AN ABSTRACT OF THE THESIS OF

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Title: Experimental Evolution of Roundup^{TM} Resistance in Outcrossing Populations of *Saccharomyces cerevisiae*

Abstract approved: _____

Molly K. Burke

Evolve & Resequence (E&R) experiments subject laboratory populations to environments controlled by investigators, who then document the phenotypic and genomic changes that take place over many generations. These experiments provide powerful tools for testing of a wide variety of evolutionary questions, especially questions about the nature of adaptive traits. While any organism could be studied in this context, microbes are most practical, due to their quick reproductive cycles. One drawback to using microbes in E&R studies is that they reproduce asexually, which generally leads to slower adaptation that is mutation-limited. By comparison, E&R experiments with sexually-reproducing populations that maintain high levels of standing genetic variation have an improved ability to dissect the genetics of complex adaptive traits. My thesis takes advantage of this powerful experimental technique by subjecting highly diverse, sexually-reproducing popu-

lations of the yeast *Saccharomyces cerevisiae* to a ten week E&R experiment to investigate the genetics of resistance to the herbicide Roundup^{TM}, a trait with significant ecological relevance for a variety of species. The first major objective of my thesis was to identify specific genomic regions that might underlie Roundup^{TM} resistance by tracking allele frequencies over time in evolving populations. A second objective was to determine whether two different selection treatments – a typical treatment involving strong constant selection pressure versus an increasing selection treatment starting with a low dose of Roundup^{TM} that increased over time – led to different evolutionary outcomes. Our results uncovered several potential candidate regions that may underlie Roundup^{TM} resistance and warrant further investigation. While the two selection treatments led to similar evolved phenotypes, and similar ability to localize potentially causative genes, we did observe evidence for a unique trait architecture that suggests that different regions may be selected for depending on the strength of the Roundup^{TM} dose used. Our results provide insight into the consequences of chronic Roundup^{TM} exposure on non-target organisms, and they also inform the general design strategies of future E&R experiments.

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Experimental Evolution of Roundup^{TM} Resistance in Outcrossing Populations of *Saccharomyces cerevisiae*

by

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Chloe C. Hanson, Author

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Chapter 1: Introduction

Experimental evolution provides a powerful framework for testing a variety of evolutionary questions by investigating how model organisms evolve under controlled laboratory conditions. *Saccharomyces cerevisiae* is an important model organism for biology research for many reasons, and is particularly well-suited for experimental evolution. It can easily be reared in the lab and has rapid generational turnover, such that dozens of generations of evolution can occur in a matter of weeks. Typically, evolution experiments with yeast feature an isogenic strain as the ancestor, and track the accumulation of *de novo* mutations that occur over time; this allows the identification of large-effect alleles underlying fitness (e.g. Kao and Sherlock (2008)). While yeast typically reproduce asexually, sexual reproduction can also be induced in the lab with specific media. This allows the creation of recombinant populations for experimental evolution in which genetic variation is initially high, and can be maintained over many generations of evolution (e.g. Linder et al. (2020)). This approach provides several advantages over the traditional approach featuring isogenic, asexual yeast (reviewed by Long et al. (2015)). Chief among these advantages is the ability to track how standing genetic variation evolves over time under different environmental conditions (Burke et al., 2014; Phillips et al., 2020). Sexual reproduction also promotes faster adaptation by decoupling deleterious alleles from causative sites that are under selection (Mc-

Donald et al., 2016). In sexually-reproducing eukaryotic organisms, adaptation is generally driven by the evolution of standing genetic variation, and not beneficial *de novo* mutations reviewed by (reviewed by Burke (2012)). Thus, experimental evolution with initially recombinant, outcrossing yeast populations is emerging as a powerful tool for studying the effects of particular environmental influences (e.g. stresses brought about by climate change, or chemicals in pollutants), that may apply generally to a variety of organisms.

Roundup^{TM} is a widely used, glyphosate-based broad-spectrum herbicide that was first introduced commercially in 1974. Since the advent of genetically modified Roundup^{TM} resistant crops in the mid-1990s, global Roundup^{TM} use has increased 15-fold (Benbrook, 2016). The increased use worldwide has prompted questions about the effects of Roundup^{TM} on organisms other than plants. While manufacturers have claimed that glyphosate, the main active ingredient in Roundup^{TM} has few or no effects on non-target species, multiple studies suggest it is toxic (Lévesque and Rahe, 1992; Baier et al., 2016; Aristilde et al., 2017). There is also evidence to suggest that the undisclosed, non-active ingredients added to commercial formulas to enhance their efficacy might also be more toxic than glyphosate alone (Janssens and Stoks, 2017; Mesnage et al., 2019). There is a need for more studies of the effects of Roundup^{TM} on organisms other than plants to gain a better understanding of the general consequences of its widespread use.

Previous work investigating the effects of glyphosate-based herbicides on yeast have revealed varying levels of toxicity when testing wild isolates and lab strains (Barney et al., 2020). In plants, glyphosate targets the shikimate pathway by in-

hibiting the 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) enzyme, which is a necessary part of amino acid production (Amrhein et al., 1980; Powles and Preston, 2006). It is well established that yeast and many other microorganisms share this same pathway; however, it is not present in any known animal species (Herrmann and Weaver, 1999). In yeast it has been shown that different variants within the yeast *ARO1* gene, which is homologous to the EPSPS gene in plants, cannot fully explain the differential growth of yeast in the presence of glyphosate based herbicides (Rong-Mullins et al., 2017). Further work implementing classic Quantitative Trait Locus (QTL) mapping (Rong-Mullins et al., 2017), RNA-seq and experimental evolution experiments in clonal strains (Ravishankar et al., 2020) has identified several other putative regions that may contribute to improved growth and viability in the presence of commercial glyphosate formulas. Specifically, Rong-Mullins et al. (2017) highlight *DIP5*, an amino acid permease, and *PDR5*, an ABC multiple drug transporter, as potential genes that may contribute to resistance, and the authors hypothesized that these two genes modulate how Roundup^{TM} gains entry into yeast cells. In addition, Ravishankar et al. (2020) identified over a thousand additional potential genetic candidates, so it is clear that more work needs to be done to dissect the complex genetics of Roundup^{TM} resistance in yeast.

Evolution experiments, particularly those that feature whole-genome sequencing of evolved populations (also known as Evolve & Resequence or E&R experiments) offer a unique platform for investigating the genetics of complex phenotypes, and recently, these experiments have emerged as a popular complement or

alternative to classic QTL mapping (Schlötterer et al., 2014; Long et al., 2015). In model systems, distinct genotypes (i.e. strains or inbred lines) representative of the species' natural genetic variation can be crossed to create an ancestral population for an E&R study that is highly diverse; in theory this allows opportunities for efficient and high-resolution trait mapping (Baldwin-Brown et al., 2014). In addition to shedding light on the genetics of the phenotype of interest, imposing artificial selection on replicates of a diverse starting population can reveal insight into the evolutionary dynamics that are at play over the course of an evolution experiment (Phillips et al., 2020). Pooled sequencing techniques allow affordable surveying of allele frequencies in large numbers of individuals, providing a snapshot of the genetics of a whole population at any time point during a population's evolutionary trajectory (Futschik and Schlötterer, 2010; Schlötterer et al., 2014).

For my master's thesis, I use an E&R experiment in outcrossing yeast to investigate the complex phenotype of Roundup^{TM} resistance in a model organism. Over the course of a ten week evolution experiment in which experimental populations were cultured in media supplemented with Roundup^{TM} I documented the changes in phenotypes that occurred with high-throughput growth rate assays, and I also documented the changes in allele frequencies that occurred with pooled-population genome sequencing. I evaluated how evolved populations become differentiated from the ancestor and one another over time. I also assessed the degree to which experimental populations differed from controls, which were handled under identical laboratory conditions as the experimental populations, but without the addition of Roundup^{TM} to the media. In this work, I use the term

"Roundup resistance" to describe improved growth in the presence of Roundup^{TM} compared to a reference population, in this case the ancestral population, but it should be noted that my work technically cannot distinguish whether this phenotypic change might be due to improved tolerance or resistance. Similar work published by other investigators also uses this term (Rong-Mullins et al., 2017; Ravishankar et al., 2020), and I have chosen to follow this convention in my thesis.

Previous simulation work shows that when performing E&R experiments with sexually-reproducing organisms, the ability to detect genomic regions underlying adaptive phenotypes can be enhanced by slowly elevating the selection pressure over time (Vlachos and Kofler, 2019). Strong selection leads to high levels of genetic hitchhiking around selected sites, which in turn reduces the ability to localize candidate genes underlying adaptive phenoytpes; in other words, strong selection leads to large genomic regions, potentially harboring many genes, to increase in frequency. By contrast, weaker selection is expected to lead to decreased hitchhiking and an improved ability to localize individual candidate genes underlying adaptive change. My research has two major objectives: i) to identify potential candidate genomic regions that confer Roundup^{TM} resistance in *S. cerevisiae*; and ii) to compare the genomic signatures left by two different types of selection regimes for Roundup^{TM} resistance – incrementally increasing versus constant selection pressures. Both of these objectives were carried out with a single E&R experiment in sexually outcrossing yeast.

Chapter 2: Materials and Methods

2.1 Experimental evolution of *S. cerevisiae* in media supplemented with Roundup^{TM}

The ancestor of this ten week E&R experiment was a highly recombinant *S. cerevisiae* population called "12X", which was created by crossing 12 geographically and genetically distinct haploid strains (see Table 2.1 for founder strain information and Burke et al. (2020) for details of how this population was created). All strains have been previously modified with an identifying barcode at the *URA3* locus and with the deletion of the *HO* gene to prevent mating type switching (Linder et al., 2020). The *MAT**a*** founder strains have been modified with an insertion of *nat*MX expression cassette at the *YCR043C* pseudogene near the mating type locus. This insertion provides *MAT**a*** mating types with resistance to nourseothricin (*MAT**a***, *HO* Δ, *URA3::KanMX-Barcode*, *YCR043C::nat*MX). Similarly, *MAT**α*** strains are resistant to hygromycin B due to an insertion of the *hph*MX cassette, which replaces *YCR043C* pseudogene (*MAT**α***, *ho* Δ, *URA3::KanMX-Barcode*, *YCR043C::hph*MX). When grown on selective media (YPD + 300mg/mL hygromycin B + 100mg/mL nourseothricin sulfate + 200mg/mL G418) only diploids that carry both resistant cassettes at the *YCR043C* locus and the *KanMX-barcode* are selected.

Table 2.1: Founding strains of the 12X ancestor population, from the Saccharomyces Genome Resequencing Project (SGRP). Strains were previously modified to facilitate crossing (Cubillos et al., 2009; Linder et al., 2020).

SGRP Strain	Origin	Genotype
DBVPG6765	*European (Wine)*	*MAT***a**, *ho Δ, ura3::Kan*MX-*Barcode ycr043C::nat*MX
DBVPG6044	*West African (Palm Wine)*	
BC187	*North American (Wine)*	
SK1	*West African (Laboratory)*	
L_1374	*South American (Wine)*	
YJM975	*European (Clinical- Vaginal)*	
YPS128	*North American (Oak Tree Soil)*	*MAT***a**, *ho Δ, ura3::Kan*MX-*Barcode ycr043C::hph*MX
Y12	*Japanese (Sake)*	
273614N	*European (Clinical- Fecal)*	
L_1528	*South American (Wine)*	
UWOPS05_217_3	*Southeast Asian (Bertam Palm Nectar)*	
YJM981	*European (Clinical-Vaginal)*	

Over the course of the experiment, replicate populations derived from the ancestral 12X population were maintained in a rich medium (1% peptone, 2% dextrose, 2% yeast extract, or YPD) supplemented with Roundup Weed & Grass Killer III$^{\text{TM}}$ (2% glyphosate). The two different selection treatments consisted of either: i) a "constant" treatment involving a continuous culture at a single dose (1% of the commercial formula in the final concentration) or ii) an "increasing" treatment which incrementally increased the dose of Roundup^{TM} added to the media each week, starting at a low dose of 0.1% and ending at a high concentration of 1% (Table 2.2). Both experimental treatments experienced complex life-history involving extended periods of competitive asexual growth in liquid media that were frequently interrupted by induced outcrossing (Figure 2.1). Control populations, derived from the same ancestral population and handled following identical protocols, were also maintained in YPD without Roundup^{TM}. For practical reasons, these control populations were generated as part of a previous experiment and were

not run in parallel with the Roundup^{TM} selection. All populations were cryopreserved weekly (at -80^{o}C in 25% glycerol), such that populations could be revived at any evolutionary time point for fitness and/or genomic surveys. Comparing phenotypes observed in the experimental populations to those in the ancestor and control populations allows determination of the trait(s) that shifted as a result of selection for Roundup^{TM} resistance, and comparing allele frequencies among these populations may reveal genomic regions harboring causative genomic variants underlying these traits. Examining allele frequency differences between experimental populations (e.g. between the constant and increasing treatments) allows evaluation of the prediction that gradually increasing the strength of selection in an E&R experiment improves the ability to localize candidate regions.

Figure 2.1 shows the weekly sequence of events that involve both growth in Roundup^{TM} supplemented media and frequent outcrossing. To begin, the ancestral population was sampled 36 times to create independent replicate populations, 18 for each treatment. Two replicate populations from each treatment were excluded from the analysis due to sample loss and/or documented experimental mishaps that may have led to contamination. The 16 control populations were generated previously and experienced all steps of the protocol shown in Figure 2.1 except for Roundup^{TM} exposure.

Populations grew in liquid batch culture for a total of 48 hours in the corresponding Roundup^{TM} treatment each week, with a dilution halfway through to increase the total number of generations of growth. During this phase, all cultures were incubated at 30°C and shaken at 200 rpm. After 48 hours of competitive

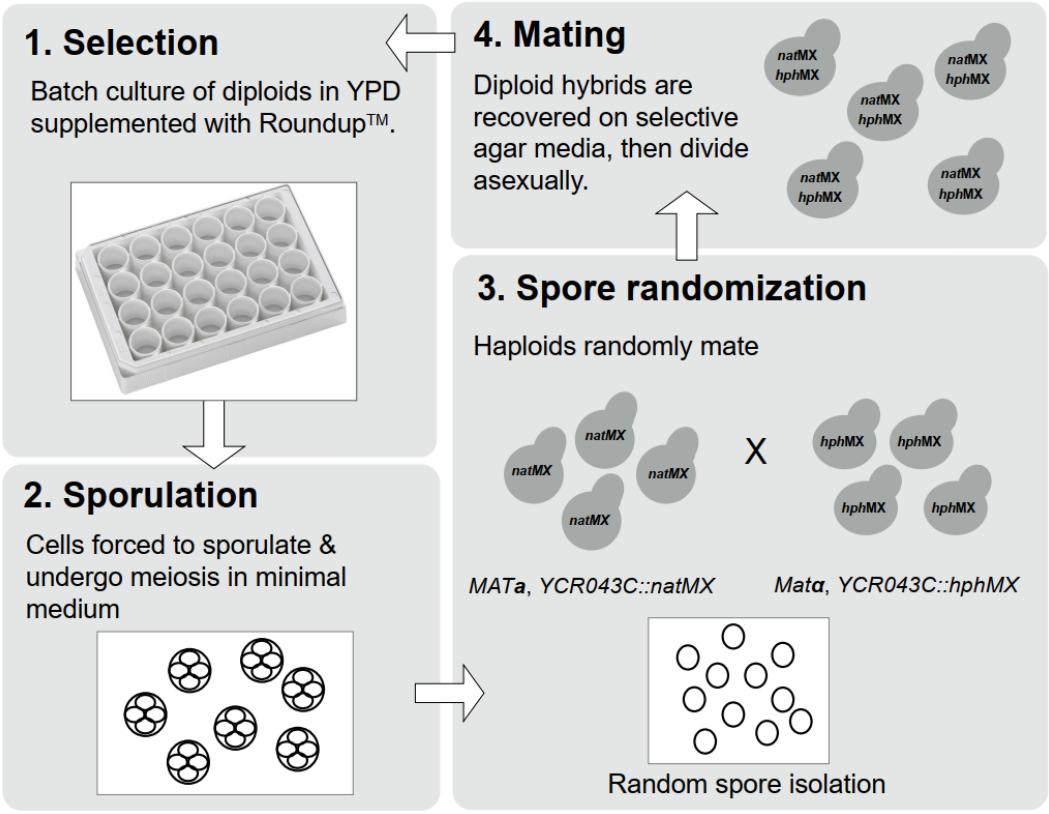

Figure 2.1: The Burke Lab's weekly selection protocol for evolution experiments in outcrossing yeast. The protocol involves 48 hours of liquid culture in YPD media (constant and increasing treatments were supplemented with Roundup^{TM} as per Table 2.2) followed by steps for inducing sporulation, spore isolation and random mating. Selective media will ensure that only randomly-mated diploids that have both the *nat*MX and *hph*MX cassettes continue on to the next week of competitive growth in Roundup^{TM}. The *nat*MX and *hph*MX expression cassettes, which replace the *ycr043C* pseudogene provide *MAT**a*** founder strains with resistance to nourseothricin and *MAT**α*** founders with resistance to hygromycin B respectively.

Table 2.2: An overview of the constant and increasing Roundup^{TM} selection treatments. The percentage of Roundup^{TM} mixed in rich liquid media (YPD), and the total estimated number of cell doublings (including both competitive growth in liquid media and the diploid recovery stage on solid media) per week are listed below. Each week one round of induced outcrossing occurs.

Week	**Constant Roundup (Replicates 1-18)**				**Increasing Roundup (Replicates 19-36)**			
	% Roundup in YPD	Estimated asexual generations			% Roundup in YPD	Estimated asexual generations		
		Competitive	Non-competitive	Total		Competitive	Non-competitive	Total
1	1.00%	5.8	7.6	13.4	0.10%	9.6	2	11.6
2	1.00%	8.9	4.4	13.3	0.20%	10.3	1.9	12.2
3	1.00%	9.2	3.5	12.7	0.30%	10.7	2.7	13.4
4	1.00%	9	3.6	12.6	0.40%	11.6	2.9	14.5
5	1.00%	9.1	2.8	11.9	0.50%	11.2	2.6	13.8
6	1.00%	9.1	3.7	12.8	0.60%	10.6	2	12.6
7	1.00%	8.6	3.7	12.3	0.70%	9.7	3.1	12.8
8	1.00%	8.8	3.7	12.5	0.80%	9.6	0.9	10.5
9	1.00%	9	2.1	11.1	0.90%	9.2	3.5	12.7
10	1.00%	9	2.8	11.8	1.00%	9.1	1.8	10.9
	Total estimated asexual generations	86.5	37.9	124.4	Total estimated asexual generations	101.6	23.4	125

growth in liquid media, sporulation was induced by transferring populations to minimal sporulation medium (0.1% potassium acetate solution) and incubated for $\sim$ 72 hours (30°C/200 rpm). After sporulation, lytic enzymes and mechanical agitation were used to break down the yeast ascus walls, so that spores could be isolated and mixed to ensure random mating. Mating occurred on selective media agar plates (YPD + 300 mg/mL hygromycin B + 100 mg/mL nourseothricin sulfate + 200 mg/mL G418) and diploids that had successfully mated were allowed to grow for $\sim$ 48 hours. Recovered diploids then underwent the next week's Roundup^{TM} treatment in batch culture, repeating the process again. At every

transfer step, cell density was estimated and standardized, as detailed in the next section.

2.2 Estimating population size and number of evolved generations

Before starting the selection experiment, I assayed growth rates and cell viability of the ancestral population in YPD and in 1% Roundup^{TM} in parallel. The data from these assays were used as benchmarks to predict the number of viable cells present in the evolving populations at any point during the selection experiment. The 16 replicate populations from each treatment were standardized to an OD_{600} $\sim$ 0.1 and grown for 48 hours in 1 mL liquid batch culture at 30°C and shaken at 200 rpm. Every $\sim$8 hours manual OD_{600} readings were taken from each population and a sample dilution of all replicate populations were grown on YPD agar plates. Plated samples were diluted (10 or 100 fold) such that colony forming units could be counted after 48 hours of growth to calculate the estimated number of viable cells in the population at each time point. Linear least squares regression (R^2= 0.9304) was performed to model the relationship between an OD_{600} value and the number of viable colonies recovered from the same time point. This predictive model allowed us to use OD_{600} measurements to approximate viable cell density at specific phases of the experiment. OD_{600} was measured in all populations at all transfer points in an attempt to standardize cell density in experimental populations, thus maintaining consistent population demographics and avoiding bottlenecking genetic diversity; the minimum number of cells transferred always exceeded 10^6.

The cell count estimates derived from this model over the course of the ten week selection experiment were also used to predict the number of cell doublings that occurred during growth in liquid media and diploid recovery after the weekly induced outcrossing. Competitive asexual generations were assessed by measuring the OD_{600} of all populations before and after Roundup^{TM} exposure (Figure 2.1, Step 1). Solving for Equation 2.1 below, (where y is the starting cell density, x is the final cell density, and z is the resulting number of cell doublings), I estimate that over 86 asexual generations occurred in the constant regime and more than 100 generations in the increasing regime over the full ten week experiment (Table 2.2).

$$z = \log_2(\frac{x}{y}) \tag{2.1}$$

Non-competitive asexual generations were also estimated with this equation, though less directly, by comparing estimates of cell counts before and after random mating and diploid recovery (Figure 2.1, Step 4). I estimated the number of viable spores entering the mating step by plating dilutions (10^{-5} & 10^{-6}) of isolated spores immediately following isolation so that the spores did not have time to mate (i.e. cultures at the end of Step 3) on YPD agar media and counting colonies after 48 hours of growth. These spore estimates were divided by 2, since in the experiment two spores would have to mate to survive the experimental protocols, and then these numbers were used as estimates of the "initial" plate cell density. "Final" plate cell density was determined from each experimental replicate population after

48 hours of growth on the agar plates; lawns of cells from each plate were scraped into liquid media and OD_{600} was measured to estimate the total number of cells on the plate.

Using these prediction methods, I estimate that if 100% of the spores successfully mated and survived there would be $\sim$ 38 additional asexual generations in the constant regime and $\sim$ 24 generations in the increasing regime over the course of the experiment. While these are indirect estimates, they can be viewed as conservative lower bounds on the range of generations that likely occurred, since they assume that rates of spore viability and mating were 100%. In reality, we expect these rates to be far lower; meaning, if either spore viability and/or mating efficiency was $<$ 100%, this would decrease the number of viable cells that could initiate growth on the plate, which would require a larger number of cell doublings to have occurred to achieve the observed cell density after 48 hours.

2.3 Relative growth rate in evolved populations

My general strategy for phenotyping evolved populations and determining how they may have diverged from the ancestor involved high throughput growth rate assays using a Tecan Spark multimodal plate reader. These assays allowed simultaneous, replicated assessments of evolved populations (from both constant and increasing selection treatments), the control populations, and the 12X ancestor. All populations were revived from frozen stocks and handled in parallel, including standardization to a starting OD_{600} of 0.05 in 200 μL 1% Roundup^{TM} YPD.

Growth rate assays in 96-well plates were carried out over 48 hours at an incubation temperature of 30°C (without agitation), and OD_{600} was measured every 30 minutes. Identical assays were carried out in Roundup^{TM}free YPD media to observe evidence of potential phenotypic trade-offs that may have resulted from long-term adaptation to Roundup^{TM}. Growth rate assays were performed using two technical replicates for each of the 16 evolved populations from each Roundup^{TM} treatment and 13 of the control populations alongside three biological replicates (independent overnight cultures) of the ancestor population. All evolved populations (of the Roundup^{TM} treatments and control treatment) were measured after ten weeks of selection.

Plate reader data were analyzed using the R package Growthcurver to assess differences in growth dynamics between assayed populations (Sprouffske and Wagner, 2016). The following logistic growth equation (Eq. 2.2) was fitted to the absorbance data to generate estimates of carrying capacity and population doubling rate (Kimura, 1971). Carrying capacity (K) refers to the maximum population size that can grow based on the environmental conditions. The intrinsic rate of growth of the population (r) is also referred to as doubling time. The population size at the beginning of the growth assay is (N_0). The logistic equation describes the population size at a given time (t) as N_t using;

$$N_t = \frac{K}{1 + (\frac{K - N_0}{N_0})e^{-rt}} \tag{2.2}$$

Growthcurver uses the OD_{600} measurements from the growth curve data to

find the best values of growth rate (r) and the carrying capacity (K). These estimated parameters were used to compare phenotypes among the treatment groups, controls and the ancestor. Assumptions of the one way ANOVA normality and homogeneity of variance were tested using Shapiro-Wilks Tests and Levene's F Tests for Equality of Variances, respectively. Both assumptions were not consistently met, so a nonparametric approach was implemented using the Kruskal Wallis test by ranks to see if there is stochastic dominance in at least one of the groups when analyzing the phenotypes of doubling time and carrying capacity. Pair wise comparisons followed using Mann-Whitney tests with Bonferroni correction.

2.4 DNA Extraction, Library Prep and Sequencing

All populations from this study were sampled for pooled population genomic DNA sequencing (Pool-SEQ). DNA was extracted and sequenced from the initial ancestral population before selection was implemented as described in Burke et al. (2020). Extractions from the 16 extant replicate populations from both the increasing and constant Roundup^{TM} treatment groups were then performed after 3, 6, and 10 weeks of selection. The 16 control populations were generated previously and sequenced after 1, 7 and 15 weeks of selection; in other words, they were not handled in parallel with the Roundup^{TM} treatments, and existing genome data happen not to correspond to the same generations at which the Roundup^{TM} treatments were sampled. All DNA extractions were performed using 1 mL of saturated overnight cultures in liquid YPD media ($\sim 10^9$ diploid cells) using the Qiagen Gentra Pure-

gene Yeast/Bacteria kit (25U zymolase replaced 1.5 μL Lytic Enzyme Solution). 25ng from each sample (quantified using a Qubit 3.0 Fluorometer) was used to prepare Illumina Nextera libraries, with slight modifications to the manufacterer's protocol to increase throughput, and samples were dual-indexed to facilitate combination into multiplexed libraries. Pooled sequencing was performed at OSU's Center for Genomic Research and Biocomputing using the Illumina HiSeq3000. Libraries were run on 1-2 PE150 lanes to achieve a minimum average genome-wide coverage of 50X per replicate population.

2.5 Variant Calling and Coverage

The Burke Lab has published pipelines for characterizing population genetic patterns from Pool-SEQ data, at the level of individual alleles, and linked haplotypes (Burke et al., 2014; Phillips et al., 2020). DNA sequencing data were first processed by aligning raw reads to the *S.cerevisiae* S288C reference genome (R64-2-1) and calling variants across all biological replicates using GATK v.4.0 (McKenna et al., 2010; Poplin et al., 2018; Van der Auwera, GA and O'Connor, BD, 2020). Base quality was also calibrated by indexing a reference VCF file that catalogs SNP information for a group of natural *S. cerevisiae* isolates (Bergström et al., 2014). The resulting VCF was used to create a SNP frequency table that was used for subsequent analysis. Annotation of genetic variants and the effect that they have on genes and their resulting proteins was investigated using SnpEff v.5.0e (Cingolani et al., 2012). Additional filtering was performed to include only SNPs

that had sufficient coverage ($> 10X$ per site per population). Patterns of variation in coverage for individual populations were assessed as this might reveal evidence for potential structural variants (i.e. large scale duplications or deletions) changing in frequency over time. I looked for potential structural variation by finding the coefficient of variation of the genome wide coverage for each population. This was calculated by dividing the standard deviation of coverage by the mean coverage within each population. A low coefficient of variation (< 1) suggests that there is not strong evidence for large scale structural variants (Phillips et al., 2020; Feng et al., 2015).

2.6 Characterizing allele frequency differentiation

I used a number of methods to describe patterns of genetic variation within and among populations, with the goal of understanding how the control and Roundup^{TM} treatments diverged from the ancestor over the three sequencing time points. First, a principal component analysis (PCA) was performed to broadly investigate patterns of population-level variation and how this changed over the course of the experiment. This PCA was performed using the prcomp() function in R (Stats v.3.6.2) including all polymorphic SNPs common to the ancestor and evolved populations at each sequencing time point. Polymorphic SNPs were defined as all SNPs that had a frequency > 0 and < 1 in the ancestor population and had a coverage > 10 in all replicate populations. To investigate more specifically how standing variant frequencies changed, Cochran Mantel Haenszel (CMH)

tests were performed to assess allelic differentiation between pairs of experimental treatments (e.g. replicate populations from two treatments at the same timepoint) using R package lawstat v.3.4. In the context of an E&R study, the p-values from CMH tests can be used to identify regions of the genome that putatively underlie the adaptive shifts occurring during experimental evolution (Kofler et al., 2011).

Compared to other approaches, CMH tests have been identified as an effective and computationally expedient tool for identifying genomic targets of selection in E&R experiments (Vlachos et al., 2019). For each pairwise CMH test a permutation-based approach, similar to the methods used in Burke et al. (2014), was implemented to determine a significance threshold. A null distribution was created by scrambling the associated sample identifiers for each SNP position such that the associated coverage and allele counts were randomized between the two groups being tested. A CMH test was performed on this null data set generating p-values that would result from genetic drift rather than selection. The most significant p-value across the full genome was taken from the permutation data and this process was repeated 1000 times. The threshold associated with a 5% false positive rate was then determined from the thousand lowest p-values by using the quantile function in R.

To characterize allele frequency differentiation among populations of our three treatments, we carried out a total of three separate CMH tests, each revealing genomic regions where differentiation was high for a specific pair. Two of the tests identify regions of the genome potentially underlying adaptation to Roundup^{TM}: comparing allele frequencies from the terminal time points between 1) the con-

stant treatment and the control treatment and 2) the increasing treatment and the control. "Peaks" of -log10(p) values exceeding the relevant significance threshold in each test are strong candidates for regions harboring genes and/or variants with functional consequences for Roundup^{TM} resistance (i.e. "Roundup-specific" regions). A third CMH test was carried out to assess allele frequency differentiation between populations of the constant and increasing treatments. The objective of this third test is to provide insight into regions of the genome that exhibit a specific signature of adaptation in these two regimes. If a specific peak presents in two of the three CMH test results, the region can be viewed as specific to the treatment common to those two tests. Meaning, significant peaks that occurred in only one treatment compared to the control were identified as either a "constant-specific" or "increasing-specific" peak, and then were further verified by checking that these same regions also showed significant differences when comparing the constant and increasing treatments directly. Peak regions that present in a single test are less informative; therefore for relevancy to the study objectives, our analysis is restricted to these so-called treatment-specific peaks shared by two of the three treatment comparisons. Individual SNPs above the significance threshold in each peak were investigated to see if these variants fell within coding or noncoding regions, if they are synonymous or nonsynonymous, and what the downstream effects of these SNPs might be. The Saccharomyces Genome Database (SGD) YeastMine tool was also used to identify the total number of genes that fell under each peak and the GO Term Finder (Version 0.86) was used to discover potential ontological categories that are shared among these verified genes (Balakrishnan

et al., 2012; Hong et al., 2008). The genomic signatures of the Roundup-specific peaks in each treatment– in other words, the size, and location of each peak - were also compared to see if the incrementally increasing regime led to different genomic patterns, and perhaps increased resolution to identify putative causative genomic sites, compared to the constant selection regime.

In addition to characterizing allele frequency differentiation at polymorphic SNPs segregating among experimental populations, I also filtered the dataset to search for potential beneficial *de novo* mutations that arose after the experiment started. This was done by first filtering for SNPs that were not present in the initial ancestor population, but then exceeded a frequency of 25% in at least one experimental replicate after the final week of selection. Further filtering was done to identify SNPs where this frequency change was only observed in a single replicate, while absent from the other replicates. It is likely that SNPs with frequency change occurring in more than one replicate were already existing in the ancestor population at a low frequency and were not detected during sequencing. These were omitted since they likely are not true *de novo* mutations that arose over the course of the selection experiment.

Chapter 3: Results

3.1 Relative growth rate in evolved populations

After ten weeks of evolution all the evolved populations were tested alongside the ancestor in rich media with and without Roundup^{TM}. Figure 3.1A shows the 48 hour growth curves generated by plotting the log transformed OD_{600} measurements taken every 30 minutes of growth in 1% Roundup^{TM} media. Nonparametric pair-wise comparisons showed that doubling time was significantly slower in the ancestor compared to both Roundup^{TM} treatment groups, constant ($p = 0.002$) and increasing ($p = 0.002$), and the control populations ($p = 0.004$). The doubling time estimates (Figure 3.1.B) suggested no significant difference between the two Roundup^{TM} selection regimes ($p = 0.381$), but did exhibit slower growth in the control compared to the constant ($p = 0.001$) and the increasing ($p = 0.015$) regimes. There was a significantly higher carrying capacity (Figure 3.1.C) in the ancestor compared to both the constant ($p = 0.002$), increasing ($p = 0.002$) and control ($p = 0.004$) populations. The control also demonstrated a higher carrying capacity compared to both the constant ($p = 0.002$) and increasing ($p = 0.0003$) treatments, however, no significant difference in carrying capacity was observed between the constant and increasing regimes ($p = 0.669$).

The growth rates from the plate reader assay in rich YPD media are shown

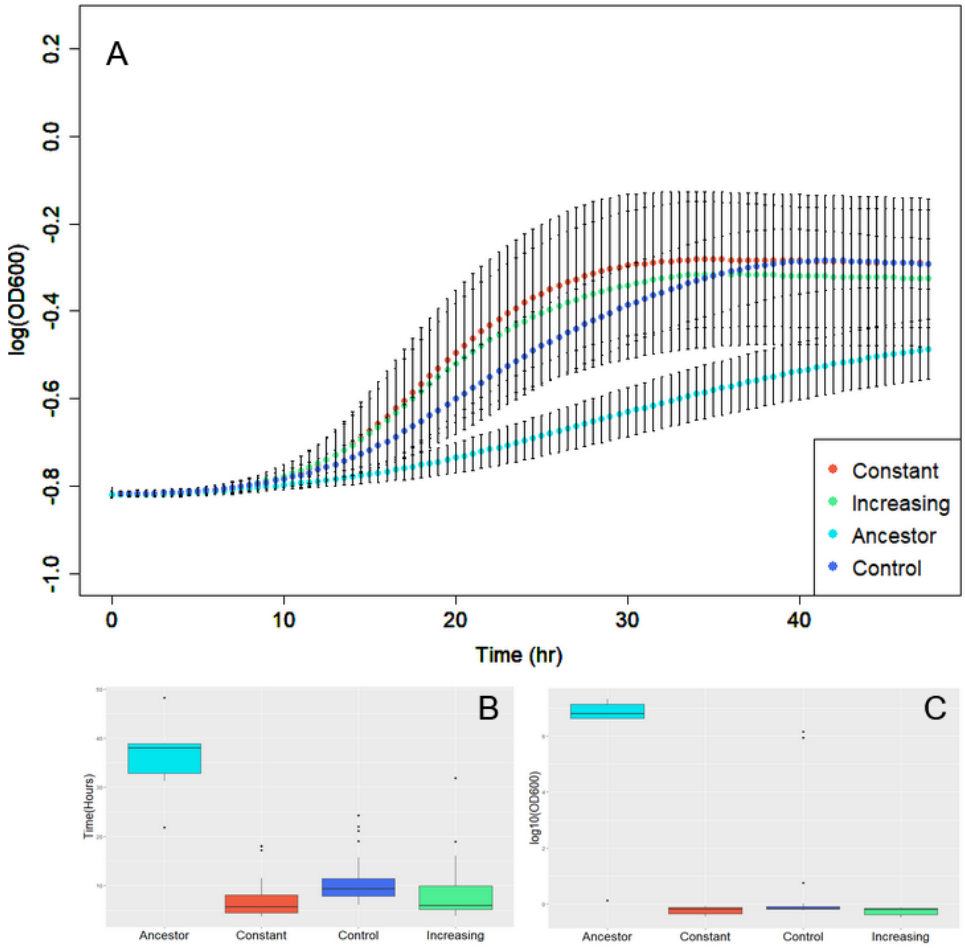

Figure 3.1: Growth in 1% Roundup^{TM} media. Evolved Roundup^{TM} resistance is evidenced by growth curves (3.1.A) generated from microplate reader OD_{600} measurements taken every 30 minutes for 48 hours. Points represent the average of two technical replicates of each population per treatment: constant (red, N=16), increasing (green, N=16), control (blue, N=13) and the ancestor (turquoise, N=3). The average doubling times (3.1.B) are significantly shorter in all experimental populations compared to the ancestor, with the two Roundup^{TM} treatments growing the fastest. Average carrying capacity (3.1.C), however, appears to be higher in the ancestor compared to both Roundup^{TM} treatments and the evolved control. No significant phenotypic differences were observed between Roundup^{TM} treatment groups with regards to either growth rate or carrying capacity.

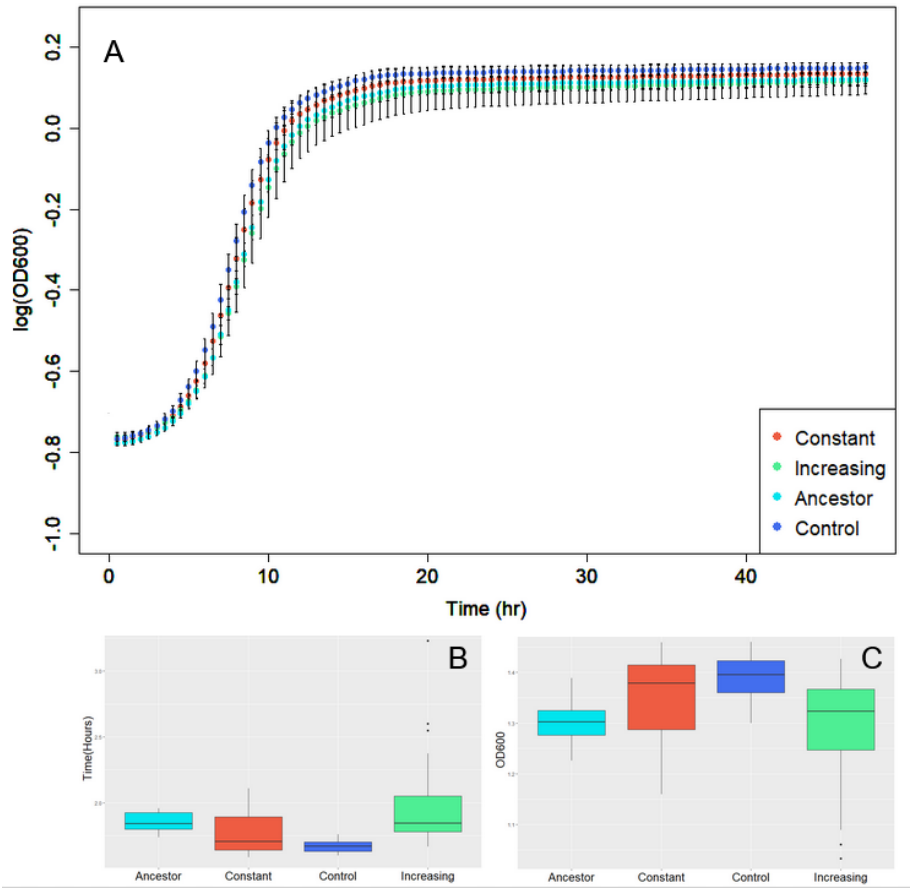

Figure 3.2: Growth in YPD media. Growth curves (3.2.A) were produced for all populations following the same protocols used in Figure 3.1, using YPD without Roundup^{TM} as the culture medium. The average doubling times (3.2.B) provide evidence of improved growth in the control, compared to the ancestor and both Roundup^{TM} treatments. There was no observed difference in doubling time between the Roundup^{TM} treatments and the ancestor. Similarly, average carrying capacity (3.2.C) estimates were slightly higher in the control compared to the increasing and ancestor populations, but no significant differences were found between the ancestor and the Roundup^{TM} treatments.

in Figure 3.2.A. Estimates of doubling time(Figure 3.2.B) demonstrate that the control populations had significantly faster growth compared to the ancestor (p = 0.004), constant (p = 0.008) and increasing (p = 2.947e-08) treatments. There were not significant differences between the ancestor and the constant (p = 0.210) or the increasing (p = 0.5593) populations; however, populations of the constant treatment had slightly higher growth rates compared to those of the increasing treatments (p = 0.004). The controls also had higher carrying capacities (Figure 3.2.C) than the ancestor (p = 0.007) and increasing treatment group (p = 1.326e-06); however, populations of the constant treatment exhibited no significant difference from the controls (p = 0.0683). The carrying capacity of the ancestor was similar to both the constant (p = 0.3591) and increasing (p = 0.7926) populations, and there were no significant differences between the two Roundup^{TM} treatments (p = 0.1835).

3.2 SNP identification, average coverage and *de novo* mutations

A total of 90,510 biallelic SNPs were identified relative to the reference genome in all evolved populations prior to any sort of filtering. The mean genome-wide SNP coverages of individual populations ranged from 10X to 82X (Appendix A). The coefficient of variation of each sample fell below 0.4 demonstrating low variation of coverage depth across the full genome. Pool-SEQ data regions with atypically high or low coverage relative to the mean may indicate potential structural variants (e.g., duplications or deletions) that may have arisen over the course of selection.

We found no such regions, and therefore report no obvious evidence for large scale structural variation in any population. Investigation into putative *de novo* mutations also found no strong candidates within the two Roundup^{TM} treatments that met the criteria outlined in section 2.5 and achieved a frequency change of $> 25\%$.

3.3 Allele frequency characterization in evolved populations

To evaluate how standing genetic variation evolved over the course of the experiment, we first ran a PCA using allele frequencies in a subset of SNPs that were polymorphic in the ancestral population, and at which we observed high depth of coverage. There were 32,298 polymorphic SNPs, which met the following quality filters across the entire dataset: 1) SNPs were at a frequency > 0 in the ancestral population and 2) coverage was $> 10X$ in all populations at all sequencing time points.

The PCA of these polymorphic SNP allele frequencies suggests that the replicates of each treatment group (control and both Roundup^{TM} treatments) are more genetically similar to one another than to populations of different treatment groups, and that the populations of each group diverge over time (Figure 3.3). In both the constant and increasing treatments, we observe that replicates are tightly clustered and shift in a consistent direction over time, while the replicates of the control treatment are more loosely clustered, potentially implicating the effects of genetic drift rather than selection.

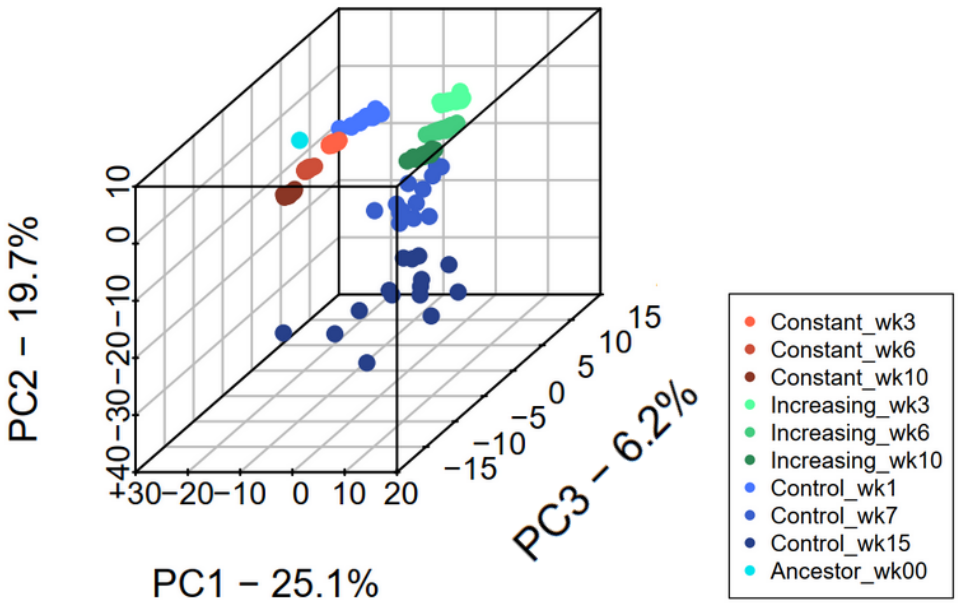

Figure 3.3: PCA of high-coverage standing variants. Each color in this PCA represents an independent replicate population belonging to one of the three experimental treatments (N=16 per color, except for the ancestor) at a specific timepoint. Replicates of the two Roundup^{TM} treatments cluster tightly together at each timepoint, which is indicative of parallel evolution within each treatment. Replicates of the control treatment do not cluster tightly, which is indicative of genetic heterogeneity produced among them.

Next, we carried out CMH tests comparing allele frequencies at the terminal time points of the experiment (week ten for the Roundup^{TM} treatments, and week 15 for the controls) in order to identify regions of the genome that are differentiated by treatment. A total of 55,105 polymorphic, high coverage SNPs were used to perform each CMH test. Only polymorphic SNP allele frequencies from the final sequencing time point were used in the CMH test and filtered for coverage $> 10X$;

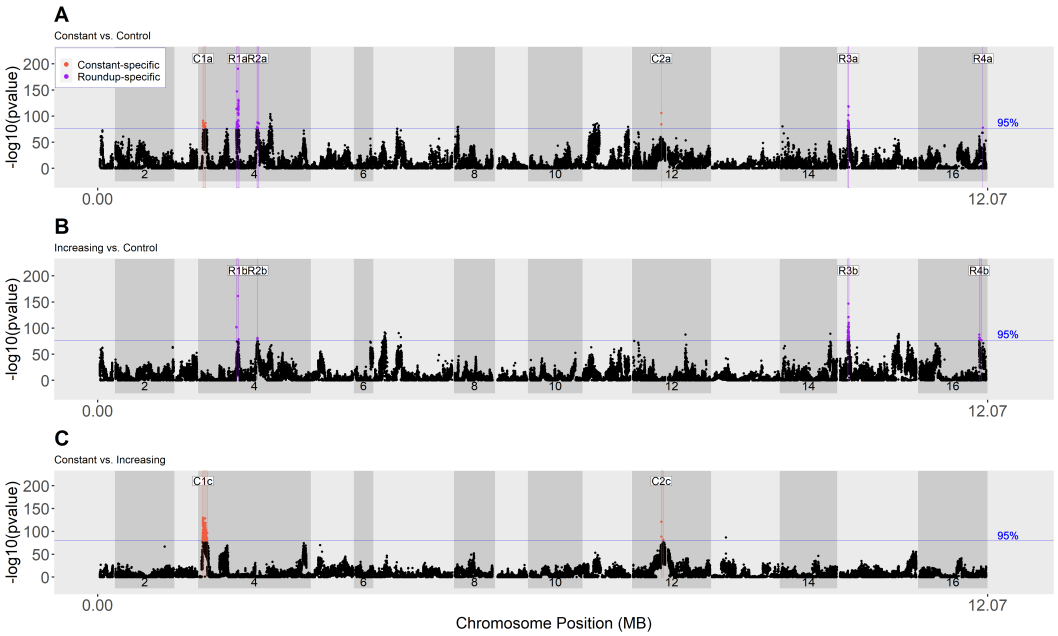

Figure 3.4: (A) Final week comparisons of the allele frequencies from the constant vs. control (A), increasing vs. control (B) and constant vs. increasing (C). Roundup-specific peaks that showed significant changes in plots A and B, but not in C are highlighted in purple. Constant Roundup^{TM} selection specific peaks that are observed in plots A and C are highlighted in orange. Peak names are designated with the first letter (C or R) corresponding to the either a Roundup- or constant-specific peak, the number matches common peaks between plots and the final letters (i.e. a, b or c) signify the CMH test that each test is from as described above.

therefore, more SNPs were included in the CMH analysis compared to the PCA. As CMH tests require pairs, we conducted three independent CMH tests: constant treatment versus control treatment (Figure 3.4.A), increasing treatment versus control treatment (Figure 3.4.B), and the constant versus increasing treatment (Figure 3.4.C). Peaks of significant SNPs were identified from each pairwise test by independently determining significance thresholds that correspond to a genome wide

Table 3.1: A summary of the treatment-specific peaks implicated by the experiment. Each peak listed in this table technically exists as a pair (e.g. C1a and C1c), as to be considered a peak, a particular genomic location must include significant SNPs in two out of three CMH tests. While peak regions share a general genomic location, the width and number of SNPs implicated differs for each pair. Two constant-specific peaks, four Roundup-specific peaks, and zero increasing-specific peaks were observed. Considering only the Roundup-specific peaks, width, SNP number, and gene count suggest that neither treatment type is better than the other in terms of the ability to detect candidate regions.

Peak Type	Peak Name	Position	Peak Width (kb)	Number of genes under each peak	Number of significant SNPs under each peak
Constant-specific	C1a	Chr4:67141-101651	34.51	19	13
	C1c	Chr4:64076-124453	60.38	32	198
	C2a	Chr12:399483-399513	0.03	0	2
	C2c	Chr12:399483-424103	24.62	12	3
Roundup-specific	R1a	Chr4:520955-557161	36.21	19	41
	R1b	Chr4:520955-549376	28.42	14	4
	R2a	Chr4:801527-826151	24.62	13	6
	R2b	Chr4:806513-806514	0.00	0	2
	R3a	Chr15:143876-155276	11.40	5	26
	R3b	Chr15:138539-158650	20.11	9	54
	R4a	Chr16:881189-881189	0.00	0	1
	R4b	Chr16:832084-865361	33.28	17	6

alpha of 0.05 using the permutation methods described in Section 2.6. Figure 3.4 shows all significant peaks that were identified above the corresponding threshold from each pairwise comparison; however, further discussion will only focus on the peaks that we identified as treatment-specific, in other words the peaks that were identified in two of the three CMH tests. We identified four "Roundup-specific" peaks where allele frequencies significantly diverged from the control in both the constant and increasing Roundup^{TM} treatments (i.e. peaks in both Figures 3.4.A & 3.4.B). We identified an additional two "constant-specific" peaks where allele frequencies in the constant treatment significantly diverged from both the control

and increasing treatment (i.e. peaks in both Figures 3.4.A & 3.4.C). There were no peaks that could be clearly labeled as specific to the increasing Roundup^{TM} treatment. Table 3.1 provides a summary of the Roundup-specific and constant-specific peaks including their position within the genome, number of SNPs per peak, the width with regards to the significance thresholds and the number of genes that fell under each peak. A more detailed summary of all individual SNPs reported in each Roundup- and constant- specific peak are provided in Appendix B including their potential functional consequences as predicted by the program SNPEff.

The GO term analysis of all of the verified genes under all six peaks identified three functional categories relating to sodium and calcium transport as mechanisms of phosphorylation. It should be noted that two of the three GO terms ("sodium-exporting ATPase activity, phosphorylative mechanism" and "calcium-transporting ATPase activity") included only genes that occur within a single peak all clustered together (R1), and these happen to be from the same family (*ENA1*, *ENA2*, and *ENA5*). Therefore, there is no basis to conclude that these two GO terms are enriched from a genome-wide perspective. By contrast, the third and most significantly enriched term, "sodium ion transmembrane transporter activity" (corrected p-value = 1.07 x 10^{-5}), identified five genes occurring on two different chromosomes in three separate peaks. One of the genes linked to this term was *ENA1*, a P-type ATPase sodium pump that is involved in Na+ and Li+ efflux contributing to salt tolerance (Mendizabal et al., 1998). Also on chromosome 15 the most significant nonsynonymous SNP fell within *HAL9*, a putative transcription factor of the C6 zinc finger class. *HAL9* activates *ENA1* and several ABC

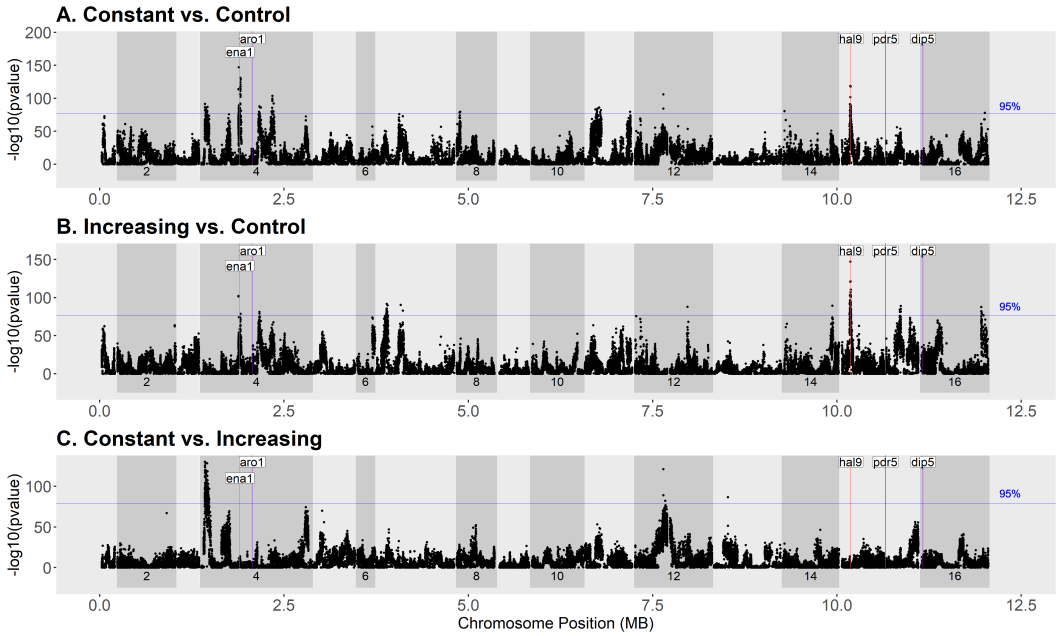

Figure 3.5: Significant genomic changes were not observed in *a priori* genes *ARO1*, *PDR5* and *DIP5* (highlighted in purple) as these genes did not fall within significant peaks of the final week comparisons of the allele frequencies from the constant vs. control (A), increasing vs. control (B) and constant vs. increasing (C). We did, however identify two genes *ENA1* shown in chromosome 4 and *HAL9* on chromosome 15 (highlighted in red) that fell within Roundup-specific peaks. These genes were of interest since *HAL9* activates both *ENA1* and several transcription factors including *PDR5*.

multidrug transporters including *PDR5*, which was previously identified as a gene potentially underlying glyphosate resistance (Rong-Mullins et al., 2017; Mendiz-abal et al., 1998). Figure 3.5 shows the location of these two putative candidate genes falling within significant peaks, while *a priori* candidates *ARO1*, *PDR5* and *DIP5* did not experience significant genomic changes.

3.4 Annotations and allele frequency trajectories of most significantly differentiated SNPs in each peak region

3.4.1 Roundup-specific peaks

The four pairs of Roundup-specific peaks (R1a/b, R2a/b, R3a/b and R4a/b) identify regions with significantly different allele frequencies in both the constant treatment compared to the controls, and the increasing treatment compared to the the controls (see Figure 3.4.A & B). While these peaks were identified because their position along the genome overlapped, we observe some meaningful differences between the a/b peaks of each pair. As shown in Table 3.1, the two peaks often implicated different numbers of significant SNPs, and had different widths. While any of the significant SNPs under any peak is worthy of further scrutiny (e.g. for functional validation with *in-vivo* experiments), for simplicity, we point out the SNP we observed to exhibit the most significant differentiation under each inclusive peak region (meaning the a/b peaks considered together). Below, we enumerate the predicted functional effects of each of these SNPs, and visualize their frequencies over the course of the experiment. For peak R1 on chromosome 4, the SNP with the highest -$\log_{10}$(p-value) is a synonymous variant within the *RSM10* gene. This gene codes for an essential protein that mediates translation within mitochondria (Saveanu et al., 2001) and was previously identified in a QTL study investigating the effects of glyphosate based herbicides on yeast (Ravishankar et al., 2020). For peak R2 on chromosome 4 the most significant SNP is a syn-

onymous variant in *HSP42*, which codes for heat shock proteins responsible for cytoskeleton maintenance (Wotton et al., 1996; Haslbeck, Martin et al., 2004). For peak R3 on chromosome 15, the most significant SNP is a synonymous variant in *MSH2*, which assists in DNA mismatch repair (Earley and Crouse, 1998), and was also previously associated wth Roundup^{TM} resistance by Ravishankar et al. (2020). For peak R4 on chromosome 16 the most significantly differentiated SNP is a modifier upstream of the gene *MRP2*, a component of the small subunit of the mitochondrial ribosome, which mediates translation in the mitochondrion (Desai et al., 2017).

Figure 3.6.A-D shows, for the four most-significant SNPs per peak outlined above, the observed changes in allele frequencies over the course of the experiment, starting from the ancestral frequency. When we looked at these allele frequency trajectories, we observe relatively little change from the original ancestor frequency in both the constant and increasing treatments; however, the control populations appear to deviate strongly from both treatments decreasing in frequency. There is one peak (R3) where this pattern is more complicated, and the SNP frequency appears to marginally increase over time in the two Roundup^{TM} treatment groups while the SNP frequency in the control decreases.

3.4.2 Constant-specific peaks

The two pairs of constant-specific peaks (C1a/b, C2a/b) identify regions with significantly different allele frequencies in both the constant treatment compared to

the controls, and the constant treatment compared to the increasing treatment (see Figure 3.4.A & C). For peak C1 on chromosome 4, the most most significant SNP is a synonymous variant in the gene *TIM22* that is critical for yeast survival and encodes a subunit of the Tim22 complex inside the mitochondrial inner membrane. This complex mediates the transport of carrier proteins into the inner membrane space of mitochondria (Sirrenberg et al., 1996). For peak C2 on chromosome 12, the most significant SNP is not in a gene, but occurs upstream of open reading frame YLR126C within a promoter region, and is predicted to modify its expression. While this gene encodes a protein of unknown function, it has been implicated in metal ion homeostasis (Freitas et al., 2004).

The allele trajectories of the most significant SNPs from each constant-specific peaks is shown in Figure 3.6.E-F. For both SNPs, the constant and increasing treatments appear to have imposed opposite selection pressures on these alleles, leading to their divergence. The frequency of each the SNP in the control populations also diverged from the constant populations, though not as dramatically.

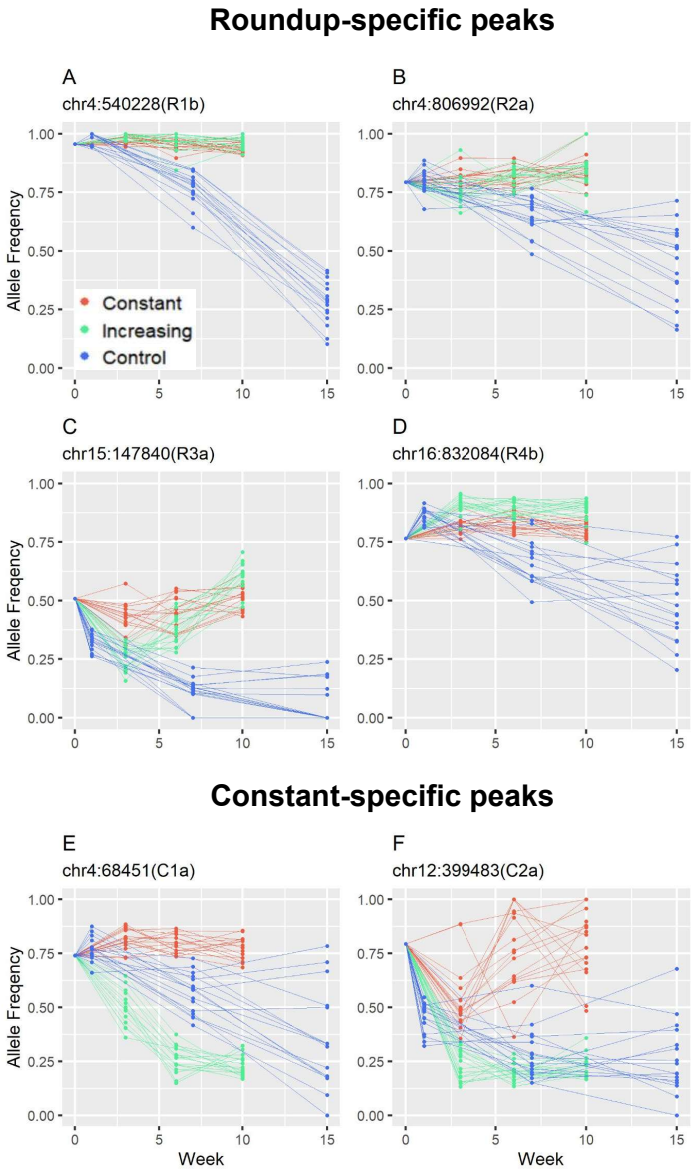

Figure 3.6: Allele trajectories of the most significant SNP from the four Roundup-specific peaks (3.6.A-D) show stasis (or slight increase) in the constant (red) and increasing (green) treatments, and a decrease in the controls (blue). The allele change shown in the most significant SNP of the two constant-specific peaks (3.6.E-F) demonstrate how the effects of Roundup^{TM} selection on the genome may be dose dependent as the increasing treatment diverges from the constant regime with an adaptive signature independent from the control replicates. The SNP being plotted here is the non-reference allele, relative to the S288C canonical sequence.

Chapter 4: Discussion

4.1 Evolved populations grow faster than the ancestor in the presence of Roundup^{TM} with no obvious phenotypic trade-offs

After ten weeks of selection, populations of both Roundup^{TM} treatments and the control treatment showed evolved Roundup^{TM} resistance when compared to the ancestor population (see Figure 3.1). Although carrying capacity was also measured, it was not a valuable metric for measuring evolved resistance as all treatments were comparable to the ancestor. Evolved resistance was determined by estimating the average doubling time from 48 hour growth experiments in 1% Roundup^{TM} media. These assays demonstrated that there was no significant difference in average growth rates between the two Roundup^{TM} treatments. While there was some variation between biological replicates of the same treatment group, this variation was marginal and not consistent across multiple plate reader assays. We therefore judged that this variation was more likely an artifact of methodological inconsistencies in individual plate reader runs and not reflective of true phenotypic differences, so we view the average doubling time across the biological replicates as an accurate representation of treatment-specific phenotypes. The similar evolved phenotypes in both Roundup^{TM} treatments are consistent with the idea put forth by Vlachos and Kofler (2019) that although implementing an increasing selection

treatment may enhance the resolution in which we can detect causative genomic loci, it will not necessarily produce a different phenotype compared to a constant selection treatment, at least in experiments with many generations (Vlachos and Kofler, 2019).

After ten weeks of selection we did detect significantly faster growth in the control populations compared to the ancestor in Roundup^{TM} media. The average growth rate of the control populations was intermediate between that of the ancestor and that of populations of the two Roundup^{TM}evolved treatments. This suggests that the trait of Roundup^{TM} resistance might be related to a broad stress response that our experimental yeast handling protocols are also imposing selection for. The weekly sexual outcrossing protocols impose a number of significant and distinct stresses on populations; these include i) culture in sporulation media which lacks nutrients, ii) exposure to heat, mechanical agitation, enzymes and chemicals to isolate individual spores and iii) batch culture which includes periods of alternating high and low nutrient availability as populations grow and reach saturation over time.

A specific aspect of our protocols that may be playing a role in this phenotypic outcome is the use of Y-PER^{TM}(Yeast Protien Extraction Reagent^{TM}, Thermo Fisher Scientific) a detergent used to kill unsporulated diploid yeast cells during the weekly sexual outcrossing protocol (detailed protocol in Burke et al. 2020). This chemical is of specific interest since it might be similar to undisclosed surfactants that are added to the commercial Roundup^{TM} formulas in order to increase the coverage, penetration and overall effectiveness of the herbicide. Some of the

surfactants that are commonly used in herbicides have varying tested levels of toxicity (Mesnage et al., 2019; Benbrook, 2016). The acute Y-PER^{TM} exposure may be targeting similar physiological pathways as the non-active ingredients in the commercial Roundup^{TM} formula during our weekly selection protocols. It is unlikely that diploid cells are evolving complete resistance to Y-PER^{TM} as it has been thoroughly tested as an effective methods for killing diploid cells; however, it could be causing spores or the asci to adapt under this selection pressure. Variation in spore viability resulting from acute Y-PER^{TM} exposure might explain some of the Roundup^{TM} resistance we are observing in our control populations.

To test the idea that selection in Roundup^{TM} media might have led to the evolution of a fitness trade-off, we measured the growth of all evolved populations alongside the ancestor in liquid YPD media without Roundup^{TM}. Overall we report no obvious evidence for such a trade-off as we observed no significant difference between the growth rate of the two Roundup^{TM} treatments and the ancestor. If selection in Roundup^{TM} media resulted in a major trade-off for growth rate, we might expect to see populations of either the constant and/or increasing treatments growing slower than the ancestor in plain YPD media. The control populations did show a slight improvement in growth in YPD media compared to the ancestor ($p = 0.004$) and constant treatment ($p = 0.008$), and a more dramatic improvement compared to the increasing ($p = 2.947e\text{-}08$) treatment. It is perhaps unsurprising to see this slight improvement in growth, given that these control populations experienced regular batch culture in YPD media, which involves similar circumstances as the growth rate assay itself. The fact that we did not see this

improvement in populations of either of the constant or increasing Roundup^{TM} treatments could be interpreted as suggestive evidence for a trade-off underlying the phenotype; in other words, the Roundup^{TM}evolved populations did not grow as fast as they potentially could have, were a trade-off not constraining them.

4.2 Putative regions identified that may improve yeast growth in Roundup^{TM} media

We identified six genomic regions that are likely to harbor alleles that confer increased Roundup^{TM} resistance. Four Roundup-specific peaks, on three different chromosomes, revealed alleles with frequencies in both the constant and increasing treatments that significantly diverged from the control. There are 58 genes falling under these peaks, warranting further investigation of whether these genes may be contributing to evolved Roundup^{TM} resistance in our two treatment groups (see Appendix C). The allele frequency trajectories of the most significant SNPs in each Roundup-specific peak suggest that the allele frequencies of the constant and increasing treatment are being maintained, while the allele frequencies of the control populations are changing dramatically. We also observe that the variance in allele frequencies at the end of the experiment is generally higher in the control populations compared to the Roundup^{TM} treatments. We interpret these results as evidence that these particular alleles (and/or those linked to them) may be under stabilizing selection in the two Roundup^{TM} treatments, but under weak directional selection in the control populations. While the action of genetic drift could also

explain the higher variance in allele frequencies in the control populations, we still see a clear average decrease in allele frequencies in the control populations across all replicate populations; therefore, we invoke weak directional selection as the most likely agent of change, rather than drift alone. Since these alleles are maintained at intermediate frequency under Roundup^{TM} conditions, but they decrease in frequency when populations are cultured in YPD media, it appears that these alleles may have pleiotropic consequences, depending on the environment

We also identified two peaks showing significantly different allele frequencies in the constant treatment compared to both the control and the increasing populations. These two peaks highlight an additional 44 genes that might specifically relate to improved resistance to high Roundup^{TM} exposure (Appendix C). The allele trajectories of the most significant SNPs from the constant-specific peaks visually demonstrate a striking difference in outcomes of selection from the two different Roundup^{TM} treatments. In peak C1, the allele frequencies in the populations of the constant treatment remain high, not deviating much from the ancestral frequency of $\sim$0.75, while the frequencies in the populations of the increasing treatment sharply decrease to $\sim$0.25 in all the replicates, followed by a plateau (see Figure 3.6.E). At this same SNP the allele frequencies of the control populations decrease less dramatically, and again we observe more heterogeneity among the control replicates, which may implicate weak selection or drift. In peak C2, we observe more noise in the constant replicates over time; however, the average allele frequency across replicate populations at the end of the experiment is similar to that of the ancestor, so we again suggest stabilizing selection as a possible

mechanism underlying this maintenance in allele frequency. The populations of the control and increasing treatments decrease, and we invoke directional selection as a likely mechanism. If the populations of the increasing treatment were not responding to Roundup^{TM}imposed selection, we would not expect to see such a pattern; instead, we would expect to see the populations of the increasing treatment following similar patterns comparable to the control group treatment. While we observe distinct allele trajectories in the control and increasing treatment in peak C1, there is more overlap in peak C2. This peak region should undergo further interrogation before it can clearly be identified as a constant-specific candidate. The constant-specific peaks provide evidence that the phenotype of Roundup^{TM} resistance likely has a complex genetic architecture as it appears that different alleles respond to varying strengths of selection.

Previous research exploring the affect of Roundup^{TM} on *S. cerevisiae* using QTL and RNA-Seq approaches highlighted over a thousand genes that may be correlated with Roundup^{TM} resistance (Ravishankar et al., 2020). We observed 24 of these genes within our peak regions, nine of which were identified within constant-specific peaks and 15 within Roundup-specific peaks (see Appendix C). Although there was some overlap in our findings, we did not see a significant allele frequency change in either *PDR5* or *DIP5*, the two genes that were thought to be involved in yeast uptake of glyphosate (Rong-Mullins et al., 2017). It is not surprising that we did not see changes in *DIP5*, since this gene was identified in experiments that used minimal media instead of YPD. While we did not directly see changes in *PDR5* (a candidate observed in experiments that used YPD), we

did observe significant changes in *HAL9*, which plays a role in activating *PDR5*.

The methods previously used by Rong-Mullins et al. (2017) and Ravishankar et al. (2020) focused on testing strains with much less genetic diversity compared to our highly diverse, ancestral population. The classic QTL study of Rong-Mullins et al. (2017), investigated crosses between two isogenic strains, one that was known to exhibit a resistant phenotype in the presence of glyphosate based herbicides and another that was more susceptible. Similarly, a handful of other isogenic strains were used to perform RNA-seq and other experimental evolution experiments. This difference in standing genetic variation may explain why we do not see more overlap between the sets of genes identified. These experiments also used a different commercial formula, Credit41^{TM}, which contains a higher percentage of glyphosate (41%) and not necessarily the same inactive ingredients. So, while there are many reasons why our experiment may have identified different candidate genes than prior work, the genes that do overlap are especially strong candidates for future examination.

4.3 Increasing treatment does not enhance ability to detect candidate regions

Testing two different Roundup^{TM} selection treatments allowed us to investigate the hypothesis that a treatment that increases the strength of selection over time leads to greater resolution in detecting causative genomic regions compared to a treatment featuring constant, strong selection. Vlachos and Kofler (2019) showed with

forward-in-time simulations that strong, constant selection is more likely to identify genomic regions containing neutral hitchhikers that are linked to causative sites compared to an increasing selection regime, which is optimized to detect causative loci. Hitchhikers provide excess noise and make it difficult to narrow down what might be causative alleles driving change in an E&R experiment, therefore an approach that leads to reduced hitchhiking is desirable, Vlachos and Kofler (2019) showed that implementing an increasing selection treatment, which decreases the number of selected individuals over time, reduces such hitchhiking and leads to higher power to detect specific alleles underlying adaptive traits. The increasing treatment, which started at a low dose of 0.1% Roundup^{TM} in YPD media and slowly increased to a high dose of 1% over ten weeks of selection did not improve our ability to detect potential causative regions compared to a constant, high selection pressure of 1% Roundup^{TM}. We expected that comparing allele frequencies between the two Roundup^{TM} treatments and controls, would lead to narrower peak regions in the increasing treatment, with fewer linked SNPs falling under those significant peaks, compared to the same analysis featuring the constant treatment. We did not observe a consistent pattern that aligned with this expectation as two of the pairs of Roundup-specific peaks were narrower and identified fewer SNPs in the increasing treatment comparison to the control (i.e. peaks R1a/b & R2a/b), while the two other peak pairs revealed the opposite pattern (i.e. peaks R3a/b & R4a/b, see Table 3.1).

There are several possibilities for why we did not observe obviously narrower peaks from the analysis featuring the increasing treatment. One reason might be

that while the dose of Roundup^{TM} steadily increased over the weeks of the experiment, this may not have led to concomitant increases in selection pressure each week, since over time we would expect the populations to become more resistant to Roundup^{TM} with repeated exposure. A more pronounced weekly increase in the dose of Roundup^{TM} might have alleviated this concern, but would have been difficult to design in advance. As it stands, we chose 1% Roundup^{TM} as the "high" dose as it resulted in approximately 90% reduction in cell viability after acute exposure, and we observed a nearly linear relationship between cell viability and Roundup^{TM} dose; in other words, we reasoned that the 0.1% starting "weak" dose incurred about 1/10 the selection pressure as the final strong dose. But, this linear dose-response may not have been realized in the context of a selection experiment, where adaptation rapidly occurs. Measuring such adaptation in real time during the experiment, and adjusting the Roundup^{TM} dose accordingly, would have been difficult to achieve, and to justify since it was not obvious which aspect of life-history (e.g. growth, sporulation, mating) to track. It is possible that since we were not able to precisely measure these population dynamics, the increasing treatment may have been more similar to a constant selection pressure in terms of the percent of the population's standing genetic variation that was maintained in the population after each weekly treatment. Similarly, the constant treatment may have imposed a stronger selection pressure at first, but as the populations evolved resistance, the selection pressure may have actually decreased over time. In short, while there are many ways in which our experiment was imperfect, the simplest design we could imagine did not lead to an obvious pattern. Therefore,

we conclude that implementing an experimental design that increases the strength of selection over time provides no obvious benefit, compared with a simpler design featuring constant selection, to an investigator whose primary interest is identifying potentially causative regions.

Another important aspect to consider is the number of generations that occurred under Roundup^{TM} selection. The simulation work by Vlachos and Kofler (2019) investigating the effect of the number of generations in E&R experiments in *Drosophila melanogaster* suggest that more than 40 generations of selection are needed to see an enhanced resolution in an increasing selection treatment compared to a constant selection treatment. The yeast populations in this study system have much more complex life-histories compared to fruit flies, so it is difficult to relate this generation threshold directly to our experiment. The populations in the experiment reproduced both asexually and sexually, which brings up the question—what constitutes a generation? While our experiment featured over 100 asexual generations in the Roundup^{TM} selection treatments only ten discrete cycles of sexual recombination occurred. As yeast have much higher average recombination rates (by approximately 100-fold) than *Drosophila*, it is not appropriate to equate a sexual cycle in our yeast system with a single generation in flies. But it is also possible that with a longer experiment, we might have observed larger and more consistent differences between the two treatments.

Implementing two different selection regimes revealed that Roundup^{TM} resistance is a more complex trait than we expected, with an underlying genetic architecture that likely depends on the strength of selection. Our constant-specific

peaks revealed allele frequencies that were maintained in the populations experiencing constant, high Roundup^{TM} but that changed dramatically in populations experiencing gradually increasing Roundup^{TM} treatment (a pattern distinct from the control populations). In spite of not seeing an improved ability to detect candidate variants in the increasing regime, we did, however, provide evidence for this complex and interesting trait architecture. These constant-specific peaks suggest that certain alleles may be selected for under high Roundup^{TM} conditions, but selected against at low doses.

Chapter 5: Conclusion

This study identified several genomic regions that may contribute to improved Roundup^{TM} resistance in yeast, and these regions provide a useful resource for future investigations into the effects of Roundup^{TM} on eukaryotic organisms. While we cannot definitively say that any of the genes in these regions are causative based on the experiments performed to date, they are promising candidates and further add to the body of work that is seeking to understand how non-target organisms evolve in response to chronic exposure to this herbicide. Next steps would include performing functional genomic assays of these strong candidates to further verify their potential contribution to improved Roundup^{TM} resistance, especially the genes that overlap with results from similar studies in other laboratories. If naturally-occuring allelic variants in genes can be definitively linked to a functional increase in resistance to Roundup^{TM} these variants could have value in an applied context. Herbicides like Roundup^{TM} appear to affect natural microbial communities, particularly in soils whose health is critical for large-scale agriculture. By identifying alleles and that allow microbes to thrive in the presence of this product and potentially also directly metabolize it, our basic research may inform applied bioengineering efforts to optimize soil microbial communities for increased crop yield, and for bioremediation of soil toxins (such as broad-spectrum herbicides). These efforts could lead to improved farming practices that increase yields and

safety.

With regards to the objective of distinguishing between evolutionary outcomes of different types of selection treatments, we observed no evidence that a treatment that incrementally increases selection pressure over time in an E&R study has a higher power to detect potentially causative variants compared to a high, constant selection pressure. We also observe no different phenotypic outcomes between the two types of selection regimes. We conclude that implementing an increasing selection regime does not offer obvious benefits in terms of genomic resolution that outweigh the challenges of implementation; we observed no clear pattern of narrower peaks that might suggest fewer false positives in the increasing selection treatment. It is important to note that the results comparing the effectiveness of the constant vs. increasing treatments are limited to our yeast model system and perhaps should not be extrapolated to E&R experiments using organisms where it is easier to measure the strength of selection (i.e. the viability and death of individuals within each population over generations of selection). Simultaneously implementing both a constant and increasing treatment did, however, offer some unexpected benefits as it uncovered a unique trait architecture of Roundup^{TM} resistance that implies that the Roundup^{TM} exposure dose influences selection outcomes and different dosage levels may lead to different alleles being favored and rising in frequency. It is possible, however, that exposing yeast to a constant, low dose of Roundup^{TM} would have also revealed this pattern. Regardless, this observation of dose-dependent genetic responses to selection may have applications for fostering healthy microbial populations in agricultural settings that are exposed

to high amounts of Roundup^{TM} directly within the spray path and also for populations exposed to spray drift and runoff where the exposure is far less. Ultimately, our results support the idea that implementing multiple types of selection regimes in an E&R experiment could be valuable for revealing complexities underlying particular quantitative traits, such as pleiotropic variants that respond differently depending upon the strength of selection. Such pleiotropic consequences could not possibly be detected from a classic E&R design featuring a single, constant selection regime.

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APPENDICES

Appendix A: Sample Coverage

Table A.1: Average genome wide sequence coverage depth across 90,510 SNPs of the ancestor and each treatment population from each sequencing time point. The standard deviation of coverage was used to calculate the coefficient of variation within samples. Low variation in coverage was observed across the genome in every sample, providing no clear evidence for large-scale structural variation in any population

Replicate	Treatment Group	Week	Mean Coverage	Standard Deviation	Coefficient of Variation
	Ancestor	0	92.71	13.05	0.14
rep02	Constant	3	67.46	11.55	0.17
rep02	Constant	6	35.81	7.39	0.21
rep02	Constant	10	59.06	11.45	0.19
rep03	Constant	3	83.59	14.87	0.18
rep03	Constant	6	48.01	9.29	0.19
rep03	Constant	10	66.55	12.51	0.19
rep04	Constant	3	67.63	12.35	0.18
rep04	Constant	6	37.11	8.08	0.22
rep04	Constant	10	70.03	12.56	0.18
rep05	Constant	3	61.34	11.30	0.18
rep05	Constant	6	58.95	10.76	0.18
rep05	Constant	10	61.19	11.54	0.19
rep06	Constant	3	64.80	11.73	0.18
rep06	Constant	6	38.05	7.77	0.20
rep06	Constant	10	67.73	12.09	0.18
rep07	Constant	3	64.00	12.06	0.19
rep07	Constant	6	41.47	8.89	0.21
rep07	Constant	10	61.60	12.28	0.20
rep08	Constant	3	65.60	12.46	0.19
rep08	Constant	6	58.35	10.81	0.19
rep08	Constant	10	80.05	13.72	0.17
rep10	Constant	3	75.75	13.03	0.17
rep10	Constant	6	48.47	9.06	0.19
rep10	Constant	10	76.68	13.06	0.17
rep11	Constant	3	64.42	12.36	0.19
rep11	Constant	6	56.02	10.20	0.18
rep11	Constant	10	49.00	9.92	0.20
rep12	Constant	3	28.02	7.90	0.28
rep12	Constant	6	75.36	12.26	0.16
rep12	Constant	10	62.20	11.29	0.18
rep13	Constant	3	51.29	11.10	0.22
rep13	Constant	6	72.55	11.75	0.16
rep13	Constant	10	54.10	10.08	0.19

Replicate	Treatment Group	Week	Mean Coverage	Standard Deviation	Coefficient of Variation
rep14	Constant	3	58.46	12.35	0.21
rep14	Constant	6	65.04	11.53	0.18
rep14	Constant	10	73.62	12.71	0.17
rep15	Constant	3	55.86	11.80	0.21
rep15	Constant	6	58.00	10.41	0.18
rep15	Constant	10	66.61	12.17	0.18
rep16	Constant	3	69.03	13.09	0.19
rep16	Constant	6	72.63	12.17	0.17
rep16	Constant	10	65.49	12.18	0.19
rep17	Constant	3	59.57	12.49	0.21
rep17	Constant	6	37.42	8.27	0.22
rep17	Constant	10	66.56	12.19	0.18
rep18	Constant	3	60.12	12.44	0.21
rep18	Constant	6	61.10	10.94	0.18
rep18	Constant	10	82.26	13.27	0.16
rep01	Control	1	35.23	8.82	0.25
rep01	Control	7	43.90	9.43	0.21
rep01	Control	15	22.95	7.13	0.31
rep02	Control	1	37.41	9.40	0.25
rep02	Control	7	48.68	9.83	0.20
rep02	Control	15	34.72	8.96	0.26
rep03	Control	1	36.33	9.10	0.25
rep03	Control	7	44.28	9.19	0.21
rep03	Control	15	25.57	6.49	0.25
rep04	Control	1	63.82	11.77	0.18
rep04	Control	7	52.19	10.21	0.20
rep04	Control	15	48.81	10.37	0.21
rep05	Control	1	55.60	11.18	0.20
rep05	Control	7	63.47	11.17	0.18
rep05	Control	15	50.91	10.43	0.20
rep06	Control	1	40.27	9.56	0.24
rep06	Control	7	67.18	11.75	0.17
rep06	Control	15	58.07	10.90	0.19
rep07	Control	7	55.19	10.51	0.19
rep07	Control	15	51.74	11.48	0.22

Replicate	Treatment Group	Week	Mean Coverage	Standard Deviation	Coefficient of Variation
rep08	Control	1	49.82	10.28	0.21
rep08	Control	7	69.17	11.49	0.17
rep08	Control	15	62.92	12.64	0.20
rep09	Control	1	23.41	6.51	0.28
rep09	Control	7	54.58	10.46	0.19
rep09	Control	15	33.81	9.13	0.27
rep10	Control	1	15.82	5.32	0.34
rep10	Control	7	27.74	7.21	0.26
rep10	Control	15	22.59	7.26	0.32
rep12	Control	1	44.99	10.28	0.23
rep12	Control	7	25.22	6.42	0.25
rep12	Control	15	19.90	6.33	0.32
rep13	Control	1	45.78	10.00	0.22
rep13	Control	7	33.55	7.82	0.23
rep13	Control	15	48.61	9.88	0.20
rep14	Control	1	63.34	13.57	0.21
rep14	Control	7	49.69	9.66	0.19
rep14	Control	15	58.34	10.48	0.18
rep15	Control	1	54.03	10.58	0.20
rep15	Control	7	34.64	7.94	0.23
rep15	Control	15	42.12	9.01	0.21
rep16	Control	1	48.55	9.62	0.20
rep16	Control	7	50.08	9.75	0.19
rep16	Control	15	44.43	10.69	0.24
rep17	Control	1	39.72	8.67	0.22
rep17	Control	7	50.41	9.85	0.20
rep17	Control	15	40.48	10.09	0.25
rep19	Increasing	3	18.51	5.79	0.31
rep19	Increasing	6	55.55	10.05	0.18
rep19	Increasing	10	50.66	9.98	0.20
rep20	Increasing	3	10.63	4.07	0.38
rep20	Increasing	6	56.01	11.01	0.20
rep20	Increasing	10	31.72	7.57	0.24
rep21	Increasing	3	66.96	12.23	0.18
rep21	Increasing	6	54.15	10.20	0.19
rep21	Increasing	10	30.55	6.94	0.23

Replicate	Treatment Group	Week	Mean Coverage	Standard Deviation	Coefficient of Variation
rep22	Increasing	3	64.98	11.90	0.18
rep22	Increasing	6	70.29	11.39	0.16
rep22	Increasing	10	58.95	10.80	0.18
rep23	Increasing	3	71.30	12.83	0.18
rep23	Increasing	6	72.86	12.03	0.17
rep23	Increasing	10	52.11	9.78	0.19
rep24	Increasing	3	62.70	12.25	0.20
rep24	Increasing	6	64.69	11.58	0.18
rep24	Increasing	10	53.24	10.23	0.19
rep25	Increasing	3	52.69	10.69	0.20
rep25	Increasing	6	78.67	11.91	0.15
rep25	Increasing	10	70.97	11.89	0.17
rep26	Increasing	3	57.90	11.37	0.20
rep26	Increasing	6	63.61	11.16	0.18
rep26	Increasing	10	60.52	11.95	0.20
rep28	Increasing	3	36.84	8.61	0.23
rep28	Increasing	6	30.79	7.39	0.24
rep28	Increasing	10	28.00	6.73	0.24
rep29	Increasing	3	58.08	12.40	0.21
rep29	Increasing	6	59.10	11.01	0.19
rep29	Increasing	10	33.86	7.59	0.22
rep30	Increasing	3	62.29	11.85	0.19
rep30	Increasing	6	31.09	6.85	0.22
rep30	Increasing	10	25.81	6.60	0.26
rep31	Increasing	3	58.22	11.15	0.19
rep31	Increasing	6	63.08	11.21	0.18
rep31	Increasing	10	45.96	9.70	0.21
rep32	Increasing	3	52.53	10.34	0.20
rep32	Increasing	6	50.69	9.88	0.19
rep32	Increasing	10	40.95	8.50	0.21
rep33	Increasing	3	70.48	12.28	0.17
rep33	Increasing	6	58.45	10.75	0.18
rep33	Increasing	10	55.79	10.44	0.19
rep34	Increasing	3	56.01	10.94	0.20
rep34	Increasing	6	65.46	11.37	0.17
rep34	Increasing	10	72.10	11.86	0.16
rep35	Increasing	3	63.50	11.64	0.18
rep35	Increasing	6	51.25	9.37	0.18
rep35	Increasing	10	56.17	10.44	0.19

Appendix B: SNPeff annotations for significant SNPs

Table B.1: **SNPeff annotations for significant SNPs under the 6 major peaks of this study**. Below is the output from the program SNPeff, which predicts the functional consequences of single-nucleotide variants, for all SNPs we observed to be significantly differentiated by treatment. The significance score, which is the -log10(*p*) value of a CMH test between two treatments, is provided for each SNP. Some SNPs are listed twice because they were identified as significant in more than one CMH test. SNPs are organized by peak type (C = constant-specific; R= Roundup-specific) as well as by CMH test type (a=constant vs. control; b=increasing vs. control; c=constant vs. increasing). The "alternate allele" listed here is the non-canonical allele with respect to the canonical S288C reference genome (R64-2-1).

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(*p*)
C1a	4	101651	A	missense_variant	MODERATE	YDL199C	p.Thr568Ile	87.10836
C1a	4	67141	A	missense_variant	MODERATE	YDL218W	p.Ala217Thr	86.79157
C1a	4	68400	G	synonymous_variant	LOW	TIM22	p.Tyr69Tyr	81.84333
C1a	4	68451	C	synonymous_variant	LOW	TIM22	p.Thr52Thr	91.46759
C1a	4	69812	T	missense_variant	MODERATE	RRI1	p.Asp170Asn	82.1319
C1a	4	76252	A	missense_variant	MODERATE	PRR2	p.Lys98Asn	84.42698
C1a	4	76541	A	missense_variant	MODERATE	PRR2	p.Ser2Leu	77.76351
C1a	4	79782	C	missense_variant	MODERATE	YDL211C	p.Thr211Ala	81.18436
C1a	4	79801	G	synonymous_variant	LOW	YDL211C	p.Gly204Gly	79.33519
C1a	4	79852	G	synonymous_variant	LOW	YDL211C	p.Ile187Ile	85.25125
C1a	4	84201	C	upstream_gene_variant	MODIFIER	YDL211C		82.584
C1a	4	97693	T	synonymous_variant	LOW	ACK1	p.Gly87Gly	78.99633
C1a	4	98318	T	upstream_gene_variant	MODIFIER	HEM3		81.07375
C1c	4	101608	A	synonymous_variant	LOW	YDL199C	p.Thr582Thr	95.38333
C1c	4	101651	A	missense_variant	MODERATE	YDL199C	p.Thr568Ile	118.6153
C1c	4	101704	T	synonymous_variant	LOW	YDL199C	p.Lys550Lys	92.16795
C1c	4	101707	T	synonymous_variant	LOW	YDL199C	p.Leu549Leu	88.22815
C1c	4	101871	A	synonymous_variant	LOW	YDL199C	p.Leu495Leu	90.52772
C1c	4	102076	G	synonymous_variant	LOW	YDL199C	p.Tyr426Tyr	92.33782
C1c	4	102955	T	synonymous_variant	LOW	YDL199C	p.Leu133Leu	103.6339
C1c	4	102979	T	synonymous_variant	LOW	YDL199C	p.Ser125Ser	107.5467
C1c	4	103111	T	synonymous_variant	LOW	YDL199C	p.Pro81Pro	87.79947
C1c	4	103444	T	upstream_gene_variant	MODIFIER	MGT1		82.86369
C1c	4	103617	T	upstream_gene_variant	MODIFIER	MGT1		80.49314
C1c	4	103768	T	missense_variant	MODERATE	GGC1	p.Val262Ile	95.76027
C1c	4	104069	G	synonymous_variant	LOW	GGC1	p.Ile161Ile	80.77012
C1c	4	105260	C	missense_variant	MODERATE	ASF2	p.Glu412Gly	84.31649

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C1c	4	105585	C	missense_variant	MODERATE	ASF2	p.Thr304Ala	85.79166
C1c	4	105914	G	missense_variant	MODERATE	ASF2	p.Ser194Thr	99.89424
C1c	4	105955	C	synonymous_variant	LOW	ASF2	p.Gly180Gly	92.76538
C1c	4	105958	C	synonymous_variant	LOW	ASF2	p.Glu179Glu	90.13848
C1c	4	106288	G	synonymous_variant	LOW	ASF2	p.Arg69Arg	82.68602
C1c	4	107148	T	upstream_gene_variant	MODIFIER	YDL199C		91.09892
C1c	4	107516	G	synonymous_variant	LOW	SEC31	p.Arg103Arg	96.67132
C1c	4	109620	T	synonymous_variant	LOW	SEC31	p.Leu805Leu	89.04228
C1c	4	109991	A	synonymous_variant	LOW	SEC31	p.Lys928Lys	98.23539
C1c	4	110312	C	synonymous_variant	LOW	SEC31	p.Ala1035Ala	83.63056
C1c	4	110318	A	synonymous_variant	LOW	SEC31	p.Ser1037Ser	83.33399
C1c	4	110492	A	synonymous_variant	LOW	SEC31	p.Ser1095Ser	103.0113
C1c	4	111373	A	upstream_gene_variant	MODIFIER	ASF2		93.1062
C1c	4	111482	T	upstream_gene_variant	MODIFIER	ASF2		90.04221
C1c	4	111501	C	upstream_gene_variant	MODIFIER	SNF3		85.91892
C1c	4	111567	A	upstream_gene_variant	MODIFIER	SNF3		90.97977
C1c	4	111617	A	missense_variant	MODERATE	SNF3	p.Arg13His	93.72558
C1c	4	111870	C	synonymous_variant	LOW	SNF3	p.Ser97Ser	80.9846
C1c	4	112446	A	synonymous_variant	LOW	SNF3	p.Arg289Arg	88.96968
C1c	4	112746	A	missense_variant	MODERATE	SNF3	p.Asn389Lys	83.79882
C1c	4	112773	C	synonymous_variant	LOW	SNF3	p.Tyr398Tyr	89.24403
C1c	4	112776	C	synonymous_variant	LOW	SNF3	p.Ala399Ala	86.29913
C1c	4	112788	T	synonymous_variant	LOW	SNF3	p.Val403Val	86.20207
C1c	4	113338	C	synonymous_variant	LOW	SNF3	p.Leu587Leu	87.45449
C1c	4	113472	T	synonymous_variant	LOW	SNF3	p.Pro631Pro	84.80433
C1c	4	113961	G	synonymous_variant	LOW	SNF3	p.Leu794Leu	86.68146
C1c	4	114047	A	missense_variant	MODERATE	SNF3	p.Arg823Gln	83.13558
C1c	4	115328	C	synonymous_variant	LOW	NUS1	p.Ala219Ala	80.28218
C1c	4	116425	T	synonymous_variant	LOW	ARF1	p.Tyr35Tyr	82.46053
C1c	4	121548	A	synonymous_variant	LOW	UFD2	p.Ser15Ser	83.23875
C1c	4	122341	G	synonymous_variant	LOW	RBS1	p.Ser42Ser	85.50197
C1c	4	122724	A	missense_variant	MODERATE	RBS1	p.Ser170Asn	80.22689
C1c	4	123113	A	missense_variant	MODERATE	RBS1	p.Leu300Ile	84.05637
C1c	4	124453	G	synonymous_variant	LOW	PPH22	p.Leu182Leu	96.94155
C1c	4	64076	T	missense_variant	MODERATE	CDC13	p.Val315Ile	90.37548
C1c	4	64539	G	synonymous_variant	LOW	CDC13	p.Thr160Thr	81.81745
C1c	4	65027	A	upstream_gene_variant	MODIFIER	HBT1		87.82216

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C1c	4	65327	C	upstream_gene_variant	MODIFIER	HBT1		96.24896
C1c	4	65570	A	missense_variant	MODERATE	DTD1	p.Pro86Gln	85.59418
C1c	4	66328	T	upstream_gene_variant	MODIFIER	FMP45		92.5163
C1c	4	66345	C	upstream_gene_variant	MODIFIER	FMP45		86.97193
C1c	4	66726	A	synonymous_variant	LOW	YDL218W	p.Ala78Ala	91.4698
C1c	4	66834	T	synonymous_variant	LOW	YDL218W	p.Pro114Pro	117.0377
C1c	4	66837	T	synonymous_variant	LOW	YDL218W	p.Tyr115Tyr	116.1999
C1c	4	66843	T	synonymous_variant	LOW	YDL218W	p.Gly117Gly	119.2777
C1c	4	66855	G	synonymous_variant	LOW	YDL218W	p.Ala121Ala	112.4893
C1c	4	66945	G	synonymous_variant	LOW	YDL218W	p.Leu151Leu	123.4119
C1c	4	67141	A	missense_variant	MODERATE	YDL218W	p.Ala217Thr	119.788
C1c	4	67386	T	synonymous_variant	LOW	YDL218W	p.His298His	111.6387
C1c	4	67426	G	missense_variant	MODERATE	YDL218W	p.Asn312Asp	88.57478
C1c	4	67491	C	upstream_gene_variant	MODIFIER	CDC13		87.39902
C1c	4	67588	A	upstream_gene_variant	MODIFIER	CDC13		87.25601
C1c	4	68097	G	synonymous_variant	LOW	TIM22	p.Ala170Ala	97.79272
C1c	4	68175	T	synonymous_variant	LOW	TIM22	p.Glu144Glu	84.51647
C1c	4	68400	G	synonymous_variant	LOW	TIM22	p.Tyr69Tyr	129.5166
C1c	4	68451	C	synonymous_variant	LOW	TIM22	p.Thr52Thr	130.253
C1c	4	68729	T	upstream_gene_variant	MODIFIER	CDC13		104.2771
C1c	4	68799	T	upstream_gene_variant	MODIFIER	CDC13		98.56802
C1c	4	68897	A	upstream_gene_variant	MODIFIER	CDC13		125.7352
C1c	4	68918	C	upstream_gene_variant	MODIFIER	CDC13		111.4532
C1c	4	69069	C	synonymous_variant	LOW	RRI1	p.Lys417Lys	85.61611
C1c	4	69114	G	synonymous_variant	LOW	RRI1	p.Asp402Asp	88.38434
C1c	4	69176	T	missense_variant	MODERATE	RRI1	p.Phe382Ile	82.74795
C1c	4	69384	T	synonymous_variant	LOW	RRI1	p.Arg312Arg	90.02683
C1c	4	69812	T	missense_variant	MODERATE	RRI1	p.Asp170Asn	119.4274
C1c	4	70149	C	synonymous_variant	LOW	RRI1	p.Leu57Leu	101.1863
C1c	4	70167	A	synonymous_variant	LOW	RRI1	p.Ser51Ser	96.56958
C1c	4	70481	T	upstream_gene_variant	MODIFIER	TIM22		82.90808
C1c	4	70556	G	upstream_gene_variant	MODIFIER	TIM22		91.47015
C1c	4	70614	A	upstream_gene_variant	MODIFIER	TIM22		93.55984
C1c	4	70706	G	synonymous_variant	LOW	GDH2	p.Asp1071Asp	96.84985
C1c	4	70790	A	synonymous_variant	LOW	GDH2	p.Val1043Val	83.25105
C1c	4	70859	A	missense_variant	MODERATE	GDH2	p.Leu1020Phe	91.03789
C1c	4	71525	G	synonymous_variant	LOW	GDH2	p.His798His	90.00318

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C1c	4	71743	A	synonymous_variant	LOW	GDH2	p.Leu726Leu	86.95275
C1c	4	72173	A	synonymous_variant	LOW	GDH2	p.Gly582Gly	80.06366
C1c	4	72266	C	synonymous_variant	LOW	GDH2	p.Lys551Lys	80.30042
C1c	4	72953	T	synonymous_variant	LOW	GDH2	p.Glu322Glu	88.14998
C1c	4	74013	G	upstream_gene_variant	MODIFIER	RRI1		91.47491
C1c	4	74079	C	upstream_gene_variant	MODIFIER	RRI1		97.32866
C1c	4	74090	C	upstream_gene_variant	MODIFIER	RRI1		95.08527
C1c	4	74428	G	upstream_gene_variant	MODIFIER	RRI1		99.66641
C1c	4	74440	C	upstream_gene_variant	MODIFIER	RRI1		91.89538
C1c	4	75577	G	synonymous_variant	LOW	PRR2	p.Ser323Ser	103.1674
C1c	4	75618	C	missense_variant	MODERATE	PRR2	p.Ile310Val	98.47564
C1c	4	75662	G	missense_variant	MODERATE	PRR2	p.Asn295Thr	97.79535
C1c	4	75880	A	missense_variant	MODERATE	PRR2	p.Glu222Asp	120.0276
C1c	4	76252	A	missense_variant	MODERATE	PRR2	p.Lys98Asn	99.04071
C1c	4	76418	T	missense_variant	MODERATE	PRR2	p.Gly43Asp	79.54663
C1c	4	76541	A	missense_variant	MODERATE	PRR2	p.Ser2Leu	83.20264
C1c	4	78578	A	synonymous_variant	LOW	SHR3	p.Leu51Leu	91.91313
C1c	4	78587	T	synonymous_variant	LOW	SHR3	p.Tyr54Tyr	91.58407
C1c	4	78617	C	synonymous_variant	LOW	SHR3	p.Val64Val	87.25537
C1c	4	78644	A	synonymous_variant	LOW	SHR3	p.Gly73Gly	98.19852
C1c	4	78728	T	synonymous_variant	LOW	SHR3	p.Tyr101Tyr	97.42586
C1c	4	78875	A	synonymous_variant	LOW	SHR3	p.Val150Val	81.0627
C1c	4	79541	A	missense_variant	MODERATE	YDL211C	p.Thr291Ile	95.47002
C1c	4	79660	A	missense_variant	MODERATE	YDL211C	p.Lys251Asn	90.00475
C1c	4	79687	A	synonymous_variant	LOW	YDL211C	p.Ile242Ile	89.20881
C1c	4	79782	C	missense_variant	MODERATE	YDL211C	p.Thr211Ala	116.0041
C1c	4	79801	G	synonymous_variant	LOW	YDL211C	p.Gly204Gly	115.6847
C1c	4	79852	G	synonymous_variant	LOW	YDL211C	p.Ile187Ile	128.8134
C1c	4	79934	A	missense_variant	MODERATE	YDL211C	p.Thr160Ile	91.67378
C1c	4	80089	A	synonymous_variant	LOW	YDL211C	p.Phe108Phe	100.1219
C1c	4	80130	C	missense_variant	MODERATE	YDL211C	p.Ile95Val	110.2294
C1c	4	80264	T	missense_variant	MODERATE	YDL211C	p.Thr50Lys	101.7757
C1c	4	80419	A	upstream_gene_variant	MODIFIER	PRR2		80.58384
C1c	4	80483	G	upstream_gene_variant	MODIFIER	PRR2		84.83409
C1c	4	80506	C	upstream_gene_variant	MODIFIER	PRR2		91.26576
C1c	4	80546	G	upstream_gene_variant	MODIFIER	PRR2		90.05691
C1c	4	80679	C	upstream_gene_variant	MODIFIER	PRR2		92.96599

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C1c	4	80753	C	upstream_gene_variant	MODIFIER	PRR2		109.8948
C1c	4	80784	A	upstream_gene_variant	MODIFIER	PRR2		99.40504
C1c	4	80820	A	upstream_gene_variant	MODIFIER	PRR2		107.4414
C1c	4	80852	G	upstream_gene_variant	MODIFIER	PRR2		102.8571
C1c	4	80858	A	upstream_gene_variant	MODIFIER	PRR2		100.5412
C1c	4	80893	C	upstream_gene_variant	MODIFIER	PRR2		103.7933
C1c	4	80961	A	upstream_gene_variant	MODIFIER	PRR2		97.71987
C1c	4	80964	A	upstream_gene_variant	MODIFIER	PRR2		97.72402
C1c	4	81001	G	upstream_gene_variant	MODIFIER	PRR2		98.72643
C1c	4	81145	C	upstream_gene_variant	MODIFIER	PRR2		94.72701
C1c	4	81156	G	upstream_gene_variant	MODIFIER	PRR2		93.90474
C1c	4	81181	G	upstream_gene_variant	MODIFIER	PRR2		101.0106
C1c	4	81257	G	upstream_gene_variant	MODIFIER	PRR2		85.54431
C1c	4	81648	A	upstream_gene_variant	MODIFIER	NOP6		85.0362
C1c	4	81963	A	upstream_gene_variant	MODIFIER	NOP6		88.55808
C1c	4	82113	T	upstream_gene_variant	MODIFIER	NOP6		87.37944
C1c	4	82114	T	upstream_gene_variant	MODIFIER	NOP6		87.97663
C1c	4	82548	C	upstream_gene_variant	MODIFIER	NOP6		82.73204
C1c	4	82807	C	upstream_gene_variant	MODIFIER	NOP6		106.9218
C1c	4	82949	C	upstream_gene_variant	MODIFIER	NOP6		107.4386
C1c	4	83002	A	upstream_gene_variant	MODIFIER	YDL211C		94.17113
C1c	4	83205	T	upstream_gene_variant	MODIFIER	YDL211C		97.4871
C1c	4	83270	G	upstream_gene_variant	MODIFIER	YDL211C		104.317
C1c	4	83299	T	upstream_gene_variant	MODIFIER	YDL211C		109.7696
C1c	4	83327	T	upstream_gene_variant	MODIFIER	YDL211C		96.42072
C1c	4	83501	A	upstream_gene_variant	MODIFIER	YDL211C		95.55448
C1c	4	83513	C	upstream_gene_variant	MODIFIER	YDL211C		102.318
C1c	4	83830	T	upstream_gene_variant	MODIFIER	YDL211C		84.33744
C1c	4	84159	C	upstream_gene_variant	MODIFIER	YDL211C		91.22244
C1c	4	84201	C	upstream_gene_variant	MODIFIER	YDL211C		100.4255
C1c	4	84392	C	synonymous_variant	LOW	UGA4	p.Thr41Thr	86.81913
C1c	4	84422	C	synonymous_variant	LOW	UGA4	p.Ala51Ala	114.4966
C1c	4	84632	T	synonymous_variant	LOW	UGA4	p.Leu121Leu	100.6232
C1c	4	84857	T	synonymous_variant	LOW	UGA4	p.Val196Val	97.95503
C1c	4	85163	C	synonymous_variant	LOW	UGA4	p.His298His	82.8825
C1c	4	85610	A	synonymous_variant	LOW	UGA4	p.Leu447Leu	92.65552
C1c	4	85677	C	synonymous_variant	LOW	UGA4	p.Leu470Leu	100.2152

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C1c	4	85721	C	synonymous_variant	LOW	UGA4	p.Pro484Pro	105.1331
C1c	4	85842	G	missense_variant	MODERATE	UGA4	p.Ile525Val	83.17814
C1c	4	86153	T	upstream_gene_variant	MODIFIER	NHP2		90.18666
C1c	4	86178	G	upstream_gene_variant	MODIFIER	NHP2		80.40926
C1c	4	86337	C	missense_variant	MODERATE	CWC2	p.Glu297Gly	92.60865
C1c	4	86372	T	synonymous_variant	LOW	CWC2	p.Val285Val	85.26164
C1c	4	86660	C	synonymous_variant	LOW	CWC2	p.Gln189Gln	82.06951
C1c	4	86767	T	missense_variant	MODERATE	CWC2	p.Ala154Thr	91.58603
C1c	4	86774	T	synonymous_variant	LOW	CWC2	p.Leu151Leu	90.24543
C1c	4	87011	A	synonymous_variant	LOW	CWC2	p.Phe72Phe	105.9608
C1c	4	87023	A	synonymous_variant	LOW	CWC2	p.Gly68Gly	117.8206
C1c	4	87260	G	upstream_gene_variant	MODIFIER	CWC2		103.3205
C1c	4	87919	T	missense_variant	MODERATE	NHP2	p.Lys136Asn	85.59221
C1c	4	88611	A	missense_variant	MODERATE	GLE1	p.Pro122Thr	81.45873
C1c	4	90266	G	missense_variant	MODERATE	YDL206W	p.Ile31Val	90.28123
C1c	4	90700	G	synonymous_variant	LOW	YDL206W	p.Ala175Ala	85.05766
C1c	4	91651	T	synonymous_variant	LOW	YDL206W	p.Tyr492Tyr	84.57039
C1c	4	91968	C	missense_variant	MODERATE	YDL206W	p.Leu598Ser	82.57616
C1c	4	92098	C	synonymous_variant	LOW	YDL206W	p.Asp641Asp	86.42096
C1c	4	94319	T	upstream_gene_variant	MODIFIER	HEM3		90.5734
C1c	4	94411	A	upstream_gene_variant	MODIFIER	HEM3		81.39285
C1c	4	94690	G	missense_variant	MODERATE	RTN2	p.Asn29Ser	98.99651
C1c	4	95036	A	synonymous_variant	LOW	RTN2	p.Leu144Leu	87.3768
C1c	4	96799	C	synonymous_variant	LOW	ACK1	p.Pro385Pro	84.33925
C1c	4	97156	T	synonymous_variant	LOW	ACK1	p.Leu266Leu	99.83955
C1c	4	97262	A	missense_variant	MODERATE	ACK1	p.Ser231Phe	92.20715
C1c	4	97285	C	synonymous_variant	LOW	ACK1	p.Ser223Ser	99.06126
C1c	4	97330	A	synonymous_variant	LOW	ACK1	p.Asn208Asn	98.54586
C1c	4	97693	T	synonymous_variant	LOW	ACK1	p.Gly87Gly	128.4447
C1c	4	97828	G	synonymous_variant	LOW	ACK1	p.Pro42Pro	86.08574
C1c	4	97927	C	synonymous_variant	LOW	ACK1	p.Pro9Pro	92.50729
C1c	4	98318	T	upstream_gene_variant	MODIFIER	HEM3		91.3215
C1c	4	98562	C	missense_variant	MODERATE	MRPL11	p.Phe30Leu	112.715
C1c	4	99630	G	missense_variant	MODERATE	TRM8	p.Asn24Asp	89.94002
C1c	4	99781	G	missense_variant	MODERATE	TRM8	p.Gln74Arg	96.23752
C1c	4	99908	A	synonymous_variant	LOW	TRM8	p.Ala116Ala	82.4918
C2a	12	399483	A	upstream_gene_variant	MODIFIER	YLR126C		106.1753

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
C2a	12	399513	A	upstream_gene_variant	MODIFIER	YLR126C		84.47153
C2c	12	399483	A	upstream_gene_variant	MODIFIER	YLR126C		121.2158
C2c	12	399513	A	upstream_gene_variant	MODIFIER	YLR126C		88.95991
C2c	12	424103	G	missense_variant	MODERATE	RRN5	p.Thr141Ala	82.33565
R1a	4	520955	G	upstream_gene_variant	MODIFIER	YDR034C-D		114.1554
R1a	4	520956	T	upstream_gene_variant	MODIFIER	YDR034C-D		113.8651
R1a	4	523585	A	missense_variant	MODERATE	EHD3	p.His377Tyr	78.19606
R1a	4	526558	C	synonymous_variant	LOW	KRS1	p.Ile373Ile	79.697
R1a	4	526864	G	synonymous_variant	LOW	KRS1	p.Leu475Leu	77.16579
R1a	4	526927	A	synonymous_variant	LOW	KRS1	p.Lys496Lys	77.85547
R1a	4	526942	A	synonymous_variant	LOW	KRS1	p.Ala501Ala	81.79141
R1a	4	527002	G	synonymous_variant	LOW	KRS1	p.Gln521Gln	79.01902
R1a	4	527056	C	synonymous_variant	LOW	KRS1	p.Ala539Ala	147.2882
R1a	4	527080	C	synonymous_variant	LOW	KRS1	p.Thr547Thr	85.46554
R1a	4	527095	C	synonymous_variant	LOW	KRS1	p.Cys552Cys	84.19345
R1a	4	527108	T	synonymous_variant	LOW	KRS1	p.Leu557Leu	89.16248
R1a	4	527122	A	synonymous_variant	LOW	KRS1	p.Leu561Leu	84.82915
R1a	4	527128	T	synonymous_variant	LOW	KRS1	p.Asp563Asp	84.97659
R1a	4	527134	T	synonymous_variant	LOW	KRS1	p.Asn565Asn	86.41589
R1a	4	527179	C	synonymous_variant	LOW	KRS1	p.Val580Val	78.39516
R1a	4	539061	T	upstream_gene_variant	MODIFIER	ENA2		78.37517
R1a	4	539068	G	upstream_gene_variant	MODIFIER	ENA2		81.93324
R1a	4	539123	T	upstream_gene_variant	MODIFIER	ENA2		83.33778
R1a	4	540228	T	synonymous_variant	LOW	RSM10	p.Phe142Phe	190.6178
R1a	4	540965	C	missense_variant	MODERATE	YDR042C	p.Thr80Ser	85.43279
R1a	4	542170	T	upstream_gene_variant	MODIFIER	ENA1		106.1966
R1a	4	543278	C	missense_variant	MODERATE	NRG1	p.Glu31Gly	116.4681
R1a	4	543502	T	upstream_gene_variant	MODIFIER	YDR042C		92.28692
R1a	4	545265	T	upstream_gene_variant	MODIFIER	YDR042C		117.5308
R1a	4	545330	C	upstream_gene_variant	MODIFIER	YDR042C		113.1582
R1a	4	547565	C	synonymous_variant	LOW	HEM13	p.Ala308Ala	124.3932
R1a	4	547646	A	upstream_gene_variant	MODIFIER	NRG1		120.9063
R1a	4	547728	G	upstream_gene_variant	MODIFIER	NRG1		110.8768
R1a	4	547747	T	upstream_gene_variant	MODIFIER	NRG1		103.1199
R1a	4	547789	C	upstream_gene_variant	MODIFIER	NRG1		90.99699
R1a	4	547794	T	upstream_gene_variant	MODIFIER	NRG1		91.63565
R1a	4	547848	A	upstream_gene_variant	MODIFIER	NRG1		102.2006

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
R1a	4	547907	A	upstream_gene_variant	MODIFIER	NRG1		131.1624
R1a	4	548255	T	missense_variant	MODERATE	RPC11	p.Ser19Asn	106.1803
R1a	4	549184	T	missense_variant	MODERATE	BAP3	p.Ser465Thr	129.7086
R1a	4	549263	A	synonymous_variant	LOW	BAP3	p.Phe438Phe	114.1688
R1a	4	549376	G	missense_variant	MODERATE	BAP3	p.Met401Leu	128.1969
R1a	4	549536	G	synonymous_variant	LOW	BAP3	p.Asp347Asp	103.9459
R1a	4	549744	T	missense_variant	MODERATE	BAP3	p.Gly278Asp	118.1393
R1a	4	557161	G	synonymous_variant	LOW	DET1	p.Pro300Pro	81.34738
R1b	4	520955	G	upstream_gene_variant	MODIFIER	YDR034C-D		102.2389
R1b	4	520956	T	upstream_gene_variant	MODIFIER	YDR034C-D		101.4919
R1b	4	540228	T	synonymous_variant	LOW	RSM10	p.Phe142Phe	161.7614
R1b	4	549376	G	missense_variant	MODERATE	BAP3	p.Met401Leu	78.64063
R2a	4	801527	T	synonymous_variant	LOW	SEC7	p.Glu232Glu	79.77403
R2a	4	801845	A	synonymous_variant	LOW	SEC7	p.Ser126Ser	76.86367
R2a	4	806992	C	synonymous_variant	LOW	HSP42	p.Tyr124Tyr	88.14499
R2a	4	808113	G	upstream_gene_variant	MODIFIER	SUP35		77.69971
R2a	4	812053	A	upstream_gene_variant	MODIFIER	ARG82		86.47141
R2a	4	826151	A	missense_variant	MODERATE	SAS4	p.Asn402Ile	86.21115
R2b	4	806513	C	upstream_gene_variant	MODIFIER	SEC7		81.30189
R2b	4	806514	C	upstream_gene_variant	MODIFIER	SEC7		79.06495
R3a	15	143876	G	upstream_gene_variant	MODIFIER	COQ3		91.11845
R3a	15	143890	C	upstream_gene_variant	MODIFIER	COQ3		91.24689
R3a	15	143915	G	upstream_gene_variant	MODIFIER	COQ3		81.50943
R3a	15	146782	G	synonymous_variant	LOW	SPO21	p.Glu483Glu	82.0003
R3a	15	147591	T	synonymous_variant	LOW	MSH2	p.Asp70Asp	90.00039
R3a	15	147729	T	synonymous_variant	LOW	MSH2	p.Ile116Ile	83.04413
R3a	15	147735	T	synonymous_variant	LOW	MSH2	p.Ser118Ser	86.0322
R3a	15	147750	T	synonymous_variant	LOW	MSH2	p.Asn123Asn	90.2056
R3a	15	147783	C	synonymous_variant	LOW	MSH2	p.Asn134Asn	101.7991
R3a	15	147840	G	synonymous_variant	LOW	MSH2	p.Gly153Gly	118.9555
R3a	15	148362	A	synonymous_variant	LOW	MSH2	p.Thr327Thr	78.71812
R3a	15	150501	G	missense_variant	MODERATE	HAL9	p.Gly997Ala	81.16249
R3a	15	150554	G	synonymous_variant	LOW	HAL9	p.Thr979Thr	81.97963
R3a	15	150756	A	missense_variant	MODERATE	HAL9	p.Glu912Val	76.93276
R3a	15	150803	A	synonymous_variant	LOW	HAL9	p.Ala896Ala	80.23927
R3a	15	151265	G	synonymous_variant	LOW	HAL9	p.Ser742Ser	88.96558
R3a	15	151772	G	synonymous_variant	LOW	HAL9	p.Asp573Asp	86.87204

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(p)
R3a	15	152215	C	missense_variant	MODERATE	HAL9	p.Ile426Val	117.9997
R3a	15	152922	T	missense_variant	MODERATE	HAL9	p.Gly190Glu	89.39567
R3a	15	153116	G	synonymous_variant	LOW	HAL9	p.Gly125Gly	84.21021
R3a	15	153242	T	missense_variant	MODERATE	HAL9	p.Met83Ile	87.71814
R3a	15	153362	A	synonymous_variant	LOW	HAL9	p.Thr43Thr	80.8655
R3a	15	153722	C	upstream_gene_variant	MODIFIER	HAL9		79.3022
R3a	15	153761	G	upstream_gene_variant	MODIFIER	HAL9		78.58049
R3a	15	154310	C	missense_variant	MODERATE	MPD2	p.Lys146Glu	81.048
R3a	15	155276	T	upstream_gene_variant	MODIFIER	HAL9		87.38068
R3b	15	138539	A	synonymous_variant	LOW	COQ3	p.Asp169Asp	77.97469
R3b	15	139013	G	synonymous_variant	LOW	COQ3	p.Ile11Ile	79.76749
R3b	15	139015	C	missense_variant	MODERATE	COQ3	p.Ile11Val	79.58557
R3b	15	139408	C	missense_variant	MODERATE	HMI1	p.Asn647Ser	91.78263
R3b	15	141731	C	synonymous_variant	LOW	RFC4	p.Lys275Lys	92.4815
R3b	15	142001	T	synonymous_variant	LOW	RFC4	p.Lys185Lys	83.63171
R3b	15	142645	G	upstream_gene_variant	MODIFIER	VRS1		78.52592
R3b	15	142732	A	upstream_gene_variant	MODIFIER	VRS1		81.09175
R3b	15	142835	G	missense_variant	MODERATE	TRM10	p.Asn7Lys	83.49645
R3b	15	143022	G	missense_variant	MODERATE	TRM10	p.Ile70Val	83.52279
R3b	15	143401	G	missense_variant	MODERATE	TRM10	p.Lys196Arg	91.59821
R3b	15	143405	A	synonymous_variant	LOW	TRM10	p.Leu197Leu	93.37912
R3b	15	143696	A	splice_region_variant & stop_retained_variant	LOW	TRM10	p.Ter294Ter	86.2191
R3b	15	143787	T	upstream_gene_variant	MODIFIER	COQ3		80.77236
R3b	15	143876	G	upstream_gene_variant	MODIFIER	COQ3		102.5584
R3b	15	143890	C	upstream_gene_variant	MODIFIER	COQ3		102.5628
R3b	15	143915	G	upstream_gene_variant	MODIFIER	COQ3		95.9893
R3b	15	144354	A	missense_variant	MODERATE	YPQ1	p.Val511Ile	90.99127
R3b	15	146782	G	synonymous_variant	LOW	SPO21	p.Glu483Glu	96.92236
R3b	15	147162	A	splice_region_variant & stop_retained_variant	LOW	SPO21	p.Ter610Ter	82.77074
R3b	15	147591	T	synonymous_variant	LOW	MSH2	p.Asp70Asp	96.46809
R3b	15	147729	T	synonymous_variant	LOW	MSH2	p.Ile116Ile	90.02517
R3b	15	147735	T	synonymous_variant	LOW	MSH2	p.Ser118Ser	93.93212
R3b	15	147750	T	synonymous_variant	LOW	MSH2	p.Asn123Asn	104.0361
R3b	15	147783	C	synonymous_variant	LOW	MSH2	p.Asn134Asn	120.867
R3b	15	147840	G	synonymous_variant	LOW	MSH2	p.Gly153Gly	147.1585

Peak	Chr	Position	Alternate allele	Annotation	Effect	Gene	Amino acid substitution	-log10(*p*)
R3b	15	148176	T	synonymous_variant	LOW	MSH2	p.Cys265Cys	94.9254
R3b	15	148362	A	synonymous_variant	LOW	MSH2	p.Thr327Thr	89.36349
R3b	15	148704	T	synonymous_variant	LOW	MSH2	p.Pro441Pro	77.07497
R3b	15	150501	G	missense_variant	MODERATE	HAL9	p.Gly997Ala	94.40134
R3b	15	150554	G	synonymous_variant	LOW	HAL9	p.Thr979Thr	91.99713
R3b	15	150756	A	missense_variant	MODERATE	HAL9	p.Glu912Val	88.98567
R3b	15	150803	A	synonymous_variant	LOW	HAL9	p.Ala896Ala	86.3389
R3b	15	150859	A	synonymous_variant	LOW	HAL9	p.Leu878Leu	83.67315
R3b	15	151265	G	synonymous_variant	LOW	HAL9	p.Ser742Ser	107.2426
R3b	15	151772	G	synonymous_variant	LOW	HAL9	p.Asp573Asp	89.75271
R3b	15	152215	C	missense_variant	MODERATE	HAL9	p.Ile426Val	121.468
R3b	15	152922	T	missense_variant	MODERATE	HAL9	p.Gly190Glu	103.3301
R3b	15	153116	G	synonymous_variant	LOW	HAL9	p.Gly125Gly	101.4599
R3b	15	153242	T	missense_variant	MODERATE	HAL9	p.Met83Ile	110.6201
R3b	15	153362	A	synonymous_variant	LOW	HAL9	p.Thr43Thr	103.3218
R3b	15	153516	A	upstream_gene_variant	MODIFIER	HAL9		80.7814
R3b	15	153722	C	upstream_gene_variant	MODIFIER	HAL9		101.5538
R3b	15	153733	A	upstream_gene_variant	MODIFIER	HAL9		80.51727
R3b	15	153740	G	upstream_gene_variant	MODIFIER	HAL9		80.53226
R3b	15	153761	G	upstream_gene_variant	MODIFIER	HAL9		100.8128
R3b	15	154056	C	synonymous_variant	LOW	MPD2	p.Arg230Arg	90.55246
R3b	15	154073	A	synonymous_variant	LOW	MPD2	p.Leu225Leu	85.12242
R3b	15	154310	C	missense_variant	MODERATE	MPD2	p.Lys146Glu	93.18563
R3b	15	155276	T	upstream_gene_variant	MODIFIER	HAL9		101.6321
R3b	15	157540	A	synonymous_variant	LOW	DUF1	p.His366His	82.7514
R3b	15	158074	C	synonymous_variant	LOW	DUF1	p.Lys188Lys	79.13539
R3b	15	158575	T	synonymous_variant	LOW	DUF1	p.Ala21Ala	84.53663
R3b	15	158650	G	upstream_gene_variant	MODIFIER	MPD2		78.59774
R4a	16	881189	T	upstream_gene_variant	MODIFIER	MRP2		77.9704
R4b	16	832084	C	missense_variant	MODERATE	URN1	p.Asn459Asp	87.74308
R4b	16	833382	T	missense_variant	MODERATE	URN1	p.Arg26Gln	82.07888
R4b	16	837416	C	upstream_gene_variant	MODIFIER	URN1		81.07874
R4b	16	839499	C	synonymous_variant	LOW	TPO3	p.Leu93Leu	78.75222
R4b	16	841702	G	missense_variant	MODERATE	TDA6	p.His146Arg	78.48837
R4b	16	865361	T	synonymous_variant	LOW	SGV1	p.Arg354Arg	76.88374

Appendix C: Genes that fall under the six major peaks of this study

Table C.1: **Genes that fall under the 6 major peaks of this study.** We observed a total of 58 genes under the Roundup-specific peaks, and 44 genes under the constant-specific peak. Peaks are organized by peak type (C = constant-specific; R= Roundup-specific) as well as by CMH test type (a=constant vs. control; b=increasing vs. control; c=constant vs. increasing). When a gene is included in more than one peak (e.g. C1a/c), it is indicated as such. Bold font indicate genes under peaks that were identified in our study and also by Ravishankar et al. (2020) in their classic QTL and/or RNA-seq experiments, but did not contain a significant SNP within the gene bounds in our results. Red font indicates genes that were identified again in both our experiment and the previously mentioned study, but did contain a significant SNP within the gene bounds. Gene details were provided by the Saccharomyces Genome Database (SGD) YeastMine tool.

Peak	Chr	Start Position	End Position	Systematic Gene Name	Gene Feature Type	Standard Gene Name	Full Gene Name
C1a/c	4	100501	101067	YDL200C	ORF	MGT1	O-6-MethylGuanine-DNA methylTransferase
C1a/c	4	99561	100421	YDL201W	ORF	TRM8	Transfer RNA Methyltransferase
C1a/c	4	98475	99224	YDL202W	ORF	MRPL11	Mitochondrial Ribosomal Protein, Large subunit
C1a/c	4	96082	97953	YDL203C	ORF	ACK1	Activator of C Kinase 1
C1a/c	**4**	**94605**	**95786**	**YDL204W**	**ORF**	**RTN2**	**ReTiculoN-like**
C1a/c	4	92762	93745	YDL205C	ORF	HEM3	HEMe biosynthesis
C1a/c	4	90176	92464	YDL206W	ORF		
C1a/c	4	88248	89864	YDL207W	ORF	GLE1	GLFG (glycine-leucine-phenylalanine-glycine) LEthal
C1a/c	4	87512	87982	YDL208W	ORF	NHP2	Non-Histone Protein
C1a/c	4	86207	87226	YDL209C	ORF	CWC2	Complexed With Cef1p
C1a/c	4	84270	85985	YDL210W	ORF	UGA4	Utilization of GAba
C1a/c	4	79294	80412	YDL211C	ORF		
C1a/c	4	78426	79058	YDL212W	ORF	SHR3	Super high Histidine Resistant
C1a/c	4	77289	77966	YDL213C	ORF	NOP6	NucleOlar Protein
C1a/c	**4**	**74446**	**76545**	**YDL214C**	**ORF**	**PRR2**	**Pheromone Response Regulator**
C1a/c	4	70640	73918	YDL215C	ORF	GDH2	Glutamate DeHydrogenase
C1a/c	4	68997	70319	YDL216C	ORF	RRI1	Regulator of Rub1 specific Isopeptidase
C1a/c	4	67983	68606	YDL217C	ORF	TIM22	Translocase of the Inner Mitochondrial membrane
C1a/c	4	83548	83618	YNCD0001W	tRNA gene		
C1c	4	122216	123589	YDL189W	ORF	RBS1	RNA-Binding Suppressor of PAS kinase
C1c	4	118707	121592	YDL190C	ORF	UFD2	Ubiquitin Fusion Degradation
C1c	4	117664	118517	YDL191W	ORF	RPL35A	Ribosomal Protein of the Large subunit
C1c	**4**	**116321**	**116866**	**YDL192W**	**ORF**	**ARF1**	**ADP-Ribosylation Factor**

Peak	Chr	Start Position	End Position	Systematic Gene Name	Gene Feature Type	Standard Gene Name	Full Gene Name
C1c	4	114672	115799	YDL193W	ORF	NUS1	Nuclear Undecaprenyl pyrophosphate Synthase
C1c	4	111580	114234	YDL194W	ORF	SNF3	Sucrose NonFermenting
C1c	4	107208	111029	YDL195W	ORF	SEC31	SECretory
C1c	4	106741	107070	YDL196W	ORF		
C1c	4	104917	106494	YDL197C	ORF	ASF2	Anti-Silencing Function
C1c	4	103649	104551	YDL198C	ORF	GGC1	GDP/GTP Carrier
C1c	4	101290	103353	YDL199C	ORF		
C1c	4	66493	67446	YDL218W	ORF		
C1c	4	65242	65765	YDL219W	ORF	DTD1	D-Tyr-tRNA(Tyr) Deacylase
C2c	12	399657	402488	YLR129W	ORF	DIP2	DOM34 Interacting Protein
C2c	**12**	**402794**	**404062**	**YLR130C**	**ORF**	**ZRT2**	**Zinc-Regulated Transporter**
C2c	12	404510	406822	YLR131C	ORF	ACE2	Activator of CUP1 Expression
C2c	**12**	**407283**	**408155**	**YLR132C**	**ORF**	**USB1**	**U Six Biogenesis**
C2c	**12**	**408445**	**410193**	**YLR133W**	**ORF**	**CKI1**	**Choline KInase**
C2c	**12**	**410723**	**412414**	**YLR134W**	**ORF**	**PDC5**	**Pyruvate DeCarboxylase**
C2c	12	413281	415527	YLR135W	ORF	SLX4	Synthetic Lethal of unknown (X) function
C2c	12	415801	416658	YLR136C	ORF	TIS11	similar to the mammalian TPA Induced Sequence gene family
C2c	12	417006	418109	YLR137W	ORF	RKM5	Ribosomal lysine (K) Methyltransferase 5
C2c	12	418437	421394	YLR138W	ORF	NHA1	Na+/H+ Antiporter
C2c	12	421542	423473	YLR139C	ORF	SLS1	Synthetic Lethal with SSM4
C2c	12	423474	423800	YLR140W	ORF		
R1a/b	**4**	**521816**	**522928**	**YDR035W**	**ORF**	**ARO3**	**AROmatic amino acid requiring**
R1a/b	4	523211	524713	YDR036C	ORF	EHD3	
R1a/b	4	525440	527215	YDR037W	ORF	KRS1	Lysyl (K) tRNA Synthetase
R1a/b	4	527422	530697	YDR038C	ORF	ENA5	Exitus NAtru (Latin, "exit sodium")
R1a/b	4	531307	534582	YDR039C	ORF	ENA2	Exitus NAtru (Latin, "exit sodium")
R1a/b	4	535192	538467	YDR040C	ORF	ENA1	Exitus NAtru (Latin, "exit sodium")
R1a/b	**4**	**539803**	**540414**	**YDR041W**	**ORF**	**RSM10**	**Ribosomal Small subunit of Mitochondria**
R1a/b	4	540601	541203	YDR042C	ORF		
R1a/b	4	542674	543369	YDR043C	ORF	NRG1	Negative Regulator of Glucose-repressed genes
R1a/b	4	546642	547628	YDR044W	ORF	HEM13	HEMe biosynthesis
R1a/b	4	547978	548310	YDR045C	ORF	RPC11	RNA Polymerase C
R1a	4	548762	550576	YDR046C	ORF	BAP3	Branched-chain Amino acid Permease
R1a	**4**	**551860**	**552948**	**YDR047W**	**ORF**	**HEM12**	**HEMe biosynthesis**

Peak	Chr	Start Position	End Position	Systematic Gene Name	Gene Feature Type	Standard Gene Name	Full Gene Name
R1a	4	553084	553398	YDR048C	ORF		
R1a	**4**	**553254**	**555152**	**YDR049W**	**ORF**	**VMS1**	**VCP/Cdc48-associated Mitochondrial Stress-responsive**
R1a	**4**	**555726**	**556472**	**YDR050C**	**ORF**	**TPI1**	**Triose-Phosphate Isomerase**
R1a/b	4	541602	541700	YNCD0010C	snoRNA gene	SNR47	Small Nucleolar RNA
R1a/b	4	520972	521043	YNCD0009W	tRNA gene		
R1a/b	4	521314	521469	YDR034W-B	ORF		
R2a	4	806621	807748	YDR171W	ORF	HSP42	Heat Shock Protein
R2a	4	808324	810381	YDR172W	ORF	SUP35	SUPpressor
R2a	4	810565	811632	YDR173C	ORF	ARG82	ARGinine requiring
R2a	4	812110	812850	YDR174W	ORF	HMO1	High MObility group (HMG) family
R2a	4	813193	814152	YDR175C	ORF	RSM24	Ribosomal Small subunit of Mitochondria
R2a	4	814452	816560	YDR176W	ORF	NGG1	
R2a	4	816878	817525	YDR177W	ORF	UBC1	UBiquitin-Conjugating
R2a	4	817950	818495	YDR178W	ORF	SDH4	Succinate DeHydrogenase
R2a	**4**	**818708**	**819196**	**YDR179C**	**ORF**	**CSN9**	**Cop9 SigNalosome subunit**
R2a	4	819433	820824	YDR179W-A	ORF	NVJ3	Nucleus-Vacuole Junction
R2a	4	821295	825776	YDR180W	ORF	SCC2	Sister Chromatid Cohesion
R2a	4	802731	802802	YNCD0016C	tRNA gene		
R2a	4	803195	804517	YDR170W-A	transposable element gene		
R3a/b	15	153912	154745	YOL088C	ORF	MPD2	Multicopy suppressor of PDI1 deletion
R3a/b	15	150398	153490	YOL089C	ORF	HAL9	HALotolerance
R3a/b	15	147382	150276	YOL090W	ORF	MSH2	MutS Homolog
R3a/b	15	145334	147163	YOL091W	ORF	SPO21	SPOrulation
R3a/b	15	144204	145130	YOL092W	ORF	YPQ1	Yeast PQ-loop protein
R3b	15	155287	156637	YOL087C	ORF	DUF1	DUB-associated Factor 1
R3b	15	142815	143696	YOL093W	ORF	TRM10	Transfer RNA Methyltransferase
R3b	15	141584	142555	YOL094C	ORF	RFC4	Replication Factor C
R3b	15	139227	141347	YOL095C	ORF	HMI1	Helicase in Mitochondria
R4b	16	833689	834245	YPR153W	ORF	MAY24	genetic interaction profile similarity to MTC Annotated Yeast genes MTC2 and MTC4
R4b	16	834565	835212	YPR154W	ORF	PIN3	Psi+ INducibility
R4b	16	835563	837413	YPR155C	ORF	NCA2	Nuclear Control of ATPase
R4b	16	837909	839777	YPR156C	ORF	TPO3	Transporter of POlyamines
R4b	16	841266	842669	YPR157W	ORF	TDA6	Topoisomerase I Damage Affected

Peak	Chr	Start Position	End Position	Systematic Gene Name	Gene Feature Type	Standard Gene Name	Full Gene Name
R4b	16	843262	844020	YPR158W	ORF	CUR1	Curing of [URe3]
R4b	16	857583	859745	YPR159W	ORF	KRE6	Killer toxin REsistant
R4b	16	861306	864014	YPR160W	ORF	GPH1	Glycogen PHosphorylase
R4b	16	856902	856974	YNCP0022W	tRNA gene		
R4b	16	860379	860449	YNCP0023W	tRNA gene		
R4b	16	854935	856257	YPR158C-C	transposable element gene		
R4b	16	850989	856257	YPR158C-D	transposable element gene		
R4b	16	844709	846031	YPR158W-A	transposable element gene		
R4b	16	844709	849980	YPR158W-B	transposable element gene		
R4b	16	860314	860415	YPR159C-A	ORF		
R4b	16	862577	862861	YPR160C-A	ORF		
R4b	16	861934	862014	YPR160W-A	ORF		