F. Sottas, C., Jiang, H., Michels, C. (1995). Characterization of AGT1 encoding a general alpha-glucoside transporter from *Saccharomyces*. Molecular Microbiology, 17(6), 1093-1107.

Lancaster, S., Payen, C., Smukowski, H.C., Dunham, M., (2019). Fitness benefits of loss of heterozygosity in *Saccharomyces* hybrids. Genome Research, 29(10), 1685-1692.

Magditch, D., Liu, T., Xue, C., Idnurm, A., (2012). DNA Mutations Mediate Microevolution between Host-Adapted Forms of the Pathogenic Fungus *Cryptococcus neoformans*. PLoS Pathogens, 8(10).

Naranjo-Ortiz, M., and Gabaldon, T., (2020). Fungal evolution: cellular, genomic and metabolic complexity. Biological Reviews, 95(5), 1198-1232.

Todd, R., Forche, A., Selmecki, A., (2017). Ploidy Variation in Fungi: Polyploidy, Aneuploidy, and Genome Evolution. Microbiology Spectrum, 5(4).